Environmental Health Criteria 241

DDT IN INDOOR RESIDUAL SPRAYING: HUMAN HEALTH ASPECTS

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

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ACRONYMS AND ABBREVIATIONS

ΣDDT	sum of DDT and its derivatives as measured in any particular study ("total DDT")
a.i.	active ingredient
AIC	Akaike's information criterion
ADI	acceptable daily intake
ALAT	alanine aminotransferase
AR	androgen receptor
ASAT	aspartate aminotransferase
BMD	benchmark dose
BMD_{10}	benchmark dose for a 10% response
BMDL	lower 95% confidence limit on the benchmark dose
$BMDL_{10}$	lower 95% confidence limit on the benchmark dose for a 10% response
BMI	body mass index
BNBAS	Brazelton Neonatal Behavioural Assessment Scale
bw	body weight
cAMP	cyclic adenosine monophosphate
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CFV	control flow valve
CI	confidence interval
CYP	cytochrome P-450
DAT	dopamine transporter
DDA	2,2-bis(*p*-chlorophenyl) acetic acid
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDMU	1-chloro-2,2-bis(*p*-chlorophenyl)ethene
DDOH	2,2-bis(4-chlorophenyl)ethanol
DDOH-PA	DDOH-palmitic acid
DDT	dichlorodiphenyltrichloroethane
DES	diethylstilbestrol
df	degrees of freedom
DNA	deoxyribonucleic acid
dUTP	deoxyuridine-5′-triphosphate
E1C	estrone conjugate
ER	estrogen receptor
F	female; filial generation
FAO	Food and Agriculture Organization of the United Nations
FSH	follicle stimulating hormone

GD	gestation day
GGT	gamma-glutamyltransferase; gamma-glutamyl transpeptidase
GST-P	glutathione *S*-transferase placental form
HCDD	1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin
HCH	hexachlorocyclohexane
IARC	International Agency for Research on Cancer
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL-1R1	interleukin-1 receptor type 1
IL-4	interleukin-4
IPCS	International Programme on Chemical Safety (WHO)
IQ	intelligence quotient
IRS	indoor residual spraying
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
M	male
MDI	Mental Developmental Index
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
NBAS	Neonatal Behavioural Assessment Scale
NCI	National Cancer Institute (USA)
ND	not determined; not detected
NHANES	National Health and Nutrition Examination Survey (USA)
NHATS	National Human Adipose Tissue Survey (USA)
NHL	non-Hodgkin lymphoma
NOAEL	no-observed-adverse-effect level
NS	not significant
OR	odds ratio
P	probability
P	parental generation
PCB	polychlorinated biphenyl
PCB-153	2,2′,4,4′,5,5′-hexachlorobiphenyl

PdG	pregnanediol-3-glucuronide
PDI	Psychomotor Developmental Index
PMR	proportionate mortality ratio
PND	postnatal day
PPE	personal protective equipment
PXR	pregnane X receptor
Q	quartile
ROS	reactive oxygen species
RR	relative risk
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SMR	standardized mortality ratio
T_3	triiodothyronine
T_4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDE	dichlorodiphenyldichloroethane (DDD)
TDI	tolerable daily intake
TNFR1	tumour necrosis factor-alpha receptor type 1
TSH	thyroid stimulating hormone
TTP	time to pregnancy
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
UF	uncertainty factor
USA	United States of America
USEPA	United States Environmental Protection Agency
VMAT2	vesicular monoamine transporter
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
WP	wettable powder

INTRODUCTION

The World Health Organization (WHO) re-evaluates the pesticide dichlorodiphenyltrichloroethane (DDT) periodically, through either the Environmental Health Criteria programme or the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues (JMPR).

The Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants evaluates the continued need for DDT for disease vector control approximately every 2 years in consultation with WHO (as required by the Stockholm Convention).

Following the availability of new scientific information since the JMPR assessment of DDT in 2001 and in order to provide specific advice to the Conference of the Parties to the Stockholm Convention relating to the use of DDT in disease vector control, a decision was taken by WHO to prepare an updated human health risk assessment focusing on DDT use in indoor residual spraying (IRS).

In order to inform the process for the WHO risk assessment, a risk assessment model for public health pesticides used in IRS was developed jointly by the WHO Department of Public Health and Environment and the WHO Pesticide Evaluation Scheme (WHOPES). The process for development of the risk assessment model is described in the resulting document (WHO, 2010).

The WHO risk assessment was conducted in a number of steps:

1) *Hazard assessment*: A draft document was prepared by L. Goldman. The draft was released via the Internet for public and peer review in the first half of 2009. A WHO expert consultation was convened on 2–4 June 2009 at WHO Headquarters, Geneva, Switzerland. The expert consultation considered and agreed on the hazard assessment. Participants in the expert consultation are listed in Annex 1. Following this expert consultation, additional dose–response modelling was carried out by the Secretariat. The dose–response modelling was independently reviewed by W. Setzer of the United States Environmental Protection Agency.

2) *Exposure assessment*: A draft document was prepared by S. Dobson. The draft was released via the Internet for public and peer review in mid-2009. A WHO expert consultation was convened on 15–16 December 2009 at the University of Bradford, Bradford, England. The expert consultation considered and agreed on the exposure assessment. Participants in the expert consultation are listed in Annex 2.

3) *Risk characterization*: A WHO expert consultation was convened on 29–30 November 2010 at WHO Headquarters, Geneva, to prepare the risk characterization, based on the already prepared hazard and exposure assessments, along with additional new information described in section 2.2 of Part A. Participants in the expert consultation are listed in Annex 3.

In this publication, the meeting report of the WHO expert consultation on DDT risk characterization is presented first, in Part A, followed by the hazard and exposure assessments on which the risk characterization was based, in Part B.

PART A

REPORT OF WHO EXPERT CONSULTATION ON DDT RISK CHARACTERIZATION

1. INTRODUCTION

Two World Health Organization (WHO) expert consultations held in 2009 resulted in a dichlorodiphenyltrichloroethane (DDT) hazard assessment and an exposure assessment for the use of DDT in indoor residual spraying (IRS). These two assessments, agreed by the experts present at those consultations and presented in Part B of this publication, provided a basis for the WHO expert consultation on DDT risk characterization, which was conducted for the purpose of assessing human health risks arising from the use of DDT in IRS.

The WHO expert consultation on DDT hazard assessment requested that additional modelling be conducted following that meeting, as part of the hazard characterization step of the hazard assessment, to develop benchmark doses (BMDs) for a number of studies. These BMDs were to be made available for the risk characterization expert consultation to use as appropriate. The additional dose–response modelling was prepared by the Secretariat and reviewed by an external expert. In addition, the Secretariat provided relevant dose metrics, expressed as micrograms per gram lipid, to facilitate the comparison between experimental animal and human data. Details of the methods used to convert the data to a common metric are presented in section 11.2.1 of Part B, along with details of the dose–response modelling that was conducted to derive BMDs for a 10% response (BMD_{10} values) and lower 95% confidence limits on the BMDs for a 10% responses ($BMDL_{10}$ values).

2. CONSENSUS STATEMENT[1]

2.1 Data considerations

While recognizing the inherent differences between exposure estimates in controlled animal studies and the stratification of exposures in epidemiological studies based on geometric means of data arranged in tertiles, quartiles or quintiles of measured serum concentrations of DDT and/or dichlorodiphenyldichloroethylene (DDE), the expert consultation considered that conversion of the exposure estimates to a common metric (μg/g lipid) facilitated the comparison of the laboratory animal–based and epidemiological data and provided a basis for making comparable risk estimates (BMD_{10} and $BMDL_{10}$) in the two types of data sets.

The expert consultation noted that where the epidemiological data were not categorical in nature or did not allow for calculation of disease incidence from crude or adjusted estimates of relative risk (odds ratios [ORs]), the alternative approach of using a "one standard deviation" change in a continuous variable allowed for a risk estimate roughly comparable to a BMD_{10}.

2.2 Evaluation of studies post-dating the hazard assessment

Numerous potential hazards of DDT and DDE were identified in the hazard assessment (Part B); all of this evidence was evaluated for use in the risk characterization.

In addition, from the literature post-dating the expert consultations on hazard assessment and exposure assessment, the expert consultation on risk characterization selected the following articles for closer review: Purdue et al. (2009), Turyk et al. (2009), Bornman et al. (2010) and Cohn et al. (2010). Two developmental neurotoxicity investigations (Pan et al., 2009; Torres-Sánchez et al., 2009) were not selected for closer review as they did not show positive effects or showed that effects identified in a cohort at 12 months of age were no longer found at 30 months of age; developmental neurotoxicity was not considered further by the expert consultation. The outcomes of considering new data for testicular

[1] This consensus statement represents the agreed conclusions of the WHO expert consultation on DDT risk characterization, convened in November 2010.

cancer and diabetes are discussed below. Other new data are addressed in the overall risk characterization.

2.2.1 Testicular cancer

In the review of the literature for the hazard assessment, a single epidemiological study with a prospective exposure measure provided some evidence for an association between DDE and testicular germ cell tumours at levels above 0.39 µg/g lipid (McGlynn et al., 2008). Since this review, two new studies on the association between DDT and/or DDE and testicular cancer have been published. The first one is a case–control study nested in the Janus Serum Bank from Norway, which reported a positive, but not statistically significant, association (OR = 2.2 for tertile 3 vs tertile 1 of *p*,*p*′-DDE; 95% confidence interval [CI] = 0.7–6.5) between DDE and testicular cancer. The study was small (only 49 case–control pairs), and similar associations were observed for chlordane, selected polychlorinated biphenyl (PCB) congeners and other insecticides (Purdue et al., 2009).

The second study examined maternal serum levels of DDT-related compounds in relation to sons' risk of testicular cancer 30 years later. Among 9744 liveborn sons, only 15 informative cases with germ cell testicular cancer were diagnosed. Mothers of testicular cancer cases had lower levels of *p*,*p*′-DDT, *o*,*p*′-DDT and *p*,*p*′-DDE, but a higher DDT/DDE ratio, than their matched controls (Cohn et al., 2010).

Taking into account the recently published studies, the expert consultation did not feel that the evidence was strong enough to warrant the use of the data on testicular cancer in the risk characterization.

2.2.2 Diabetes

In the review of the literature for the identification of hazards from DDT/DDE, several cross-sectional studies were identified that demonstrated an association between type 2 diabetes and DDT and/or DDE. Uncertainty in these findings and the weaknesses of the individual studies led the expert consultation on hazard assessment to conclude that the results were inconclusive. Since the meeting of the expert consultation on hazard assessment, two additional studies have been published, one of which (Turyk et al.,

2009) could alter the interpretation of the body of evidence. This is a study of a cohort that was established in the early 1990s and followed through 2005. Serum levels for DDE and PCB congeners were assessed at the start of the study and at two other points in time. At several points in time, Turyk et al. (2009) assessed diabetes (doctor diagnosed as assessed by questionnaire), various risk factors for diabetes and fish consumption. Associations of diabetes incidence with exposures were examined prospectively in participants without diabetes in 1994–1995, followed through 2005. Annual per cent changes in DDE and PCB-132/153 from 1994 to 2005 were examined by diabetes status. Turyk et al. (2009) found that DDE levels in the range of 0.37–7.9 μg/g lipid were associated with incident diabetes. Incident diabetes was not associated with mono-ortho PCB-118, total PCBs (sum of PCB congeners 74, 99, 118, 146, 180, 194, 201, 206, 132/153, 138/163, 170/190, 182/187 and 196/20) or years of sport fish consumption. Annual per cent change in DDE and PCB-132/153 did not differ significantly by diabetes status. This study strengthens the plausibility of an association of DDE internal dose with diabetes and highlights the need for further study in this area. Because this study has a prospective design and shows that those diagnosed with diabetes did not change their metabolism of DDE, it is stronger than earlier studies. However, there are limitations in interpretation of these data because of possible confounding by unmeasured persistent compounds that are suspected to be related to diabetes (i.e. dioxins), the possibility of metabolic changes predating the diabetes diagnosis and also affecting DDE metabolism and the absence of supporting data from experimental animal and mechanistic studies. The expert consultation did not feel that the association was strong enough in this group of studies to warrant its use in the risk characterization.

2.3 Risk characterization

2.3.1 Exposures for use in risk characterization

Table 1 presents a summary of the exposure levels that were reported in the DDT exposure assessment (Part B). The table displays ranges of exposures that have been observed in occupational studies of IRS workers, residents in IRS-treated homes and the general population living in areas where IRS is used extensively. Exposures are given as concentrations of total DDT and DDE in blood serum and umbilical cord blood. Not in the table are

measurements of DDT and DDE in breastfeeding infants' blood, because there were very few studies that made this determination; however, these levels were approximated to about 2-fold the levels in maternal serum.

Table 1. Exposures of different populations, measured as total DDT or DDE concentrations[a]

Tissue	Occupational exposure (IRS spray operators)		Residents in IRS-treated homes[b]		General population[c]	
	Total DDT	DDE	Total DDT	DDE	Total DDT	DDE
Blood serum (lipid adjusted)	Mean: 77.8 (8.7–241.1) µg/g lipid	Mean: 41.8 (7.1–131.8) µg/g lipid	Mean: 9.8 (1.09–21.8) µg/g lipid	Mean: 19.7 (0.8–77.9) µg/g lipid	Mean: 5.0 (0.38–26.1) µg/g lipid	Mean: 1.0 (0.2–3.18) µg/g lipid
	Median: 58.6 µg/g lipid	Median: 25.6 µg/g lipid	Median: 5.18 µg/g lipid	Median: 9.7 µg/g lipid	Median: 0.93 µg/g lipid	Median: 0.77 µg/g lipid
Umbilical cord blood	—	—	Mean: 29.9 µg/l	Mean: 60.3 µg/l	Mean: 15.0 µg/l	Mean: 3.1 µg/l
	—	—	Mean: 4.6 µg/g lipid	Mean: 9.3 µg/g lipid	Mean: 0.44 µg/g lipid	Mean: 0.36 µg/g lipid

[a] Mean is the arithmetic mean of the set of individual study estimates of central tendency values (median or geometric mean). The range is from the lowest to highest estimate of central tendency in each data set. Medians are also given for the same data sets. Details of the methods used to convert the data to a common metric are presented in section 11.2.1 of Part B.

[b] This population lives in areas where IRS is used extensively; they have been sampled at local clinics, and many, but not all, studies specifically indicate that they were selected because they lived in IRS-treated homes.

[c] The "general population" is people living in malarial countries where IRS is used, but they are identified in the studies as having no direct exposure through IRS; they live in unsprayed areas and will be subject to general environmental exposure.

2.3.2 Uncertainty factors

Estimation of health-based guidance values using data from studies in experimental animals or humans requires the use of uncertainty factors (UFs). Internationally agreed procedures to address UFs have been used in this risk characterization (IPCS, 2005). UFs are used to allow for interspecies differences and/or human variability and are based on differences or variability in toxicokinetics and toxicodynamics. A default UF of 10, consisting of subfactors of 4.0 for toxicokinetics and 2.5 for toxicodynamics, is

normally applied to account for interspecies differences. A default UF of 10, divided equally between toxicokinetics and toxicodynamics, is normally applied to account for human variability. These default factors can be modified if chemical-specific data are available for relevant parameters. For example, if data on the internal dose metric in experimental animals are available, then the toxicokinetic subfactor can potentially be modified (e.g. reduced from 4.0 to 1.0, leaving an interspecies UF of 2.5). These principles have been applied where possible in this risk characterization.

2.3.3 Conclusions on human health risks

2.3.3.1 Acute poisoning

The expert consultation noted that DDT was clearly identified as an acute poisoning hazard to children, with high doses associated with convulsions and even deaths following acute ingestion.

2.3.3.2 Carcinogenicity

As noted in the hazard assessment (Part B), a few human studies have suggested that liver cancer is associated with internal doses of DDT and/or DDE, but only one study provides strong evidence for an association of DDT, but not DDE, in serum with liver cancer (McGlynn et al., 2006). In this study, no significant change in response was seen for DDT in serum in three of the quintile groups; the highest non-significant group ranged from 0.522 to 0.787 μg/g lipid (no-observed-adverse-effect level [NOAEL]), but the highest group (> 0.787 μg/g lipid) saw a significant risk (lowest-observed-adverse-effect level [LOAEL]), and the test for trend was significant. When the liver cancer results were adjusted for DDE levels, the LOAEL dropped to 0.522–0.787 μg/g lipid and the NOAEL became 0.383–0.521 μg/g lipid. As the ORs seen in this study were elevated in every group, we chose to use a slope factor to describe the results; the slope factor was 3.0×10^{-6} for the analysis not adjusted for DDE and 3.6×10^{-6} for the adjusted analysis. This slope factor is a conservative measure of risk per unit dose in the studied population and can be used to evaluate risks in the general population. At an exposure of 10 μg/g lipid, as calculated from the experimental animal evidence (see below), this would suggest a risk at or below 3.6×10^{-5}. The expert consultation noted that the background incidence of liver cancer in this cohort was quite high,

possibly because of a higher rate of hepatitis B infection. Adjustments were made for this risk factor, and for other possible confounders, as described in section 8.2.2.3 of the hazard assessment (Part B).

Liver tumours have been seen in both sexes in several different mammalian species under chronic exposure to internal doses of both DDT and DDE. NOAELS were available for DDT exposure in mice but not for other species (or for DDE), in which the lowest doses used resulted in increased tumour incidence. The two NOAELS from two separate studies (estimated from original dose to lipid-adjusted serum levels in units of μg/g lipid) were 344 and 3438 μg/g lipid. LOAELS for DDT ranged from 344 μg/g to approximately 43 000 μg/g, and for DDE, the two values were 3847 μg/g and 25 444 μg/g. Geometric means of BMD_{10}s ($BMDL_{10}$s) from multiple models (see Table 19 in Part B) ranged from 1590 to 96 200 μg/g (1190–55 200 μg/g) for DDT and from 1370 to 18 000 μg/g (910–6000 μg/g) for DDE, with almost half of the BMDs (BMDLs) in the range of 1200–4000 μg/g (900–2500 μg/g).

The lowest exposure for which tumours were seen was for hepatomas in male mice, with a LOAEL of 344 μg/g lipid in a very large multigeneration study (Turusov et al., 1973). These tumours were not malignant, and the same increase was not seen in females for this exposure. For malignant liver tumours, the LOAEL was 42 980 μg/g lipid, with a NOAEL of 8596 μg/g. No NOAELS were observed for DDE, but the lowest LOAEL was 3847 μg/g. The lowest $BMDL_{10}$s for all of the data modelled were 1190 μg/g for DDT and 910 μg/g (hepatoma) for DDE. Given all of these analyses, a reasonable point of departure from the rodent studies to humans would be a $BMDL_{10}$ of approximately 1000 μg/g lipid. Given that this is a $BMDL_{10}$, that conversions have already been made to correct for species differences in half-lives by using body burdens and that both DDT and DDE work through secondary pathways unlikely to be linear, a UF of 100 should provide adequate protection for humans, leading to a negligible risk estimated from the experimental animal data at 10 μg/g lipid. Using the slope factor calculated from the human data, this would yield a risk in humans of less than 4 in 100 000.

The risk described above represents the fraction of incident cases seen in the study population over a 16-year period (based on

follow-up time for the cohort study) for liver cancer in adults aged 40–69. Hence, it does not represent a lifetime risk. A crude adjustment[1] for lifetime cancer risks can be used to adjust these risks. This adjustment results in approximate lifetime risks below 5 per million at 1 μg/g lipid, 26 per million at 5 μg/g lipid and 53 per million at 10 μg/g lipid.

Given this adjustment and the range of exposures provided in Table 1, we would conclude the following. The majority of the general population in Table 1 are expected to experience risks below 10^{-5}, with virtually all below 10^{-4}. The exposures seen by residents in sprayed homes, while higher, generally are expected to yield risks below 10^{-4} for liver cancer, with at least half of the population exposures yielding a potential risk below 6×10^{-5}. Workers spraying in homes appear to have potential risks 3–10 times higher.

Although age-specific cancer risks are highly speculative, it is possible to set some bounds on these numbers. For an exposure of 1 μg/g lipid, it is unlikely that the annual risks will exceed 1 per million in the general population. At 10 μg/g lipid, the risks will also generally be below 2 per million per year. For occupational exposures in IRS where exposures could exceed 100 μg/g lipid in a small fraction of the population, the risks will generally not exceed 3 per 100 000 per year.

2.3.3.3 Developmental effects

The hazard assessment (Part B) reported few conventional developmental toxicity studies and no contemporary guideline studies; it was also noted that the studies that were performed did not identify anomalies.

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[1] If α is the slope factor and D is the dose, then αD represents the risk of getting liver cancer in a cohort of adults followed for 16 years with ages ranging from 40 to 69. Using their average age of 55, we can calculate this as αD = Prob(Cancer < Age 71 | Cancer > Age 55) = 1 − Prob(Cancer > Age 71 | Cancer > Age 55) = 1 − Prob(Cancer > Age 71) / Prob(Cancer > Age 55). If the age-specific risk is proportional to dose times age to the third power and dose is at steady state, this can be calculated as Prob(Cancer > Age x) = $\prod_{i=1}^{x} 1 - \beta D i^3$, and β can be solved. For $\alpha = 3.6 \times 10^{-6}$, $\beta = 8.6 \times 10^{-13}$. Lifetime risk is then calculated at 70 years of exposure.

The onset of puberty in female dogs was accelerated by DDT exposure, with a $BMDL_{10}$ of about 100 µg/g serum lipid (Ottoboni et al., 1977). To extrapolate to humans, a UF should be applied for species differences in toxicokinetics and toxicodynamics. For the interspecies UF, the toxicokinetic component has been accounted for by use of an internal dose metric, leaving the toxicodynamic UF of 2.5. For accounting for interindividual differences, another factor of 10 is required, for a total UF of 25. The corresponding level of exposure to DDT for no effects on puberty in humans would therefore be 4 µg/g serum lipid, which is in the range for median levels among residents in IRS-treated homes.

Gestational exposures of rats to DDE are positively associated with a decrease in anogenital distance in male offspring at birth (You et al., 1998), an outcome consistent with an anti-androgenic mode of action (e.g. a positive finding in the Hershberger assay). The $BMDL_{10}$s for the effects were calculated to be at relatively high levels (approximately 1000 µg/g serum lipids; see Table 15 in Part B). Nipple retention in male offspring (You et al., 1998), a more sensitive measure of androgen function disruption in rats, had $BMDL_{10}$s for DDE in the range of 150 µg/g serum lipid. Reduced anogenital distance is considered to be more relevant to humans, and application of a 25-fold UF yields a corresponding no-effect level for DDE of 40 µg/g serum lipid, which is well above median levels observed in umbilical cord blood for newborns of residents of IRS-treated homes.

In epidemiological studies, a cross-sectional study did not find an association of DDE level with anogenital distance in boys (highest internal dose level for DDE of 56 µg/g serum lipid; Longnecker et al., 2007), whereas a nested case–control study reported that a doubling of first-trimester DDE levels significantly reduced the anal position index (90th percentile internal dose of DDE was 6 µg/g serum lipid; Torres-Sánchez et al., 2008). A recent study by Bornman et al. (2010) of urogenital malformations in newborn boys suggests increased rates of malformations among those whose mothers lived in DDT-treated areas; however, no dose–response information was available. These data do not provide sufficient information for determination of a quantitative risk to humans, although the biological plausibility is strengthened by the animal findings and the mode of action (anti-androgenic) data for DDE.

2.3.3.4 Reproductive effects: males

Effects of DDT on spermatogenesis in the testes and on sperm count and motility have been observed in treated rats (Ben Rhouma et al., 2001), and associations with DDE have been observed among men with recent or current DDT use and exposures.

Semen quality is used as a measure of male fecundity in clinical andrology, male fertility, reproductive toxicology and epidemiology (Cooper et al., 2010).

Low sperm numbers, impaired progressive sperm motility and low number of sperm with normal morphology per se may be contributing to male infertility.

There are recent WHO reference values for semen parameters from a fertile population, which may assist in the diagnosis of infertility (Cooper et al., 2010; reproduced in Table 2). However, Andersson et al. (2008) concluded that a sperm count above 40 million per millilitre distinguishes between an optimal sample and one with reduced ability to conceive, indicating that impaired fertility may occur at levels above the WHO cut-off value for a diagnosis of infertility (Andersson et al., 2008). These values, as well as a summary of results from the De Jager et al. (2006) study in Chiapas, Mexico, and the Aneck-Hahn et al. (2007) study in Limpopo, South Africa, are shown in Table 2.

Men in the Chiapas study group (De Jager et al., 2006) had mean sperm motility within the normal range, even though there was a trend of declining sperm motility with increasing DDE level and evidence for a number of men with low values. In the Aneck-Hahn et al. (2007) study, the number of subjects with low sperm number and low progressive sperm motility increased with higher DDE levels (15%, 20%, 23% and 30% per quartile DDE starting at 0–43 μg/g), and the DDE levels were considerably higher than in Mexico. Although the average sperm parameters are in the "normal" range for WHO, many individual values are lower, and the average value is at a level considered by Andersson et al. (2008) to indicate impaired male fertility. These effects are observed at internal doses of DDE occurring in areas of active or recent DDT IRS; studies in populations with lower internal dose levels did not report these effects. For example, Hauser et al. (2003a) published a cross-

sectional study of 212 male partners of subfertile couples with a median DDE level of 0.2 µg/g serum lipid and found no association between DDE levels and sperm parameters.

Table 2. Sperm parameters: epidemiological studies

Sperm parameter	De Jager et al. (2006)	Aneck-Hahn et al. (2007)	< 12 months TTP[a] (WHO, 2010)	"Optimally fertile" (Andersson et al., 2008)
Volume (ml)	Mean (SD): 1.8 (1.0) Median: 1.6	Mean (SD): 1.9 (1.3) Median: 1.5	1.4–1.7	—
Sperm concentration (× 10^6 sperm per millilitre)	Mean (SD): 76.2 (60.0) Median: 60.0	Mean (SD): 51.8 (48.2) Median: 39	12–16	40
Total count (× 10^6 sperm per ejaculate)	Mean (SD): 137 (123) Median: 109	Mean (SD): 101.6 (159.3) Median: 59	33–46	—
Progressive motility (% grades a + b)	Mean (SD): 52.7 (14.3) Median: 56	Mean (SD): 48.1 (21.1) Median: 55	38–42	—
Morphology (% normal)	Mean (SD): 8.4 (6.2) Median: 7.5	Mean (SD): 4.1 (2.7) Median: 4	3–4	—
DDE levels (µg/g serum lipid)	Mean (SD): 45.0 (31.0) Median: 41	Mean (SD): 215.5 (210.6) Median: 134	—	—

SD, standard deviation; TTP, time to pregnancy

[a] Based on men with partners known to have a time to pregnancy up to and including 12 months.

BMD analysis identified a BMD_{10} and $BMDL_{10}$ for rats in the range of 1000 µg/g lipid for DDT for decreased sperm count and motility (Ben Rhouma et al., 2001) and for the two human studies in the range of 100 µg/g serum lipid for DDE for sperm count, motility and sperm tail abnormalities. Using a UF of 25 for the experimental animal studies and 5 for the human studies (De Jager et al., 2006; Aneck-Hahn et al., 2007) suggests negligible risk for lowered male fertility at and below 40 µg/g serum lipid for DDT and 20 µg/g serum lipid for DDE.

2.3.3.5 Reproductive effects: females

Isolated epidemiological studies have found a range of effects on female reproductive function, including early menarche, spontaneous abortion, preterm birth and earlier menopause, as a function of DDT or DDT + DDE levels. The $BMDL_{10}$ values for these findings were calculated to be low concentrations (1–10 μg/g serum lipid). Because most of these studies were not prospective in nature and because confounding by other factors, such as age, body mass index and other risk factors, could not always be controlled, caution should be applied in using these effect levels in quantitative risk assessment. Collectively, however, the positive dose–response relationships for these endocrine-regulated end-points do raise concern for effects of exposure to DDT on female reproductive health. In raising this concern, it is important to bear in mind that for nearly every instance of a positive association, other studies (of varying quality and sensitivity) did not report positive associations for the same end-points. For example, in the case of spontaneous abortion, only four of nine studies reported a positive association (and the BMD approach could be applied to only one of the positive studies). Likewise, of nine reviewed studies of gestational age/preterm birth, only three showed a significant association with DDT/DDE levels (and again, only one was sufficient for BMD modelling). Additional research is required to confirm whether such effects are occurring in the context of IRS.

As rodents do not provide biologically relevant models (e.g. differences in early pregnancy maintenance and in the mechanisms of menarche) for the effects seen on female reproduction, it is not surprising that similar findings have not been observed in those studies. In general, no adverse effects on fertility were observed in multigeneration studies with rats, mice or dogs.

In a multigeneration study in rats, the duration of fertility was extended at 0.5 and 5 mg/kg body weight (bw) (equivalent to 68.5 and 685 μg/g lipid; Ottoboni, 1969). In a follow-up experiment, females exposed to 1 mg/kg bw had a higher reproductive livespan (Ottoboni, 1972). Further, in a study with rabbits dosed with 3 mg/kg bw 3 times per week for 12 weeks (upper bound of DDT concentration 450 μg/g lipid), a decrease in ovulation rate was observed (Lindenau et al., 1994), which was not associated with an effect on fertility (Seiler et al., 1994).

2.3.4 Overall conclusions of the risk characterization

Numerous potential hazards of DDT and DDE were identified in the hazard assessment (Part B); all of this evidence was evaluated for use in the risk characterization.

The expert consultation concluded that there is an acute poisoning hazard for children with accidental ingestion of DDT.

Carcinogenicity, developmental toxicity and both male and female reproductive toxic effects were assessed in association with IRS-related internal dose levels of DDT and DDE.

In terms of relevant exposure scenarios for the general population in countries using IRS, evidence to date does not point to concern about levels of exposure for any of the end-points that were assessed. In terms of potential risks at levels of exposure of the general population in countries using IRS, research is needed on reproductive effects in females and certain child developmental effects to better evaluate risks that were suggested in the studies that were reviewed.

For households where IRS is undertaken, there was a wide range of DDT and DDE serum levels between studies. Generally, these levels are below potential levels of concern for populations. Considering the ranges of exposures in treated households that are summarized in Table 1, in some areas, the exposures in treated residences have been higher than potential levels of concern. Efforts are needed to implement best practices to protect residents in treated households from exposures arising from IRS. Of particular concern would be women of childbearing age who live in DDT IRS-treated dwellings and transfer of DDT and DDE to the fetus in pregnancy and to the infant via lactation.

Reported exposures of IRS workers, as summarized in Table 1, have greater overlap with serum levels of DDT and DDE within a range that in some studies are associated with carcinogenic and male reproductive effects. Efforts are needed to implement best practices to protect IRS workers.

PART B

HAZARD AND EXPOSURE ASSESSMENTS

1. SUMMARY AND CONCLUSIONS

1.1 Introduction

The World Health Organization (WHO) decided to prepare an updated human health risk assessment focusing on the use of dichlorodiphenyltrichloroethane (DDT) in indoor residual spraying (IRS) as a result of new scientific information that had become available since the 2001 assessment of DDT by the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues (JMPR) and in order to provide specific advice to the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants relating to the use of DDT in disease vector control.

1.2 Hazard identification[1]

DDT is the common name for a human-made chemical and does not occur naturally in the environment. Chemically, technical DDT is a mixture, the main components of which are *p*,*p*′-DDT (63–77%) and *o*,*p*′-DDT (8–21%), with trace amounts of *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE). Experimental studies often use pure *p*,*p*′-DDT and *p*,*p*′-DDE, whereas humans are exposed to mixtures of the compounds, as described above. This hazard assessment focuses on the health effects of *p*,*p*′-DDT and *p*,*p*′-DDE.

DDE is the major metabolite of DDT in biological systems. Studies comparing groups of people occupationally exposed to the DDT product show elevated concentrations of DDT and DDE in both blood serum and adipose tissues. Elevated DDT levels in both blood and adipose tissue are indicative of recent exposure to the pesticidal product. Ratios of DDE to total DDT (i.e. DDT and its derivatives) typically rise with time following exposure to DDT; ratios of about 0.8 and above suggest no recent exposure to the parent compound.

Lipid adjustment of serum levels of DDT, DDE and other persistent compounds is frequently done. The aim of this hazard assessment was to perform dose–response analyses using lipid-

[1] The text in this section was agreed by the participants of the expert consultation convened to peer review the hazard assessment of DDT (held in June 2009).

adjusted DDT/DDE concentrations. Where only non-adjusted values were reported, they were corrected using the ratio of average serum DDT/DDE concentrations expressed in µg/g lipid and µg/l: 0.160. The same ratio is used to describe the ratio of serum DDT/DDE per volume serum to adipose tissue DDT/DDE per mass lipid.

Exposure to DDT causes liver enlargement in rats and induction of cytochrome P-450 (CYP) enzymes via constitutive androstane receptor (CAR)–pregnane X receptor (PXR) binding. In humans, induction of CYP and elevation of gamma-glutamyltransferase (GGT) activity in serum have been observed.

At dose levels above 6–8 mg/kg bw per day, DDT caused tremors and convulsions in adult mice and rats; similar effects have been observed in children who have accidentally ingested DDT.

On the basis of available data, it is not possible to conclude whether serum DDT and/or dichlorodiphenyldichloroethylene (DDE) levels are associated with immunotoxicity.

Results from studies on possible diabetogenic effects of DDT are inconclusive.

DDT, while negative in most genetic toxicity assays, can induce DNA damage in cultured rodent cells and in human lymphocytes. The mechanism of this damage has not been elucidated.

Several studies demonstrate that DDT induces tumours in rodents, notably tumours of the liver, but also lung tumours and leukaemia.

A large, well-conducted prospective study in China where there is a relatively high background rate of liver cancer in an area with high exposure to DDT demonstrated strong evidence for an association of serum DDT with liver cancer. This association was not observed in several human studies that suffered from limited statistical power and poorer exposure assessment. The concordance for liver tumours in experimental animals and humans strengthens the plausibility of the single positive human study.

Of many studies looking at exposures to DDT and breast cancer, most were negative, and exposures of adults to DDT are not demonstrably linked to breast cancer. One study suggested that

prepubertal exposure to DDT may be associated with breast cancer. This finding is consistent with estrogenic actions of DDT in experimental animals as well as with findings (described below) of possible menstrual cycle alterations induced by DDT, which in turn could change breast cancer risks by altering estrogen hormone levels in menstrual cycles. Overall, the association between DDT and breast cancer is inconclusive.

A single human study provided evidence for an association between DDE and testicular germ cell tumours. Such a relationship is consistent with the observed hormonal properties of DDE. Overall, the associations between DDE and testicular cancer are inconclusive. A short-term study on testicular cancer in rabbits was inconclusive.

A number of studies linked DDT exposure to non-Hodgkin lymphoma (NHL) but did not adequately control for potential confounding by other pesticide exposures. It was concluded that the data are inadequate to assess the association of DDT with NHL.

Data are inadequate to assess associations between DDT/DDE and lung, pancreatic, prostate or endometrial cancers.

There are several potential modes of action for DDT and DDE that may be relevant to carcinogenesis. Generally, it is thought that genotoxicity is unlikely to be a mode of action for DDT carcinogenicity. DDT is known to bind to the CAR, which may mediate cancer effects. Also, after initiation with a nitrosamine, DDT can induce the formation of preneoplastic liver lesions. Thus, there is a strong possibility that DDT promotes the progression of cancer in rodents. For some tumour types (e.g. breast and testes), effects of DDT/DDE on hormonal receptors may be of relevance.

Overall, the human studies for DDT/DDE and thyroid hormones are inconclusive.

Multigeneration studies on reproductive function in several mammalian species have generally not revealed effects on fertility, fecundity or pregnancy after exposure to DDT. However, effects on spermatogenesis in the testes and on sperm count and motility have been observed in treated rats. In exposed human males, studies are inadequate to directly assess fertility and fecundity. Associations between exposure to DDT and abnormalities in sperm character-

istics have been reported, particularly among men with recent or current DDT use and exposures. These studies do not convincingly demonstrate causality. They are possibly consistent with the ability of DDT and DDE to alter hormonal status via receptor binding or aromatase induction.

DDE is anti-androgenic and *o,p'*-DDT weakly estrogenic in vitro, and effects related to endocrine disruption (reduced anogenital distance, nipple retention, cryptorchidism, possibly hypospadias) have been reported in rodents and/or rabbits after high exposures. The expression of hormonal effects in experimental animals depends on exposure during particular critical phases, exposure levels and duration and the hormonal status of the animal. The human studies addressing hypospadias, anogenital distance and cryptorchidism are too limited for evaluation.

Treatment with DDT significantly reduced ovulation rate in the rabbit. In human females, associations between DDT exposure and menstrual cycle alterations have been described in studies of high quality. These changes are consistent with hormone-like properties of DDT. While one human study showed earlier age of menopause, an animal study (in rats) showed an older age of cessation of fertility.

Two cohort studies indicated a possible association between DDT and DDE levels and fetal loss in women in countries with recent usage of DDT. However, analogous results were generally not observed in the multigeneration animal studies.

The available human data support a possible association between exposure to DDT/DDE and reduced gestational age and increased rates of preterm birth.

Available evidence supports a possible association between prenatal or early life exposures to DDE and reduced childhood growth. They do not support an association between fetal growth restriction and DDT/DDE. None of the animal multigeneration studies reported decreased growth.

Studies from one laboratory provide evidence that exposure of neonatal mice to DDT on postnatal day 10 induces significant neurochemical and functional neurodevelopmental changes.

Studies in humans provide consistent evidence for perinatal exposure having neurocognitive effects in children, particularly for DDT.[1] Moreover, given the differences in timing of various developmental sequences in the mouse compared with the human and the very long half-lives of DDT and DDE in humans compared with those in mice, the experimental animal and human data are consistent. There is no evidence that this effect is mediated by thyroid function.

1.3 Hazard characterization[2]

The hazard characterization uses both experimental animal and human studies to provide reference levels for DDT/DDE toxicity. The same basic methods were used for both cancer and non-cancer assessments.

The preferred method for determining reference levels for various end-points was the benchmark dose (BMD). However, BMD modelling could be performed only for studies for which the relevant input data could be identified and that met certain selection criteria, such as having a minimum of three dose groups. For studies and end-points not amenable for BMD analysis, the traditional approach was used to identify a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL). A further consideration is that BMD modelling could be performed only on studies that showed a positive association between the end-point and DDT or DDE exposure, without taking into account other studies that might have been identified that did not show a positive association. The quantitative dose–response analysis therefore shows an automatic bias towards positive results.

BMD modelling was done using the United States Environmental Protection Agency's BMD software, version 2.1.2.60. The modelling was performed after conversion of the dose metrics to concentrations of DDT/DDE in serum/adipose tissue as micrograms per gram lipid. The BMD modelling was independently reviewed.

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[1] See evaluation of later published studies considered by the expert consultation on the risk characterization (Part A, section 2.2).

[2] The hazard end-points and studies selected for dose–response modelling were agreed by the expert meeting convened to peer review the hazard assessment of DDT. The finalized results of the dose–response modelling were not available to those experts but were subject to a separate peer review.

1.4 Exposure assessment[1]

Current human exposure to DDT in countries where the insecticide is no longer used in agriculture or public health is expected to be through food; residues in adipose tissue (the major storage site of lipophilic DDT metabolites) have been falling, together with concentrations in blood serum. In countries still using DDT for IRS, concentrations remain stable or, in some cases, are rising. Remaining use of DDT globally is restricted to public health application for the control of endophilic vectors of the parasitic diseases malaria and leishmaniasis. IRS with DDT is almost always carried out using 75% wettable powder products at an application rate of 2 g/m^2 on indoor walls of dwellings.

This review concentrates on human exposure via IRS but puts this into the context of residues of both DDT and its metabolites in people exposed via previous agricultural and public health use of the insecticide. Exposure is reviewed only in terms of concentrations of DDT and its metabolites in human body tissues (adipose tissue, blood serum and breast milk); although a large literature exists measuring DDT and its metabolites in biota and food items, these data do not allow estimation of the specific route of exposure leading to body residues in the context of IRS. Exposure is assessed for spray operators and others occupationally exposed in the IRS programmes, members of the general population inhabiting sprayed dwellings, the wider general population and infants exposed via breast milk. Exposure of the fetus is another important concern, but the data are limited and have to be developed from data on cord blood.

The literature was searched up to November 2009. Studies that included time series were selected preferentially to illustrate trends over time in countries that had stopped all use of DDT for either agriculture or public health.

Manufacture, trade and use of DDT are severely restricted under the Stockholm Convention on Persistent Organic Pollutants. The use of DDT continues to be permitted in some developing countries only in accordance with WHO recommended guidelines,

[1] The text in this section was agreed by the participants of the expert meeting convened to peer review the exposure assessment of DDT (held in December 2009).

and countries are required to develop regulatory and other mechanisms to ensure that use of DDT is restricted only to the control of endophilic vectors of the parasitic diseases malaria and leishmaniasis. Misuse for applications in agriculture is outside the scope of this document because it cannot be quantified.

Following the WHO guidelines on application methodology and safety will keep exposure of operators to a practical minimum. Engineering controls to minimize exposure of spray personnel should be implemented; use of personal protective equipment (PPE) is recommended.

Residue levels of both DDT and its lipophilic metabolites in human tissues are highly variable in all studies. Data sets are typically highly skewed, with a small proportion of the population carrying higher concentrations in body tissues and a large proportion carrying much lower concentrations. Normal distribution can often be achieved by log transformation. Arithmetic means are not appropriate measures for such data sets; medians or geometric means express the mid-range more appropriately. Many of the studies report means, not medians.

There are a number of uncertainties associated with the data available. There are few controlled studies available for any of the settings relevant for this document (occupational exposure, residential exposure or infant exposure via breast milk). Diversity in patterns of application occur in different parts of the world, and changes of equipment have occurred over time. Differing cultures demonstrate different child-bearing and child-rearing practices, and fetal exposure can be determined by extrapolation only. Also, there is a lack of studies on DDT in total diet.

There have been no published reports of detailed operational field studies of exposure of spray operators during IRS. A generic model has been developed by WHO to estimate exposure of both operators and residents in houses treated under IRS. The model estimates the total exposure for workers at 0.16 mg active ingredient (a.i.)/kg bw per day for a realistic scenario without PPE and 0.016 mg a.i./kg bw per day for the safest scenario with PPE. Total exposure of residents is estimated at 0.05, 0.07, 0.359 and 0.0988 mg a.i./kg bw per day for adults, children, toddlers and breastfed infants, respectively. Ingestion represents approximately 40% of

estimated total exposure for both adults and children, with the remaining 60% from dermal exposure; ingestion in toddlers (from both foodstuffs and hand-to-mouth activity) represents 25% of total exposure.

As expected, all studies comparing occupationally highly exposed groups with others showed elevated concentrations in both blood serum and adipose tissues. A higher proportion of DDT in both blood and adipose tissue is indicative of recent exposure to the pesticide. Ratios of DDE to total DDT typically rise with time following exposure to DDT, stabilizing at around 0.8.

Studies attempting to show relationships between duration of occupational exposure and concentrations of DDT or DDE did not always succeed. Most studies showed increased residues of DDT and/or DDE in body tissues with longer time periods working with the insecticide for IRS for malaria control. This relationship is not straightforward. Correlation with years worked in spraying operations has been reported in some studies. More complex indices of exposure, scoring not only years worked but other measures of exposure (relative time on different jobs, etc.), have correlated well with measured concentrations in other studies. One major study failed to demonstrate a relationship and attributed this to poor information on short-term and medium-term exposure. Detailed exposure information is needed to establish correlations, and this has seldom been available. Jobs performed by personnel involved in IRS tend to rotate, and this is true from studies in many different parts of the world (although not all); job rotation is a major factor contributing to difficulties in establishing relative exposure. In one study, clear correlation was established between tonnage used in different regions of a country and concentrations in spray operators' tissues.

All occupational studies indicated that referents (often administrative workers from the IRS programmes) showed lower exposure. "Controls" from agriculture, spraying other pesticides, also showed very low residues of DDT or its metabolites.

In the single occupational study measuring DDT in abdominal adipose tissue of spray operators, the geometric mean concentration was 104.5 μg/g. The mean concentration of total DDT in adipose tissue in residents of areas where spraying of DDT for IRS occurs is 25.33 μg/g fat (range 5.1–38.6 μg/g fat), with the median value at

25.92 µg/g fat and the highest maximum reported as 176.5 µg/g fat. Much of the DDT stored in fat is converted to DDE within 2 years, as evidenced by measurements made following cessation of the use of DDT in countries.

For occupational exposure, both high exposure and "control" categories show a highly skewed distribution of total DDT concentrations in blood serum. "Controls" in these studies are spray programme personnel who are not directly exposed through IRS (technicians in laboratories, administration workers). The arithmetic mean value of the study estimates of central tendency (median or geometric mean) is 517.5 µg/l (median 521.9 µg/l; range 56.5–1572 µg/l) for the highest exposure group. The highest exposure groups had all been exposed through the use of DDT for disease vector control; all but one had definitely used DDT in IRS.

Exposure of residents is via the immediate environment of treated houses, most likely through skin contact with contaminated surfaces and ingestion via contamination of food in homes, food produced in the homesteads and hand-to-mouth activity (house walls, floor and soil outside the dwelling).

Those living in houses treated with DDT by IRS showed elevated DDT and DDE concentrations in blood serum compared with residents in untreated houses, although concentrations were lower than those of spray operators. The relationship between application of DDT sprays for IRS and subsequent accumulation of residues by exposed populations may not be a simple one; a longitudinal study showed changes over a year, but no direct correlation between spraying time and concentrations in those living in sprayed houses. Elevated residues in blood and adipose tissue, where measured, are restricted to those intimately associated with sprayed housing. Even residents in adjacent communities can show markedly different concentrations of DDT and DDE.

For the general population living in malarial countries but not directly exposed through IRS, the mean concentration of total DDT in blood serum is 31.9 µg/l (not adjusted for lipid content; median 18.8 µg/l; range 2.49–170 µg/l). For residents of IRS-treated homes, the mean concentration of total DDT in blood serum is 63.7 µg/l (not adjusted for lipid content; median 82.8 µg/l; range 7.1–142 µg/l). Newborn infants had blood concentrations approximately

40% of those in maternal blood for both DDT and DDE. Only three studies reported concentrations of DDE in umbilical cord blood in areas likely to be exposed via IRS, too few to give reliable mean estimates for risk assessment. However, the mean DDE level in cord blood relative to maternal blood is 47% (median 42%; range 32–62%) across eight studies globally where paired samples are reported; therefore, cord blood levels can be estimated as 47% of serum levels.

Concentrations of DDE tend to increase with age; this has particularly been noted in highly exposed populations. In young children, blood levels can fall throughout childhood from a high attained during breastfeeding. However, this has been studied systematically only in one area, South Africa, with extended periods of breastfeeding (up to 2 years).

Models attempting to link concentrations in exposed populations with factors outlined above have failed to explain more than about 40–50% of the variation, so other factors must be involved. Many study authors assume that the remaining DDT exposure is via food, often citing studies from developed countries as evidence. It seems reasonable to suppose that exposure through food is a factor. However, no direct evidence is available to prove that food exposure is a significant factor in overall DDT/DDE residues, as studies have not concurrently measured both concentrations in body tissues and concentrations in total diet. Although food should be removed from houses prior to IRS treatment, food introduced into houses after IRS may become contaminated. As well, food produced adjacent to treated houses in gardens, including free-ranging chickens foraging there, becomes significantly contaminated with DDT and its metabolites.

Where studies have been done on residents of treated homes, IRS is the major source of the DDT in breast milk. Infants are exposed to DDT and its metabolites through breast milk. Lactation is a means for excretion of the DDT body burden of mothers. The concentration of DDT and its metabolites tends to be greater in breast milk produced by primiparous mothers, especially at high exposure levels, exposing the first-born infants to higher levels compared with the following siblings. In one study, the concentrations of total DDT and DDE were twice as high for milk from primiparous mothers as for milk with subsequent infants. Although having more children does further reduce the DDT

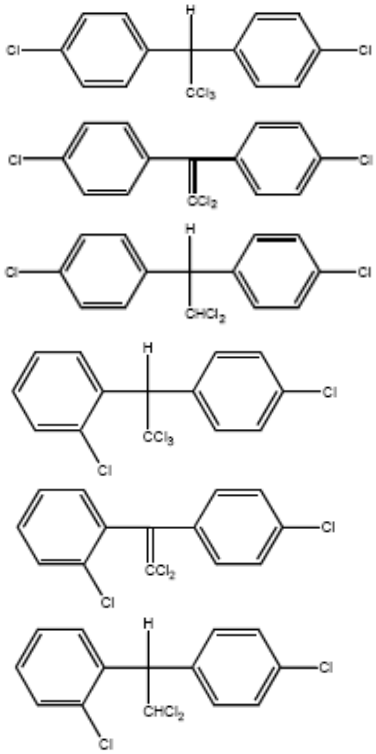
concentration in milk, the effect is substantially lower than for first-borns. Concentrations of total DDT and of DDE in infants can be reasonably accurately predicted solely from concentrations in breast milk, whether or not a child is first-born and infant age.

For highly exposed mothers in controlled IRS studies, an average total DDT concentration of 12.8 (range 0.2–76.8; median 6.24) mg/kg milk fat is found (equivalent to 640 µg/l whole milk, assuming a value of 5% for fat content of the milk; range 10–3840 µg/l; median 312 µg/l). For the general population in malarial countries without specific exposure to IRS, a mean total DDT concentration is 2.8 (range 0.47–15.8; median 1.6) mg/kg milk fat (equivalent to 140 µg/l whole milk, assuming a value of 5% for fat content of the milk; range 23.5–790 µg/l). The mean of reported maxima is 72.5 (range 0.57–370.4; median 26.12) mg/kg milk fat (equivalent to 3625 µg/l whole milk; range 28.5–18 520 µg/l) for total DDT.

2. CHEMICAL IDENTITY

DDT is the common name for a human-made chemical and does not occur naturally in the environment. Chemically, technical DDT is a mixture, the main components of which are *p*,*p*′-DDT (63–77%), *o*,*p*′-DDT (8–21%) and *p*,*p*′-DDE (0.3–4%). Differences in the mixtures of DDT reflect variations in the manufacturing process. DDE is also the major metabolite of DDT in biological systems. DDT and especially DDE are very resistant to degradation and, especially in cold and temperate climates, tend to be very persistent in the environment. Therefore, this assessment will focus on DDT and DDE (IPCS, 1979).

The main components of technical DDT and the primary metabolites of DDT, together with their Chemical Abstracts Service (CAS) registry numbers, are illustrated in Figure 1.



p,*p*′-DDT, *p*,*p*′-dichlorodiphenyl-trichloroethane
CAS No. 50-29-3

p,*p*′-DDE, *p*,*p*′-dichlorodiphenyl-dichloroethylene
CAS No. 72-55-9

p,*p*′-DDD (*p*,*p*′-TDE), *p*,*p*′-dichlorodiphenyldichloroethane
CAS No. 72-54-8

o,*p*′-DDT, *o*,*p*′-dichlorodiphenyl-trichloroethane
CAS No. 789-02-6

o,*p*′-DDE, *o*,*p*′-dichlorodiphenyl-dichloroethylene
CAS No. 3424-82-6

o,*p*′-DDD (*o*,*p*′-TDE), *o*,*p*′-dichlorodiphenyldichloroethane
CAS No. 53-19-0

Fig. 1. Main components of technical DDT and the primary metabolites of DDT

There are differences between the chemicals used in experimental studies and the mixtures to which humans are exposed. Experimental studies often use pure *p,p′*-DDT and *p,p′*-DDE, whereas humans are exposed to mixtures of the compounds described above. This is relevant to understanding some of the differences between the results of experimental studies and those of observational studies. This DDT hazard assessment focuses on the health effects of *p,p′*-DDT and *p,p′*-DDE.

3. EXPOSURE SOURCES AND METRICS

This section discusses exposure to the extent necessary to interpret the toxicological and epidemiological studies under consideration. An exposure assessment of the use of DDT in IRS has been conducted separately (see section 12).

3.1 Sources of exposure

Residues of DDT and DDE are found in the environment, in biota and in food supplies globally as a result of releases during production and formulation and past use as an insecticide for agriculture and vector control. Studies of humans everywhere in the world find some background level of these compounds resulting from global transport and the long biological half-lives of these compounds. Because of global management of DDT under the Stockholm Convention on Persistent Organic Pollutants, the production and use of DDT are now restricted to the control of public health disease vectors. Today, DDT is among a suite of tools that are available for reducing the transmission of malaria. In areas with malaria outbreaks, IRS of DDT is used to decrease the incidence and spread of the disease (Roberts et al., 1997; Attaran & Maharaj, 2000), not only by killing mosquitoes but also by repelling them from interior surfaces, thus decreasing the odds that people will be infected. DDT-impregnated bednets have also been studied for use in malaria control (Loong et al., 1985; Philavong et al., 2000). Through the Stockholm Convention, it is the obligation of countries to monitor the use of DDT to ensure that uses are restricted to vector control.

When assessing the hazards from exposure to DDT and DDE, it is important to note that the occupationally exposed cohorts (in manufacture, processing and application of DDT) were exposed mainly to technical DDT (i.e. the ratio of *p*,*p*′-DDT to *p*,*p*′-DDE is in the order of approximately 10), with a substantial exposure also to *o*,*p*′-DDT and often other insecticides as well.

The general population today, especially in countries where DDT use for agricultural purposes has been banned for decades, is exposed mainly to DDE; in those situations, the concentration of this congener is by far highest in dietary items as well as in blood, lipid and milk specimens (ATSDR, 2002). Population subgroups exposed to DDE in the diet also tend to be exposed to other lipid-

soluble environmental contaminants, such as other chlorinated insecticides as well as polychlorinated biphenyls (PCBs).

3.2 Metrics of exposure

In toxicological studies, exposures are controlled by investigators, and the metric for exposure is given in terms of the *external exposure* or *administered dose*, expressed as milligrams of substance per kilogram of body weight per dose or per day over some period of time. However, in epidemiological studies, the metric of exposure is generally given in terms of the *internal dose*, which is the quantity of DDT or DDE measured in tissue samples, usually serum. Thus, whereas toxicologists assay doses in terms of intake, epidemiologists utilize measures of storage of DDT compounds. Generally, this has been workable.

While there are technical challenges associated with extrapolating intake rates from experimental animals to humans, results of toxicological studies have been useful in setting acceptable daily intakes (ADIs) or tolerable daily intakes (TDIs), which are then used as reference standards for the establishment of maximum residue limits (MRLs) and drinking-water guidelines to promote good practices in the use of *p*,*p*′-DDT. While it is known qualitatively that the metabolism of DDT is different between laboratory rodents and humans, quantitative data on the differences are limited. Furthermore, validated models on the relationship between oral dose and concentrations in blood or adipose tissue are not available. The relationship would be very different in short-term compared with long-term studies, in which steady state is approached. In this document, a simple equation involving intake, absorption and disappearance half-life is used to describe the relationship between the dose rate and the body burden (see section 11.2.1).

Evidence suggests that whereas DDT and DDE have structural similarities and share some modes of action for toxicity, they also have distinct modes of action. Therefore, it is important to distinguish between independent and synergistic effects of DDT and DDE on disease risks. Although the insecticidal product DDT is predominantly composed of *p*,*p*′-DDT, it contains a significant amount of *o*,*p*′-DDT and traces of *p*,*p*′-DDE as well as other related chemicals. When active exposure to the DDT pesticide product ceases, DDE becomes predominant over time, because it has a

longer half-life than DDT in most biological systems and because of the metabolic conversion of DDT to DDE in many species (including humans) and in the environment. This has important implications for extrapolation of risk from epidemiological or toxicological studies to specific exposure scenarios.

Studies comparing groups of people occupationally exposed to the DDT product show elevated concentrations of DDT and DDE in both blood serum and adipose tissues. Elevated DDT levels in both blood and adipose tissue are indicative of recent exposure to the pesticidal product. Ratios of DDE to total DDT[1] typically rise with time following exposure to DDT; ratios of about 0.8 and above suggest no recent exposure to the parent compound. Likewise, higher ratios of DDE to total DDT are observed in environmental samples taken and food grown in locations without recent usage of DDT. Higher ratios of DDE to total DDT in humans are found among individuals with exposure to the DDT product only in the remote past, but they are also commonly found among individuals who have never been exposed directly to the DDT product but rather have been exposed to low concentrations of DDE and DDT in environmental media and food in areas where the DDT product has not been used for many years.

In epidemiological studies, one factor may confound or modify the effects of another factor on risk. In the case of DDE and DDT, individuals in the same population may have variable exposures to DDE relative to DDT depending on their personal history of exposure to the DDT product (amount and timing), past residential history and recency of use of the DDT product in various locations, locations of sources of food and interindividual variability in metabolic conversion of DDT to DDE and DDT and DDE half-lives. As such, the accuracy of concentrations of DDT and DDE in blood as indicators of what is probably the most important toxicological parameter, body burden, will depend on the complex interplay of a number of factors: 1) exposures over a lifetime, in that people who have resided in countries with more recent use of DDT have been found to have both higher levels and also higher DDT to DDE ratios in blood; 2) differences in the relative concentrations of DDT and DDE in the environmental media to which individuals are exposed, related to factors such as differences in activity patterns as well as

[1] "Total DDT" refers to the sum of all DDT-related compounds measured in a particular study.

differences in food sources (i.e. lower DDE to total DDT ratios are found in food grown in regions with more recent DDT usage); and 3) interindividual variability in DDT metabolism, most strikingly metabolic and kinetic changes that are associated with pregnancy and lactation. Epidemiological studies can (and do) attempt to address these sources of variability by controlling for factors such as residential history, age and pregnancy history. However, information about exposure sources over time is usually not obtainable, and most studies that have monitored blood or adipose tissue levels have assessed DDT and DDE levels at only one point in time.

Lipid adjustment of serum levels of DDT, DDE and other persistent compounds is frequently done. The justifications for lipid adjustment are that 1) persistent organic pollutants segregate into the lipid fraction of serum; 2) serum lipid levels may be quite variable; variation due to a meal was reported to be compensated by the correction of the DDT concentrations to serum lipid (Phillips et al., 1989); 3) lipid adjustment facilitates the comparison between concentrations in serum and adipose tissue, as the figures tend to be similar (Haddad et al., 2000), especially when both are corrected to total lipid concentration (Patterson et al., 1988); and 4) lipid adjustment facilitates comparison of doses in human and experimental animal studies. In contrast, whether lipid adjustment (or lack thereof) introduces bias into the estimates of the effects of persistent organic pollutants depends on the statistical model chosen to represent the causality scenario (Schisterman et al., 2005).

Schisterman et al. (2005) recommended that investigators conduct hypothesis testing both with and without lipid adjustment. In some of the rare cases where models were performed and reported using both exposure metrics, major differences were identified. Although lipid-corrected serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) values gave the best prediction of adipose tissue TCDD concentrations (Patterson et al., 1988), and whereas lipid correction essentially made the meal-induced variation in serum *p*,*p*′-DDT concentrations disappear (Phillips et al., 1989), one should note that a substantial portion of DDT and DDE in serum is associated with albumin, not lipid (Morgan et al., 1972; Norén et al., 1999). Moreover, the formula that uses serum total cholesterol, free cholesterol, triglycerides and total phospholipids to calculate "total lipids" for lipid adjustment (Phillips et al., 1989) requires four separate analyses (all with their own uncertainties) and in many

studies is replaced by the simpler formula that sums only total cholesterol and triglycerides, which is less accurate (Phillips et al., 1989). No single correction factor is likely to function properly in different situations, such as different DDT levels, different ages and different sexes (Dalvie et al., 2004c).

The best information on the relationship between DDT (DDE) concentration in serum, expressed in terms of either wet weight (µg/l) ("non-adjusted") or lipid weight (µg/g lipid) ("adjusted"), in the general population comes from the National Health and Nutrition Examination Survey (NHANES) of the United States of America (USA) (CDC, 2009). In Annex 4, it is shown that the median of the ratio of DDT (µg/g lipid) to DDT (µg/l) is 0.160. For DDE, the ratio is 0.168, and for *o,p′*-DDT, 0.155. The arithmetic means are 0.164, 0.172 and 0.158, respectively. The numbers of pairs of observations were 1400, 1951 and 115 for *p,p′*-DDT, *p,p′*-DDE and *o,p′*-DDT, respectively. Other, smaller studies (Polishuk et al., 1977; Kanja et al., 1992; López-Carillo et al., 1997; Hoppin et al., 2000; Romieu et al., 2000; Dalvie et al., 2004c) converge towards the same value of 0.160.

The aim of this hazard assessment was to perform dose–response analyses using lipid-adjusted DDT/DDE concentrations. Where non-adjusted results were also provided by study authors, these are provided as well; where only non-adjusted values were reported, they were corrected using the ratio of average serum DDT/DDE concentrations expressed in micrograms per gram lipid and micrograms per litre: 0.160.

From an analysis of existing studies of DDT, human adipose tissue:serum partition coefficients range between 0.14 and 0.41 for DDT and its metabolites (Haddad et al., 2000). The average ratio, weighted by the number of serum/adipose tissue pairs analysed, is 0.165: 0.21, n = 29 (Wolff et al., 1979a); 0.23, n = 2 (Morgan & Roan, 1972); 0.375, n = 12 (Mussalo-Rauhamaa, 1991); 0.193, n = 27, and 0.157, n = 235 (Wolff et al., 1979b); 0.310, n = 44 (Robison & Hunter, 1966); and 0.147, n = 52 (Brown & Chow, 1975).

Theoretically, the two ratios (serum DDT per volume serum / serum DDT per mass lipid and serum DDT per volume serum / adipose tissue DDT per mass lipid) should be similar, but not necessarily identical, because of the different lipid compositions in adipose tissue and serum and because DDT is also transported

bound to albumin. The most clear-cut study on this issue (Patterson et al., 1988) seems to roughly support the similarity of the two. The two seem to converge in the published studies cited above; therefore, in the present document, a single value of 0.160 is used to describe both ratios. However, this number does not reflect the variation that is observed in human studies or that would be predicted based on what is known about the shifts that occur in lipid adipose:blood ratio by sex, across the life course and with pregnancy.

4. KINETICS AND METABOLISM

Approximately 70–90% of ingested DDT is absorbed from the gastrointestinal tract in the rat when it is administered in vegetable oil. The rate of absorption is dependent on the vehicle; absorption is more effective when DDT is dissolved in oil compared with other media. Oral absorption of DDT in mice and hamsters was approximately 50% (Gingell & Wallcave, 1974). Quantitative data are not available for dermal absorption, but the ratio of oral to dermal median lethal dose (LD_{50}) values is approximately 10 (ATSDR, 2002). Quantitative data are also lacking for absorption via inhalation exposure, but the extent of absorption is apparently governed by the aerodynamic characteristics of the DDT aerosol.

DDT and DDE have very high octanol–water partition coefficients, meaning that they are very soluble in lipids and tend to accumulate in lipid compartments in biological systems. In humans and other mammals, DDT is stored in adipose tissue.

There is marked variability among species in the accumulation of DDT and DDE over time. In particular, these compounds have much longer half-lives in humans than in other mammals (Table 1), so that a much higher rate of intake (per unit body weight) of DDT and DDE in experimental animals is needed to achieve tissue levels that are equivalent to human exposure levels. Half-lives of DDT and DDE in humans have been estimated to range between 6 and 10 years (Wolff, 1999), so that even short-term exposures that raise body burdens are associated with long-term exposures as DDT and DDE are slowly released from fat.

In humans and other mammalian species, DDT is readily transferred from mother to fetus and is excreted in breast milk.

Interspecies differences in the elimination of DDT from fat were summarized by Morgan & Roan (1972). Rate of loss of DDT from lipid stores varies markedly among humans, monkeys, dogs and rats, with humans showing a significantly slower rate of elimination. The kinetics of DDT in humans after oral exposure are complex. Immediately after oral dosing, the concentration of the major urinary metabolite of DDT, 2,2-bis(*p*-chlorophenyl) acetic acid (DDA), decreases rapidly in the urine (half-life very roughly 1–2 days). During the months following the cessation of dosing, the concentrations of DDT in serum and adipose tissue decrease with a

very approximate half-life of 1 year, but at steady state, the half-life of DDT in adipose tissue and breast milk is in the order of 5 years (Morgan & Roan, 1971; Wolff, 1995, 1999; Chen et al., 2009). Humans have a very long retention of DDE in body fat (approximate half-life 13–15 years), with many individuals in whom no disappearance could be detected over a (median) follow-up time of 25 months (Wolff et al., 2000).

Table 1. Half-lives for DDT and DDE in different species

Species	*p*,*p*′-DDT	Compartment	Reference	*p*,*p*′-DDE	Compartment	Reference
Mouse	165 days	Adipose	Gingell & Wallcave (1974)	—	—	—
Rat	19 days	Adipose	Morgan & Roan (1972)	120 days	Adipose	Muhlebach et al. (1991)
Hamster	20 days	Adipose	Gingell & Wallcave (1974)	—	—	—
Rabbit	79 days	Serum	Lindenau et al. (1994)	—	—	—
Dog	5 days	Adipose	Morgan & Roan (1972)	—	—	—
Human	5.7 years	Adipose	Kutz et al. (1991); Wolff (1999)	13–15 years	Serum	Wolff et al. (2000)
	6.3 years	Breast milk	Craan & Haines (1998); Wolff (1999)			

Morgan & Roan (1972) gave three volunteers technical DDT orally at three dose rates (20 mg/day for 183 days, 10 mg/day for 183 days and 5 mg/day for 50 days). The rate of elimination of *o*,*p*′-DDT from adipose tissue was much slower at lower dose levels than at higher dose levels, demonstrating a dose-related effect on elimination and indicative of several consecutive half-lives and thus distribution in several compartments.

As has been noted, outside of the laboratory, there is no such thing as a "pure" DDT exposure or internal dose, because the technical compound is a mixture, because DDE is more persistent in humans than DDT and because of metabolism of DDT to DDE. Although there are clearly interspecies differences in the rate of

elimination of DDT from adipose tissue and in the metabolism of DDT to DDE (ATSDR, 2002), the data available do not allow direct prediction of human toxicity from comparisons between laboratory animals and humans.

Likewise, there are interspecies differences in the metabolism of DDT to DDE. This means not only that experimental animals and humans would be expected to have different levels of exposure with identical dosing rates, but also that there would be different DDT to DDE ratios, which would in turn affect toxicity.

The issue of dose-dependent differences in DDT and DDE half-lives was discussed above. This issue bears on the interpretation of toxicological studies at various levels of exposure. Long-term levels of these chemicals at target tissues are likely to be lower in relation to dosing when dose levels are higher. This means that higher dosing levels will generally tend to overestimate the "actual" exposure or internal dose over time.

These metabolic differences may at least in part explain the differences in the toxicity of DDT, with reported oral LD_{50} values varying widely: rats, 113–4000 mg/kg body weight (bw) per day; mice, 152.3–1466 mg/kg bw per day; rabbits, 300 mg/kg bw per day; and guinea-pigs, 400 mg/kg bw per day (ATSDR, 2002).

Two different pathways for the metabolism of DDT have been proposed in rodents (Fig. 2). The major urinary metabolite of DDT, DDA, is produced by a sequence involving reductive dechlorination, dehydrochlorination, reduction, hydroxylation and oxidation of the aliphatic portion of the molecule. DDT is initially metabolized in the liver to two intermediary metabolites, DDE and DDD. In rats, DDE is slowly converted in the liver to 1-chloro-2,2-bis(*p*-chloro-phenyl)ethene (DDMU), and then to DDA. In hamsters and mice, the DDD to DDMU pathway seems to be a minor pathway. The main difference between mice and hamsters is the relative inability of the latter to form DDE.

The identified metabolites of DDT in humans include DDD, DDA (Roan et al., 1971) and DDE (Morgan & Roan, 1971). However, the quantitative proportions of the different metabolic pathways observed in rodents are not well known for humans, and most of the intermediates have not been positively identified.

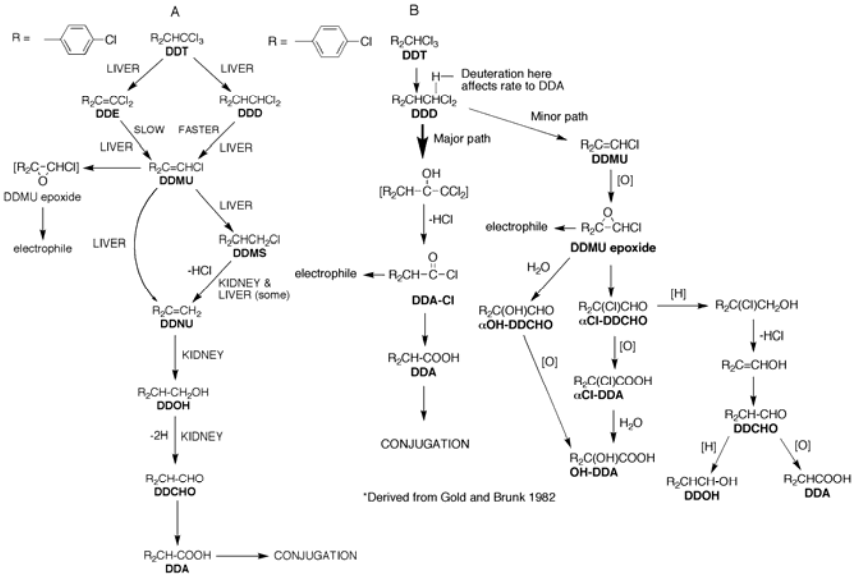


Fig. 2. Metabolic schemes for DDT in rodents: (A) rats; (B) mice and hamsters (ATSDR, 2002)

However, it seems clear that the conversion of DDT to DDE is very limited in humans in comparison with that in rodents and that DDT and DDD but not DDE are metabolized to DDA in humans (Morgan & Roan, 1971; Roan et al., 1971; Chen et al., 2009). The concentration of DDA in urine thus reflects exposure to DDT, not storage in fat of DDE (Chen et al., 2009). People with recent exposure to DDT have a higher ratio of DDT to DDE compared with those who do not (see section 12).

5. HEPATIC EFFECTS AND ENZYME INDUCTION

5.1 Laboratory animals and in vitro systems

Fitzhugh & Nelson (1947) reported on chronic animal feeding studies in which Osborne-Mendel rats were given diets containing a commercial preparation of DDT added to the food in corn oil at levels of 100, 200, 400, 600 and 800 mg/kg or DDT added dry to the food at levels of 600 and 800 mg/kg for 2 years starting at age 21 days. The dosages received from the age of 4 months onwards, as estimated from the graphs presented in the paper, were approximately 4.5, 8.5–10.5, 20–25, 30–35 and 40–50 mg/kg bw per day, respectively. Females generally had somewhat higher dosages using this regimen. Rats had liver enlargement and damage at and above 200 mg/kg diet for females and 400 mg/kg diet for males and increased liver tumours, which are discussed in section 8.1.2 (Fitzhugh & Nelson, 1947).

Laug et al. (1950) reported a study in which rats (strain unspecified) were given DDT, beginning on day 21, at five levels in the diet: controls (0.12 mg/kg), 1, 5, 10 and 50 mg/kg. Exposures were terminated at 23 weeks, and further evaluation was done over a 3-month period to study the elimination of DDT from adipose tissue. There were 15 rats of each sex in each dose group. No changes in growth or "gross toxic" effects were observed. The authors described "minimal" hepatic changes (centrilobular hepatic cell enlargement, increased cytoplasmic oxyphilia and a more peripheral location of the basophilic cytoplasmic granules, with little increase in nuclear size and no evidence for nonspecific degenerative changes such as necrosis) in rats given diets with DDT at 5 mg/kg and above. Changes were dose related and more prominent in males than in females. These changes are consistent with the photomicroscopic appearance of hypertrophic endoplasmic reticulum typically observed with phenobarbital-type hepatic enzyme induction. Levels of DDT in perirenal fat were in the range of 3–9 μg/g for controls, 11–38 μg/g at 1 mg/kg diet, 45–107 μg/g at 5 mg/kg diet, 65–178 μg/g at 10 mg/kg diet and 217–642 μg/g at 50 mg/kg diet. Dietary levels of 1 mg/kg (approximately 0.05 mg/kg bw per day, with levels in fat below 40 μg/g) were not associated with hepatocellular changes.

It has long been observed that DDT induces the synthesis of CYP enzymes (Morello, 1965) that metabolize not only DDT, but

also other drugs. In rats, both DDT and DDE are phenobarbital-type CYP inducers, causing induction of hepatic CYP2B and CYP3A, but not CYP1A. In primary cultures of adult rat hepatocytes, DDE and DDT induced half-maximal CYP2B induction at nominal concentrations of 0.83 and 0.93 µmol/l, respectively (Nims et al., 1998).

In the rat, DDT at 1000 mg/kg in the diet [approximately 50 mg/kg bw per day][1] for 77 days induced the hepatic phenobarbital-type CYP enzyme activities and caused hepatomegaly (Flodström et al., 1990).

In experiments on male Sprague-Dawley rats, You et al. (1999a) found that DDE administration (100 mg/kg bw per day for 7 days) caused liver enlargement, endoplasmic reticulum proliferation and elevated levels of CYP2B1 and CYP3A1 and their hydroxylated testosterone products in both adult and developing rats and elevated levels of CYP2C11 and CYP2A1 among developing but not adult rats.

Administration of DDE (100 mg/kg bw by daily gavage for 7 days) to rats induced hepatic CYP2B1 and aromatase, a member of the CYP family that catalyses the conversion of C19 steroids to estrogens (You et al., 2001).

Daily gavage doses of DDE at 100 mg/kg bw induced CYP3A1 and CYP2B1 in the Sprague-Dawley rat. Exposure of developing rats to DDE (25 or 250 mg/kg in the diet) during gestation and lactation resulted in increased hepatic expression of CYP3A1, but not CYP2B1, which persisted into adulthood. The transcriptional activities of both CAR and PXR were significantly enhanced by DDE. Induction of hepatic CYP3A1 and CYP2B1 by DDE may therefore be mediated through the activation of CAR and PXR (Wyde et al., 2003).

Three doses of *o,p′*-DDT at 300 mg/kg bw also induced the CAR/PXR receptors and CYP2B2 and CYP3A2 in immature ovariectomized Sprague-Dawley rats. Estrogen receptor–regulated genes *Cyp17a1* and *Cyp7b1* were induced in immature ovariectomized

[1] Notes in square brackets […] come from the expert consultation, not from the original paper.

C57BL/6 mice but not in Sprague-Dawley rats by the *o,p′*-DDT treatment (Kiyosawa et al., 2008a,b).

5.2 Humans

Ortelee (1958) reported on a study of 40 DDT workers whose DDT exposures were assessed via urinary levels of the major urinary metabolite, DDA. Although exposure levels were substantial, as modelled via an unspecified method, no chronic health conditions were identified by a physical examination and haematological workup. One worker had an enlarged liver, but that individual had a history of malaria. Morgan & Roan (1974) found a weak association between serum DDT and DDE levels and serum lactate dehydrogenase (LDH) activity, but no relationships with other measures of liver function (aspartate aminotransferase [ASAT], alanine aminotransferase [ALAT], alkaline phosphatase) or with creatine phosphokinase or urinary glucaric acid excretion (used to measure enzyme induction), in 71 pesticide-exposed workers compared with 56 controls (mean serum *p,p′*-DDT concentrations for all participants ranged from less than 1 to 167 µg/l; and mean serum *p,p′*-DDE concentrations, from 8 to 538 µg/l). Laws et al. (1973) reported on a study of 31 men aged 37–64 years who were occupationally exposed to DDT between 16 and 25 years of age. Dose estimates were based on work histories, but serum concentrations of *p,p′*-DDT for 10 of the men ranged from 280 to 1170 µg/l [approximately 45–190 µg/g lipid]. They found no liver abnormalities among these men.

Hayes et al. (1971) published a study in which 24 male volunteers were dosed with *p,p′*-DDT at 3.5 or 35 mg/day for 21.5 months. After following the men for 2–5 years, they identified "no definite clinical or laboratory evidence of injury". Men dosed with DDT had a "slight tendency" towards weight loss. One man receiving the higher dose had a myocardial infarction, which was attributed to prior atherosclerosis. A second man with prior gall bladder surgery developed serious jaundice, which was not explained, but could have been due to viral hepatitis. Interestingly, three of four men in the highest dose group had eosinophilia. No relationship was found between level of DDT dosing and serum ASAT level.

Poland et al. (1970) reported on a study of 18 DDT factory workers and 18 working controls matched by age, ethnicity, smoking, alcohol consumption and medication use. The investigators did not use paired tests, but rather assessed differences in group means. Not surprisingly, the workers had much higher adipose DDT levels on average (0.57 µg/g) than the comparison group (0.012 µg/g). The exposed workers had somewhat higher levels of 6β-hydroxycortisol in urine and somewhat shorter phenylbutazone half-lives, indicating an effect on hepatic metabolism. This study differed from the Morgan & Roan (1974) and Hayes et al. (1971) studies in that exposure was of longer-term duration and measures of hepatotoxicity were more sensitive. The expert consultation noted that, like earlier worker studies (Ortelee, 1958; Laws et al., 1973; Morgan & Roan, 1974), this study suffers from survivor bias, in that only active, long-term workers were evaluated; in other words, these cohorts may be lacking workers who perhaps could have become ill due to the toxicity of DDT.

In a study of 499 persons living near a defunct DDT plant, the geometric mean total DDT serum level was 76.2 µg/l [approximately 12 µg/g lipid]; the national geometric mean at the time was given as 15.0 µg/l [approximately 2.4 µg/g lipid]. DDE was on average 86.7% of total DDT. DDT levels were not associated with specific illness or ill health but were positively associated with levels of serum cholesterol, triglyceride and gamma-glutamyltransferase (GGT). The authors noted that the associations with elevated cholesterol and triglycerides may be related to DDT's fat solubility and not to a true increase in exposure (Kreiss et al., 1981).

Likewise, in a study of 23 DDT applicators in malaria control operations in Natal (now known as KwaZulu-Natal), South Africa, with mean serum DDT and DDE levels of 61.7 and 129.3 µg/l, respectively [approximately 10 and 21 µg/g lipid, respectively], Bouwman et al. (1991a) reported that serum albumin, alkaline phosphatase, ASAT and GGT levels did not differ between exposed applicators and an age-matched control group, but the mean GGT value for the applicators was higher than the maximum of the laboratory normal range. No clinical significance was ascribed to this difference.

Bouwman et al. (1991b) assessed serum concentrations of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in association with liver function

measures among members of households of two different areas of KwaZulu, one area with an annual household application of DDT for malaria control and the other a control area. The two areas were comparable with respect to age but not sex, with a higher proportion of females in the control area. Serum levels varied with age. Total DDT (DDT + DDE + DDD) levels were significantly higher among 71 subjects in the treatment area than among 77 subjects in the control area (mean total DDT 140.9 µg/l and 6.04 µg/l, respectively), with a higher percentage of DDT in the treatment group. Several liver function parameters were evaluated; with the exception of the concentration of total protein, the levels were, on average, higher among the DDT-exposed population, but this was statistically significant only for GGT. Multivariate models on people more than 20 years of age (not fully presented) showed that alcohol consumption was a stronger predictor of GGT than DDT, which was not statistically significant in the model. On the one hand, this points to alcohol consumption in the exposed group as explaining this difference; on the other hand, GGT was also higher (but still within the normal range) among the younger, non-drinking members of the exposed group than among controls. Either this study had insufficient statistical power to independently assess the effect of DDT on GGT, controlling for alcohol, or there is no effect.

***Summary*:** In rats, at doses at and above 5 mg/kg bw per day, DDT caused liver enlargement. At similar and higher dose levels, DDT and DDE caused an increase in hepatic expression of a number of CYP enzymes. It has been demonstrated in rodents that *o,p'*-DDT, like phenobarbital, binds to CAR-PXR and induces xenobiotic metabolizing enzymes.

None of the studies in humans indicated hepatotoxicity by the usual clinical chemistry. One occupational study suggested an effect on enzyme induction in the liver. Three studies in humans at levels greater than 50 µg/l serum, representing active use or occupational human exposure, noted an increase in GGT, but it is unclear whether this is an adaptive or adverse effect.

6. NEUROTOXICITY

Developmental neurotoxicity is discussed in section 10.3.

6.1 Laboratory animals and in vitro systems

In the study of Fitzhugh & Nelson (1947) described in section 5.1, Osborne-Mendel rats were given diets containing a commercial preparation of DDT added to the food in corn oil at 100, 200, 400, 600 and 800 mg/kg or DDT added dry to the food at 600 and 800 mg/kg for 2 years starting at age 21 days. Higher dose levels (mostly 600 and 800 mg/kg; 30–35 and 40–50 mg/kg bw per day, respectively) resulted in marked neurological symptoms, including tremors, convulsions and death, and lower doses produced "irritability", which is possibly a sign of less severe neurotoxicity. Neurotoxic effects were more severe in females and, in both sexes, were aggravated by starvation of the animals, which would have raised the serum levels of DDT (Fitzhugh & Nelson, 1947).

Kashyap and co-workers (1977) carried out an 80-week study in which inbred Swiss mice were treated with technical DDT via several routes beginning at age 6 weeks: orally in the diet (100 mg/kg [approximately 13 mg/kg bw per day]), by intubation (10 mg/kg bw per day), subcutaneously (0.25 mg, twice per month) and by skin painting (0.25 mg, twice per week). There were 30 males and 30 females in each dose group and the control group. There was no difference in body growth or mortality between the experimental and control groups. Toxic manifestations of DDT were observed in mice treated by oral intubation or subcutaneously in the form of tremor, convulsions and corneal opacity, usually after 40 weeks of treatment.

Hyperactivity, tremor and hunched appearance were observed in Osborne-Mendel rats treated with DDT in the United States National Cancer Institute (NCI) bioassay described in section 8.1. The symptoms began to appear in the 5th week of the study in the high-dose group of females (630 mg/kg in the diet [approximately 32 mg/kg bw per day]) and were apparent in both dose groups and both sexes by week 26 (at week 26, males were receiving 500 and 1000 mg/kg in the diet [approximately 25 and 50 mg/kg bw per day, respectively] and females were receiving 315 and 630 mg/kg in the diet [approximately 16 and 32 mg/kg bw per day, respectively]). Because of the toxicity, the doses were halved for both sexes after

week 26 (500 and 250 mg/kg for males [approximately 25 and 12.5 mg/kg bw per day, respectively] and 315 and 158 mg/kg for females [approximately 16 and 8 mg/kg bw per day, respectively]); neurological symptoms disappeared, but they reappeared after another approximately 30 weeks. Dose levels for the study overall were reported as time-weighted averages (NCI, 1978).

The Turusov et al. (1973) multigeneration study of CF-1 mice (described below in section 8.1.1) identified gross signs of neurotoxicity, including tremors and convulsions followed by death, in 1–2 days among 2 male and 4 female animals in the 50 mg/kg [approximately 6.5 mg/kg bw per day] group and among 29 males and 47 females in the 250 mg/kg [approximately 33 mg/kg bw per day] group. Neurotoxicity was not observed in the groups dosed with 10 mg/kg [approximately 1.3 mg/kg bw per day] or less. Most mice developed tremors at the age of 50 weeks and older. The highest rates occurred in the parental and F_5 generations (Turusov et al., 1973).

A study was conducted to evaluate whether DDT and DDE could damage the dopamine system. In vitro studies of mouse synaptosomes and vesicles demonstrated that DDT and DDE inhibit the plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2). However, mice exposed to either DDT or DDE (1, 2 or 3 mg/kg bw per day for 30 days) did not show evidence of nigrostriatal damage or changes in the open field activity or stride length (Hatcher et al., 2008).

6.2 Humans

At very high doses, DDT has been associated with convulsions and even death, including among children with access to containers of DDT in household environments (Cunningham & Hill, 1952; Hsieh, 1954).

One small study reported that concentrations of DDE were higher in the substantia nigra of patients with Parkinson disease than in healthy referents. In a larger study, no such difference was observed in brain specimens mainly from the frontal and occipital cortex (Fleming et al., 1994; Corrigan et al., 1998, 2000). A case–control study of 136 cases of essential tremor and 144 referents reported no associations between DDT and DDE serum levels or

DDT occupational exposure and total tremor score (Louis et al., 2006).

A study of 36 retired Costa Rican malaria control workers and 31 non-occupationally exposed individuals found that the exposed workers had a statistically significant decline in neurobehavioural function and an increase in neuropsychological and psychiatric symptoms (Van Wendel de Joode et al., 2001). Statistically significant decrements in cognitive visuomotor test results were also observed with each increasing year of DDT application. Years of DDT application were estimated by questionnaire and health ministry records and were used as a proxy for exposure. Exposure to cholinesterase-inhibiting pesticides was estimated similarly and controlled for in the analysis.

Summary: DDT caused tremors and convulsions in mice and rats at doses above 6–8 mg/kg bw per day. Similar effects have been observed in children following acute accidental ingestion. No such effects have been reported for occupational and environmental exposure.

7. IMMUNOTOXICITY

Developmental immunotoxicity is discussed in section 10.3.

7.1 Laboratory animals and in vitro systems

In a study in which adult rats were dosed with DDT and DDE at high levels for 6 weeks (200 mg/kg in the diet [approximately 10 mg/kg bw per day]), both DDT and DDE increased albumin to globulin ratios, reduced immunoglobulin M (IgM) and immunoglobulin G (IgG) levels and decreased ovalbumin-induced antibody responses. There was also evidence for inhibition of several functions related to cell-mediated immunity (inhibition of leukocyte and macrophage migration and suppression of delayed-type hypersensitivity reactions) (Banerjee et al., 1996).

In the endocrine modulator screening assay (O'Connor et al., 2002) described in section 10.3.2 using oral doses of *p,p′*-DDE of 50, 100, 200 and 300 mg/kg bw per day administered to mature intact male Sprague-Dawley rats for 15 days, immune system end-points (humoral immune function, spleen and thymus weights and spleen cell number) were not modified.

7.2 Humans

A study in North Carolina, USA, assessed serum DDE levels among 302 persons living near a Superfund site near Aberdeen containing organochlorine pesticides, volatile organic compounds and metals. Blood specimens were analysed for 20 organochlorine pesticides; only DDE was detected, and age-adjusted mean plasma DDE levels were 4.05 µg/l for Aberdeen residents and 2.95 µg/l ($P = 0.01$) for residents of neighbouring communities. Individuals with higher plasma DDE levels (> 7.6 µg/l [approximately 1.2 µg/g lipid]) had lower mitogen-induced (pokeweed mitogen, concanavalin A, phytohaemagglutinin) lymphoproliferative activity, which reached statistical significance for concanavalin A ($P = 0.03$), as well as modestly increased total lymphocytes (range 2.0–2.3 × 10^3/µl; $P = 0.05$) and immunoglobulin A (IgA) levels (range 2.1–2.5 g/l; $P = 0.04$). There was no consistent relationship between plasma DDE level and response to the skin tests with bacterial and yeast antigens (which are tests of the ability to launch an immune response) (Vine et al., 2001).

A study of 44 immunological parameters and several organochlorine compounds in 49 farmers reported an association between interleukin-4 (IL-4) plasma levels and plasma levels of *p,p′*-DDT ($P = 0.0001$) and *p,p′*-DDE ($P = 0.001$) (Daniel et al., 2002).

Summary: On the basis of the available data, it is not possible to conclude whether DDT or DDE exposures of adults are associated with immunotoxicity.

8. CARCINOGENICITY

8.1 Laboratory animals

The International Agency for Research on Cancer (IARC) has classified DDT and DDE as probable carcinogens (Group 2B) (IARC, 1991). The evidence is reviewed here with a particular emphasis on studies relevant to the development of dose–response models. The relevant animal studies are summarized in Table 2.

8.1.1 Mice

Innes et al. (1969) carried out a study in which two hybrid strains of mice were dosed with 130 compounds at their maximum tolerated doses (MTDs). Of these, 11 compounds, including DDT, demonstrated increased rates of tumorigenesis. The MTD for DDT was 46.4 mg/kg bw per day, and this dose was maintained from 7 days of age until about 18 months for 18 male and 18 female animals from each hybrid strain. In comparison with control mice (with 70–90 mice per group), males of both strains and females of one strain had elevated rates of hepatoma; females of the other strain had elevated rates of lymphoma.

Tarján & Kemény (1969) carried out a six-generation study in which BALB/c mice were fed diets containing DDT at 2.8–3.0 mg/kg (0.4–0.7 mg/kg bw per day); in all, 1063 dosed and 2097 control mice were studied. Study authors measured DDT at concentrations between 0.2 and 0.4 mg/kg in the basic diet fed to control mice. Control mice had DDT levels between 1.2 and 1.5 µg/g in fat tissue samples pooled from 10 animals; dosed mice in the F_3–F_5 generations had reached "steady state" at DDT concentrations between 6.3 and 7.7 µg/g in fat tissue pools. Overall rates of tumours (11.3% and 17.4% in treated males and females vs 0.49% and 2.7% in controls) and of leukaemia (3.1% and 9.4% in treated males and females vs 0.74% and 1.7% in controls) were higher in the DDT-exposed group, especially in females. Spontaneous leukaemias were not found in this strain in this laboratory. Other cancers that were increased were adenocarcinomas, especially of the lung.

Table 2. Carcinogenicity studies in mice and rats

Study	Chemical	Strain/sex	Dose group sizes/ages treated	Route	Doses (mg/kg bw per day)[a]	Liver	Lung	Leukaemia/ lymphoma	Other
Mice									
Innes et al. (1969)	DDT	C57BL/6 × C3HANF	70–90 C, 18 T 7 days–18 Mo	Gavage and feed	46.4	M+, F+	M−, F−	M−, F−	Total tumours M+, F+
Innes et al. (1969)	DDT	C57BL/6 × AKR	70–90 C, 18 T 7 days–18 Mo	Gavage and feed	46.4	M+, F−	M−, F−	M−, F+	Total tumours M+, F+
Tarján & Kemény (1969)	DDT	BALB/c	30 C, 28–30 T Multigeneration[b]	Feed	0.4–0.7	M−, F−	M+, F+, F_2–F_5	M+, F+, F_4–F_5	Total tumours M+, F+, F_2–F_5
Shabad et al. (1973)	DDT	A-strain	206[c] C, 264[c] T Multigeneration[b]	Gavage	6.5 F_0 1.3 F_1–F_5	M−, F−	M+, F+	M−, F−	—

Study	Chemical	Strain/sex	Dose group sizes/ages treated	Route	Doses (mg/kg bw per day)[a]	Liver	Lung	Leukaemia/ lymphoma	Other
Terracini et al. (1973)	DDT	BALB/c	54–62 C, 47–69 T Multigeneration[b]	Water	0.26, 2.6, 33	M+, F+ at 33	M−, F−	M−, F−	—
Thorpe & Walker (1973)	DDT	CF-1	45 C, 30 T 4 W–2 Y	Feed	13	M+, F+	M−, F−	NR	Total tumours M−, F−
Turusov et al. (1973)	DDT	CF-1	60 C, 60 T Multigeneration[b]	Feed	0.26, 1.3, 6.5, 33	Hepatoma M+ (all), F+ (all except 0.26) Hepatoblastoma M+, F− at 33	M−, F−	M−, F−	—
Tomatis et al. (1974)	DDE	CF-1	90–100 C, 60 T 6–130 W	Feed	33	M+, F+	M−, F−	M−, F−	Total tumours M−, F−
Kashyap et al. (1977)	DDT	Swiss	30 C, 30 T 6–86 W	Feed Gavage Dermal Subcutaneous	13 (f) 10 (g) 0.25[d] (d) 0.25[e] (s)	M+ (f, g, s) M− (d) F+ (f, g, s, d)	M+ (f, g) M− (s, d) F+ (f, g, s, d)	M+ (f, g, s, d) F+ (f, g, s, d)	—

Study	Chemical	Strain/sex	Dose group sizes/ages treated	Route	Doses (mg/kg bw per day)[a]	Liver	Lung	Leukaemia/ lymphoma	Other
NCI (1978)	DDT	B6C3F1	20 C, 50 T 6–84 W (+ 15 W[f])	Feed	2.9, 5.7 (M) and 11, 23 (F)	M−, F−	M−, F−	M−, F+ (trend only)	—
NCI (1978)	DDE	B6C3F1	20 C, 50 T 6–84 W (+ 15 W[g])	Feed	19, 34 (M, F)	Hepatic carcinoma M+, F+	M−, F−	M−, F−	—
Vesselinovitch et al. (1979)	DDT	C57BL/6J × C3HeB/FeJ (male only)	50 C, 49–50 T 1–90 W	Gavage and feed	0.23 mg/day 1–4 W (gavage); 18 mg/kg bw per day 5–90 W (feed); combined group received both	M+ (feed and combined groups)	NR	NR	—
Rats									
Fitzhugh &	DDT	Osborne-Mendel	24 C, 24 T	Oil in feed	4.5, 8.5–10.5,	+; sex not	NR	NR	—

Study	Chemical	Strain/sex	Dose group sizes/ages treated	Route	Doses (mg/kg bw per day)[a]	Liver	Lung	Leukaemia/ lymphoma	Other
Nelson (1947)			3 W–2 Y		20–25, 30–35 and 40–50	specified			
Rossi et al. (1977)	DDT	Wistar	35–36 C, 35–37 T 7–152 W	Feed	25	M+, F+	M−, F−	M−, F−	—
NCI (1978)	DDT	Osborne-Mendel	20 C, 50 T 7–85 W (+ 35 W[h])	Feed	16, 32 (M) and 11, 21 (F)	M−, F−	M−, F−	M−, F−	F+ (thyroid follicular cell adenoma and carcinoma) low dose only
NCI (1978)	DDE	Osborne-Mendel	20 C, 50 T 7–85 W (+ 35 W[i])	Feed	22, 42 (M) and 12, 23 (F)	M−, F−	M−, F−	M−, F−	M−, F−
Cabral et al. (1982a)	DDT	MRC Porton	38 C, 30–38 T 6–144 W	Oil in feed	6, 12, 24	M−, F+ (trend only)	M−, F−	M−, F−	M− (thyroid adenomas; possible trend not tested), F−

C, controls; d, dermal; F, female; f, feed; f_x, xth filial generation; g, gavage; M, male; Mo, months; NR, no information reported;
s, subcutaneous; T, treated groups; TWA, time-weighted average; W, weeks; Y, years

[a] Unless otherwise provided. Dosages calculated as time-weighted averages where relevant.

[b] Treatment commenced with parental animals and was maintained continuously in parents and offspring through multiple generations.

[c] Sum of animals in all generations; individual group sizes not reported.

[d] 0.25 mg per animal applied twice per week.

[e] 0.25 mg per animal applied twice a month.

[f] Following the 78-week treatment period, there was a 14- to 15-week observation period during which mice received control diet.

[g] Following the 78-week treatment period, there was a 14- to 15-week observation period during which mice received control diet. High dose group mice were treated 4 weeks out of every 5 weeks from week 37 onwards. Dosages are calculated as time-weighted averages.

[h] Following the 78-week treatment period, there was a 35-week observation period during which rats received control diet.

[i] Following the 78-week treatment period, there was a 35-week observation period during which rats received control diet. High dose group rats were treated 4 weeks out of every 5 weeks from weeks 56 (females) or 60 (males) onwards. Dosages are calculated as time-weighted averages.

Shabad and co-workers (1973) fed strain A mice with DDT at 10 or 50 mg/kg [approximately 1.3 and 6.5 mg/kg bw per day] in the diet; the lower dosage was continued in five further generations. Lung adenomas were significantly increased at the higher dose and also at the lower dose in generations F_0, F_2, F_3 and F_4. No other cancers were increased.

Thorpe & Walker (1973) carried out a 2-year oral toxicity study on CF-1 mice using DDT at 100 mg/kg in the diet [approximately 13 mg/kg bw per day]. There were 30 male and 30 female mice in each exposed group (45 mice in control groups), and dosing commenced at age 4 weeks. DDT-treated mice reportedly showed no signs of intoxication. Liver enlargement was observed in females at 60 weeks and in males at 68 weeks. There were significant increases in the incidence of liver tumours in both females and males; these were classified as either type a, hyperplastic foci, or type b, papilloform adenoid growths. Type b lesions were found to metastasize to lungs.

Terracini et al. (1973) reported a two-generation cancer study in BALB/c mice using three dose levels of DDT (2, 20 and 250 mg/kg in the diet [approximately 0.26, 2.6 and 33 mg/kg bw per day]) as well as a control group. Treatment of parent mice started at weaning, at 4–5 weeks of age. Survival in males was poor because of fighting, so that results were reported for females, for which the highest dose levels of DDT produced liver cell tumours in 44% of the parent mice and 74% of first-generation mice; no liver cell tumours were found in controls or those mice on lower doses. Liver cell tumours were not metastatic; however, they were able to grow after transplantation to syngeneic mice. Malignant lymphomas occurred in about 50% of the mice in all colonies given DDT at 0, 2 or 20 mg/kg in the diet and with lower incidences (14–36%) in the groups given DDT at 250 mg/kg in the diet.

Turusov and co-workers (1973) fed six consecutive generations of CF-1 mice (parents, F_1–F_6) with technical DDT mixed into the diet at dose levels of 0 (controls), 2, 10, 50 and 250 mg/kg [approximately 0, 0.26, 1.3, 6.5 and 33 mg/kg bw per day] over their lifespans. The experiment included 3987 mice. Mice were kept until natural death or killed at 130–140 weeks for examination, which included a thorough necropsy and histological examinations. Exposure to DDT significantly increased liver tumours (hepatomas)

in males at all dose levels; in females, however, hepatoma incidence did not increase at the 2 mg/kg diet dose level, and the increase reached statistical significance only at 50 mg/kg diet. No progressive increase of hepatoma incidence from generation to generation was noted in DDT-treated mice. Only one metastatic hepatoma in controls and 13 in DDT-treated groups were reported. Malignant liver tumours, hepatoblastomas, were slightly increased in the 10 and 50 mg/kg diet groups and significantly increased in the 250 mg/kg diet group.

Tomatis et al. (1974) reported on a study in which CF-1 mice were dosed over a lifetime with *p*,*p*′-DDE or *p*,*p*′-DDD at a dose level of 250 mg/kg [approximately 33 mg/kg bw per day] or the two chemicals combined at a dose level of 125 mg/kg diet [approximately 16 mg/kg bw per day], each mixed into the diet. Exposure to *p*,*p*′-DDE resulted in a high incidence and early appearance of liver tumours, especially in female mice. Exposure to *p*,*p*′-DDD resulted in (slightly) increased liver tumours among males only. A marked increase and earlier appearance of liver tumours were observed among both sexes exposed to the combination of *p*,*p*′-DDE plus *p*,*p*′-DDD.

The 80-week study by Kashyap et al. (1977), in which Swiss mice were treated with technical DDT via several routes, is described in section 6.1 above. Oral and subcutaneous DDT treatment (but not skin painting) resulted in a significant increase in the incidence of tumours, mainly of lymphoid tissues, lung and liver. The highest tumour incidence was recorded in the group of mice receiving DDT by subcutaneous injection. Males and females were equally susceptible. Lung tumours as well as lymphomas and leukaemias were significantly increased in the groups with oral dietary (100 mg/kg diet [approximately 13 mg/kg bw per day]) and oral intubation DDT dosing and were increased (but not significantly) in the groups with subcutaneous and skin painting exposures. Liver tumours were described as well-differentiated liver cell carcinomas and were significantly increased only among the group with subcutaneous injections.

The NCI (1978) carried out bioassays of technical-grade DDT and *p*,*p*′-DDE for possible carcinogenicity using B6C3F1 mice (and

Osborne-Mendel rats; see below). Starting at about 6 weeks of age, DDT was administered in the feed to groups of 50 mice at 22 and 44 mg/kg diet [approximately 2.9 and 5.7 mg/kg bw per day] for males and 87 and 175 mg/kg diet [approximately 11 and 23 mg/kg bw per day] for females; there were 20 controls. The time-weighted average dietary concentrations of DDE were 148 and 261 mg/kg diet [approximately 19 and 34 mg/kg bw per day] for male and female mice. Mice were observed for up to 15 weeks after a 78-week dosing period. Female mice dosed with DDT and DDE had significantly accelerated mortality compared with controls. Male mice, including controls, had poor survival because of fighting (70-week survival 12/20, 20/50 and 37/50 in the control DDT, low-dose DDT and high-dose DDT groups; 5/20, 31/50 and 35/50 in the control DDE, low-dose DDE and high-dose DDE groups). Hepatocellular carcinomas occurred in 2/19, 1/49 and 1/48 control, low-dose and high-dose males administered DDT and in 0/20, 1/22 and 3/27 females administered DDT. There was a significant positive association between the concentration of DDE administered and the incidences of hepatocellular carcinomas in male (0, 7/41, 17/47) and female (0, 19/47, 34/48) mice. No histopathological organ damage attributable to DDT or DDE was observed in the treated mice. No significant increases in tumour rates were observed with DDT (NCI, 1978).

Vesselinovitch et al. (1979) carried out a study with several agents, including DDT, to investigate the role of early life exposure in DDT-related hepatocarcinogenesis among a hybrid strain of male mice. There were four dosing groups of 49–50 mice each: Group I received DDT in weeks 1–4 at 230 µg/animal daily by stomach tube; Group II was dosed in weeks 1–90 at 140 mg/kg [approximately 18 mg/kg bw per day] in food, Group III received the doses for both Group I and Group II and Group IV was the controls. One of 50 controls, 5 of 49 Group I mice, 8 of 49 Group II mice and 10 of 49 Group III mice developed hepatic cancers. Rates of cancer were highest in the group with both perinatal and later life exposure. However, in the design of the study, this group was also the most highly exposed, so that the role of age in hepatocarcinogenesis with DDT cannot be determined from the results of this study.

Summary: In conclusion, all nine studies of DDT in eight different mouse strains, including two multigenerational studies, demonstrated some degree of increased tumour incidence caused by DDT. The lowest dose producing increased tumour

incidence was approximately 0.26 mg/kg bw per day. Six of these nine studies showed an increase in liver tumours, including two of the three studies with multiple dose groups. Lung tumours were seen in three of the studies in three different strains, but were somewhat inconsistent for BALB/c mice, for which a multigeneration study with low doses demonstrated an increase and an adult exposure higher-dose study did not. Four studies showed increases in leukaemia and/or lymphomas. Both studies using DDE were positive for liver tumours in males and females using two different strains.

8.1.2 Rats

The Fitzhugh & Nelson study (1947) (see section 5.1) was perhaps the first to identify evidence for hepatic tumours with high doses of DDT. Where they would have expected one or two hepatic cell tumours, they identified eight tumours that occurred later in life and appeared not to be metastatic. They also identified 11 rats with precancerous lesions described as hepatic nodular adenomatoid hyperplasia. No information was provided as to the dosage groups or sex for the rats with tumours and other lesions. Other findings included liver enlargement and damage (see section 5), neurotoxicity (see section 6) and kidney pathology, as well as increased size of adrenal glands, ovarian stromal fibrosis and increased interstitial cellularity of the testes at the 600 mg/kg diet dose level. At the highest dose, abnormalities in other organs were also noted.

Rossi and co-workers (1977) fed 213 male and female Wistar rats a diet containing technical DDT at either 0 (controls) or 500 mg/kg [approximately 25 mg/kg bw per day] over their lifespan. Liver cell tumours developed in both sets of treated animals, at similar incidence levels, but not in controls. The tumours were described as nodular growths that did not metastasize. Females were more susceptible than males to DDT.

The aforementioned NCI (1978) bioassay dosed groups of 50 Osborne-Mendel rats with DDT at time-weighted average doses of 321 and 642 mg/kg diet [approximately 16 and 32 mg/kg bw per day] for male rats and 210 and 420 mg/kg diet [approximately 11 and 21 mg/kg bw per day] for female rats, with 20 controls. Dosing started at about 7 weeks of age. The dietary concentrations of DDE

were 437 and 839 mg/kg for male rats [approximately 22 and 42 mg/kg bw per day] and 242 and 462 mg/kg for female rats [approximately 12 and 23 mg/kg bw per day]. Rats were observed for up to 35 weeks after the 78-week dosing period. DDT did not affect rat mortality; however, DDE was significantly associated with earlier mortality in both male and female rats. No histological non-tumour findings (for neurotoxicological findings after DDT treatment, see section 6) in the DDT-treated animals were related to the dosage, but *p*,*p*′-DDE was hepatotoxic in Osborne-Mendel rats. There were no consistent statistically significant differences in the incidence of any tumour type between the groups treated with DDT or DDE and the controls. In the DDT-treated rats, the incidence of thyroid follicular adenomas and carcinomas combined was 9/19, 20/45 and 22/49 in control, low-dose and high-dose males and 1/19, 14/45 and 11/43 in control, low-dose and high-dose females (the increase was statistically significant at the low dose but not at the high dose in females). There were no positive tumour findings in the DDE-exposed rats (NCI, 1978).

Cabral et al. (1982a) dosed 6- to 7-week-old male and female Porton rats with technical-grade DDT dissolved in 3% olive oil and mixed in feed at doses of 0 (controls), 125, 250 and 500 mg/kg, which the authors stated was equivalent to dietary DDT doses of 0, 6, 12 and 24 mg/kg bw per day (in addition to what would have been in the background). There were 38 males and 38 females in the control and high-dose groups and 30 of each in the other groups. DDT dosing did not affect survival and was associated with a small, non-significant decrease in body weight; at 52 weeks, exposure was associated with liver cell hypertrophy. Females dosed with DDT had a dose-dependent, significantly higher incidence of liver tumours compared with controls. Histologically, the liver cell tumours ranged from a trabecular type to ones showing an irregular arrangement in which the lobular architecture was difficult to recognize. These tumours regularly compressed the surrounding parenchyma. No metastases were observed. Male rats had an increasing incidence of thyroid tumours that was not significant in any one dose, but may demonstrate a trend (untested).

> ***Summary***: In conclusion, three of the four studies using three different strains of rats showed an increase in liver tumours. One of these studies had positive effects only in females, and another was negative when repeated by NCI using similar doses (but with shorter duration), the same strain, larger

sample sizes, better reporting and complete histopathology. One study also showed an increase in thyroid follicular cell adenomas and carcinomas in female rats at the low dose, but not at the high dose, and another had a suggestion of a trend in thyroid tumours in males, but statistical significance was not assessed. The one study of DDE in rats was negative.

8.1.3 Other laboratory animals

Studies in which DDT (≤ 500 mg/kg in the diet [approximately 40 mg/kg bw per day]) was administered to Syrian golden hamsters have not found increased tumour incidence (Agthe et al., 1970; Cabral et al., 1982b; Rossi et al., 1983); however, hamsters, unlike mice and humans, do not metabolize DDT to DDE (Gingell & Wallcave, 1974).

A study in Syrian golden hamsters utilizing DDE confirms the notion that DDE may be the proximate carcinogen for DDT exposure. The Rossi et al. (1983) study was a lifetime dosing study of male and female Syrian golden hamsters beginning at 8 weeks of age, using DDE (99% pure) in the diet at a dose of 500 or 1000 mg/kg [approximately 40 or 80 mg/kg bw per day], technical-grade DDT at 1000 mg/kg diet and a control group. Each group had at least 40 hamsters per sex. Animals exposed to DDE had hepato-cellular tumours, classified as neoplastic nodules, later in life. These tumours did not appear in untreated or DDT-treated animals. Some of the animals treated with DDE or DDT at 1000 mg/kg diet had hyperplastic foci of the liver.

Studies of DDT in dogs (Lehman, 1951, 1965) did not show a carcinogenic effect, and studies in monkeys were inconclusive (Adamson & Sieber, 1979, 1983; Takayama et al., 1999); these studies were limited because of small numbers of animals and/or short duration.

Veeramachaneni et al. (2007) dosed rabbit does from gestation day (GD) 15 through postnatal day (PND) 4 to provide 25 µmol (low) or 250 µmol (high) DDT. After 12 or 24 weeks, in five animals treated with the high DDT dose, cells suggestive of testicular carcinoma in situ were seen, whereas they occurred in

none of the controls or low-dose animals. It should be noted that in this study, the exposure was perinatal.

> ***Summary***: Studies in other test species (dogs and monkeys) have been too small and/or short term to be conclusive. A short-term study on testicular cancer after perinatal exposure in rabbits was inconclusive.

8.1.4 Conclusions for laboratory animals

DDT and DDE have tested positive for cancer in rodent species (and perhaps also rabbits). Studies in other test species (e.g. dogs and monkeys) have been too small and/or short term to be conclusive. The most prominent and consistent findings across species are the progression of effects ranging from hyperplasia (mice, rats, hamsters) to benign tumours over dose and time. Hepatocellular cancer has been observed in mice. Mice have also developed lung tumours and leukaemia in response to DDT.

8.2 Humans

8.2.1 Ecological and cohort studies

Historically, a number of cohort studies have evaluated the relationship between exposure to DDT, occupationally or in the general environment, and subsequent cancer incidence and/or mortality, looking across a broad swath of cancers. Usually, these studies were conducted among occupational cohorts. Most of these have had limited power because of small numbers of specific cancer types and/or limited exposure assessments; however, they are presented in this review to give a full picture of what has been observed in human populations. As these limited studies assessed rates of multiple cancer diagnoses, they are not described and critiqued in the sections below. For reference, the cohort and ecological studies are summarized in Table 3.

Table 3. Summary of ecological and cohort studies of cancer incidence or mortality due to DDT exposure

Study	Population	Risk metric	Years of observation	End-point	Exposure	Comments	Cancer sites increased	Cancer sites not increased
Ditraglia et al. (1981)	354 workers manufacturing DDT and other chemicals ("Plant 4")	SMR, comparison with general population	Employed ≥ 6 months prior to January 1964; follow-up through 1976; 7601 person-years	Cancer mortality	All workers in plant employed ≥ 6 months	10% of Plant 4 cohort lost to follow-up; numbers too small to assess individual cancer types	All cancer SMR > 20 years of employment 132 (NS); NS SMR for stomach, pancreas, respiratory	Oesophagus, intestine, rectum, liver, bladder and urinary, lymphatic and haematopoietic
Wong et al. (1984)	740 white male workers with potential exposures to DDT	SMR, comparison with general population	Employed between 1935 and 1976 with vital status in 1976; 17 187 person-years for DDT	Cancer mortality	Work area and department	5% of overall cohort and 6% of deaths lost to follow-up; very small numbers of individual	NS for buccal cavity and pharynx, rectum, lung, testis, bladder, leukaemia	Overall cancer, stomach, large intestine, respiratory

Study	Population	Risk metric	Years of observation	End-point	Exposure	Comments	Cancer sites increased	Cancer sites not increased
			subcohort			cancers		
Austin et al. (1989)	304 white males, 327 white females, 204 black males and 84 black females from South Carolina, USA	(1) RR (2) SMR (South Carolina and USA)	10-year prospective follow-up	Cancer mortality	Serum DDT and DDE levels 1974	99% follow-up; very small numbers of individual cancers	(1) and (2) NS increase in respiratory cancer deaths; (1) NS gastrointestinal cancer	Other cancers not reported
Cocco et al. (1997)	1043 deaths among male DDT sprayers in anti-malarial campaign in Sardinia, Italy	PMR	Work in 1940s; deaths occurring 1956–1992	Cancer mortality	Employees listed as having applied DDT or inspected DDT applications	Very small numbers of some individual cancers	Increased liver and biliary tract cancers (PMR = 228; 95% CI = 143–345); multiple myeloma (PMR = 341; 95% CI = 110–795); NS for oral and pharynx, oesophageal, kidney, haemolymphatic, myeloid leukaemia	Stomach, colon, rectal, pancreatic, laryngeal, lung, bladder, brain and central nervous system, NHL

Study	Population	Risk metric	Years of observation	End-point	Exposure	Comments	Cancer sites increased	Cancer sites not increased
Cocco et al. (2000)	Population in 22 states of USA in 1968	RR	Ecological, cross-sectional	Cancer mortality 1975–1994	State adipose tissue DDE levels from USEPA NHATS 1968		Liver (white males and white females)	Liver (black males and black females), pancreas, breast (reverse association), corpus uteri (reverse white females), multiple myeloma, NHL (reverse all but black males)
Cocco et al. (2005a)	4552 male workers in DDT anti-malarial operations in Sardinia, Italy,	(1) SMR, Sardinia comparison population; (2) RR, unexposed	Occupational cohort	Cancer mortality	Estimates of average and cumulative exposure from DDT usage	0.5% lost to follow-up; 10% of death causes undefined; very small	Stomach cancer, for highest exposure quartile, RR = 2.0; 95% CI = 0.9–4.4; (1) NS for bladder; (2) NS for stomach and	(1) stomach, leukaemia; (1) and (2) liver, pancreatic, lung, prostate, lymphatic;

Study	Population	Risk metric	Years of observation	End-point	Exposure	Comments	Cancer sites increased	Cancer sites not increased
	1946–1950	subcohort				numbers of individual cancers	leukaemia	(2) bladder
Purdue et al. (2007)	25 291 pesticide applicators	RR	Occupational cohort	Cancer incidence	DDT use report on take-home questionnaire	Very small numbers of some individual cancers	Prostate RR = 1.2 (95% CI = 1.0–1.4) and no evidence for dose–response relationship with total insecticide use; NS for lung, rectum, leukaemia	Colon, bladder, NHL, melanoma

CI, confidence interval; NHATS, National Human Adipose Tissue Survey; NHL, non-Hodgkin lymphoma; NS, not significant; PMR, proportionate mortality ratio; RR, relative risk; SMR, standardized mortality ratio; USEPA, United States Environmental Protection Agency

Ditraglia et al. (1981) reported a retrospective occupational cohort mortality study of workers employed in four plants manufacturing a number of organochlorine pesticides, including chlordane, DDT, heptachlor and aldrin/dieldrin/endrin. Each cohort included all workers employed for at least 6 months prior to January 1964; cancer mortality was ascertained through the end of 1976. The DDT subcohort, from a plant in California, USA, had only 354 workers. Given the timing of the study, there were very few deaths attributed to cancer. Overall standardized mortality ratios (SMRs) between 66 and 82 were consistent with the "healthy worker effect". No significant relationships between cancer mortality and employment in the DDT plant were observed other than an increase in the overall cancer SMR for those employed at least 20 years. Given small numbers and long latency periods for cancer, this is not surprising.

In another occupational cohort mortality study, Wong et al. (1984) determined the vital status in 1976 for 740 white male workers employed between 1935 and 1976 with potential exposures to DDT by virtue of employment in a manufacturing plant in California, USA. For the study population analysed (of which the 740 DDT workers were a subgroup), the study authors had been able to determine the vital status of 95% of the cohort, with death certificates (allowing coding of cause of death) obtained for 94% of those who had died. SMRs were used to compare death rates with those of white males of the same age and during the same time in the USA. Worker exposures were classified by their work areas or departments. As might be expected, given the healthy worker effect, overall mortality and SMRs for most causes of death, including diseases of the digestive system, were low. For DDT, there were a few non-significant SMRs for individual cancer types (Table 3), but these were based on very small numbers.

Austin et al. (1989) reported results of a 10-year prospective mortality study of 919 male and female subjects with serum DDT and DDE levels measured in 1974 and 1975. Of 209 who had died in the interim, 54 had cancer. Total mortality and cancer mortality were not associated with DDT levels. There were non-significant positive relationships between respiratory and gastrointestinal cancer mortality and serum DDE and DDT levels. Other cancer

types were not reported as a result of small numbers. This study was limited by low statistical power and a short follow-up interval.

Cocco et al. (1997) published a proportionate mortality study of 1043 deaths that occurred between 1956 and 1992 among men who used mainly DDT in an anti-malarial campaign in Sardinia, Italy, during the late 1940s. They reported that workers directly exposed to DDT had a significant increase in risk for liver and biliary tract cancers (proportionate mortality ratio [PMR] = 228; 95% confidence interval [CI] = 143–345) and multiple myeloma (PMR = 341; 95% CI = 110–795). Supervisors, drivers, laboratory staff and administrative staff of the project, considered not to be exposed, also had an elevated PMR for liver cancer (PMR = 210; 95% CI = 117–346), casting doubt on the role of DDT exposures as opposed to exposures shared among all of the workers. Indeed, subsequent to that, Cocco et al. (2005a) conducted a more thorough mortality follow-up study of 4552 male workers from this same cohort who worked around DDT during anti-malarial operations in Sardinia, Italy, in 1946–1950. For this study, they used information about DDT use to develop individual estimates of average and cumulative exposure. In comparison with the Sardinian population, there was no increase in cancer mortality. Using Poisson regression analysis to calculate relative risk (RR) in an unexposed subcohort as the reference, the only evidence for increased mortality with DDT exposure was for stomach cancer in the highest quartile of cumulative exposure (RR = 2.0; 95% CI = 0.9–4.4). The actual DDT exposure levels are unknown. There was no evidence for a linear dose–response trend across exposure categories. Non-significant elevations of bladder cancer and leukaemia were reported. Risks of liver cancer, pancreatic cancer and cancers of the lung, prostate and lymphatic system were not elevated among DDT-exposed workers in the follow-up study.

Cocco et al. (2000) reported an ecological study that analysed the levels of DDE as measured in the population in 22 states of the USA in 1968 and age-adjusted cancer mortality rates between 1975 and 1994 for multiple myeloma, NHL and cancer of the breast, corpus uteri, liver and pancreas. Stratifying by sex and race, they used multiple regression models to control for average per capita income, per cent metropolitan residents and population density. States with higher adipose DDE levels had increased liver cancer mortality for whites, but not for African Americans. No associations

were observed for pancreatic cancer or multiple myeloma. States with higher adipose DDE levels had lower breast cancer mortality rates for women of both races and lower uterine cancer rates for white women only. An inverse relationship was also reported for NHL in all but African American males.

In the Agricultural Health Study of the USA, no relationship was found between self-reported use of DDT and cancer of the colon, bladder cancer, NHL or melanoma among 25 291 pesticide applicators (1100 exposed cases for all cancers combined) who completed a take-home questionnaire. Actual exposure levels are unknown. An elevated risk (RR = 1.2; 95% CI = 1.0–1.4) was reported for prostate cancer. However, prostate cancer was not related to "total insecticide usage", a metric including organochlorines other than DDT, so the authors discounted this finding. Non-significant relationships were reported for cancers of the lung and rectum and leukaemia (Purdue et al., 2007).

Summary: Ecological and cohort studies for DDT do not provide convincing evidence of patterns of associations between DDT and cancer incidence or mortality. However, these studies all have severe limitations. Most, except for Austin et al. (1989), lack individual DDT or DDE exposure measures and instead rely on occupational history, self-report or ecological measures of exposure, which are likely to be unreliable. Some, such as Austin et al. (1989), are too small to have adequate statistical power to observe an increased incidence of cancer associated with DDT. Of the others, all but Purdue et al. (2007) examine cancer mortality but not cancer incidence. Also, most of those studies did not include pathological confirmation of cancer case status.

8.2.2 Case–control and nested case–control studies

8.2.2.1 Breast cancer

Many studies have been conducted on the relationship between DDT exposure and breast cancer risk, with associations between DDE body burdens and breast cancer frequently being investigated. A meta-analysis published in 2004 formally evaluated 22 epidemiological studies published through January 2001 and

evaluating associations between DDE internal doses and breast cancer risk. The authors used DerSimonian and Laird's method for random effects models and the Q-statistic to identify heterogeneity in breast cancer rates across studies. The summary odds ratio (OR) for selected studies was 0.97 (95% CI = 0.87–1.09), and the gradient of exposure ranged from 0.084 to 12.95 μg/g serum lipid weight. No overall heterogeneity in the OR was observed (χ^2 = 27.93; degrees of freedom [df] = 23; P = 0.218). The authors concluded that this analysis provided strong evidence to reject the hypothesized relationship between DDE internal dose and breast cancer risk (López-Cervantes et al., 2004).

Subsequent to that time, there have been several published studies of the relationship between internal doses of DDE and DDT in adult women and breast cancer. Pavuk et al. (2003) reported a hospital-based case–control study of breast cancer in eastern Slovakia. Cases diagnosed between May 1997 and May 1999 were recruited, along with 88 controls who were participants in a cross-sectional study conducted in the same district. Serum levels of DDE and DDT were significantly higher in cases than in controls. ORs for breast cancer risk were estimated using unconditional logistic regression and tertiles of DDT and DDE internal doses, adjusting for age, height, body mass index (BMI), number of full-term pregnancies, menopausal status, age at menarche, alcohol consumption, smoking, marital status and education. Serum levels of DDE in the third tertile, in the range of 4.389–19.912 μg/g, were associated with increased odds of breast cancer (OR = 3.04; 95% CI = 0.65–14.3), but there was no association for DDT.

A second study in Eastern Europe by Charlier et al. (2004) reported that serum levels of DDE in 231 cases at the time of breast cancer diagnosis were significantly higher (3.46 [SD 3.48] μg/l; 580 [SD 580] ng/g lipid) than among 290 age-matched healthy controls (1.85 [SD 2.099] μg/l; 310 [SD 3509] ng/g lipid) (P < 0.0001). In the cancer group, no differences in serum levels of DDE were found in relation to estrogen receptor (ER) status, Bloom stage or lymph node metastasis.

Raaschou-Nielsen et al. (2005) reported a prospective nested case–control study of 409 postmenopausal women who developed breast cancer and 409 controls selected from the 29 875 women enrolled in the Danish Diet, Cancer, and Health cohort between

1993 and 1997. They measured levels of DDT and DDE and a number of other persistent substances in adipose tissue collected at enrolment, and they used conditional logistic regression to compute an "estimated relative risk (RR)". The median DDE concentration of the referents was 0.477 µg/g lipid. Raaschou-Nielsen et al. (2005) found no association between pesticide levels and breast cancer. The RR for the highest quartile of DDE concentration was 0.7 (95% CI = 0.5–1.2). They observed a lower risk of ER-negative breast cancer in association with higher levels of pesticides, including DDE (RR = 0.1; 95% CI = 0.0–0.5); this was unexplained.

More recently, a retrospective case–control study in Alaska evaluated serum DDE levels in 63 Native women who had developed breast cancer in comparison with 63 age-matched control women who remained cancer-free. The serum samples from cases and controls had been collected and banked in the period 3–10 years prior to the date of diagnosis. The geometric mean serum DDE level among case women was 8.67 µg/l (95% CI = 7.48–10.04); among control women, the geometric mean concentration was 7.36 µg/l (95% CI = 6.53–8.30) [these are approximately 1.4 and 1.2 µg/g lipid, respectively]. Using conditional logistic regression analysis to adjust for potential confounders (e.g. ethnicity, family history of breast cancer, parity), Rubin et al. (2006) found an OR of 1.43 (95% CI = 0.46–4.47) for the highest tertile of serum DDE level.

Li et al. (2006) reported on a population-based case–control study of 90 newly diagnosed breast cancer cases and 136 healthy community controls (information obtained from an English abstract; the original paper was written in Chinese). Assessment of DDT exposure was via analysis of serum levels. Multivariate logistic regression models adjusting for confounding factors showed significant positive associations between exposure to DDT and breast cancer, most strongly among premenopausal women (adjusted OR = 3.59; $P < 0.05$).

Gatto et al. (2007) conducted a population-based case–control study of 355 African American breast cancer case patients diagnosed between 1995 and 1998 and 327 controls identified by random digit dialling methods. Serum levels of DDE (mean for controls, 1.25 µg/g lipid) were adjusted for total lipid content. Using

multivariate unconditional logistic regression controlling for age, BMI and breastfeeding, breast cancer risk was not associated with increasing quintiles of lipid-adjusted DDE levels (highest versus lowest quintile OR = 1.02; 95% CI = 0.61–1.72). Risk was not modified by strata of BMI, breastfeeding, parity, menopausal status or tumour receptor status.

The Japan Public Health Center–based Prospective Study enrolled 24 226 female subjects 40–69 years of age who responded to the baseline questionnaire and provided blood in 1990–1995. With follow-up until December 2002, 144 cases of breast cancer were diagnosed and two matched controls selected for each case. Plasma levels of DDT and DDE were measured. The adjusted OR for breast cancer risk associated with plasma DDT level was less than 1. For DDE (median plasma *p*,*p*′-DDE level, 6.08 µg/l), the adjusted OR for the highest versus lowest quartile was 1.48 (95% CI = 0.70–3.13) (Iwasaki et al., 2008).

Of recent interest is a study by Cohn et al. (2007), the first to examine prepubertal exposure to DDT and breast cancer risk. The period of thelarche (breast maturation) would be expected to be a time when developing breast tissue is more vulnerable to carcinogens, so it is important to evaluate the effects of early exposure. Cohn et al. (2007) conducted a prospective, nested case–control study that was based on an existing longitudinal cohort study in California, USA, called the Child Health and Development Studies. This is a study of the mothers of the children who were in this study and the subsequent development (or not) of breast cancer among these women. The median time to diagnosis of a breast cancer among the mothers in this cohort was 17 years. Blood samples were taken between 1959 and 1967 1–3 days after the women gave birth (mean age, 26 years). The investigators identified a total of 129 women from among this cohort of mothers who developed breast cancer before the age of 50 years. They matched these cases to 129 control mothers who were also in this cohort study by year of birth. The average serum DDT level was 1.4 µg/g lipid, and the approximate low and high bounds were 3 and 48 µg/l (Cohn et al., 2003). Higher serum levels of DDT predicted a 5-fold increase in risk of breast cancer among women who were born after 1931, but not among women born prior to 1931. Women born after 1931 would have been under 14 years of age in 1945, when DDT came into widespread use, and mostly less than 20 years of age as

DDT use peaked. Women born earlier than 1931, who could not have had early life (prior to age 14) exposure to DDT, showed no association between serum DDT levels and breast cancer. Within the younger group of women who had DDT exposure in childhood, there is evidence for a dose–response relationship; women in the second tertile of exposure (8.09–13.90 µg/l [approximately 1.1–1.8 µg/g lipid, calculated from the ratio of the central estimates of *p,p'*-DDT expressed in µg/l and µg/g lipid in this population]) had an OR of 2.8 (95% CI = 1.1–6.8); those in the third tertile of exposure (> 13.90 µg/l) had an OR of 5.4 (95% CI = 1.7–17.1). The authors concluded that DDT exposure early in life may increase breast cancer risk. Of note is that the Cohn et al. (2007) study includes women with much higher levels of exposures than most other studies.

Summary: For breast cancer epidemiological studies, exposures to DDT and DDE as ascertained at the time of diagnosis or during adulthood are generally not associated with breast cancer; there are some positive studies, but they are outweighed by an overwhelming number of negative studies, as is clear from the López-Cervantes et al. (2004) meta-analysis and a number of the studies completed since that time. Most of the ecological and cohort studies (Table 3) included males only; one (Cocco et al., 2000) did not support a relationship between DDT and breast cancer. A single study of prepubertal exposure to DDT suggested that early life (prior to age 14) exposure, but not later, may be associated with breast cancer.

8.2.2.2 Testicular cancer

Hardell et al. (2006) conducted a case–control study of 58 cases with testicular cancer and 61 age-matched controls; 44 case mothers were also matched with 45 control mothers. They measured serum DDE levels in case and control mothers. Using unconditional logistic regression adjusting for age and BMI at the time of serum analysis (smoking was evaluated and was reported not to be a confounder), they calculated an OR of 1.3 (95% CI = 0.5–3.0) using the median concentration for the controls as a cut-off value. This study did not report the actual serum DDE levels in this population, nor did the authors assess serum DDT levels. This study was limited

by the rather oblique assessment of exposure via measurement of levels in mothers years after their adult sons were diagnosed with cancer.

A population-based case–control study of testicular germ cell cancer was conducted among 18- to 44-year-old male residents of three counties in the state of Washington, USA. Investigators recruited 246 cases diagnosed in 1999–2003 with a first, primary testicular germ cell cancer and 630 controls of similar age from the same population and recruited through random digit dialling. A blood specimen taken well after the time of diagnosis was used to measure levels of DDE and a number of other organochlorine pesticides and for genotyping. There was no clear association between risk of testicular germ cell cancer and serum DDE levels or other compounds that were studied, even after controlling for androgen receptor (AR) CAG (glutamine) or GGN (glycine) genotypes (Biggs et al., 2008). This study was limited by the assessment of serum DDE levels years after diagnosis of and treatment for cancer.

McGlynn et al. (2008) published a case–control study with 754 cases of testicular germ cell tumours and serum levels of persistent organic pollutants, including DDT and DDE. The subjects were participants in the United States Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study and consisted of cases who were 45 years of age or younger at the time of diagnosis and had donated at least one serum sample to the Department of Defense Serum Repository prior to diagnosis. Controls were healthy men who had donated serum during the same time and were matched on birth year (within 1 year), race/ethnicity (white, black, other) and date of available serum sample (within 30 days). The authors reported a strong and significant relationship between serum DDE levels in the upper quartile (> 0.39 µg/g lipid) and subsequent development of testicular germ cell tumours (RR = 1.71; 95% CI = 1.23–2.38). When they considered risk of the two major subtypes of testicular germ cell tumour separately (seminoma and non-seminoma), both risks were significantly elevated for DDE and of similar magnitude (RR = 1.91 and 1.63, respectively). There were no significant relationships between testicular germ cell tumour and testicular germ cell tumour subtypes and serum DDT level. This study provides very strong evidence for an association between serum DDE level and testicular germ cell cancers in men.

Summary: For testicular cancer epidemiological studies, a single study with a prospective exposure measure provided evidence for an association between DDE exposure and testicular germ cell tumours at levels above 0.39 µg/g lipid.

8.2.2.3 Liver cancer

Hardell and co-workers (1984) investigated the etiological factors behind primary liver cancer in a case–control study of 102 cases and 205 controls in northern Sweden. From the figures given in the paper, IARC (1991) estimated an OR for exposure to DDT in agriculture to be 0.4 (95% CI = 0.1–1.1), and in forestry, 1.3 (95% CI = 0.3–4.0).

In another study, McGlynn et al. (2006) reported a nested case–control study among the participants of the Nutritional Intervention Trials in Linxian, China. Subjects were 168 cases who developed liver cancer during the trials, and controls included 385 controls matched on age and sex who were alive and well at the end of the study. Geometric mean serum DDT and DDE levels did not differ between cases and controls in bivariate analyses, but, as might be expected, cases had a much higher rate of hepatitis B surface antigen positivity than did controls (25% vs 4%). Of note is that this population has a high background level of liver cancer; crude annual liver cancer rates were 26 liver cancers per 100 000 persons in the lowest quintile of serum DDT concentration (< 0.265 µg/g) versus 46 liver cancers per 100 000 persons in the highest quintile of serum DDT concentration (> 0.787 µg/g). In multivariable-adjusted models that controlled for age, sex, hepatitis B surface antigen status and commune of residence, the risk of developing liver cancer increased with increased serum DDT concentration (OR for quintile 1 vs quintile 5 = 3.8; 95% CI = 1.7–8.6; *P* for trend = 0.0024), but there was no statistically significant association between liver cancer and serum DDE concentration. Serum DDE levels were not associated with liver cancer, and higher levels of DDE in serum seemed to attenuate the DDT–liver cancer relationship. This effect modification was not explained but had a strong influence on the dose–response relationship between serum DDT level and liver cancer. The dose–response relationship was interesting and consistent with

a trend across the entire range of serum DDT levels monitored in this study (Table 4).

The cohort and ecological studies (see Table 3) frequently included liver cancer, but most studies had too few subjects to evaluate the cancer incidence. One study of Italian anti-malaria workers initially reported elevated liver and biliary tract cancers (Cocco et al., 1997), but a larger and more thorough follow-up of that same cohort did not replicate this finding (Cocco et al., 2005a). A United States ecological study that categorized states by average DDE levels in adipose tissue did report significant associations between DDE and liver cancer in white (but not African American) males and females (Cocco et al., 2000).

Table 4. Odds of liver cancer by category of DDT exposure (without and with adjustment for DDE levels)

Serum DDT level (µg/g lipid)	OR (95% CI)	OR (95% CI) adjusted for DDE
< 0.265	Referent	Referent
0.265–0.382	1.3 (0.7–2.5)	1.5 (0.8–2.7)
0.383–0.521	1.4 (0.7–2.6)	1.7 (0.9–3.3)
0.522–0.787	1.4 (0.7–2.7)	2.1 (1.0–4.3)
> 0.787	2.0 (1.1–3.9)	3.8 (1.7–8.6)
	(*P* for trend = 0.049)	(*P* for trend = 0.002)

Source: McGlynn et al. (2006)

Summary: A single incidence study provides strong evidence for an association between DDT (geometric mean 0.49 µg/g) but not DDE (geometric mean 2.9 µg/g in cases, 3.0 µg/g in controls) levels in serum and liver cancer among relatively highly exposed populations. Other studies have not been positive, but this is the only study with individual prospective measures of DDT exposure and complete ascertainment of cancer incidence.

8.2.2.4 Lymphocytic cancers

A number of epidemiological studies have assessed possible relationships between DDT/DDE exposures/internal doses and lymphoma. In a population-based case–control study among 576 NHL cases diagnosed between 1981 and 1984 and 694 randomly selected referents without cancer on the association between chlorophenol and phenoxyacid herbicide exposure and NHL (and

soft tissue sarcoma) in Washington state, USA, NHL was associated with several occupational and lifestyle factors, including reported DDT exposure (OR = 1.82; 95% CI = 1.04–3.2) (Woods et al., 1987).

Zahm et al. (1990) conducted a case–control study of 201 cases with NHL and 725 controls among men in farming communities in eastern Nebraska, USA; they did not find associations with reported DDT exposures.

Cantor et al. (1992) interviewed 622 white men with newly diagnosed NHL and 1245 population-based controls in Iowa and Minnesota, USA. Elevated risks were found, with ORs generally 1.5-fold or greater, for several individual insecticides, including DDT. Associations were generally stronger for first use prior to 1965 than more recently and when protective clothing or equipment was not used. However, the findings lacked specificity.

To extend this work, Baris et al. (1998) published a pooled analysis of three prior studies of NHL and DDT use among male farmers enrolled in three case–control studies from four Midwestern states in the USA (Nebraska, Iowa, Minnesota, Kansas), for a total of 993 cases and 2918 non-farmer controls. Information on exposure to DDT was based on interviews about agricultural pesticide use. In total, 161 cases and 340 controls reported DDT use; the OR was 1.2 (95% CI = 1.0–1.6). There was evidence for a dose–response relationship, in that farmers who had used DDT for 15 years or longer had an OR of 1.5 (95% CI = 1.0–2.3); those who used DDT for 5 days a year or more had an OR of 2.6 (95% CI = 1.1–5.9). However, adjustment for use of other pesticides attenuated or completely eliminated the excess risk, suggesting that the apparent relationship between DDT and NHL was due to confounding by other pesticide use.

A population-based case–control study in southern Italy compared pesticide exposures by job matrix to cases with leukaemia, lymphoma and myeloma compared with controls with other neoplasms. For these cancers combined, the OR for exposure to DDT and creolin was 4.11 (95% CI = 1.16–14.55) (Assennato et al., 1995).

A nested case–control study examined serum DDT levels measured between August and November 1974 among 74 cases diagnosed between January 1975 and May 1994 and 147 matched controls from a prospective cohort of 25 802 adults. They found no associations between serum concentrations of total DDT (median 2.8 µg/g lipid in referents) and risk of NHL (Rothman et al., 1997).

McDuffie et al. (2001) studied 517 incident cases of NHL and 1506 population controls in Canada; exposure information was elicited via a postal questionnaire followed by a telephone interview of those who reported annual pesticide exposure of 10 hours or more and a 15% random sample of the remainder. Adjusted ORs were computed using conditional logistic regression stratified by the matching variables of age and province of residence. For DDT, the OR for NHL was 1.73 (95% CI = 1.08–2.76). However, there were significant associations between NHL and past usage of many other pesticides, so confounding could not be ruled out in this study.

Spinelli et al. (2007) performed a population-based case–control study in British Columbia, Canada, among 422 cases (serum drawn prior to treatment) and 460 controls. Serum levels of DDE but not of DDT were higher among cases than among controls, but so were levels of a variety of other organochlorine pesticides and PCBs; concentrations of all measured organochlorine compounds were intercorrelated. This study did not provide strong evidence for an association.

Cocco et al. (2008) measured the concentration of DDE (median *p*,*p*′-DDE level in different countries 200–800 µg/l) and numerous other organochlorines in plasma samples of 174 NHL cases and 203 controls from France, Germany and Spain. The risk of NHL and its major subtypes associated with increasing blood levels of DDE was calculated using unconditional logistic regression. Risk of NHL, diffuse large B cell lymphoma and chronic lymphatic leukaemia did not increase with plasma levels of DDE in the overall study population.

Multiple myeloma was assessed in a population-based case–control study of 173 cases and 650 controls that was conducted in Iowa, USA. Exposure was determined via a questionnaire. History of use of DDT (OR = 1.1; 95% CI = 0.6–1.9) and other pesticides

was not associated with risk of multiple myeloma (Brown et al., 1993). One study of Italian anti-malaria workers initially reported elevated multiple myeloma with DDT exposure (Cocco et al., 1997), but a larger and more thorough follow-up of that same cohort did not replicate this finding (Cocco et al., 2005a).

> ***Summary***: Cohort and ecological studies did not find relationships between DDT and lymphocytic cancers (Table 3). Most studies linking pesticide exposure to NHL did not distinguish DDT from other chemicals as the causative agent. A single case–control study that adjusted for other pesticide exposures did not show a significant association with DDT exposure.

8.2.2.5 Lung cancer

There are no case–control studies examining the association between DDT/DDE and lung cancer.

A prospective cohort study was reported of 919 subjects with serum DDT and DDE measurements in 1974 and 1975 (see Table 3). Over 10 years, 209 deaths occurred, among whom 54 had cancer. There was no relationship between total cancer mortality and DDT levels. However, there was a positive relationship with respiratory cancer mortality; the ORs for the medium and high serum DDT tertiles were 1.5 (95% CI = 0.5–4.9) and 1.8 (95% CI = 0.5–6.2), respectively. This study was limited by relatively small numbers and years of follow-up and the fact that only cancer mortality, and not cancer incidence, was ascertained (Austin et al., 1989).

> ***Summary***: Data are inadequate to assess any possible associations between DDT/DDE and lung cancer.

8.2.2.6 Pancreatic cancer

Garabrant et al. (1992) conducted a case–control study nested within a cohort of 5886 chemical manufacturing workers. A prior cohort mortality study (Ditraglia et al., 1981; see Table 3) had shown increased mortality due to pancreatic cancer. They verified 28 cases of pancreatic cancer that were matched with 112 controls.

Information about lifestyle factors (tobacco, alcohol and coffee consumption) was collected from next of kin, and information about exposures was gathered from work records and co-worker interviews. DDT exposure was associated with pancreatic cancer (RR for ever vs never exposed = 4.8; 95% CI = 1.3–17.6), and there was evidence for a dose–response relationship with duration of exposure and with latency. No DDT levels were available. In both this study and the study reported by Beard et al. (2003) (see below), the exposure assessment left open the possibility of random misclassification and/or recall bias.

An Australian study assessed the mortality of 1999 outdoor staff working as part of an insecticide application programme during 1935–1996 compared with that of 1984 outdoor workers not occupationally exposed to insecticides and with the Australian population. DDT exposure was determined via questionnaires. Mortality from pancreatic cancer was more frequent in subjects exposed to DDT (SMR = 5.27 [95% CI = 1.09–15.40] for subjects working for less than 3 years, but SMR = 1.37 [95% CI = 0.37–3.44] for those exposed for more than 3 years) (Beard et al., 2003).

Porta et al. (1999) conducted a hospital-based case–case study with 51 pancreatic cancer cases (17 with wild-type K-*ras* and 34 with K-*ras* mutation) compared with 26 hospital-based controls. Serum concentrations of *p*,*p*′-DDT and *p*,*p*-DDE were significantly higher in pancreatic cancer cases with a K-*ras* mutation than in cases without a mutation or in controls (*P* for trend = 0.005 and 0.031 for DDT and DDE, respectively). A specific association was observed between a glycine to valine substitution at codon 12 and both the DDT and DDE concentrations.

Summary: Studies linking pesticide exposure to pancreatic cancer generally did not distinguish DDT from other chemicals as the causative agent. One small study indicates a possible association between both DDT and DDE and a subtype of pancreatic cancer with a specific K-*ras* mutation. In conclusion, data are inadequate to assess associations between DDT/DDE and pancreatic cancer.

8.2.2.7 Other cancers

(a) Prostate cancer

A pilot study of 58 incident prostate cases and 99 matched controls found no relationship with DDT exposure (median serum *p*,*p*′-DDE concentration 0.27 µg/g lipid in referents) (Ritchie et al., 2003). Settimi et al. (2003) reported on a hospital-based case–control study in rural areas in Italy between 1990 and 1992. One hundred and twenty-four new cases of prostate cancer and 659 controls were interviewed; DDT exposure was estimated by a team of agronomists. Using multiple regression analysis, use of DDT was associated with prostate cancer (OR = 2.1; 95% CI = 1.2–3.8); this was true for dicofol as well (OR = 2.8; 95% CI 1.5–5.0). These two were often used together, and their effects could not be separated.

One of the cohort studies in Table 3 (Purdue et al., 2007) also provided an indication of a possible relationship between past DDT use and prostate cancer (RR = 1.2; 95% CI = 1.0–1.4); the study design did not allow for an estimate of DDT exposure levels.

(b) Endometrial cancer

A United States case–control study of 90 cases of endometrial cancer and 90 controls did not show any association between DDT and DDE levels in serum and endometrial cancer (Sturgeon et al., 1998). A study of endometrial cancer in Sweden measured serum concentrations of 10 chlorinated pesticides and 10 PCB congeners in 154 endometrial cancer cases and 205 population controls. They found no significant associations between endometrial cancer and serum DDE level for the highest compared with the lowest quartile of DDE concentration (OR = 1.0; 95% CI = 0.6–2.0) (Weiderpass et al., 2000).

Summary: Data are insufficient to assess any possible association between DDT/DDE exposures and either prostate or endometrial cancer.

8.3 Mode of action

Exposure to DDT has been shown to cause liver cancer in mice and rats, lung tumours and leukaemias in mice and testicular carcinoma in situ in rabbits. In humans, single studies suggest that DDT or DDE is associated with breast cancer when exposure is prepubertal (DDT), liver cancer (DDT, but not DDE) and testicular germ cell carcinomas (DDE). From a mechanistic point of view, a number of non-exclusive modes of action could contribute to the development of DDT-associated cancers. These include initiation (including oxidative damage), promotion (including apoptosis) and interaction with a number of nuclear receptors (CAR, PXR, ER and AR). Determination of the relevance of the mode of action is dependent on measuring the key events in the target tissues during the process of cancer formation. This has generally not been the case for mechanistic studies.

Both DDT and DDE might be involved in initiation of tumours. Both DDT and DDE have been shown to be genotoxic in human lymphocytes both in vitro and in vivo. Bacterial assays are generally negative for genotoxicity (see section 9). Evidence for initiation is derived from recently published studies reporting DNA damage in exposed populations. In addition, it was shown in vitro that *p,p′*-DDT, *p,p′*-DDE or *p,p′*-DDD at concentrations of 60 mg/l and above increased the generation of reactive oxygen species (ROS) in human blood mononuclear cells (Perez-Maldonado et al., 2005). Although a clear link with ROS could not be established, deoxyribonucleic acid (DNA) damage and apoptosis in lymphocytes were observed in a population of 57 children living in an area where indoor spraying of DDT to control malaria had been performed. Levels of total DDT in serum were approximately 50–70 µg/l in the three different locations, and there was a significant association between DNA damage in lymphocytes and DDT and DDE blood levels (Perez-Maldonado et al., 2006).

DDT is a promoter in two-stage initiation–promotion studies in rat liver. In an initiation–promotion study, the potential for DDT as a carcinogen at low doses via assessment of preneoplastic (glutathione *S*-transferase placental form [GST-P]) lesions in livers in F344 rats was studied (Sukata et al., 2002). GST-P-positive foci in the liver, assessed at the end of the 16-week dosing schedule, increased in a dose-dependent fashion, the increase reaching statis-

tical significance for rats dosed with 20 mg/kg (0.6 mg/kg bw per day) or higher levels of DDT for 16 weeks. At the lowest doses, 0.005 and 0.01 mg/kg diet, the responses were lowered compared with controls, but this decrease was not statistically significant. CYP3A2 and CYP2B1 protein expression showed the same pattern of decrease at the low doses and increase at the high doses; none of these changes were statistically significant.

DDT (500 mg/kg in the diet) given to rats after initiation with diethylnitrosamine and partial hepatectomy increased the number and volume of preneoplastic foci in the liver (Ito et al., 1983). DDT also acted as a promoter after initiation with acetylaminofluorene and acetamidophenanthrene in rats (IARC, 1991). DDT (1000 mg/kg diet [approximately 50 mg/kg bw per day]) in the diet for 77 days enhanced the development of GGT-positive altered hepatic foci in nitrosamine-initiated male Sprague-Dawley rats (Flodström et al., 1990). DDT, like phenobarbital, is an inducer of CYP enzymes. This induction is mediated by CAR (Wyde et al., 2003; Kiyosawa et al., 2008a,b). CAR also regulates cell proliferation–related genes (Yamamoto et al., 2004; Columbano et al., 2005; Kiyosawa et al., 2008a). The tumour promotion activity of phenobarbital and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (the most potent phenobarbital-type inducer known) is also abolished in CAR-null mice (Yamamoto et al., 2004; Huang et al., 2005). Consequently, DDT may act as a phenobarbital-type hepatic tumour promoter through CAR activation in rats and mice. As noted previously, liver enzyme induction was also observed in workers occupationally exposed to DDT.

In healthy individuals, DDT and DDE at concentrations of 80 mg/l and above induced apoptosis in isolated peripheral blood mononuclear cells. A preliminary study in 15 volunteer children with environmental exposure to DDT and 6 controls between the ages of 6 and 12 years found that exposed children had higher DDT and DDE levels than controls and also had a higher frequency of apoptosis. In the exposed children, a weak positive association was found between the frequency of apoptosis and the DDT and DDE levels (Perez-Maldonado et al., 2004). DDT and DDE at concentrations of 60 mg/l and above increased ROS generation in human blood mononuclear cells in vitro; this was followed by an increase

in apoptosis. The authors proposed that the mechanism for apoptosis may be via the formation of ROS (Perez-Maldonado et al., 2005).

Among 57 children living in an area where indoor spraying of DDT to control malaria had been performed, the total DDT concentration in serum was approximately 50–70 μg/l in the three different locations. A significant association with apoptosis frequencies was found only with blood DDE levels. The associations between DDT or DDE and oxidative DNA damage (see section 9 on genotoxicity) and between oxidative damage and apoptosis were not significant (Perez-Maldonado et al., 2006).

For some tumour types (e.g. breast and testes), effects of DDT/DDE on hormonal receptors may be of relevance. Both *o,p′*-DDT and *p,p′*-DDT are weakly estrogenic, whereas *p,p′*-DDE acts as an anti-androgen (see section 10.3.1). DDT and DDE have not been tested in the usual animal models for breast cancer. Testicular cancers in rodents have a different pathology compared with those in humans, so models are lacking.

DDT (10 μmol/l) inhibited gap junctional intercellular communication both in the Chinese hamster V79 metabolic cooperation assay and in the scrape-loading/dye transfer assay in WB-F344 rat liver epithelial cells (Flodström et al., 1990). In another study, DDT (≥ 25 mg/kg bw per day for 2 weeks) also inhibited hepatic gap junctional intercellular communication in rats (Tateno et al., 1994).

In a highly exposed Inuit population, persistent organic pollutants, including DDT, have been associated with hypomethylation of DNA isolated from whole blood (Rusiecki et al., 2008).

Summary: DDT causes cancer in several species of animals and in several organ systems. There are likely multiple modes of action for tumour induction that may be operational simultaneously, particularly when chronic exposures are involved. Because many of the mechanistic studies were not conducted under conditions of the bioassays, the form of the dose–response curve cannot be well defined at the molecular level. DDT is known to bind to CAR, which may mediate the hepatocarcinogenic effects observed in rodents. In initiation and promotion studies, DDT is a promoter of rat liver foci. Disruption of cellular communication would be expected to promote progression of cancer development. Finally, although DDT and DDE have not generally been considered to be genotoxic, there is evidence, particularly in human and

mammalian systems, for DNA damage (and apoptosis) in lymphocytes with higher level exposures. The possibility that such exposures, even transiently, are involved with carcinogenesis cannot be dismissed. Endocrine disruption per se can be a mechanism for carcinogenicity of endocrine organs, either by promoting growth of hormone receptor–positive tumours or via epigenetic mechanisms. No mode of action has been proposed for DDT carcinogenicity that is specific to animals and would not be relevant to humans (see IPCS, 2007).

9. GENOTOXICITY

9.1 Summary of past studies

Dozens of genotoxicity studies with DDT and its metabolites have been summarized by IARC (1991) and ATSDR (2002). Based on data available in 1990, IARC concluded that whereas conflicting data were obtained with regard to some genetic 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.

9.2 Recent studies

9.2.1 Laboratory animals and in vitro systems

DDE was evaluated for genotoxicity in cultured human lymphocytes by scoring the frequency of micronuclei in the cytokinesis block micronucleus assay. *p,p′*-DDE induced significant increases in the frequency of micronucleated binucleates (but not total micronuclei). The effect was seen only at the nominal concentration of 80 mmol/l (26 g/l) (the highest concentration of DDE added) (Ennaceur et al., 2008).

Peripheral blood mononuclear cells from healthy donors and incubated in either *p,p′*-DDT (≥ 40 mg/l) or *p,p′*-DDE (40 or 80 mg/l) were found to have significant DNA damage, as shown by the single-cell electrophoresis assay (comet assay). Such cells showed a significant increase in the percentage of hypodiploid cells compared with untreated peripheral blood mononuclear cells (Yáñez et al., 2004).

9.2.2 Humans

Rupa et al. (1991) evaluated the frequency of sister chromatid exchange, mitotic index and cell cycle kinetics in peripheral lymphocytes from the blood of 61 male pesticide applicators who worked in cotton fields and regularly sprayed with DDT but also a number of other pesticides, including hexachlorocyclohexane (HCH), endosulfan, malathion, methyl parathion, phosphamidon, dimethoate, monocrotophos, quinalphos fenvalerate and cypermethrin. All study subjects were non-smokers and non-drinkers. Subjects were matched with 45 control males with no known pesticide exposures. The description of the study does not allow for

the evaluation of whether controls were appropriate. The frequency of sister chromatid exchange was significantly higher among the pesticide applicators at all durations of exposure when compared with controls. Subjects exposed to pesticides also showed cell cycle delay and decrease in mitotic index when compared with the control group. However, the design of the study did not provide an opportunity to separately evaluate the contribution of DDT exposure to mutagenesis.

The Vine et al. (2001) study described in section 7.2 above, of 302 persons living around a contaminated site near Aberdeen, North Carolina, USA, included peripheral blood lymphocyte micronuclei counts. Neither serum DDE level nor residential location was associated with frequency of micronuclei per 1000 cells or the percentage of cells with micronuclei after controlling for age, pack-years of current smoking, sex and assay scorer.

Nagayama et al. (2003) reported a study to examine the frequency of sister chromatid exchange in cultured lymphocytes from 10-month-old infants in relation to lactational exposure to DDT, HCH and chlordane, as measured in maternal breast milk. Cumulative lactational exposures to DDT ranged from 33 to 1361 mg/kg bw, with a median of 272 mg/kg bw. No significant association between lactational exposure to DDT and the frequency of sister chromatid exchange was observed.

Nagayama et al. (2003) also studied the frequency of sister chromatid exchange in cultured lymphocytes taken from 10-month-old infants (as above) in response to benzoflavone treatment in vitro. There was a borderline significant ($P = 0.05$) negative association between the frequency of benzoflavone-induced sister chromatid exchange and exposure to DDT from maternal breast milk.

Peripheral blood mononuclear cells from women with varying serum levels of DDT and DDE (*p*,*p*′-DDT, 0.02–20.7 µg/l; and *p*,*p*′-DDE, 0.04–39.1 µg/l) were examined; blood levels of DDT and DDE were associated with increased DNA damage as assessed using the comet assay, controlling for nutritional status, smoking habits, alcohol ingestion and reported exposure to other pesticides (Yáñez et al., 2004).

A study of 57 children living in communities sprayed with DDT examined DNA damage and oxidative DNA damage in peripheral blood mononuclear cells using the comet assay. The association between exposure to DDT or DDE and DNA damage was significant (P = 0.004 and P = 0.005 for DDT and DDE, respectively) (Perez-Maldonado et al., 2006).

Summary: DDT is inactive in most genetic toxicity assays, although it has been shown in some studies to induce DNA damage in human lymphocytes, both in vitro and in bio-monitoring studies, and in cultured rodent cells. These data do not allow discrimination between primary and secondary genotoxic effects. There is also a lack of consistency between findings from experimental animal and in vitro studies.

10. ENDOCRINOLOGICAL AND REPRODUCTIVE EFFECTS

10.1 Diabetes mellitus

A potential association between exposures to organochlorines and subsequent development of type 2 diabetes was first identified by Morgan et al. (1980).

A cross-sectional study of 196 men (median age 60 years) and 184 women (median age 64 years) in a fishing community assessed serum DDE and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) levels and medical history of type 2 diabetes. Multiple logistic regression was used to control for confounders; the OR for DDE (by units of 0.1 μg/g lipid) was 1.05 (95% CI = 1.01–1.09; $P = 0.006$). However, there was a somewhat stronger relationship for PCB-153 (OR = 1.16). In this population, DDE and PCB-153 were highly (and significantly) correlated (women, $r = 0.68$; men, $r = 0.64$), so that it was not possible to assess the effects of these two compounds independently. The study could not exclude other possibilities, such as 1) DDE and PCB-153 being biomarkers for some other persistent compound that is a diabetogenic agent or 2) reverse causality (Longnecker & Michalek, 2000)—that is, that the profound metabolic changes associated with type 2 diabetes might result in higher serum levels of persistent compounds like DDE and PCB-153 generally (Rylander et al., 2005).

Cox et al. (2007) evaluated the prevalence of self-reported type 2 diabetes in relation to serum DDT and DDE levels among 1303 Mexican Americans participating in the Hispanic Health and Nutrition Examination Survey from 1982 to 1984. Using logistic regression analysis, they found that self-reported diabetes was significantly associated with serum levels of DDT and DDE, even when adjusting for total serum lipids. However, a number of other persistent compounds (i.e. *trans*-nonachlor, oxychlordane and β-HCH) were also associated with self-reported diabetes. (This study was not able to assess PCBs.) DDT and DDE were highly correlated with each other, and their effects could not be separated (Spearman correlation coefficient = 0.53). *Trans*-nonachlor and oxychlordane, both metabolites of chlordane, were also highly correlated, and both had higher ORs than either DDT or DDE. For the same reasons stated above, the cross-sectional design did not provide strong evidence for causality.

Lee et al. (2006) assessed the prevalence of type 2 diabetes (as determined by prior diagnoses of diabetes by a physician and current use of insulin and oral hypoglycaemic agents) and serum levels of several persistent organic pollutants—DDT, PCB-153, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, octachlorodibenzo-*p*-dioxin, oxychlordane and *trans*-nonachlor—among 2016 study participants who had been enrolled in the NHANES studies in 1999–2000 and 2000–2001. Correlations were found among all the persistent organic pollutants that were measured in this study (see above), especially PCB-153, oxychlordane and *trans*-nonachlor, but also (to a lesser extent) DDT. In multiple regression analysis controlling for a number of potential confounders, diabetes was associated with all of the organochlorines, including DDT; the highest ORs were for PCB-153, oxychlordane and *trans*-nonachlor. The study had the same limitations as discussed above.

Everett et al. (2007) studied diagnosed type 2 diabetes, undiagnosed diabetes (glycohaemoglobin > 6.1%) and total diabetes in relation to serum levels of DDT, PCB-126 and 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HCDD) among 1830 participants 20 years of age and older in the 1999–2002 NHANES. DDT and PCB-126, but not HCDD, were significantly associated with diagnosed diabetes. For DDT, serum levels between 0.021 and 0.027 µg/g lipid had an OR of 2.52 (95% CI = 1.26–5.02) for an association with diagnosed diabetes, and levels above 0.027 µg/g lipid had an OR of 2.74 (95% CI = 1.44–5.23); this model was adjusted for PCBs, HCDD, age, sex, race, country of birth, education, poverty, income ratio, BMI, waist circumference and physical activity. However, the ORs for PCB-126 were quite similar (lower for the mid-range tertile and higher for the high tertile). Again, as the authors noted, the cross-sectional design could not demonstrate a causal association between DDT and diabetes.

A cross-sectional study was conducted on 544 Swedish women with a median age of 50 years. DDE and PCB-153 levels were analysed in serum, and type 2 diabetes was ascertained via self-report of whether they had diabetes, the type of diabetes, years since diagnosis and mode of treatment. There was a significant association with type 2 diabetes mellitus for DDE; the OR was 1.3 (95% CI = 1.1–1.6). However, the association for PCB-153 was similar (OR = 1.4); there was a high correlation between PCB-153

and *p,p′*-DDT levels in plasma (r = 0.69). The authors noted that these findings could not demonstrate a causal relationship between DDE and type 2 diabetes mellitus (Rignell-Hydbom et al., 2007).

Summary: Several recent cross-sectional studies of different populations have demonstrated statistical associations between type 2 diabetes mellitus and DDT and/or DDE. However, in each case, the studies also show associations that are of equivalent or greater strength for other persistent compounds, so that DDT and DDE levels may be biomarkers for other exposures (most notably PCBs) that, in turn, are more strongly associated with type 2 diabetes. All these studies did look for possible confounding by obesity (concurrently or prior to diagnosis). Alternatively, the metabolic alterations associated with diabetes are known to alter fat metabolism, which can affect serum lipid levels not only of DDT and DDE, but also of other persistent organic pollutants. Therefore, these results should be interpreted with caution, and at this point, results are inconclusive.

10.2 Thyroid effects

10.2.1 In vitro

In 2003, Santini et al. reported on an in vitro system to test substances for their ability to inhibit thyroid stimulating hormone (TSH)–stimulated cyclic adenosine monophosphate (cAMP) production. DDT (≥ 1 µmol/l) produced a dose-dependent inhibition of TSH-stimulated adenylate cyclase activity and also inhibited the cAMP production stimulated by TSH receptor antibody. It did not inhibit TSH binding to its receptor, nor did it block antibody binding to TSH. Santini et al. (2003) concluded that DDT may affect cAMP production at a post-receptor step.

10.2.2 Laboratory animals

A single dose (100 mg/kg bw by gavage) of DDT to rats was reported to cause temporary suppression of iodine uptake by and release from the thyroid (Goldman, 1981). DDT or DDE did not bind the thyroid hormone receptor, thyroglobulin or transthyretin in vitro (Cheek et al., 1999).

In the screening assay using oral doses of *p,p′*-DDE of 50, 100, 200 and 300 mg/kg bw per day for 15 days (O'Connor et al., 2002), described in section 10.3.2, higher levels of DDE were associated with reduced levels of triiodothyronine (T_3) and thyroxine (T_4), without concomitant alterations in levels of TSH. There were no histopathological changes in the thyroid glands.

10.2.3 Humans

In a study of PCBs, fish consumption and thyroid hormone status, Persky et al. (2001) also reported results for DDE (median serum level among fish consumers = 0.046 µg/g). They found no relationship between DDE levels in this range and thyroid hormone levels in adult fish consumers.

Hagmar et al. (2001) assessed levels of persistent organohalogens and hormones among 43 Swedish and 57 Latvian adult males. No relationship was identified between DDT (0.010–0.185 µg/g lipid, 10th–90th percentiles) or DDE (0.197–3.152 µg/g lipid, 10th–90th percentiles) levels and thyroid hormone levels in plasma.

Takser et al. (2005) reported a study of thyroid hormone levels among a sample of healthy pregnant women and newborns in relation to plasma levels of a number of persistent organic pollutants. DDE (in the range of 0.1–1.2 µg/l or approximately 0.028–0.34 µg/g lipid) as well as PCBs, *cis*-nonachlor (a metabolite of chlordane) and hexachlorobenzene were each individually associated with reduced maternal levels of total T_3, but not with other thyroid hormone levels or levels in infants' cord blood. This study could not separately ascertain effects associated with DDE levels in plasma.

A study in northern Thailand evaluated thyroid hormone levels in relation to levels of DDT and DDE in serum among 39 mother–infant pairs with normal delivery and full-term gestation. The geometric mean DDE level was 1.191 µg/g in maternal serum and 0.742 µg/g lipid in cord serum. The geometric mean DDT level was 0.123 µg/g in maternal serum and 0.077 µg/g lipid in cord serum. Levels of DDE and DDT in maternal and infant serum were strongly correlated. Higher DDE and DDT levels were associated with lower cord serum total T_4, but not lower free T_4 or higher TSH levels (Asawasinsopon et al., 2006a).

The Sugiura-Ogasawara et al. (2003) miscarriage study (described in section 10.3.3.3 below) found no associations between serum DDT or DDE levels and TSH and free T_4 levels. However, as noted below, the study was underpowered (Sugiura-Ogasawara et al., 2003).

In the USA, 341 adult men recruited from an infertility clinic between 2000 and 2003 were studied in a cross-sectional study of levels of DDT, DDE (average plasma DDE concentration, 0.2 µg/g lipid) and other persistent organochlorines and thyroid hormones, as measured by free T_4, total T_3 and TSH levels. In multivariate linear regression, there were positive associations between DDE and both free T_4 and total T_3 and an inverse association between DDE and TSH (Meeker et al., 2007).

A study of youth from the Akwesasne Mohawk Nation (*n* = 232) in the USA investigated DDE levels (medians 0.3–0.4 µg/g blood, maximum 2.93 µg/g blood) with respect to levels of TSH, T_3, total T_4 and free T_4. DDE levels were not related to levels of thyroid hormones (Schell et al., 2008).

The relationship between prenatal DDT and DDE internal doses and thyroid function during pregnancy was evaluated among 334 pregnant women living in the Salinas Valley, California, USA, between 1999 and 2000. Outcome measures were TSH, total T_4 and free T_4 measured in serum samples collected during pregnancy. DDT and DDE levels were not associated with thyroid hormone levels (Chevrier et al., 2008).

A Menorca, Spain, birth cohort study evaluated DDT and DDE levels in cord serum and TSH concentrations in plasma 3 days after birth for 387 newborns from Menorca. The TSH concentration was categorized as high or low, "low" being not detected. DDT and DDE levels were not related to TSH concentrations (Alvarez-Pedrerol et al., 2008a).

Two hundred and fifty-nine children from the Menorca birth cohort were assessed at the age of 4 years. Thyroid hormones (free T_4 and total T_3), TSH, DDT, DDE and other organochlorines were

measured in blood. Blood levels of DDT but not of DDE were related to lower total T_3 levels ($P < 0.05$), but not to levels of free T_4 or TSH (Alvarez-Pedrerol et al., 2008b).

A case–control study conducted in Japan examined the relationship between congenital hypothyroidism and prenatal exposure to DDT and a number of other persistent organochlorine compounds among 34 clinically confirmed cases and 102 controls, based on normal newborn thyroid screening tests. Concentrations of DDT and other organochlorine compounds were measured in breast milk of mothers; levels of all organochlorine compounds were about 2 times higher in cases than in controls. The crude OR for patients in the "cretinism" group to have serum "DDT" concentration higher than the median was 3.6 ($P = 0.027$), and the OR adjusted for parity and mother's age was 10.0 ($P = 0.003$); however, the relationship was no stronger for DDT [it is not clear from the paper whether DDT only or the sum of DDT metabolites was measured] than for several other organochlorines (and one, hexachlorobenzene, had a much higher OR of 22). The study was too small to control for possible confounding (Nagayama et al., 2007).

Summary: The findings on thyroid hormones and TSH are inconsistent. For adults, generally, there was only one study (Meeker et al., 2007) that showed associations between DDE levels and decreased thyroid hormones and increased TSH levels. Three studies (Sugiura-Ogasawara et al., 2003; Takser et al., 2005; Chevrier et al., 2008) looked at thyroid hormone status during pregnancy and had mixed results for DDE and either decreased T_3 or decreased T_4 or free T_4. Three studies of children (Takser et al., 2005; Asawasinsopon et al., 2006a; Alvarez-Pedrerol et al., 2008a) had mixed results, with one showing decreased T_4 with DDE and not DDT and another decreased T_3 with DDT and not DDE (the third showed that DDT and DDE levels were not related to TSH concentrations in newborns). Two studies of older children showed mixed effects. One (Alvarez-Pedrerol et al., 2008b) showed decreased T_3 with higher DDT (but not DDE) at birth, and the other (Schell et al., 2008) did not show effects with concurrent exposures. A case–control study (Nagayama et al., 2007) associated higher breast milk DDT and DDE (but also other persistent chemicals) with congenital hypothyroidism. Overall, the human studies for DDT/DDE and thyroid hormones are inconclusive.

10.3 Reproductive and developmental toxicity

10.3.1 In vitro

Kelce et al. (1995) reported that whereas *p,p′*-DDE had little ability to bind the ER, it inhibited androgen binding to the AR and androgen-induced transcriptional activity.

p,p′-DDE stimulates aromatase (CYP19) activity in ovarian granulosa cells. Human granulosa cell cultures were exposed to follicle stimulating hormone (FSH) and DDE, and aromatase activity was measured. Aromatase response to FSH was significantly stimulated at a DDE concentration of 100 µg/l (0.3 µmol/l), a level the authors considered to be similar to those present in human follicular fluid (Younglai et al., 2004).

Likewise, *p,p′*-DDE (0.17 µmol/l) has been shown to increase aromatase activity in endometrial stromal cells in culture (Holloway et al., 2005).

Placental explants treated with *p,p′*- or *o,p′*-DDT and *p,p′*- or *o,p′*-DDE (≥ 100 µg/l, approximately 0.3 µmol/l) showed reduced estradiol secretion, which was attributed to direct action on aromatase activity. *o,p′*-DDT, *o,p′*-DDE and *p,p′*-DDE, but not *p,p′*-DDT, increased progesterone secretion, which was thought to be suggestive of action on P450scc (the cholesterol side-chain cleavage enzyme) (Wójtowicz et al., 2007).

p,p′-DDE was tested to see whether it would affect the proliferation of CAMA-1 cells, a human breast cancer cell line that expresses the ERα and the AR. Treatment with DDE (≥ 2 µmol/l) diminished and, at higher concentrations, fully reversed the dihydrotestosterone-impaired proliferation of CAMA-1 cells induced by estradiol. DDE had a similar effect on the proliferation of MCF7-AR1 cells, an estrogen-responsive cell line genetically engineered to overexpress the AR. The potency of DDE to induce phase transition (cell cycle change) in CAMA-1 cells was one tenth of that of the potent anti-androgen hydroxyflutamide (Aubé et al., 2008).

p,p′-DDT has been shown to downregulate the expression of trophoblast-specific human calcium binding protein and (like diethylstilbestrol [DES]) to inhibit cell proliferation, induce apoptosis and suppress expression of several trophoblast differentiation marker genes, a possible pathway of effects on fetal development. The potency of DDT was approximately 0.1–0.01% of that of estradiol (Derfoul et al., 2003).

DDT (85:15 mixture of *p,p′*- and *o,p′*-isomers) had stimulatory effects in a number of ER-positive cell lines. At 0.3 µmol/l, it increased the growth of MCF-7 cells in the presence (but not in the absence) of insulin. The activity of cyclin-dependent kinase increased in growth-arrested T-47D and MCF-7 cells treated with β-estradiol or DDT. The relative potency of DDT in inducing cell cycle progression in such cells was 100–300 times less than that of estradiol when measured in the presence of insulin (Dees et al., 1997).

o,p′-DDT and its DDE and DDA metabolites bound specifically to the human ER with approximately 10 000-fold weaker affinities than estradiol; the binding of *p,p′*-isomers was much weaker (Oien et al., 1997).

o,p′-DDT (10 µmol/l) agonized both the ERα- and ERβ-mediated transcription with a potency of approximately 0.1% of that of estradiol. *p,p′*-DDT was considerably less potent (Lemaire et al., 2006).

10.3.2 Laboratory animals

10.3.2.1 Multigeneration studies

Sprague-Dawley rats were fed technical DDT (80% *p,p′*-DDT, 17% *o,p′*-DDT and 2% *p,p′*-DDE) at levels of 0, 20 and 200 mg/kg diet [approximately 0, 0.5 and 5 mg/kg bw per day] in a three-generation study (Ottoboni, 1969). Dosing was started with a weanling P_0 litter and terminated with weaning of the third F_1 litters. Three dose groups containing 12 females and 6 males each were bred at 16 and 24 weeks, and litters were examined after parturition and at 21 days for number, weight, sex and health of pups and daily for survival. Breeding stock was selected from second litters, and the same protocol was followed for F_1 generation animals, except

that females were bred for a third time at 52 weeks. DDT ingestion produced no apparent effects on fecundity of dams, viability of young or fertility of 16- or 24-week-old rats. However, one female in the 20 mg/kg group and one in the 200 mg/kg group failed to become pregnant in both the first and second matings; the authors suggested that this may represent an adverse effect of DDT in matings of marginal fertility. Fertility of 62-week-old DDT-treated rats was higher than that of the controls in both dose groups, indicating that DDT may prolong reproductive viability with age. The 200 mg/kg DDT diet produced a significant increase in the occurrence of a condition called ringtail among suckling rats.

As a follow-up study, Ottoboni (1972) fed 12 pairs of littermate female Sprague-Dawley rats diets containing technical DDT at 0 or 20 mg/kg [approximately 1 mg/kg bw per day] from weaning until termination of the experiment. They were bred at the age of 3 months and every 2 months thereafter until 11 breedings were accomplished. Male rats were young adults of proven fertility obtained from the same colony as the females and maintained on control diets between breeding periods. Females receiving DDT at 20 mg/kg had a significantly longer average reproductive lifespan (14.6 months) than did littermate controls (8.9 months). The number of females becoming pregnant and the number of successful pregnancies after the age of 17 months were significantly higher for the DDT-exposed group.

Tarján & Kemény (1969) carried out a multigeneration study in which BALB/c mice were fed diets containing DDT at 2.8–3.0 mg/kg (0.4–0.7 mg/kg bw per day) over six generations. The treated group in each generation contained 28–30 mice, and the control groups contained 30 mice. No generational or overall differences were observed in numbers of pregnancies, births, litters, offspring or weanlings, mean weight at weaning or survival of offspring.

The Turusov et al. (1973) multigeneration study of CF-1 mice, described in section 8.1.1, found no impairments in fertility or fecundity either overall or across generations dosed with technical DDT in food at concentrations of 2, 10, 50 and 250 mg/kg. They did identify increased preweaning mortality among pups when dams

were dosed with DDT at 250 mg/kg (equivalent to 33 mg/kg bw per day).

Ottoboni et al. (1977) conducted a multigeneration reproductive study in which Beagle dogs (135 adult females, 63 adult males and 650 pups in three generations) were administered technical DDT (80% *p*,*p*′-DDT, 17% *o*,*p*′-DDT and 2% *p*,*p*′-DDE) at doses of 1, 5 or 10 mg/kg bw per day. They found no adverse effects on fertility, success of pregnancy, length of gestation, litter size, survival at 24 hours or at weaning, sex distribution or growth of the pups, organ to body weight ratios or gross pathology or histopathology. Puberty occurred earlier (by 2–3 months) in dosed female dogs than in controls. The changes in puberty across the four dose groups are shown in the Table 5.

Table 5. Age of puberty (days) for Beagle dogs dosed with DDT

DDT dose (mg/kg bw per day)	Number of animals	Age of puberty (days)	Standard deviation (days)
0	24	379	82
1	30	332	63
5	30	311	62
10	44	303	44

Source: Ottoboni et al. (1977)

Summary: From multigeneration studies, there is little evidence for reproductive toxicity of DDT in mammalian species (mice, rats, dogs). One study reported increased mortality of mouse pups with DDT dosing of 33 mg/kg bw per day, and a second found earlier puberty in dogs dosed at 10 mg/kg bw per day.

10.3.2.2 Effects on fertility in males

Juvenile male rats treated with DDT [composition/purity not specified] (300 mg/kg bw per day on days 4–23 postpartum) showed testicular degeneration and lowered fertility (Krause, 1977). Adult male rats treated with DDT (100 mg/kg bw 3 times/week for 3 weeks) had decreased levels of testosterone in blood and in the testes, without alterations in serum FSH or luteinizing hormone (LH). There were no histological changes in the testis.

Ben Rhouma et al. (2001) assessed the reproductive toxicity of *p,p′*-DDT (purity 98%) to adult male rats dosed at levels of 50 and 100 mg/kg bw per day intraperitoneally for 10 successive days. Rats exposed to DDT had dose-dependent reductions in both testicular weight and sperm numbers and motility, as well as decreased weights of seminal vesicles, decreased testosterone production and increased serum levels of LH and FSH.

Treatment of 120-day-old male rats with *p,p′*-DDE (200 mg/kg bw per day) by gavage for 4 days significantly reduced seminal vesicle and ventral prostate weights despite high serum testosterone levels. The prostates exhibited a 13-fold increase in androgen-repressed testosterone-repressed prostatic message 2 messenger ribonucleic acid (mRNA) levels and a 35% decline in androgen-induced prostate binding protein subunit 3 mRNA levels These specific cellular responses are characteristic of the effects of anti-androgens on androgen-dependent gene expression in the rat prostate (Kelce et al., 1995).

Kelce et al. (1997) dosed castrated male Sprague-Dawley rats with DDE (200 mg/kg bw per day for 4 or 5 days). *p,p′*-DDE induced a reciprocal decline in seminal vesicle ($P < 0.01$) and prostate ($P < 0.01$) weight as well as a reduction in immuno-histochemical staining of AR in epididymal nuclei compared with vehicle-treated controls. DDE also induced testosterone-repressed prostatic message 2 mRNA and repressed testosterone-induced prostatic message (prostatein subunit C3) mRNA. The authors concluded that DDE acts as an anti-androgen by altering the expression of androgen-dependent genes.

As described in section 5 on hepatic toxicity, administration of *p,p′*-DDE (purity > 99%, 100 mg/kg bw per day) to rats induced the hepatic CYP2B1 and aromatase, a member of the CYP family that catalyses the conversion of C19 steroids to estrogens. However, the change in the enzyme activity was not accompanied by changes in the levels of 17β-estradiol in plasma (You et al., 2001).

In 2002, O'Connor et al. published results of a screening assay using oral doses of *p,p′*-DDE of 50, 100, 200 and 300 mg/kg bw per day for 15 days on mature, intact male Sprague-Dawley rats. Liver

weights, but not body and sex organ weights, were decreased in a dose-dependent manner. Dihydrotestosterone levels were decreased, and estradiol levels were increased, with no significant trends observed for testosterone, LH, FSH or prolactin level. Higher levels of DDE were associated with reduced levels of T_3 and T_4, without concomitant alterations in levels of TSH. There were no histopathological changes in testis, epididymis or thyroid glands of DDE-treated animals. Immune system end-points (humoral immune function, spleen and thymus weights and spleen cell number) were not modified by dosing with DDE (O'Connor et al., 2002).

Summary: There are adverse effects on multiple male reproductive parameters and hormones with increasing exposure to DDT (≥ 50 mg/kg bw per day for 10 days) and DDE (200 mg/kg bw per day for 4 or 5 days). DDE induces anti-androgenic effects in vitro and in vivo. Anti-androgenic effects have been described in male rats after high exposure in utero and during lactation. DDT binds to the ERα and is a weak estrogen agonist.

10.3.2.3 Effects on fertility in females

Female rabbits dosed with technical DDT (15–20% *o,p′*-DDT, 80–85% *p,p′*-DDT; 3 mg/kg bw, 3 times per week for 12 weeks) by gavage accumulated high amounts of DDT in ovarian, oviductal and uterine tissues, in follicular fluid and in uterine secretions. DDT-treated animals showed a significantly reduced ovulation rate; no decrease in the ovulation rate was observed in animals given the same dose of DDT together with γ-HCH (Lindenau et al., 1994). Further, there was a statistically non-significant decrease of the serum progesterone level during early pregnancy; in a group treated simultaneously with DDT (3 mg/kg bw per day), PCB and lindane, no effect was observed (Lindenau et al., 1994). In a follow-up study, no effect on fertilization rate or preimplantation and post-implantation loss was found after artificial insemination and further treatment (up to day 11 postcoitum) (Seiler et al., 1994).

Morozova et al. (1997) dosed 4-month-old female CBA mice with single doses of technical DDT at 500 and 375 mg/kg bw; both positive DES controls and vehicle controls were used. Mice given 500 mg/kg bw had poor survival, so examinations at that dose were more limited. Relative uterus to body weight ratios increased in both DDT dose groups but were more increased in mice dosed with DES.

At 36 and 48 hours, DES-treated mice showed pseudo-estrus; comparable levels of pseudo-estrus were observed in DDT-treated mice at 48 hours. At very high doses, potential estrogenic activity in female mice was seen as increased uterine weights and pseudo-estrus.

An early, small study did not find higher levels of DDT in cows that had aborted (Macklin & Ribelin, 1971).

> ***Summary***: There are too few studies of effects on fertility of females to make a firm conclusion.

10.3.2.4 Developmental toxicity

No developmental toxicity studies conducted according to current protocols have been identified in the literature.

(a) Fetal growth

Fabro et al. (1984) dosed 10 pregnant rabbits with DDT (1 mg/kg bw) orally on GDs 4, 5, 6 and 7; there were 22 control rabbits. Mean litter sizes were similar between the two groups. However, offspring of DDT-treated dams had significantly smaller fetal weight: 28.0 g (standard error of the mean [SEM] 2.1 g) versus 37.4 g (SEM 1.4 g). Not only did offspring manifest intrauterine growth retardation, but they also had smaller fetal brain weights. No malformations were observed (Fabro et al., 1984).

When rabbits were treated orally with *p*,*p*′-DDT (8 or 88 mg/kg bw) from pregnancy day 15 to 28 days postpartum, there was no adverse effect on maintenance of pregnancy to full term, the growth of pups as reflected by body weights at 6 weeks of age or mean weights of body, liver, left testis, left epididymis or accessory sex glands of rabbits at 12 or 24 weeks of age (Veeramachaneni et al., 2007).

> ***Summary***: The available studies are too limited to make conclusions on possible effects of DDT/DDE on overall embryo-fetal development.

(b) Developmental neurotoxicity

The aforementioned multigeneration study by Tarján & Kemény (1969) evaluated motility in 20–30 animals from each of six generations exposed to DDT (0.4–0.7 mg/kg bw per day), using the "spontaneous motility test" and also evaluating caffeine-induced motility. No effects on spontaneous or caffeine-induced motility, either overall or within generations, were identified. Therefore, there was no evidence for increased sensitivity for developmental exposures to DDT.

Eriksson and co-workers conducted a series of studies to elucidate the effects of DDT on muscarinic and nicotinic receptors in the brains of immature mice. In the first study, Eriksson et al. (1984) dosed 10-day (immature) and 60-day (adult) NMRI mice with a single oral dose of *p*,*p*′-DDT (0.5 mg/kg bw); control mice were dosed with a fat emulsion vehicle. Muscarine- and nicotine-like binding sites were measured in the cerebral cortex and in the cerebellum at 24 hours or 7 days post-treatment; in the cerebral, but not cerebellar, cortex, there was a significant decrease in the muscarine-like receptors in DDT-treated adult animals at 7 days, but no change at 24 hours. In immature rats, there was a decrease at 24 hours (not significant) and an increase at 7 days (not significant). No change was observed in nicotinic receptors (Eriksson et al., 1984).

To explore this issue further, Eriksson & Nordberg (1986) undertook a second experiment with neonatal (10-day) NMRI mice given a one-time dose of DDT at 0.5 mg/kg bw, 2,2-bis(4-chlorophenyl)ethanol–palmitic acid (DDOH-PA) (a DDT metabolite found in the liver) at 0.7 mg/kg bw or a vehicle control. At 1 and 7 days post-exposure, the cerebral cortex and hippocampus were examined. Outcome measures included muscarinic receptor density, muscarinic receptor high- and low-affinity binding sites and measure of the sodium-dependent choline uptake system. At 7 days (but not 1 day) post-dosing, there was a significant increase in muscarinic acetylcholine receptor density in the cerebral cortex (but not hippocampus) for mice treated with DDT or DDOH-PA, thus confirming the finding for DDT in the first study. The proportion of low-affinity muscarinic sites compared with high-affinity sites was increased among DDT- and DDOH-PA-treated animals. DDT and

DDOH-PA did not alter the sodium uptake system (Eriksson & Nordberg, 1986).

To assess the possibility of longer-term effects, once again neonatal (10-day-old) NMRI mice were dosed with a single low oral dose of DDT (0.5 mg/kg bw) or DDOH-PA (0.7 mg/kg bw) or a vehicle control (Eriksson et al., 1990b). At 5 months, mice receiving DDT had increased potassium-induced release of acetylcholine from cortical brain slices (Eriksson et al., 1992). When studied at 3 months, the DDT-treated (but not DDOH-PA-treated) animals had a decreased muscarinic acetylcholine receptor density in the cortex but not hippocampus or striatum brain regions. There were no changes in cholinesterase acetyl transferase activity in the same three regions for DDT or DDOH. Behavioural testing at 4 months included assessments of locomotion, rearing and total activity. For all three measures of behaviour, mice treated with DDT or DDOH had significantly higher activity levels compared with controls at 20–40 and 40–60 minutes; at 40–60 minutes, the amount of activity for DDT was generally higher than that for DDOH as well. Thus, habituation in response to the diminished novelty of the test chambers over 60 minutes was considerably attenuated in the mice treated with DDT (Eriksson et al., 1990a,b, 1992). The authors noted that the doses required to elicit neurological symptoms in adult animals in earlier studies were 50–200 times higher (Eriksson et al., 1990a). The authors also noted that when these mice were similarly administered ^{14}C-labelled DDT on PND 10, radioactivity in the brain was highest 7 days later, almost the same as the activity at 24 hours. On day 30, no radioactivity could be detected in the brain (Eriksson et al., 1990b). This implies that the changes observed at 4 months are due to altered neurological function and not continuous poisoning by DDT in brain tissue.

In further experiments, the same dose of DDT (0.5 mg/kg bw) was given to NMRI mice at 3, 10 and 19 days of age, and again behavioural testing and receptor assays were done at age 4 months. The mice that received DDT at 10 days demonstrated the same significant increases in spontaneous motor behaviour that were reported in the 1990 studies (Eriksson et al., 1990a,b). Mice tested at 3 and 19 days showed no differences in spontaneous motor behaviour from controls. Additionally, at 4 months, mice dosed at

10 but not at 3 and 19 days had a significant decrease in muscarinic acetylcholine receptor density in the cerebral cortex. At this point in time, no change in the proportions of low- and high-affinity sites was observed (Eriksson et al., 1992). This study indicates that there may be a brief window of development during which the developing mouse brain is susceptible to the neurotoxic effects of DDT. The precise "window" during which this effect is manifest is difficult to elucidate given the findings by this same group relevant to brain levels after dosing. For example, when DDT was given at day 10, there were measurable levels in the brain at 24 hours and at 7 days, and there were no measurable levels at 30 days (Eriksson et al., 1990b). Given that the observed effect seems to be bounded by the day on which the effective dose is given (day 10) and the day on which two ineffective doses are given (days 3 and 19), it seems likely that this precise window is sometime between days 10 and 19, when the brain level from a dose delivered from day 10 would be fairly high and when a brain level of a dose delivered on day 3 would be too low.

Summary: No guideline developmental neurotoxicity studies were available. The studies by Eriksson and co-workers (e.g. Eriksson et al., 1990b, 1992) provide evidence that exposure of neonatal mice to DDT (a single intragastric dose of 0.5 mg/kg bw) during a specific stage of development (for the mouse, at PND 10) caused significant neurochemical and functional neurodevelopmental changes, including variation in muscarinic acetylcholine receptor density in the cerebral cortex and increases in spontaneous motor activity, indicative of a relative inability to habituate to new surroundings.

(c) Mode of action for developmental neurotoxicity

Exposure to high doses of DDT induces tremor and convulsions in humans. Chronic neurotoxicity has not been reported in humans or in experimental animals. In contrast, developmental neurotoxicity has been reported in both humans (see section 10.3.3.3) and experimental animals.

The toxicity for DDT in insects has been attributed to "slowing the closing of voltage-sensitive sodium channel". This effect was monitored in vitro at high DDT concentrations (millimole per litre level) (Lund & Narahashi, 1983). A recent in ovo study using the chicken embryo model and lower DDT concentrations (nanomole per litre level) suggested the same mechanism for DDT based on the

electron microscopic morphological alterations that simulate the effect of sodium channel modulators (Bornman et al., 2007). However, the sodium channel mechanism does not necessarily explain DDT effects in adult mammals at high dosages (i.e. tremor and convulsion). Rather, these effects have been attributed as serotonergic outcomes (Hwang & Van Woert, 1978).

According to the Hebbian theory, the developing brain utilizes normal neuronal activities to compose proper neuronal networks (based on "plasticity" of the developing brain or synaptic competition). Any exogenous stimuli that modify the neuronal activity would have the potential to induce irreversible alterations in brain circuitry. Therefore, any chemical that triggers altered neuronal signalling is a candidate "early exposure – late effect" type neurotoxicant, affecting higher brain function. The series of studies by Eriksson and co-workers (e.g. Eriksson et al., 1992) can be biologically interpreted as a case that fits with the hypothesis.

The series of studies by Eriksson and co-workers (Eriksson et al., 1984, 1990a,b, 1992; Eriksson & Nordberg, 1986) have elucidated the effects of DDT on muscarinic and nicotinic receptors in the brains of developing mice, resulting in altered behavioural test results at 4 months of age. The doses required to elicit neurological symptoms in adult animals in earlier studies were 50–200 times higher than doses resulting in developmental effects. Changes observed at 4 months of age were attributed to altered neurological function and not to continuous exposure to DDT in brain tissue.

Another possible mode of action of DDT as a toxicant to the developing brain is by activating receptor molecules or receptive signalling pathways, thereby modifying neural activities in the developing brain. Regional neuronal apoptosis by specific signalling may be involved (Zou et al., 2009).

Information on other possible mechanisms is limited. The estrogenic effects of DDT and anti-androgenic effects of DDE may target the neurosteroid system, including ERα, ERβ and AR (Hojo et al., 2008). DDT may affect cAMP production at a post-receptor step (Santini et al., 2003). A signalling system that influences the

mRNA levels of interleukin-1 receptor type 1 (IL-1R1) and tumour necrosis factor-alpha receptor type 1 (TNFR1) has also been reported to be involved (Sukata et al., 2002). Systems that lead to oxidative stress (Sukata et al., 2002) may be considered as well. Although all of these targets/mechanisms may be found in the brain, there are no studies reported that support such mechanisms taking part in the propagation of late neurobehavioural deficits.

10.3.2.5 Reproductive endocrine effects

(a) Exposure of castrated adult males (Hershberger assay)

A number of investigators have reported on the results of using DDE in development of a screening assay called the Hershberger. To put this into perspective, the Hershberger assay had been identified as a possible short-term screening assay to identify potential endocrine-active chemicals (anti-androgens) for further testing and assessment. Because this assay was under development, assays have used varying dosing durations and testing regimens. DDE, which was "known" to be a weak anti-androgen, was often assessed as a "positive control" as a way of validating the assay.

In 2000, Sunami et al. published results of a 5-day Hershberger assay of DDE and fenitrothion (a pesticide). They dosed 12-week-old male rats castrated at 11 weeks with DDE at 100 mg/kg bw per day; DDE at this dose level induced decreases in weights of reproductive organs (seminal vesicles, ventral prostate) when testosterone propionate was given at 0.1 mg/kg bw but not with a higher dose of testosterone propionate (1 mg/kg bw) (Sunami et al., 2000).

Leavens et al. (2002) dosed adult (11–13 weeks) male Long-Evans rats with DDE at 0, 5, 12.5, 25, 50 or 100 mg/kg bw per day for 4 days. On the first day of dosing, rats were castrated and testosterone capsules implanted [dose not reported]. On day 5, the rats were euthanized and examined. The liver displayed a dose-dependent increase in weight without concomitant changes in body weight or weights of sex organs. The authors noted that the oral doses administered to the rats in this study are several orders of magnitude greater than the daily average human dietary intake of DDE (as measured by NHANES data). DDE tissue levels were quite high; at the five dose levels administered, DDE concentrations in fat

were 73.1 (standard deviation [SD] 13.3), 201.0 (SD 41.2), 378.0 (SD 51.6), 884.0 (SD 98.2) and 2620 (SD 616) µg/g, respectively (Leavens et al., 2002).

Nellemann et al. (2003) reported on a "revised" Hershberger assay that used a gonadotrophin-releasing hormone inhibitor instead of surgical castration and also added to the protocol assessment of changes in gene expression of androgen-dependent prostatic genes, as well as a higher stimulatory dose of testosterone propionate. DDE was dosed at 50, 100 and 200 mg/kg bw per day to young mature pretreated Sprague-Dawley rats. Results for DDE were quite variable, and the authors concluded that more work was needed for development of the modified assay (Nellemann et al., 2003).

Kang et al. (2004) published results of a 10-day Hershberger assay for DDE as well as a number of other anti-androgens (flutamide, vinclozolin, procymidone and linuron). They administered DDE by oral gavage at doses of 25, 50 or 100 mg/kg bw per day to immature male Sprague-Dawley rats castrated at 6 weeks of age. For DDE, seminal vesicle weights were significantly decreased at 50 mg/kg bw per day (to 66% of the control) and 100 mg/kg bw per day (to 58% of the control). Other changes in organ weights (ventral prostate, levator ani/bulbocavernosus muscles and Cowper's gland) were observed only at 100 mg/kg bw per day, and no statistically significant differences in serum testosterone or LH levels were observed. DDE significantly increased liver weight in a dose-dependent manner (Kang et al., 2004).

(b) Exposure during pregnancy

When pregnant female Long-Evans rats were given *p*,*p*′-DDE by gavage at 100 mg/kg bw on GDs 14–18, the anogenital distance in the male offspring was decreased ($P < 0.04$; litter means analysis), and thoracic nipples were retained on PND 13 (Kelce et al., 1995).

You et al. (1998) studied two strains of rats (Sprague-Dawley and Long-Evans) at two dose levels of DDE (10 and 100 mg/kg bw per day) administered prenatally to pregnant females on GDs 14–18. *p*,*p*′-DDE reduced anogenital distance, which reached statistical significance in Long-Evans but not in Sprague-Dawley rats at

100 mg/kg bw per day. Nipple retention was observed in males of both strains at 100 mg/kg bw per day and in Sprague-Dawley rats even at 10 mg/kg bw per day; female pups showed no changes (parameters measured were anogenital distance and time of vaginal opening). Increased AR expression was observed in the testis and other reproductive organs at the high DDE dose only. Similarly, serum testosterone levels were elevated in the high DDE dose groups. The investigators measured DDE concentrations in a number of organs for three dams in each group on GD 20. For Sprague-Dawley rats, at the two dose levels, serum concentrations were 0.11 (SD 0.10) and 2.45 (SD 0.52) µg/ml, respectively; for Long-Evans rats, the serum levels for the two dose groups were 0.89 (SD 0.08) and 13.5 (SD 3.4) µg/ml. Although these appear to be high dosage rates, these serum levels overlap with levels that have been observed in human populations.

In You et al. (1999a), pregnant Sprague-Dawley rats were treated with DDE at 0, 10 or 100 mg/kg bw per day during GDs 14–18. CYP enzymes that are responsible for testosterone hydroxylations were assayed (by immunoblotting and by enzyme activity assays) in livers of pups on PNDs 10 and 21. Adult male rats were also treated with DDE at 0 or 100 mg/kg bw per day for 7 days to provide comparison with animals exposed in utero. Flutamide was administered to an additional group on GDs 14–18 as a positive control. You et al. (1999a) found that, unlike flutamide administration, DDE administration caused elevated levels of CYP2B1 and CYP3A1 and their hydroxylated testosterone products in both adult and developing rats and elevated levels of CYP2C11 and CYP2A1 among developing rats but not adult rats (You et al., 1999a).

Wolf et al. (1999) published a study of the reproductive effects of 10 known or suspected anti-androgens, including *p,p′*-DDE (100 mg/kg bw on pregnancy days 14–18, the intended dosing schedule being stopped because of reduced maternal weight gain), in Sprague-Dawley and Long-Evans rats. They found 7.8% of hypospadias in Sprague-Dawley rats compared with none among controls. This difference was not statistically significant. Long-Evans rats did not exhibit hypospadias. Further, DDE induced decreased anogenital distance (statistically significant only in Sprague-Dawley rats) and development of areolae. Further, decreased weight of seminal vesicles, glans penis and cauda epididymis in Sprague-Dawley rats and elevated incidence of retained

nipples and decreased weight of ventral prostate in both Sprague-Dawley and Long-Evans rats were detected. Thus, DDE was found to have a similar toxicity profile to, but was less potent than, vinclozolin and procymidone, both of which are AR ligands.

Loeffler & Peterson (1999) dosed pregnant Holtzman rats with *p,p′*-DDE at 1–200 mg/kg bw on GDs 14–18 and examined a number of endocrine end-points among male offspring at PNDs 21 (weaning), 32 (prepuberty), 49 (puberty) and 63 (post-puberty). The treatment group had reduced anogenital distance on PND 1 at 50, 100 and 200 mg/kg bw ($P < 0.05$). However, only the 200 mg/kg bw dose group had statistically significantly reduced anogenital distance on PND 4. Cauda epididymal sperm number was reduced by DDE but not further reduced by the combination of TCDD and DDE. Age at onset of puberty, daily sperm production, testicular and accessory sex organ weight (non-prostate) and levels of prostatic androgen-regulated gene transcripts were not affected. DDE-treated animals retained nipples on PND 13.

(c) Exposure during pregnancy and postnatally

You et al. (1999b) examined the ability of prenatal exposure to anti-androgens to modify subsequent response to DDE in male rats. Pregnant Long-Evans rats were dosed with DDE from GD 14 to GD 18 at 0, 10 (low dose) or 100 (high dose) mg/kg bw per day. At about 80 days of age, the male pups from each in utero group were divided into treatment or control groups. The treatment group was administered DDE at 70 mg/kg bw 4 times daily by gavage. Whereas the treatment with DDE as adults decreased the ventral prostate weight, the in utero treatment had no significant further effect.

When rabbits were treated orally with *p,p′*-DDT (8 or 88 mg/kg bw per day) from pregnancy day 15 to 28 days postpartum (Veeramachaneni et al., 2007), there was no adverse effect on maintenance of pregnancy to full term, the growth of pups as reflected by body weights at 6 weeks of age or mean weights of body, liver, left testis, left epididymis or accessory sex glands of rabbits at 12 or 24 weeks of age. DDT was associated with cryptorchidism (undescended testicles) (1/16 and 5/16 in the low- and high-dose groups compared with none in the controls) and germ

cell atypia. DDT did not affect serum LH, FSH or testosterone levels, nor did it affect sexual interest, penile erection, ejaculation, sperm counts or sperm morphology.

(d) Exposure postnatally

To determine the effect of DDE on androgen-induced pubertal maturation in male rats, weanling (21 days) male rats were administered *p,p′*-DDE at 100 mg/kg bw until after puberty (day 57). Treatment with DDE significantly delayed the onset of puberty by 5 days compared with control rats. The onset of puberty was defined as the day on which the prepuce separated from the penis (day 43 in the control rat). DDE did not reduce serum testosterone levels in these pubertal rats, suggesting that the anti-androgenic effect was not confounded by the reported ability of DDT-related chemicals to increase steroid metabolism by increased steroid hydroxylase activity (Kelce et al., 1995).

***Summary*:** Shortened anogenital distance has been consistently observed in Long-Evans rats and in single studies in two other rat strains at dose levels of 100–200 mg/kg bw per day. A single rabbit study with DDT (at 90 mg/kg bw per day) showed increased incidence of cryptorchidism. A single study in two strains of rats showed no significant increase in hypospadias after dosing with DDE.

10.3.3 Humans

Below are reviewed human studies relevant to reproduction and development. Studies that are specifically on male or female reproduction are reviewed separately. For each sex, studies associated with fetal and prepubertal exposures are reviewed separately from studies of adults. Finally, effects on fetal and child growth and development that are not sex specific are reviewed.

10.3.3.1 Male reproductive functions and hormone levels

(a) Reproductive functions

Associations reported between adverse effects on multiple semen parameters and levels of DDE or DDT are summarized in Table 6.

Table 6. Human studies of semen quality in relation to DDT and DDE levels

Study	Population	*N*	50th percentile serum level (range)	Semen collection	Sperm count	Sperm motility	Sperm morphology
Hauser et al. (2003a,b)	Boston, Massachusetts, USA, fertility clinic	212	DDE: 0.22 (0.07–7.8) µg/g lipid	WHO protocols	Null for "low" count	OR suggestive by tertile (1.0, 1.15, 1.5)	Null
Dalvie et al. (2004b)	Limpopo, South Africa, vector control workers	48	ΣDDT: 94.3 (1.4–315) µg/g lipid	WHO protocols	*p*,*p*′-DDT negative association	Null	Null
Rignell-Hydbom et al. (2004, 2005b)	Swedish fishermen	176; 195	DDE: 0.23 (0.04–1.46) µg/g lipid; 0.334–2.25 µg/g lipid	Mobile laboratory unit	ND	Null	ND
De Jager et al. (2006)	Mexico population in malaria-endemic region with spraying until 2000	116	DDE: 41 µg/g lipid	WHO protocols	Null	DDE negative association	DDE negative association

Study	Population	*N*	50th percentile serum level (range)	Semen collection	Sperm count	Sperm motility	Sperm morphology
Stronati et al. (2006)	Mixed exposure, collaborative Arctic areas	652	DDE: 0–2.5 µg/g lipid	Collected and frozen prior to analysis	ND	ND	ND
Aneck-Hahn et al. (2007)	Limpopo, population-based in community with spraying	311	DDT: 90 (SD 102) µg/g DDE: 216 (SD 211) µg/g	WHO protocols	DDE negative association	DDE negative association	Null
Messaros et al. (2009)	Fertility clinic	336	DDT: 0.005 µg/g lipid DDE: 0.290 µg/g lipid	WHO protocols	ΣDDT negative association	ΣDDT negative association	ΣDDT negative association

ΣDDT, total DDT; ND, not determined

In a study of men from a fertility clinic population, Hauser et al. (2003b) reported on results of the neutral single-cell microgel electrophoresis assay (neutral comet assay), which is used to assess DNA integrity in sperm. Linear regression results of the comet assay were slightly below zero, but CIs were wide, consistent with lack of a relationship between concentrations of DDE and DNA damage.

Hauser et al. (2003a) published a cross-sectional study of 212 male partners of subfertile couples who visited the Massachusetts General Hospital in Boston, Massachusetts, USA. They assessed a number of semen parameters as binary outcomes: low sperm concentration (< 20 million/ml), decreased motility (< 50% motile) and strict Kruger criteria for abnormal morphology (< 4% normal). DDE levels were measured in serum (median serum DDE concentration 0.2 µg/g lipid). No association between DDE tertiles and ORs of sperm concentration below a reference value was observed; for sperm motility, there was a weak, statistically not significant inverse association between DDE level and sperm motility.

A cross-sectional study of 60 vector control workers in Limpopo, South Africa, examined a number of male reproductive parameters (sexual function, fertility and job history) by questionnaire, a physical examination of the reproductive system and semen analysis (sperm count, density and motility using the WHO criteria and morphology using the strict Tygerberg criteria) in relation to serum DDT and DDE levels. The average sum of DDT derivatives (six compounds) in the serum was 94 µg/g lipid (range 1.4–315 µg/g lipid), including 28 µg/g lipid (range 0.3–67.1 µg/g lipid) for DDT and 65 µg/g lipid (range 1.1–273.6 µg/g lipid) for DDE. Among the 48 participants with a semen sample, 84% of morphology scores were below either the WHO or the Tygerberg criteria, but semen quality was not related to DDT or DDE level. The level of *p,p′*-DDT (but not the levels of other DDT derivatives) was negatively associated with sperm count, but positively associated with sperm density in the multivariate analysis (Dalvie et al., 2004a,b).

A cross-sectional study of 195 Swedish fishermen (volunteers recruited from among a cohort of 5183 enrolled in a larger study) evaluated serum levels of DDE and PCB-153 in relation to male reproductive function. The median *p,p′*-DDE serum level was approximately 0.23 µg/g lipid (range 0.040–2.3 µg/g lipid). DDE and PCB-153 levels were strongly correlated. Whereas PCB-153 level was associated with decreased sperm motility, Rignell-Hydbom et al. (2004) found no association between DDE level and semen parameters, including semen volume, sperm concentration, sperm motility or plasma FSH, LH, testosterone, inhibin B, estradiol or plasma sex hormone-binding globulin levels.

For 176 of the subjects in the Rignell-Hydbom et al. (2004) study, there was a sufficient quantity of semen to test sperm DNA/chromatin integrity using the sperm chromatin structure assay. The semen outcomes measured were per cent DNA fragmentation and high DNA stainable cells. There was a non-significant positive relationship between higher per cent DNA fragmentation index and serum DDE level quintiles ($P < 0.10$), perhaps due to confounding, with a stronger association between per cent DNA fragmentation index and serum PCB-153 level (Rignell-Hydbom et al., 2005b).

In further work, semen from 157 of these men (with sufficient quantity) was analysed, and no relationship was found between serum DDE levels and semen levels of prostate-specific antigen, neutral α-glucosidase, fructose and zinc (Rignell-Hydbom et al., 2005a).

A study in Chiapas, Mexico, assessed plasma DDT and DDE levels and semen quality according to WHO methods among 116 men aged 27 years (SD 8.2 years) and recruited in 2000–2001. Plasma DDE levels were high in this population. The outcomes studied were semen motility, sperm chromatin condensation, α-glucosidase activity (biomarker for epididymal function) and AR CAG repeats. Participation rates were not available. Participants were men who had never been occupationally exposed to DDT but resided in a region where biannual IRS with DDT took place until 1997. The non-lipid-adjusted median DDE concentration was 227 µg/l; the lipid-adjusted median concentration was 41 µg/g. Crude regression analysis showed that several sperm motion parameters, including the percentage of motile sperm, decreased

with higher DDE concentrations (β = −8.38; $P = 0.05$ for squared motility), and the percentage of sperm with morphological tail defects increased with higher plasma DDE concentrations (β = 0.003; $P = 0.017$). Insufficient sperm chromatin condensation was observed in 46.6% of participants, and the most severe category of incomplete DNA condensation was also positively correlated with DDE concentration ($r = 0.223$; $P = 0.044$). Participants in this study worked in agriculture; although they could no longer be exposed to DDT via working on farms, they may have had other occupational exposures that could affect semen parameters (De Jager et al., 2006).

A cross-sectional study was conducted of 652 adult men in four populations (200 Inuits from Greenland, 166 Swedish, 134 Polish and 152 Ukrainian). Sperm DNA fragmentation (measured by the terminal deoxynucleotidyl transferase deoxyuridine-5′-triphosphate [dUTP] nick end labelling [TUNEL] assay) and apoptotic markers were not associated with serum DDE levels for men in any region. Several sites experienced low participation, which could have affected the findings. The authors proposed, however, that as the time to pregnancy was not different between men who gave an ejaculate and those who did not, selection bias probably was not marked. Another possible source for bias is that most eligible men came from antenatal clinics—that is, non-fertile men were excluded (Stronati et al., 2006).

A cross-sectional study of 311 healthy men between 18 and 40 years of age (mean age 23 years; SD 4.7 years) living in an endemic malaria area in Limpopo, South Africa, evaluated plasma DDT and DDE levels in relation to semen analysis according to WHO standards as well as Hamilton Thorne Computer Assisted Sperm Analysis sperm motility parameters. The mean DDT and DDE concentrations were 90 (SD 102) µg/g lipid and 215 (SD 211) µg/g lipid, respectively. The multivariate linear regression analyses showed that higher DDE levels were associated with reduced Computer Assisted Sperm Analysis motility (β = −0.02; $P = 0.001$) and reduced ejaculate volume. Higher DDT levels were associated with higher Computer Assisted Sperm Analysis beat cross-frequency (β = 0.01, $P = 0.000$) and also with a higher percentage of sperm with cytoplasmic droplets (β = 0.0014; $P = 0.014$). Plasma DDE level was associated with oligozoospermia (< 20 million

sperm/ml; OR = 1.001; $P = 0.03$). Both DDT and DDE levels were significantly associated with asthenozoospermia (Aneck-Hahn et al., 2007).

In a case–control study, investigators analysed levels of DDT, DDE, aldrin and isomers of HCH in semen of men of 50 fertile and 50 infertile couples. Higher concentrations of *p*,*p*′-DDE and *o*,*p*′-DDT but not *p*,*p*′-DDT, as well as various HCH isomers, were detected in semen samples of men from infertile couples. Sperm count, but not motility, was negatively associated with the concentration of DDE in semen (Pant et al., 2007).

Reproductive outcomes in association with exposure to DDT were studied among a cohort of 2033 workers in the anti-malaria campaign in Mexico. Occupational exposure to DDT and reproductive outcomes were determined via a questionnaire that elicited information about 9187 pregnancies. Logistic regression models included parents' age at each child's birth, history of exposure to other pesticides and chemical substances in other employment, smoking and alcohol consumption. The OR for DDT exposure and birth defects, comparing pregnancies before and after the first exposure, was 3.77 (95% CI = 1.19–9.52). Likewise, estimated DDE levels were associated with increased odds of birth defects when the highest quartile was compared with the lowest quartile. Modelled DDE levels for each quartile were < 39.68, 39.68–61.12, 61.13–103.64 and > 103.64 µg/g fat. This study was limited by the use of modelled DDT and DDE levels; such a measure of exposure via recall and modelling is known to misclassify exposure, especially in the context of a disease such as birth defects, where recall of exposures could be biased among parents of cases. Another weakness of this study is its lack of specificity, in that the outcome ("all" birth defects) would not be expected to be associated with any one exposure. Finally, there was a lack of medical verification of the birth defects (Salazar-Garcia et al., 2004). Salazar-Garcia et al. (2004) also found no significant association for spontaneous abortion or sex ratio in relation to estimates of DDT or DDE levels. In this study, the determination of sex ratio and spontaneous abortion would be more reliable than ascertainment of birth defects, as discussed above.

Messaros et al. (2009) evaluated human sperm parameters in relation to ambient exposures to DDT and its metabolites and the role of genetic polymorphisms in modifying these associations. They obtained semen from 336 male partners of couples presenting to infertility clinics and analysed concurrently collected sera for levels of DDT, DDE and other organochlorines. Mean levels were 0.05 µg/g lipid for *p,p′*-DDT and 0.29 µg/g lipid for *p,p′*-DDE. DDT and DDE levels were summed, and, for the analysis, Messaros et al. (2009) considered those men in the highest quartile to have a "high" DDT + DDE level. The range of DDT + DDE levels within this highest quartile is not given; however, the authors stated that all values were within norms for the population in the USA as established by CDC (2003). They also determined DNA polymorphisms in GSTM1, GSTT1, GSTP1 and CYP1A1. Sperm parameters were categorized as abnormal according to WHO criteria (concentration < 20 million/ml, motility < 50%, morphology < 4%). Using multivariate logistic regression analysis and adjusting for serum lipids, other organochlorine pesticides, PCBs and various demographic factors, higher DDT + DDE levels were associated with significantly increased odds of having low sperm count (OR = 2.53; 95% CI = 1.0–6.31), low sperm motility (OR = 2.91; 95% CI = 1.27–6.66) and abnormal sperm morphology (OR = 3.23; 95% CI = 1.51–6.95). The negative effects of high DDT + DDE levels in serum were exacerbated by the GSTT1 null polymorphism (for decreased motility) and by the CYP1A1 common alleles (for increased abnormal morphology).

Reproductive history was evaluated among a cohort of male vector control workers who were exposed to DDT in a 1946–1950 anti-malaria campaign in Italy. Information to support retrospective DDT exposure estimates, as well as reproductive outcomes, was ascertained via questionnaire for spouses of 105 workers. The time to pregnancy in months at the first successful conception was estimated from population registrars. Stillbirth rates were elevated and the male/female ratio in the offspring was reversed among DDT-exposed workers, particularly among DDT applicators. Among DDT applicators, the male/female ratio decreased by tertile of estimated cumulative DDT exposure. The average number of children and spontaneous abortion rate were unaffected by DDT exposure. Exposure assessment was based on a model of inhalation

(only) exposure to a volatile chemical, more than 50 years after the exposure (Cocco et al., 2005b).

(b) Hormone levels

Hagmar et al. (2001) assessed levels of persistent organo-halogens and hormones, including testosterone, among 43 Swedish and 57 Latvian adult men. DDT (0.010–0.185 μg/g lipid, 10th–90th percentiles) and DDE (0.197–3.15 μg/g lipid, 10th–90th percentiles) had no effect on serum testosterone levels.

An occupational cohort study of 107 vector control workers in Sardinia, Italy, evaluated current DDE body burdens and historical information about DDT usage between 1946 and 1950 in relation to current (circa 2000) serum levels of a number of sex hormones, including serum LH, FSH, 17β-estradiol, testosterone and sex hormone binding globulin. The median lipid-adjusted serum DDE concentration over the total study population was 0.40 μg/g; levels of DDE in serum were not associated with estimates of past or cumulative work exposures to DDT. No relationships were found between these levels of exposure and sex hormone levels; however, no information was available on hormonal status at the time of exposure. Moreover, there was potential for response bias given that 1) only men still residing in the Sardinia area were included and 2) some 39 men refused "mainly due to serious health problems", and 2 were known to have died. Lack of a relationship between serum DDE levels at the time of study and work histories during the malaria eradication campaign 50 years prior raises doubt as to the validity of this very retrospective measure of exposure (Cocco et al., 2004).

Ayotte et al. (2001) published a brief report on a study of 24 young men recruited from an area in Mexico that, at the time, had active household DDT treatment activities. Serum DDE levels were on average 78 μg/g (range 17–177 μg/g). Higher levels of DDT were associated with higher concentrations of serum sex hormone binding globulin, lower bioavailable testosterone/total testosterone ratio, lower semen volume and decreased sperm count (Ayotte et al., 2001).

Dalvie et al. (2004a) reported on a cross-sectional study of 50 current vector control workers in Limpopo, South Africa. Concentrations of the isomers of DDT, DDE and DDA were measured in serum; the average concentration of the sum of DDT derivatives was 94 µg/g lipid (range 1.4–315 µg/g lipid). LH, FSH, testosterone, sex hormone binding globulin, estradiol and inhibin were measured before and after a gonadotrophin-releasing hormone challenge (100 µg) was given. Mean baseline estradiol levels (62 ± 30 ng/l) exceeded the laboratory reference range. Investigators reported a positive relationship of baseline estradiol and baseline testosterone with total DDT. Overall, however, the associations between total DDT levels in the plasma and the hormonal parameters were weak, there were a number of inconsistencies and the authors concluded that the associations might be due to chance.

No association between DDE and plasma FSH, LH, testosterone, inhibin B, estradiol or sex hormone binding globulin levels was observed in the cross-sectional study of Swedish fishermen described above (Rignell-Hydbom et al., 2004).

A cross-sectional study in the Thailand village of Mae Sa Mai evaluated serum DDT and DDE levels and reproductive hormones, including estradiol, testosterone, LH and FSH, among 97 adult men. The median DDE level was 4.1 µg/g lipid. Higher DDE levels were associated with lower levels of estradiol ($\beta = 7.093$; standard error [SE] = 2.899; $P = 0.016$) (Asawasinsopon et al., 2006b).

A large international cohort study of Inuit and Europeans, 2269 women and their spouses, did not provide any direct evidence for relationships between serum DDE levels in adults and endogenous or exogenous estrogenic or androgenic hormone activity in serum. The mean plasma DDE levels in the different populations varied between 300 and 1000 ng/g lipid (Bonde et al., 2008).

Summary: Associations have been reported between adverse effects on multiple semen parameters and increasing exposure to DDE, particularly among men with recent or current use and higher exposure levels (Table 6). It is difficult to ascertain causality of exposure in cross-sectional studies, but the evidence suggests that several different semen parameters

were negatively affected by increasing DDE levels. Human data are inadequate to assess male fertility and fecundity with regards to DDT/DDE exposures at population levels. A recent study has indicated that there may be a relationship between occupational DDT exposure and higher serum estradiol levels in men. A single human study suggests that DDT/DDE exposures of fathers may be associated with birth defects. However, this study had major weaknesses in assessment of exposure and birth defects via parental recall.

10.3.3.2 Female reproductive functions

(a) Fertility/fecundity

Serum DDE levels and time to pregnancy were assessed among 390 pregnant women enrolled in the Collaborative Perinatal Project from 1959 to 1965. A questionnaire asked, "How long did it take you to become pregnant?" if the respondent indicated that she had been trying to become pregnant when she enrolled in the project. This is a crude measure of time to pregnancy and is likely to be subject to error. Covariates included in the Cox proportional hazards regression models a priori included triglycerides, cholesterol, study centre location, smoking status and age. Additional confounders were selected based on a 10% change in estimate criterion. PCB and DDE levels in blood collected in the prenatal and perinatal periods, as well as maternal age, did not differ among women who planned or did not plan their pregnancy, so that there was no potential confounding by "planning status" for time to pregnancy. For women in the highest internal dose category (DDE ≥ 60 µg/l [approximately 10 µg/g serum lipid]), time to pregnancy increased compared with the lowest exposure category (DDE < 14 µg/l [approximately 2.2 µg/g serum lipid]), but the relationship was not significant (fecundability OR = 0.65; 95% CI = 0.32–1.31). However, the relationship between total PCB levels and fecundability was similar, and the analysis did not control for possible confounding by PCBs, nor did it exclude the possibility that the DDE and PCB levels were biomarkers for some other persistent organic pollutant associated with time to pregnancy. When DDE results were expressed as serum lipid concentrations, the relationship with time to pregnancy was attenuated (OR for DDE ≥ 7.685 µg/g = 1.01; 95% CI = 0.52–1.95) (Law et al., 2005).

Cohn et al. (2003) measured DDT and DDE levels in maternal serum samples drawn 1–3 days after delivery between 1960 and 1963 and assessed time to pregnancy among 289 eldest daughters 28–31 years later. For each 10 µg/l [approximately 1.60 µg/g] increase in DDT in maternal serum, they reported a decreased probability of pregnancy among daughters of 32% (95% CI = 11–48%). However, the probability of pregnancy increased by 16% (95% CI = 6–27%) per 10 µg/l increase in DDE concentration (Cohn et al., 2003).

Summary: The available data are inadequate to evaluate the effect of prenatal exposure to DDT or DDE on fertility or fecundity as measured by time to pregnancy.

(b) Menstrual cycle function

Chen et al. (2005) enrolled 60 Chinese women 20–24 years of age and evaluated DDT and DDE levels in relation to menstrual cycle characteristics (cycle length, duration of menses and heaviness of flow) among 47 who did not use hormonal contraceptives. In univariate analysis, higher DDE concentration was associated with longer menstrual cycle length; however, this difference was attenuated after adjustment for age, BMI, education, occupation and resident location (0.42 day; 95% CI = −0.35 to 1.19). DDE was not associated with duration of menses or heaviness of menstrual flow. DDT was not associated with any cycle characteristics. This study relied on recall of menstrual cycle length rather than utilizing hormonal measurements to assess menstrual cycles.

Four hundred and sixty-six newly married, nulliparous female Chinese textile workers aged 20–34 years who enrolled in a cohort study between 1996 and 1998 were included in a cross-sectional study to evaluate serum DDT and DDE levels in relation to length of menstrual cycle. DDT and DDE levels were measured in nanograms per gram serum (not lipid adjusted and equivalent to micrograms per litre). Multivariate logistic regression was used to estimate DDT exposure effects on odds of experiencing short or long cycles. Relative to those in the lowest DDT quartile (5.5–19.2 µg/l), the odds of any short cycle (< 21 days) in the previous year

were higher for those in the fourth quartile (41.2–113.3 µg/l; OR = 2.78; 95% CI = 1.07–7.14). There were no associations between serum DDT concentrations and odds of experiencing a long cycle (> 40 days) (Ouyang et al., 2005). This study relied on recall of menstrual cycle length rather than utilizing hormonal measurements to assess menstrual cycles.

Additionally, Perry et al. (2006) examined serum DDT and DDE levels prospectively in relation to urinary pregnanediol-3-glucuronide (PdG) and estrone conjugate (E1C) levels among 287 of these newly married, non-smoking Chinese women who were intending to conceive. Daily menstrual diaries and urine specimens were collected for 1 year or until a clinical pregnancy was achieved. Day of ovulation was determined for each cycle, and the association of serum DDT levels with daily PdG and E1C levels in a ± 10-day window around ovulation was analysed. After adjustment for covariates, including age, BMI and occupational exposures, Perry et al. (2006) reported consistent inverse associations of levels of most DDT and DDE congeners with urine E1C levels during the periovulation phase and with urine PdG levels during the luteal phase of the menstrual cycle; results for *p,p′*-DDT and *p,p′*-DDE are shown in Table 7. These results are consistent with a negative association between DDT and DDE and estrogen and progesterone levels at times during the menstrual cycle that are critical for ovulation and early pregnancy maintenance.

Windham et al. (2005) examined menstrual function and serum DDT and DDE levels among 50 South-east Asian women who were immigrants of reproductive age in California in 1997–1999. The women collected daily urine samples that were assayed for metabolites of estrogen and progesterone; menstrual cycle parameters were also assessed. In multiple regression models adjusting for age, parity and tubal ligation status, higher levels of DDT and DDE were associated with shorter mean luteal phase lengths. With each doubling of the DDE level, cycle length decreased by 1.1 days (95% CI = −2.4 to 0.23 day) and luteal phase length decreased by 0.6 day (95% CI = −1.1 to −0.2 day). Progesterone metabolite levels during the luteal phase were consistently decreased with higher DDE concentrations. PCB levels were not associated with menstrual cycle functions and did not modify the relationships between DDT and DDE and menstrual cycles (Table 8).

Table 7. Relationship between levels of urinary pregnanediol-3-glucuronide (PdG) and estrone conjugate (E1C) and serum levels of DDT and DDE

	Log PdG				Log E1C		
	β	SE	*P*-value		β	SE	*P*-value
Follicular							
N = 3199 days				*N* = 3152 days			
DDT	−0.56	0.45	0.21		−0.15	0.29	0.61
DDE	−0.06	0.03	0.06		−0.02	0.02	0.49
Periovulation							
N = 1337 days				*N* = 1337 days			
DDT	−0.54	0.36	0.13		−0.43	0.20	0.03
DDE	−0.06	0.03	0.05		−0.05	0.02	0.03
Luteal							
N = 3927 days				*N* = 3886 days			
DDT	−0.57	0.28	0.04		−0.31	0.26	0.24
DDE	−0.06	0.03	0.03		−0.05	0.02	0.04

Source: Perry et al. (2006)

A cross-sectional study of 1494 women from Greenland, Swedish fishermen's wives and inhabitants of Warsaw in Poland and Kharkiv in Ukraine evaluated serum levels of DDE (mean plasma DDE 0.4–2.1 μg/g lipid in the different countries) in relation to menstrual cycle length and regularity. Outcomes were obtained by questionnaires. No consistent effects of DDE exposure on menstrual cycle characteristics were observed (Toft et al., 2008). However, this study utilized imprecise measures of menstrual cycling.

Summary: One study found that higher serum DDT and DDE levels were associated with decreased estrogen and progesterone levels, and another study reported decreased length of the luteal phase of menstrual cycles and lowered luteal phase progesterone metabolite levels.

Table 8. Change in luteal phase of menstrual cycles by category of DDT and DDE level

Serum level (ng/ml)	Serum level (µg/g)[a]	Luteal phase (days)	Lower 95% CI	Upper 95% CI
DDT				
< 0.5	< 0.08	Referent	Referent	Referent
0.5–0.69	0.08–0.10	−0.10	−1.5	1.3
0.7–1.39	0.11–0.22	0.30	−0.72	1.3
≥ 1.4	≥ 0.22	−1.5	−2.7	−0.30
DDE				
< 7	<1.12	Referent	Referent	Referent
7–12	1.12–1.92	−0.84	−1.9	0.25
13–24	1.93–3.84	−1.1	−2.2	−0.05
> 24	> 3.84	−1.4	−2.6	−0.20

[a] Estimated using the ratio DDT/DDE µg/g lipid = 0.16 × DDT/DDE µg/l (see section 3).

Source: Windham et al. (2005)

(c) Lactation

A study of 858 mother–child pairs in North Carolina, USA, found that higher DDE levels in breast milk were associated with shorter duration of lactation (Rogan et al., 1987). A second study conducted in Mexico followed 299 women from childbirth; DDE was measured in breast milk samples taken at birth. Median duration of lactation was 7.5 months in the lowest DDE group (< 2.5 µg DDE/g milk fat) and 3 months in the highest group (≥ 12.5 µg/g milk fat). The difference was confined to those who had lactated previously. This raises the possibility that DDE levels were higher only among those who did not previously lactate and therefore that prior lactation experience, and not lower DDE levels, is associated with longer duration of lactation (Gladen & Rogan, 1995).

In a highly exposed area of Mexico, 784 mother–son pairs were followed to determine the length of lactation in relation to DDE and DDT levels in maternal serum obtained within a day of delivery. Compared with those with DDE concentrations of 3.00 µg/g or below, mothers with DDE concentrations of 3.01–6.00, 6.01–9.00 or above 9.00 µg/g showed no evidence that DDE shortened the length

of lactation among those who had not breastfed. Similarly, concentrations of DDT showed no association with shorter lactation time (Cupul-Uicab et al., 2008).

> ***Summary***: The evidence linking duration of lactation with DDE and DDT exposure is inconsistent.

(d) Menopause

In a hypothesis-generating study in postmenopausal women, higher serum levels of DDE were associated with decreased bone mineral density (Beard et al., 2000). However, a second study did not find a relationship between DDT and DDE levels and rate of bone mineral loss (Bohannon et al., 2000).

A cross-sectional study of 219 menopausal women participating in the Hispanic Health and Nutrition Examination Survey in 1982–1984 evaluated the relationships between DDT and DDE levels measured in serum and age at natural menopause. Analysis of variance was employed to compare adjusted mean age at natural menopause among women by category of serum pesticide level. DDT was analysed both by median split and also by quartiles based on integer categorization. The median DDT level was 3.43 µg/l or approximately 0.55 µg/g, and age at menopause for those below the median to the limit of detection of 2.0 µg/l [approximately 0.32 µg/g lipid] was 47.76 years. Serum levels of DDT were associated with a younger age at menopause; women with DDT levels in the highest exposure category (serum DDT ≥ 6 µg/l [approximately 0.96 µg/g lipid]) had an adjusted mean age at menopause on average 5.7 years earlier than women with serum levels below the detection limit (Table 9). Women with serum DDE levels greater than 23.6 µg/l [approximately 3.8 µg/g lipid], the highest quintile, had an adjusted mean age at menopause 1.7 years earlier than women with levels less than 5.5 µg/l [approximately 0.9 µg/g], the lowest quintile ($P = 0.13$). No consistent dose–response effect was apparent across low, medium and high exposure categories (Akkina et al., 2004). The organochlorine concentrations in serum were measured on average 10 years after menopause; menopause thus may have affected the observed concentrations. The associations were not

controlled for parity, known to be related to late menopause, or breastfeeding, which reduces plasma DDT levels.

Summary: A single study suggests that high exposure to DDT and its metabolites is associated with earlier age at menopause. However, this study did not include any prospective measures of DDT exposure, and early menopause per se may cause increased DDT or DDE levels (e.g. by causing changes in body fat distribution).

Table 9. Change in age of menopause (referent minus exposed group)[a]

Level (µg/l)	Level (µg/g lipid)[b]	*N*	Difference between referent and exposed (years)	Lower 95% CI	Upper 95% CI
***p,p'*-DDT**[c]					
< 2.00	<0.32	142	Referent	Referent	Referent
2.00–3.43	0.32–0.55	34	1.04	−1.06	3.14
> 3.43	> 0.55	36	2.76	0.70	4.82
***p,p'*-DDT**[d]					
< 2.00	< 0.32	142	Referent	Referent	Referent
2.00–2.99	0.32–0.47	28	1.14	−1.04	3.32
3.00–3.99	0.48–0.63	16	0.27	−2.35	2.89
4.00–5.99	0.64–0.95	13	2.11	−0.74	4.96
≥ 6.00	≥ 0.96	13	5.65	2.80	8.50
***p,p'*-DDE**					
< 5.46	< 0.87	41	Referent	Referent	Referent
5.46–10.43	0.88−1.66	43	−0.59	−2.57	1.39
10.44–16.09	1.67−2.57	43	−1.16	−3.16	0.84
16.10–23.60	2.58−3.78	42	0.20	−1.81	2.21
> 23.60	> 3.78	43	1.68	−0.34	3.71

[a] The differences and 95% CIs are calculated using the ages reported in Table 4 of Akkina et al. (2004).

[b] Estimated using the ratio DDT/DDE µg/g lipid = 0.16 × DDT/DDE µg/l (see section 3).

[c] Median split: defined as 1) below the limit of detection, 2) above the limit of detection but below the median value and 3) above the median value.

[d] Integer categorization.

10.3.3.3 Developmental effects

(a) Spontaneous abortion

Spontaneous abortion, miscarriage or fetal loss is defined as the death of the fetus prior to term. Stillbirth refers to the death of the fetus prior to term but after the time it has reached viability, often defined as at least 20–24 weeks' gestational age.

An early case-series with 100 women with spontaneous abortion and 152 controls found no relationship between serum DDT levels (3–92 µg/l) and spontaneous abortion (O'Leary et al., 1970).

In a study on 50 women with stillborn babies, preterm labour or full-term pregnancies, Saxena et al. (1981, 1983) reported that serum DDT levels (and also those of aldrin, dieldrin, lindane and HCH) were higher among mothers of stillborn babies than among those of full-term babies (Saxena et al., 1983) and higher among women with spontaneous abortions than among those with preterm and full-term births (Saxena et al., 1981).

Another study did not find associations between blood DDT levels and miscarriage (Leoni et al., 1989).

Gerhard et al. (1998) examined levels of chlorinated hydrocarbons, including DDT, in 89 women with repeated miscarriages in a clinic-based sampling of cases. No correlations between recurrent miscarriage type and serum DDT levels were observed.

A case–control study of 45 patients with a history of three or more (up to 11) consecutive first-trimester miscarriages and 30 healthy women with no history of miscarriage and infertility found no difference in serum DDT or DDE levels between cases and controls. However, the levels in this population were quite low, the numbers small and the controls not necessarily appropriate (Sugiura-Ogasawara et al., 2003).

As a whole, these studies (O'Leary et al., 1970; Saxena et al., 1981, 1983; Leoni et al., 1989; Gerhard et al., 1998; Sugiura-Ogasawara et al., 2003) had small numbers and uncertain case definitions for spontaneous abortion.

A subset of the Collaborative Perinatal Project cohort (n = 1717 pregnant women) was sampled to assess the association between serum DDE levels (and levels of other organochlorines) and previous fetal loss. Their responses indicated that 15% of previous pregnancies resulted in a loss, a number that is likely underestimating the true number of losses due to the incidence of early loss before a woman recognizes she is pregnant. Analyses were adjusted for maternal levels of dieldrin and β-HCH to account for the expected decrease in levels of persistent organic pollutants with pregnancy and lactation. Increasing odds of fetal loss were seen in relation to increasing DDE levels. The adjusted ORs of fetal loss according to category of DDE level are shown in Table 10. An OR for fetal loss in a previous pregnancy of 1.4 (95% CI = 1.1–1.6) was seen per 60 μg/l [approximately 9.6 μg/g] increase in serum DDE. These results did not change when Bayesian analysis was used, when DDE was measured by serum lipid weight or after stratifying prior losses before 20 weeks' gestation and after 20 weeks' gestation. This study had certain limitations. Serum DDE levels were measured on average 6 years after the event of interest. As noted above, pregnancy and breastfeeding result in transfer of DDE and other organochlorines from the mother to the fetus and infant. Breastfeeding was reportedly infrequent in the Collaborative Perinatal Project cohort (12%). However, pregnancies going to term would be expected to lower maternal DDE levels more than pregnancies ending in fetal loss. It is probable that adjustments for dieldrin and β-HCH offset this factor; nonetheless, there is some possibility of residual confounding that would exaggerate the magnitude of the relationship between DDE and fetal loss (Longnecker et al., 2005).

A pilot investigation of the hypothesized association of DDT with spontaneous abortion was conducted as a nested case–control study from women enrolled in a longitudinal study of the reproductive effects of shift work among female Chinese textile workers. Of 412 pregnancies, 42 ended in spontaneous abortion. Fifteen spontaneous abortion cases and 15 full-term controls were

randomly selected and DDT and DDE determined via assays of serum levels. Serum levels of DDE were significantly ($P < 0.05$) higher in cases than in controls (0.022 vs 0.012 µg/g). Each nanogram per gram serum increase in DDE was associated with a 1.13 (95% CI = 1.02–1.26) increased odds of spontaneous abortion (Korrick et al., 2001).

Table 10. Fetal loss by category of DDE level

DDE level (µg/l)	DDE level (µg/g)[a]	*N*	Adjusted OR	Lower 95% CI	Upper 95% CI
< 15	< 2.4	126	Referent	Referent	Referent
15–29	2.4–4.6	336	1.1	0.9	1.5
30–44	4.7–7.1	161	1.4	1.0	1.9
45–59	7.2–9.4	93	1.6	1.1	2.4
60+	≥ 9.5	53	1.2	0.7	1.9

[a] Estimated using the ratio DDE µg/g lipid = 0.16 × DDE µg/l (see section 3).

Source: Longnecker et al. (2005)

As a follow-on, Venners et al. (2005) conducted a prospective study of fetal loss and DDT exposure of 388 newly married nulliparous, non-smoking women in China enrolled in this same prospective cohort of textile workers. After stopping contraception, the subjects provided daily urine specimens and records of vaginal bleeding until they became clinically pregnant or for a year. Researchers assayed urinary human chorionic gonadotrophin levels each day, so that they could detect conceptions as well as early pregnancy losses (those that occur after a positive urinary human chorionic gonadotrophin test and prior to 6 weeks following the last menstrual period). Pregnancies were followed to detect clinical spontaneous abortions (defined as losses occurring between 6 and 20 weeks after the last menstrual period). Among 500 conceptions, there were 128 (26%) early pregnancy losses. Among 372 clinically recognized pregnancies, there were 36 (10%) clinical spontaneous abortions. When analysed by timing of loss, total DDT level in serum was associated with early pregnancy loss, but not clinically recognized spontaneous abortions. The relative odds of early pregnancy losses associated with a 10 ng/g increase in serum total DDT were 1.17 (95% CI = 1.05–1.29) (Venners et al., 2005). The

dose–response relationships for tertiles of total DDT levels are shown in Table 11.

Table 11. Odds of spontaneous abortion (including both early pregnancy losses and clinical spontaneous abortions) by category of total DDT level

Serum total DDT level (µg/g)	*N* (women)	*N* (conceptions)	*N* (losses)	Adjusted OR	Lower 95% CI	Upper 95% CI
0.055–0.229	130	155	41	Referent	Referent	Referent
0.0230–0.0365	129	165	50	1.23	0.72	2.10
0.0366–0.1133	129	180	71	2.12	1.26	3.57

Source: Venners et al. (2005)

Sugiura-Ogasawara et al. (2003) conducted a case–control study of women with and without repetitive first-trimester miscarriage and found no associations between DDT or DDE levels and the levels of a number of hormones, including prolactin and progesterone. However, the study was too small (45 cases and 30 controls) for small differences to be detected (Sugiura-Ogasawara et al., 2003).

Summary: Two cohort studies indicated an association between increasing DDT and DDE levels and fetal loss.

(b) Gestational age/preterm birth

Prematurity, or preterm birth, is a condition in which delivery occurs significantly earlier than the expected full term of 40 weeks. Babies born before 37 weeks are referred to as "preterm", and those born at less than 33 weeks are considered to be "very preterm". In addition to examining gestational age as a bivariate variable ("preterm" or not), some studies have evaluated gestational age as a continuous variable.

A small case–control study of 54 mother–infant pairs with no occupational exposure to DDT found no difference in the DDE concentration in maternal blood with term and preterm deliveries, but a significant difference in DDT cord blood levels in term and preterm infants as well as in DDT levels in maternal and neonatal blood in both groups. DDT cord blood levels correlated negatively with infants' birth weights. The authors suggested that the

association between prematurity and high DDT cord blood levels is related to the smaller amount of fetal adipose tissue in preterm infants (Procianoy & Schvartsman, 1981).

Saxena et al. (1981) and Siddiqui et al. (1981) reported that DDT levels were higher among mothers with preterm births than among those with full-term births.

The Wasserman et al. (1982) case–control study assessed serum levels of DDT and a number of other organochlorine compounds for l7 women with premature deliveries and 10 with normal term pregnancies. Five cases had "high" DDT serum levels (119.6 µg/l vs 26.5 µg/l), but these also had higher levels of several other organochlorines. At any rate, this study was very small and underpowered (Wassermann et al., 1982).

A study of 20 cases of premature rupture of fetal membranes at term and 15 matched controls found no relationship with serum DDT and DDE levels (the mean total DDT level in serum was 16 µg/l) (Ron et al., 1988).

A small study of 20 women with preterm delivery and 20 matched controls found no substantial difference in DDE levels in blood collected during the first trimester (the median *p*,*p*′-DDE concentration for controls was 1.35 µg/l) (Berkowitz et al., 1996).

Longnecker et al. (2001) analysed a sample of 2380 stored third-trimester blood samples from the Collaborative Perinatal Project, a cohort of pregnant mothers enrolled in the USA between 1959 and 1966, with a median maternal DDE concentration of 25 µg/l (range 3–178 µg/l). The adjusted OR for preterm birth increased monotonically as DDE concentration rose and was 3.1 (95% CI = 1.8–5.4) for the top quintile (≥ 60 µg/l) compared with the referent group (≤ 15 µg/l). A test for trend of the OR of preterm birth was statistically significant ($P < 0.0001$). The OR for small for gestational age (< 10th percentile for week of gestation) for the top quintile was 2.6 (95% CI = 1.3–5.2), and a test for trend across categories of increasing maternal DDE levels was statistically significant ($P < 0.04$).

The Farhang et al. (2005) study of 420 male infants from the Child Health and Development Studies, described above, examined the relationship of DDE and DDT levels with preterm birth and gestational age. For preterm birth, the adjusted OR was 1.28 (95% CI = 0.73–2.23) for DDE and 0.94 (95% CI = 0.50–1.78) for DDT (Farhang et al., 2005).

Fenster et al. (2006) reported no relationship between maternal prenatal exposures to DDT and DDE and infants' length of gestation among a birth cohort of 385 infants in the Salinas Valley, California, USA.

Jusko et al. (2006) assessed the role of in utero maternal serum levels of DDT and DDE on fetal and early childhood growth among children born to 399 women (average serum DDE concentration 6.9 μg/g fat) from among 20 754 women who participated in the Child Health and Development Studies conducted in the San Francisco Bay area in California, USA, during the 1960s. After covariate adjustment, a small increase in gestational age was observed with increases in DDT level (mean 1.7 days; 95% CI = 0.2–3.1 days), but not DDE level.

> ***Summary***: The strongest epidemiological study (Longnecker et al., 2001) suggests an association of elevated maternal serum DDE levels with reduced gestational age and increased rates of small-for-gestational-age babies. Inconsistent results on association between DDT/DDE levels and gestational age were reported in other studies.

(c) Fetal growth

Conventionally, babies born at less than 2500 g are considered to be "low birth weight" and those less than 1500 g "very low birth weight". When considered in this way, it is easy to confuse reduced fetal growth with reduced gestational age, given the very rapid growth that occurs during the last several weeks of pregnancy. To assess growth independently of gestational age, measures of size for gestational age (usually percentiles) are used, and the condition of being below expected size for gestational age may be called "small for gestational age", "fetal growth retardation", "intrauterine growth retardation" or, most recently, "fetal growth restriction". In addition

to examining growth as a bivariate variable ("small" or not), some studies have evaluated measures of growth as continuous variables. In such cases, it is important to control for gestational age to isolate growth from term of birth.

In a study of 912 infants, serum DDE level was not associated with birth weight, head circumference or neonatal jaundice (Rogan et al., 1986a). A study in Spain reported that DDT-exposed newborns had somewhat higher birth weight and head circumference (Martinez Montero et al., 1993). A study of 197 births in cities in Ukraine (1993–1994) reported no relationship between prenatal internal doses of DDT and DDE (as measured in maternal milk taken at 4–5 days of age; median DDE concentration 2.46 [range 0.33–17.41] µg/g milk fat) and birth weight (Gladen et al., 2003). Siddiqui et al. (2003) examined the association between DDT and intrauterine growth retardation (< 10th percentile of birth weight for gestational age). DDT and DDE levels were measured in maternal blood, placenta and cord blood from 30 mothers of babies with intrauterine growth retardation and 24 mothers of babies with normal weight. Using multiple logistic regression, they reported an OR for DDE of 1.21 (95% CI = 1.03–1.42). They also found a significant negative correlation between body weight of newborn babies and DDE level in maternal blood ($r = -0.25$; $P < 0.05$) and cord blood ($r = -0.26$; $P < 0.05$) after controlling for gestational age (Siddiqui et al., 2003). In this study, the main DDT metabolite in blood was *p,p′*-DDD. Neither DDD level nor the sum of the concentrations of all DDT metabolites was related to weight gain of the infant. The association of levels of HCH isomers to intrauterine growth retardation was stronger than that for DDT metabolites; the possible covariation of the two pesticides was not studied.

For 420 male infants born between 1959 and 1967 whose mothers were enrolled in the Child Health and Development Studies, investigators assessed prenatal DDT and DDE levels in relation to small-for-gestational-age birth and birth weight. Using multivariate logistic regression for small-for-gestational-age birth, the adjusted ORs for small-for-gestational-age birth were, for DDE, 0.75 (95% CI = 0.44–1.26) and, for DDT, 0.69 (95% CI = 0.73–1.27) (Farhang et al., 2005).

Fenster et al. (2006) reported no relationship between maternal prenatal internal doses of DDT and DDE and infants' birth weight and crown–heel length among a birth cohort of 385 infants in the Salinas Valley, California, USA. Likewise, among a cohort of 722 infants born between 1993 and 1998 to mothers in New Bedford, Massachusetts, USA, there was no association between prenatal DDE levels (< 0.2–15 µg/l cord blood) and measures of birth weight, length and head circumference (Sagiv et al., 2007).

Summary: Available evidence does not suggest an association between DDT and/or DDE and fetal growth restriction.

(d) Childhood growth

A study of 858 children found that DDE levels in breast milk did not affect children's growth or health as measured by frequency of physician visits for various illnesses (Rogan et al., 1987). A follow-up study of 594 children from the North Carolina, USA, cohort found that height of boys at puberty increased with transplacental (but not lactational) exposure to DDE, as did weight adjusted for height; those with the highest exposures (maternal concentration ≥ 4 µg/g fat) were 6.3 cm taller (adjusted mean; P = 0.054) and 6.9 kg heavier (adjusted mean; P = 0.010) than those with the lowest exposure (0–1 µg/g fat). Such effects were not observed in girls (Gladen et al., 2004).

Karmaus et al. (2002) followed the growth of 343 German children from birth to 10 years of age in association with serum levels of DDE and PCB at 8 years of age. Investigators measured height between 1994 and 1997 and obtained earlier data from medical records. Among girls, growth through 8 years of age was significantly reduced by an average of 1.8 cm (P < 0.0275) among those in the highest DDE quartile (> 0.44 µg/l in blood) compared with the lowest quartile (0.08–0.2 µg/l). There were no observed effects of DDE on growth in boys or of PCBs on growth in either sex. For girls, the observed differences were smaller at 9 years of age and disappeared by age 10.

A cohort of 304 males born in Philadelphia, Pennsylvania, USA, in the early 1960s showed no relationship between anthropometrics, skeletal age, serum testosterone or serum dehydroepiandrosterone

sulfate and prenatal maternal serum DDE and DDT levels (total DDT 1.8–33.1 µg/g fat) (Gladen et al., 2004).

In the Child Health and Development Studies, Jusko et al. (2006) found that, at 5 years of age, an increase in serum DDE level from the 25th to the 75th percentile (3.90 and 8.56 µg/g lipid, respectively) was related to a 2 mm increase in childrens' head circumference (95% CI = 0–4 mm).

Childhood growth in the first 7 years of life was evaluated in relation to maternal prenatal DDE levels among children born between 1959 and 1966 in Spain and enrolled in a prospective cohort study. The highest prenatal concentrations of DDE (≥ 60 µg/l; median 78.9 µg/l, 9.5 µg/g lipid), compared with the lowest (< 15 µg/l; median 12.1 µg/l, 1.6 µg/g lipid), were associated with decreased height; children born within the lowest range of serum DDE levels were on average 0.72 (SE 0.37) cm, 1.14 (SE 0.56) cm and 2.19 (SE 0.46) cm taller than those who were born with serum levels in the highest DDE range at the age of 1, 4 and 7 years, respectively ($P < 0.05$ for all). No associations were observed among lower categories of exposure or with serum DDT concentrations (Ribas-Fito et al., 2006a).

Summary: Available evidence is mixed but supports the possibility of an association between prenatal or early life exposures to DDE and reduced childhood or pubertal growth. In only one study were the results consistent for males and females.

(e) Age of menarche and pubertal stages

The North Carolina, USA, cohort study followed children to puberty; no effect on the ages at which pubertal stages were attained was found that was attributable to DDE exposure during pregnancy or lactation (Gladen et al., 2000).

The Ouyang et al. (2005) study of 466 newly married female Chinese textile workers evaluated total DDT levels at age 20–34 years in relation to age of menarche using multivariate linear regression. Relative to those in the lowest DDT quartile, the

adjusted mean age at menarche was lower in those in the fourth quartile (−1.11 years; 95% CI = −1.50 to −0.72 year). Modelled as a continuous variable, a 10 ng/g increase in serum DDT concentration was associated with an adjusted reduction in age at menarche of 0.20 year (95% CI = −0.28 to −0.13 yea) (Ouyang et al., 2005). A limitation of this study is that total DDT levels were measured 5–20 years after age of menarche.

A cross-sectional study of pubertal status in relation to hormonally active environmental exposures recruited a multiethnic group of 174 healthy 9-year-old girls residing in New York City, USA. Plasma DDE level (geometric mean concentration approximately 0.4 µg/l) was not significantly associated with breast or pubic hair stage (Wolff et al., 2008).

> ***Summary***: Although results are mixed, there is no conclusive evidence that DDT or DDE is associated with change in age of menarche or pubertal stages.

(f) Developmental neurotoxicity

The results of human developmental neurotoxicity tests for DDT and DDE are summarized in Table 12.

In a follow-up study of 912 infants from a North Carolina, USA, cohort, the results of Brazelton Neonatal Behavioural Assessment Scales (neurodevelopmental tests administered to newborns) showed that higher DDE levels (estimated as concentration in milk fat at birth) were associated with hyporeflexia (Rogan et al., 1986a).

A later follow-up study of 858 infants from the North Carolina, USA, cohort evaluated Bayley Scales of Infant Development at 6 and 12 months of age; higher transplacental exposure to DDE was associated with higher mental scores at 6 months of age but not at 12 months. There was no relationship at either time with breast milk exposures (Gladen et al., 1988).

Table 12. Summary of human developmental neurotoxicity tests for DDT and DDE: timing, tests administered and results

Study	Age (months)							
	Newborn	1	3	6	12	18	24	48
1. Rogan et al. (1986a)	NBAS: hyporeflexia							
1. Gladen et al. (1988)				Bayley DDE ↑ mental scores	Bayley DDE ↔			
1. Gladen & Rogan (1991)								McCarthy DDE ↔
2. Rogan & Gladen (1991)						Bayley DDE ↔	Bayley DDE ↔	
3. Darvill et al. (2000)				Fagin	Fagin			

Study	Age (months)							
	Newborn	1	3	6	12	18	24	48
				DDE ↔	DDE ↔			
4. Eskenazi et al. (2006)				Bayley DDT and DDE ↓ PDI ↔ MDI	Bayley DDT ↓ PDI ↓ MDI		Bayley DDT ↔ PDI ↓ MDI	
4. Fenster et al. (2007)	BNBAS DDT and DDE ↔							
5. Ribas-Fito et al. (2006b)								McCarthy DDT ↓ verbal ↓ memory
6. Torres-Sánchez et al.		Bayley	Bayley	Bayley	Bayley			

Study	Age (months)							
	Newborn	1	3	6	12	18	24	48
(2007)		DDE ↓ PDI ↔ MDI	DDE ↓ PDI ↔ MDI	DDE ↓ PDI ↔ MDI	DDE ↓ PDI ↔ MDI			
7. Sagiv et al. (2008)	NBAS DDE ↔							

↓ means significantly lower score in association with exposure; ↔ means no difference between exposed and control group; and ↑ means significantly higher score in association with exposure

Tests: Brazelton Neonatal Behavioural Assessment Scale (BNBAS); Bayley Scales of Infant Development (Bayley); Bayley subscales (Mental Developmental Index [MDI] and Psychomotor Developmental Index [PDI]); Fagin Test of Infant Development (Fagin); McCarthy Scales of Children's Abilities (McCarthy); McCarthy subscales (verbal, memory, quantitative, perceptual performance); and Neonatal Behavioural Assessment Scale (NBAS)

Seven hundred and twelve of these children were examined with the McCarthy Scales of Children's Abilities at 3, 4 or 5 years of age; in addition, 366 school report cards were examined. There was no relationship between McCarthy scores or poorer grades and DDE exposure by the transplacental or breastfeeding route (Gladen & Rogan, 1991).

A second study by this group followed a cohort of children from birth through the age of 2 years; they used the Bayley Scale to test 676 at 18 months and 670 at 24 months and estimated transplacental and cumulative exposures to DDE from breast milk. They found no effects on psychomotor development attributable to DDE exposure (Rogan & Gladen, 1991).

Likewise, Darvill et al. (2000) found no relationship between serum DDE levels and performance on the Fagin Test of Infant Development at 6 and 12 months among 230 infants.

Eskenazi et al. (2006) reported on the results of a birth cohort study of 360 singleton births in Salinas, California, USA. DDT and DDE levels in maternal serum were measured. The ranges of the concentrations of *p,p′*-DDT and *p,p′*-DDE were 0.002–33 μg/g lipid and 0.05–159 μg/g lipid, respectively. When psychomotor development and mental development were assessed with the Bayley Scales of Infant Development at 6, 12 and 24 months, an approximately 2-point decrease was observed in Psychomotor Developmental Index scores with each 10-fold increase in DDT level at 6 and 12 months (but not 24 months) and DDE level at 6 months only. A 2- to 3-point decrease in Mental Developmental Index scores was also observed for DDT at 12 and 24 months, corresponding to 7- to 10-point decreases across the exposure range. No association was found between DDT or DDE level and performance on the Brazelton Neonatal Behavioural Assessment Scale (Fenster et al., 2007)

Ribas-Fito et al. (2006b) evaluated neurocognitive development in relation to DDT levels in cord serum among 475 children belonging to two birth cohorts in Spain, recruited between 1997 and 1999. They assessed the children at age 4 using the McCarthy Scales of Children's Abilities. DDT level at birth was inversely associated with verbal, memory, quantitative and perceptual performance skills at age 4 years (Table 13). Children whose DDT

concentrations in cord serum were greater than 0.20 µg/l [approximately 0.03 µg/g lipid] had mean decreases of 7.86 (SE 3.21) points in the verbal scale and 10.86 (SE 4.33) points in the memory scale when compared with children whose concentrations were less than 0.05 µg/l [approximately 0.008 µg/g lipid]. These associations were stronger among girls.

Torres-Sánchez et al. (2007) assessed prenatal maternal serum levels of DDE with respect to performance on the Bayley Psychomotor Developmental Index and Mental Developmental Index during the first year of life among 244 children whose mothers were residents of a zone in Mexico with endemic malaria. The Psychomotor Developmental Index and Mental Developmental Index were evaluated at 1, 3, 6 and 12 months of age. Using multivariate models adjusting for quality of the home environment and maternal intelligence quotient (IQ), only DDE levels during the first trimester of pregnancy were associated with a significant reduction in Psychomotor Developmental Index (every 2-fold increase of first-trimester DDE level reduced the Psychomotor Developmental Index by 0.5 point). The DDE level was not associated with the Mental Developmental Index.

Sagiv et al. (2008) investigated cord serum DDE levels (as well as levels of PCBs and dioxins) and measures of attention from the Neonatal Behavioural Assessment Scale in a cohort of 788 infants born between 1993 and 1998 to mothers residing in New Bedford, Massachusetts, USA. The median DDE level was 0.30 (0–10.29) µg/l serum [approximately 0.048 µg/g serum lipid]. For the 542 subjects with a Neonatal Behavioural Assessment Scale examination at 2 weeks, there were consistent inverse associations between cord serum DDE levels and Neonatal Behavioural Assessment Scale measures of alertness, cost of attention, self-quieting and motor maturity. None of these changes were statistically significant, however, and there were also consistent (and mostly stronger) associations between these measures and levels of PCBs and dioxins. The authors did not find evidence for associations with infant orientation, habituation and regulation of state, assessed as summary cluster measures. The analysis did not control for possible confounding of the results by levels of PCBs and dioxins.

Table 13. Change in scores for McCarthy scales by DDT level categories

DDT level (µg/l)	DDT level (µg/g)[a]	*N*	β (SE)		
			General cognitive	Verbal	Memory
All children					
Referent ≤ 0.05	≤ 0.008	203	104.03	98.38	88.93
0.051–0.10	0.008–0.016	86	1.45 (2.72)	1.80 (3.36)	1.64 (4.53)
0.101–0.20	0.017–0.032	74	−2.01 (2.95)	−4.02 (3.65)	−4.46 (4.92)
> 0.20	> 0.032	112	−5.87 (2.60)*	−7.86 (3.21)*	−10.86 (4.33)*
Girls					
Referent ≤ 0.05	≤ 0.008	101	104.67	97.22	88.22
0.051–0.10	0.008–0.016	48	−1.37 (3.95)	−2.26 (4.86)	−2.46 (6.61)
0.101–0.20	0.017–0.032	33	−0.44 (4.47)	−2.58 (5.51)	−4.76 (7.47)
> 0.20	> 0.032	55	−8.89 (3.89)*	−12.79 (4.80)**	−17.19 (6.51)**
Boys					
Referent ≤ 0.05	≤ 0.008	102	102.64	101.99	96.54
0.051–0.10	0.008–0.016	38	3.39 (4.09)	5.66 (5.05)	2.47 (6.82)
0.101–0.20	0.017–0.032	41	−5.15 (4.06)	−6.65 (5.01)	−6.30 (6.77)
> 0.20	> 0.032	57	−3.74 (3.63)	−3.41 (4.47)	−5.63 (6.04)

SE, standard error

* $P < 0.05$; ** $P < 0.01$

[a] Estimated using the ratio DDT µg/g lipid = 0.16 × DDT µg/l (see section 3).

Source: Ribas-Fito et al. (2006b)

Summary: The studies provide consistent evidence for perinatal exposure having neurocognitive effects on a population level (Table 12), particularly for DDT.[1] Two well-conducted prospective studies (Eskenazi et al., 2006; Ribas-Fito et al., 2006b) indicate that perinatal exposure to DDT at levels above 0.20 µg/l [approximately 0.032 µg/g lipid] (Ribas-Fito et al., 2006b) or resulting in a 10-fold increase in newborn serum DDT levels within the range of 0.002–33 µg/g lipid (Eskenazi et al., 2006) is associated with an adverse effect on neurodevelopment up to age 4. The evidence for DDE is mixed.

[1] See evaluation of later published studies considered by the expert consultation on the risk characterization (Part A, section 2.2).

(g) Developmental immunotoxicity

Rates of infectious disease episodes were compared for 98 breastfed and 73 bottle-fed Inuit infants; prenatal exposure to DDE and other organochlorines was inferred from breast milk levels. Risk of ear infection (otitis media) increased with prenatal exposure to DDE (RR = 1.87, 95% CI = 1.07–3.26, for ages 4–7 months; RR = 1.52, 95% CI = 1.05–2.22, for the first year of life). Immunological parameters were not affected (Dewailly et al., 2000).

Dallaire et al. (2004) reviewed the medical charts of a cohort of 199 Inuit infants during the first 12 months of life and evaluated the incidence rates of upper and lower respiratory tract infections, otitis media and gastrointestinal infections in relation to levels of PCBs and DDE in maternal plasma during delivery and infant plasma at 7 months. They observed a positive relationship between prenatal but not postnatal exposure to DDE and PCBs and the rates of infections. The relationship with PCB exposures was of longer duration than the relationship with DDE. No relationships were found with alterations of clinical immunological parameters. Although this study demonstrated a possible association between prenatal exposure to persistent organic pollutants and acute infections early in life, it did not clearly implicate a role for DDE.

Sunyer et al. (2006) carried out a prospective birth cohort study in Menorca, Spain. Children 6.5 years of age had DDE levels measured in cord blood (n = 402) and blood samples at age 4 (n = 285). Asthma was defined as the presence of wheezing at age 6 and during any preceding year or doctor-diagnosed asthma; a skin prick test at age 6 was used to determine atopic status. DDE level in cord blood at birth was associated with diagnosed asthma (OR = 1.18; 95% CI = 1.01–1.39) and with persistent wheezing (OR = 1.13; 95% CI = 0.98–1.30) for each 1 μg/l [approximately 0.16 μg/g lipid] increase. DDE level in blood at 4 years was not associated with wheezing or asthma.

Serum DDT and DDE levels from samples taken from mothers at delivery were evaluated with respect to estimated thymus volume in 982 newborn infants from eastern Slovakia. Neonatal thymus volume was estimated using ultrasound measurements on the third

or fourth day after birth. There was no relationship between exposure to DDT and DDE and calculated thymic index (Park et al., 2008).

> ***Summary***: Studies of developmental immunotoxicity were inconclusive.

(h) Effects on male reproductive tract

Hypospadias. Longnecker et al. (2002) conducted a nested case–control study of male infants with hypospadias (*n* = 199) within the United States Collaborative Perinatal Project. The OR for hypospadias for the highest level of DDE exposure from maternal (third trimester) serum (> 10.67 µg/g) was 1.3 (95% CI = 0.6–2.5). At lower levels, there was no association.

A group in Mexico completed a pilot study of exposures to DDT and DDE and congenital hypospadias. This was a case–control study during 1997–1999 enrolling 41 subjects with hypospadias and 28 controls. DDT and DDE levels were determined from maternal blood samples. Using multivariate logistic regression, the OR for DDT was 1.13 (95% CI = 0.24–5.29); the OR for DDE was 0.48 (95% CI = 0.15–1.60). The study was limited by small sample size and low statistical power (Flores-Luevano et al., 2003).

Bhatia et al. (2005) conducted a nested case–control study of 70 infants with hypospadias in the Child Health and Development Studies and 283 random controls and evaluated maternal serum DDT and DDE levels during pregnancy. Infants whose mothers had higher DDE levels (≥ 61.0 µg/l [approximately 10 µg/g lipid]) had an adjusted OR of 1.18 (95% CI = 0.46–3.02) compared with infants in the lowest DDE group (< 27 µg/l [approximately 4 µg/g lipid]). Infants whose mothers had higher exposures to DDT (≥ 20 µg/l [approximately 3.2 µg/g lipid]) had an adjusted OR of 0.79 (95% CI = 0.33–1.89) compared with boys whose mothers had serum DDT levels below 10 µg/l [approximately 1.6 µg/g lipid]. This study had the same limitations as the Longnecker et al. (2002) study.

There are several methodological problems that are inherent in these studies. In all of these studies, investigators relied on recording of the diagnosis of hypospadias in medical records,

without independent confirmation. Recording of diagnoses of milder forms of hypospadias (of a severity that does not require surgery) in medical records is not completely reliable. Because of lack of inclusion of mild cases of hypospadias, extensive under-ascertainment of hypospadias in these studies is likely, which could bias the results if, for example, DDT exposures and under-ascertainment of hypospadias were both more prevalent in rural communities. Additionally, given that hypospadias is a relatively rare birth defect, it is possible that none of these studies was adequately powered to assess hypospadias.

> ***Summary***: Human evidence is inadequate to conclude whether there is an association between exposure to DDT/DDE and hypospadias.

Anogenital distance. Longnecker et al. (2007) conducted a cross-sectional study of 781 newly delivered male infants in 2002–2003 in Chiapas, Mexico. Investigators measured anogenital distance and penile dimensions and assayed levels of DDE in the mothers' blood. There was no evidence that exposure in utero to DDE (minimum, median and maximum serum DDE concentrations were 0.1, 2.7 and 56.1 μg/g lipid, respectively) was related to anogenital distance or penile dimensions at birth.

Torres-Sánchez et al. (2008) conducted a nested case–control study in Morelos, Mexico, to examine the relationship between maternal serum levels of DDT and DDE during pregnancy and development of secondary sexual characteristics as measured by the anal position index, a measure of anogenital distance that does not depend on age. Maternal serum was drawn prior to pregnancy and at each trimester during pregnancy. After delivery, infants were examined at home visits. Among boys, a doubling of maternal first-trimester DDE (but not DDT) levels was associated with a significantly reduced anal position index ($\beta = -0.02$; $P = 0.02$) after adjusting for birth weight, birth length and age at evaluation. At baseline, prior to pregnancy, the ranges between the 10th and 90th percentiles of DDT and DDE levels were 0.012–7.7 μg/g and 0.21–5.89 μg/g, respectively. The study was limited by small numbers (only 37 boys and 34 girls), so there was very little statistical power to detect smaller effects (e.g. in girls) and limited generalizability.

Despite the small numbers, the study identifed a relationship between DDE exposure and anal position index.

> ***Summary***: Two well-conducted epidemiological studies gave conflicting results for DDE exposure and anogenital distance.

Cryptorchidism. Longnecker et al. (2002) conducted a nested case–control study of cryptorchidism (*n* = 219) within the Collaborative Perinatal Project. The OR for cryptorchidism for the highest level of DDE exposure from maternal (third trimester) serum was 1.4 (95% CI = 0.8–2.6).

A nested case–control study of 79 infants with cryptorchidism in the Child Health and Development Studies and 283 random controls was also reported by Bhatia et al. (2005). Using the same groupings of DDE and DDT levels from maternal serum (third trimester or immediately after delivery), the adjusted ORs for cryptorchidism were as follows: DDE, 1.34 (95% CI = 0.51–3.48); and DDT, 1.01 (95% CI = 0.44–2.28).

Damgaard et al. (2006) conducted a nested case–control study within birth cohorts in Denmark and Finland enrolled from 1997 to 2001. Twenty-nine Danish and 33 Finnish cases with crypt-orchidism were identified at birth. Spontaneous descent by 3 months of age occurred in 25 Danish and 8 Finnish infants. Controls (*n* = 38) without cryptorchidism at birth or 3 months were selected randomly in Denmark but were matched on many factors in Finland. Mothers' milk collected between 1 and 3 months postpartum was analysed for DDT and DDE; median concentrations of these as well as a number of other organochlorine pesticides were higher in case milk than in control milk. This study did not allow for separation of the effects of DDT and DDE from those of other organochlorine pesticide exposures.

Brucker-Davis et al. (2008) carried out a case–control study of cryptorchidism among babies born in the Nice area of France. DDE, one plasticizer and seven PCB compounds were assessed in 151 cord blood samples (67 cryptorchid, 84 controls matched for place and date of birth, birth weight, gestational age and, when possible, parental origin) and 125 colostrums (56 for cryptorchid and 69 for controls). Whereas the concentrations of DDE in serum or mothers'

milk were not significantly different among boys with cryptorchidism and referents, cryptorchid boys were more likely to be classified in the most contaminated groups in colostrum for total PCBs and for DDE ($P = 0.037$). The study design did not allow the possibility of controlling for co-exposures to these two sets of compounds, nor could it exclude the possibility that these persistent organic pollutants are serving as biomarkers for other persistent compounds that actually are associated with cryptorchism.

Summary: Four human studies did not show significant association between DDT/DDE and cryptorchidism.

11. HAZARD CHARACTERIZATION[1]

11.1 Summary of hazard identification for use in hazard characterization

Exposure to DDT causes liver enlargement in rats and induction of CYP enzymes via CAR-PXR binding. In humans, induction of CYP and elevation of GGT activity in serum have been observed.

At dose levels above 6–8 mg/kg bw per day, DDT caused tremors and convulsions in adult mice and rats; similar effects have been observed in children who have accidentally ingested DDT.

On the basis of available data, it is not possible to conclude whether serum DDT and/or DDE levels are associated with immunotoxicity.

Results from studies on possible diabetogenic effects of DDT are inconclusive.

DDT, while negative in most genetic toxicity assays, can induce DNA damage in cultured rodent cells and in human lymphocytes. The mechanism of this damage has not been elucidated.

Several studies demonstrate that DDT induces tumours in rodents, notably tumours of the liver, but also lung tumours and leukaemia.

A large, well-conducted prospective study in China where there is a relatively high background rate of liver cancer in an area with high exposure to DDT demonstrated strong evidence for an association of serum DDT with liver cancer. This association was not observed in several human studies that suffered from limited statistical power and poorer exposure assessment. The concordance for liver tumours in experimental animals and humans strengthens the plausibility of the single positive human study.

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[1] The text in this section was agreed to by the participants of the expert consultation convened to peer review the hazard assessment of DDT (held in June 2009). However, the finalized results of the benchmark dose (BMD) modelling were not available to this expert consultation. The results of the BMD modelling were therefore subjected to a separate peer review process.

Of many studies looking at exposures to DDT and breast cancer, most were negative, and adult exposures to DDT are not demonstrably linked to breast cancer. One study suggested that prepubertal exposure to DDT may be associated with breast cancer. This finding is consistent with estrogenic actions of DDT in experimental animals as well as with findings (described below) of possible menstrual cycle alterations induced by DDT, which in turn could change breast cancer risks by altering estrogen hormone levels in menstrual cycles. Overall, the association between DDT and breast cancer is inconclusive.

A single human study provided evidence for an association between DDE and testicular germ cell tumours. Such a relationship is consistent with the observed hormonal properties of DDE. Overall, the associations between DDE and testicular cancer are inconclusive. A short-term study on testicular cancer in rabbits was inconclusive.

A number of studies linked DDT exposure to NHL but did not adequately control for potential confounding by other pesticide exposures. It was concluded that the data are inadequate to assess the association of DDT with NHL.

Data are inadequate to assess associations between DDT/DDE and lung, pancreatic, prostate or endometrial cancers.

There are several potential modes of action for DDT and DDE that may be relevant to carcinogenesis. Generally, it is thought that genotoxicity is unlikely to be a mode of action for DDT carcinogenicity. DDT is known to bind to the CAR, which may mediate cancer effects. Also, after initiation with a nitrosamine, DDT can induce the formation of preneoplastic liver lesions. Thus, there is a strong possibility that DDT promotes the progression of cancer in rodents. For some tumour types (e.g. breast and testes), effects of DDT/DDE on hormonal receptors may be of relevance.

Overall, the human studies for DDT/DDE and thyroid hormones are inconclusive.

Multigeneration studies on reproductive function in several mammalian species have generally not revealed effects on fertility, fecundity or pregnancy after exposure to DDT. However, effects on

spermatogenesis in the testes and on sperm count and motility have been observed in treated rats. In exposed human males, studies are inadequate to directly assess fertility and fecundity. Associations between exposure to DDT and abnormalities in sperm characteristics have been reported, particularly among men with recent or current DDT use and exposures. These studies do not convincingly demonstrate causality. They are possibly consistent with the ability of DDT and DDE to alter hormonal status via receptor binding or aromatase induction.

DDE is anti-androgenic and *o,p′*-DDT weakly estrogenic in vitro, and effects related to endocrine disruption (reduced anogenital distance, nipple retention, cryptorchidism, possibly hypospadias) have been reported in rodents and/or rabbits after high exposures. The expression of hormonal effects in experimental animals depends on exposure during particular critical phases, exposure levels and duration and the hormonal status of the animal. The human studies addressing hypospadias, anogenital distance and cryptorchidism are too limited for evaluation.

Treatment with DDT significantly reduced ovulation rate in the rabbit. In human females, associations between DDT exposure and menstrual cycle alterations have been described in studies of high quality. These changes are consistent with hormone-like properties of DDT. While one human study showed earlier age of menopause, an animal study (in rats) showed an older age of cessation of fertility.

Two cohort studies indicated a possible association between DDT and DDE levels and fetal loss in women in countries with recent usage of DDT. However, analogous results were generally not observed in the multigeneration animal studies.

The available human data support a possible association between exposure to DDT/DDE and reduced gestational age and increased rates of preterm birth.

Available evidence supports a possible association between prenatal or early life exposures to DDE and reduced childhood growth. They do not support an association between fetal growth restriction and DDT/DDE. None of the animal multigeneration studies reported decreased growth.

Studies from one laboratory provide evidence that DDT exposure to neonatal mice on PND 10 induces significant neurochemical and functional neurodevelopmental changes.

Studies in humans provide consistent evidence for perinatal exposure having neurocognitive effects in children, particularly for DDT.[1] Moreover, given the differences in timing of various developmental sequences in the mouse versus the human and the very long half-lives of DDT and DDE in humans compared with those in mice, the experimental animal and human data are consistent. There is no evidence that this effect is mediated by thyroid function.

11.2 Dose–response assessment

11.2.1 Methods used for dose–response assessment

As is noted above, experimental animal studies have the advantage of providing dose-dependent data with relatively few confounding effects, but a number of disadvantages with regards to the relevance of the data to humans and the extrapolation from external exposure to serum and fat levels of DDT and DDE. This section uses both experimental animal and human studies to provide reference levels for DDT/DDE toxicity. The same basic methods were used for both cancer and non-cancer assessments.

The preferred method for determining reference levels for various end-points is the BMD approach. BMD models are considered preferable to use of a NOAEL, for a number of reasons. The NOAEL is the highest dose or exposure at which no statistically or biologically significant adverse effects are identified. Limitations of use of the NOAEL include the fact that it does not reflect the shape of the dose–response curve, it does not account for study size and variability and it assumes that there is an exposure threshold level below which there is no effect, an assumption that is difficult to prove. However, there are also a number of uncertainties associated with the use of BMD models, including quantitative

[1] See evaluation of later published studies considered by the expert consultation on the risk characterization (Part A, section 2.2).

definition of a BMD and selection of appropriate dose–response models and statistical tools for calculating BMDs.

Modelling was done using the United States Environmental Protection Agency's BMD software, version 2.1.2.60. Because of uncertainties about appropriate models for dose–response relationships, all possible models were calculated. From the models that calculated a BMD and lower 95% confidence limit on the BMD (BMDL), the final BMD/BMDL were derived as the geometric mean of the different models (with allowance for multiple models giving identical results). However, studies with an Akaike's information criterion (AIC) 10 or more times larger than the lowest AIC obtained were excluded, as were (dichotomous) models in which the chi-squared *P*-value was less than 0.05.

Several criteria were used to select studies for modelling. First, studies needed to be of sufficient quality and relevance, as reviewed above in section 11.1. Second, a study had to have at least three dose groups (e.g. controls and two other doses). Third, studies with non-monotonic dose–response relationships were excluded from the modelling exercise. Finally, only studies for which all relevant input data could be identified, in either the study or related studies, were selected for BMD modelling. For studies and end-points not amenable for BMD analysis, the traditional approach was used to identify a NOAEL or a LOAEL.

For several end-points, many studies have been published, often with discordant results. As quantitative dose–response analysis could be performed only on studies that showed an association between DDT/DDE exposure and a particular end-point and provided quantitative data on the association, this selection is automatically biased towards positive results. This is very clear for, for example, studies on breast cancer, but also for those on liver cancer; there are many negative studies, but only the positive studies are analysed quantitatively.

The dose metrics used were dose rates/concentrations of DDT/DDE in feed, concentrations in serum and concentrations in serum lipids and/or adipose tissue. Studies that used other methods of exposure assessment (e.g. surrogate measures used in some occupational and ecological studies) were not modelled. The

modelling was performed after conversion of these dose metrics to concentrations of DDT/DDE in serum/adipose tissue as micrograms per gram lipid.

In the estimation of the lipid-based concentration figures, the average animal weights were assumed to be as follows: mice, 0.03 kg; hamsters, 0.12 kg; rats, 0.3 kg; rabbits, 2 kg; and Beagle dogs, 12 kg. The food consumption was assumed to be 130, 80 and 50 g/kg bw per day for mice, hamsters and rats, respectively (USEPA, 1988). The proportion of total body weight made up of body fat was assumed to be 0.09, 0.15, 0.16, 0.31 and 0.12 for mice, hamsters, rats, Beagle dogs and rabbits, respectively (Peckham et al., 1962; Romsos et al., 1976; Edozien & Switzer, 1978; West et al., 1992, 1995; Boozer et al., 1995; Fortun-Lamothe et al., 2002; Kim et al., 2002; Carroll et al., 2004; Jeusette et al., 2006). All data that were used for the BMD analyses are available in supplementary information available on the International Programme on Chemical Safety (IPCS) web site (http://www.who.int/ipcs).

For long-term animal studies for which no DDT/DDE concentrations in adipose tissue or serum were available, doses were converted to body burden levels using a pharmacokinetic model used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for a different set of persistent chemicals, dioxins, in foods, by applying the following equation (FAO/WHO, 2002):

$$\text{Body burden at steady state (mg/kg bw)} = \frac{f \times \text{intake (mg/kg bw per day)} \times \text{half-life (days)}}{\ln(2)}$$

where f is the fraction of DDT or DDE absorbed. The oral absorption of DDT was assumed to be 80% for rats and 50% for other species (see section 4). The same assumption was used for DDE. Absorption from intraperitoneal injection is assumed to be 100%. The half-lives used are given in Table 1 of section 4. Body burdens were converted to lipid concentrations by dividing total body burden by per cent of body weight as fat, as given above.

For animal studies with single or short-term dosing (and in which, therefore, the above equation is not valid) and lacking adipose tissue concentration measures, the estimated lipid concentrations for doses were calculated as follows:

$$\text{Lipid concentration (µg/g lipid)} = \frac{f \times \text{dose (mg/kg bw per day)} \times N}{F}$$

where f is the proportion absorbed, N is the number of doses and F is fat as a proportion of body weight. Apparently, this is a rough estimation and represents an upper bound for the adipose tissue concentration, as it assumes no disappearance of DDT/DDE from adipose tissue in this short (relative to the half-life) time period.

Studies for which this procedure was used were Krause (1977), Fabro et al. (1984), Eriksson et al. (1990a,b, 1992), Kelce et al. (1995), Ben Rhouma et al. (2001) and Veeramachaneni et al. (2007).

For both categorical and continuous end-points, all available models were applied for each end-point. Only models providing unique estimates of the BMD and BMDL are reported. For categorical end-points, the BMDs for the 10% responses (BMD_{10}) as well as the lower 95% bounds on the BMDs for the 10% responses (i.e. $BMDL_{10}$) are reported. For continuous end-points, a "one standard deviation" change was used as the basis for developing a BMD. Mathematically, a 1 SD change is close to a 10% response (Crump, 1995).

For human studies, the exposure groups were computed from ranges of exposure indicators as reported in the studies (e.g. tertiles, quartiles or quintiles of, for example, serum concentrations). Given that DDT and DDE are distributed in body tissues in a lognormal fashion, medians and geometric mean concentrations are the most appropriate measures of central tendency for distributions. Therefore, when data are presented in quintiles, the geometric mean of the lowest and highest value was used to compute a measure of central tendency for an interval. Where the lowest bound of the smallest interval was not in the published paper, the limit of detection divided by the square root of 2 was used.

For human studies, investigators often present both "crude" and "adjusted" measures of risk (e.g. RRs and ORs) or regression slopes. This hazard characterization used risk estimates and measurements derived from analyses that adjusted for potential confounders. Where investigators did not report SDs, it was often possible to back-calculate them from 95% CIs. For categorical end-points, numbers of cases were computed from adjusted ORs and baseline

disease rates. At times, continuous measures were also computed from results of multivariate models.

For human studies, when given in the original paper, serum concentrations of DDT and/or DDE by lipid weight of serum or adipose tissue were used as the exposure metric. When these were not available, data on wet weight in serum were converted to lipid weight concentrations by multiplying by a factor of 0.16 (see section 3).

There are a number of uncertainties associated with BMD modelling, and no single agreed-upon approach exists for how the modelling should be carried out. This is particularly the case for BMD analysis of epidemiological studies. In all cases, it is necessary to apply scientific judgement. For this assessment, BMD modelling has been performed for all studies for which suitable data could be identified. Results of modelling that were used in the risk assessment are tabulated below. Supplementary information relating to the BMD modelling conducted is available from the IPCS web site (http://www.who.int/ipcs).

An independent review of the BMD modelling presented in this assessment has indicated that alternative approaches for deriving the input data and carrying out the modelling could be valid. For this reason, the results of the studies have in most cases been presented separately from the BMD modelling results. It is acknowledged that alternative BMD modelling approaches are possible and could give different results, depending on the scientific judgements made during the modelling process.

11.2.2 Non-cancer effects

The results of the experimental animal studies for non-cancer end-points are presented along with the BMD modelling results in Table 14, as BMD analysis of this type of data is considered less contentious. The results of the epidemiological studies for non-cancer end-points are summarized in Table 15, and the proposed BMD values modelled from those epidemiological studies are presented in Table 16.

Table 14. Summary of NOAELs, LOAELs and dose–response analysis for non-cancer end-points from experimental studies used in risk characterization[a]

Effect	Species, strain, sex	Chemical	Dose levels (mg/kg bw per day)	DDT/DDE concentration (µg/g lipid)[b]	NOAEL (µg/g lipid)[b]	LOAEL (µg/g lipid)[b]	BMD_{10} (µg/g lipid)[b]	$BMDL_{10}$ (µg/g lipid)[b]	Models applied	Study source
Sperm count	Rat, M	DDT	Control	0	—	—	1100	850	Linear	Ben Rhouma et al. (2001)[c] [section 10.3.2.2]
			50	3125						
			100	6250						
Sperm motility	Rat, M	DDT	Control	0	—	—	2200	1600	Linear	Ben Rhouma et al. (2001)[c] [section 10.3.2.2]
			50	3125						
			100	6250						
Reduced anogenital distance	Rat, Long-Evans, M[d]	DDE	Control	0	—	—	1900	1000	Linear, power	You et al. (1998)[e] [section 10.3.2.5(b)]
			10	103						
			100	2400						

Effect	Species, strain, sex	Chemical	Dose levels (mg/kg bw per day)	DDT/DDE concentration (µg/g lipid)[b]	NOAEL (µg/g lipid)[b]	LOAEL (µg/g lipid)[b]	BMD_{10} (µg/g lipid)[b]	$BMDL_{10}$ (µg/g lipid)[b]	Models applied	Study source
Reduced anogenital distance	Rat, Sprague-Dawley, M[d]	DDE	Control 10 100	0 117 1830	—	—	2100	1200	Linear, power	You et al. (1998)[e] [section 10.3.2.5(b)]
Nipple retention	Rat, Long-Evans, M[d]	DDE	Control 10 100	0 103 2400	—	—	190	130	Linear, exponential	You et al. (1998)[e] [section 10.3.2.5(b)]
Nipple retention	Rat, Sprague-Dawley, M[d]	DDE	Control 10 100	0 117 1830	—	—	240	190	Linear, exponential	You et al. (1998)[e] [section 10.3.2.5(b)]

Effect	Species, strain, sex	Chemical	Dose levels (mg/kg bw per day)	DDT/DDE concentration (µg/g lipid)[b]	NOAEL (µg/g lipid)[b]	LOAEL (µg/g lipid)[b]	BMD_{10} (µg/g lipid)[b]	$BMDL_{10}$ (µg/g lipid)[b]	Models applied	Study source
Early puberty	Dog, F	DDT	Control	0	—	—	510	110	Power, linear	Ottoboni et al. (1977)[f] [section 10.3.2.1]
			1	12			(P gen.)	(P gen.)		
			5	58			140	82		
			10	116			(F_1 gen.)	(F_1 gen.)		
Prolonged reproductive viability	Rat, Sprague-Dawley, M/F	DDT	Control	0	—	68.5	—	—	Dose–response modelling not conducted	Ottoboni (1969)[f] [section 10.3.2.1]
			0.5	68.5						
			5	685.3						
Prolonged reproductive viability	Rat, Sprague-Dawley, F	DDT	Control	0	—	137	—	—	Dose–response modelling not conducted	Ottoboni (1972)[f] [section 10.3.2.1]
			1.0	137						

Effect	Species, strain, sex	Chemical	Dose levels (mg/kg bw per day)	DDT/DDE concentration (µg/g lipid)[b]	NOAEL (µg/g lipid)[b]	LOAEL (µg/g lipid)[b]	BMD_{10} (µg/g lipid)[b]	$BMDL_{10}$ (µg/g lipid)[b]	Models applied	Study source
Decreased ovulation rate	Rabbit, F	DDT	Control 3.0	0 450	—	450	—	—	Dose–response modelling not conducted	Lindenau et al. (1994)[c] [section 10.3.2.3]

F, female; gen., generation; M, male

[a] Only studies used in the risk characterization are presented.
[b] In adipose tissue.
[c] Calculated values; dose converted to upper bound of adipose tissue concentration using method for short-term exposure.
[d] Refers to male offspring of treated dams.
[e] Measured values in adipose tissue.
[f] Calculated values; dose converted to body burden and then adjusted to adipose tissue concentrations by proportion of body fat.

Table 15. Summary of results for non-cancer end-points from epidemiological studies used in risk characterization[a]

Effect	Population description	Chemical associated with effect	Mean concentrations[b]	Concentration categories[b]	Results	Analysis of results	Study source
Oligozoospermia	311 males Non-occupational South Africa	DDE	DDT: 90 ± 102 µg/g serum lipid DDE: 216 ± 211 µg/g serum lipid	Quartiles 0.02–43 44–132 133–345 346–997 (µg/g serum lipid)	Incidence 14% 17.5% 21.5% 30.0%	Significant positive association with DDE level	Aneck-Hahn et al. (2007) [section 10.3.3.1.1]
Asthenozoospermia	311 males Non-occupational South Africa	DDE	DDT: 90 ± 102 µg/g serum lipid DDE: 215 ± 211 µg/g serum lipid	Quartiles 0.02–43 44–132 133–345 346–997 (µg/g serum lipid)	Incidence 15.0% 21.5% 23.0% 31.0%	Significant positive association with DDE level	Aneck-Hahn et al. (2007) [section 10.3.3.1.1]

Effect	Population description	Chemical associated with effect	Mean concentrations[b]	Concentration categories[b]	Results	Analysis of results	Study source
Asthenozoospermia	116 males Non-occupational Mexico	DDE	DDE: 45 ± 31 µg/g plasma lipid	Results not presented by category Median = 41 µg/g plasma lipid	Sperm motion parameters decreased with higher DDE concentrations	Significant positive association with DDE level	De Jager et al. (2006) [section 10.3.3.1(a)]
Sperm tail abnormalities	116 males Non-occupational Mexico	DDE	DDE: 45 ± 31 µg/g plasma lipid	Results not presented by category Median = 41 µg/g plasma lipid	% of sperm with morphological tail defects increased with higher DDE concentrations	Significant positive association with DDE level	De Jager et al. (2006) [section 10.3.3.1(a)]

Effect	Population description	Chemical associated with effect	Mean concentrations[b]	Concentration categories[b]	Results	Analysis of results	Study source
Earlier menopause	219 females Non-occupational USA (Hispanic)	DDT	DDT: 4.24 µg/l serum	3 intervals[c] < 0.32 0.32–0.55 > 0.55 5 intervals[d] < 2.00 2–2.99 3–3.99 4–5.99 > 6.00 (µg/l serum)	Difference (referent − exposed) referent category 1.04 years 2.76 years referent category 1.14 years 0.27 years 2.11 years 5.65 years	Significant decrease in mean age at menopause for the highest exposure categories	Akkina et al. (2004) [section 10.3.3.2(d)]

Effect	Population description	Chemical associated with effect	Mean concentrations[b]	Concentration categories[b]	Results	Analysis of results	Study source
Spontaneous abortion	388 females Non-occupational China	DDT	Total DDT[e]: 5.52–113.3 µg/l serum (range)	5.5–22.9 23.0–36.5 36.6–113.3 (µg/l serum)	Adjusted OR (95% CI) 1.00 (referent) 1.23 (0.72–2.10) 2.12 (1.26–3.57)	Higher total DDT associated with early pregnancy loss	Venners et al. (2005) [section 10.3.3.3(a)]
Preterm birth (< 37 weeks)	361 females (preterm births) Non-occupational USA	DDE	DDE: 25 µg/l serum (median)	Quintiles < 15 15–29 30–44 45–59 60–178 (µg/l serum[e])	Adjusted OR (95% CI) 1.00 (referent) 1.5 (1.0–2.3) 1.6 (1.0–2.6) 2.5 (1.5–4.2) 3.1 (1.8–5.4)	Maternal DDE concentration associated with increased odds of preterm birth	Longnecker et al. (2001) [section 10.3.3.3(b)]

Effect	Population description	Chemical associated with effect	Mean concentrations[b]	Concentration categories[b]	Results	Analysis of results	Study source
Early menarche	466 females Non-occupational China	DDT + DDE	Total DDT[f]: 32 ± 17.8 µg/l serum	Quartiles 5.5–19.2 19.2–27.8 27.8–41.2 41.2–113.3 (µg/l serum[f])	Adjusted β[g] (95% CI) Referent −0.55 (−0.91 to −0.18) −0.40 (−0.78 to −0.03) −1.11 (−1.50 to −0.72)	Adjusted mean age at menarche was younger in those in the 4th quartile	Ouyang et al. (2005) [section 10.3.3.3(e)]
Anogenital distance	781 males Infants Mexico	DDE	DDE: 2.7 µg/g serum lipid (median); 0.1–56.1 µg/g serum lipid (range)	—	No association of DDE with anogenital distance	No association with DDE level	Longnecker et al. (2007) [section 10.3.3.3(h)]
Anal position index	37 males Infants Mexico	DDE	DDE: 0.21–5.89 µg/g serum lipid (10th to 90th percentile)	—	Doubling of maternal 1st trimester DDE was associated with a significantly reduced anal position index	Significant positive association with DDE level	Torres-Sánchez et al. (2008) [section 10.3.3.3(h)]

[a] Only studies used in the risk characterization are presented.

[b] µg/l serum refers to µg/l of fresh weight serum.
[c] Defined as 1) below the limit of detection, 2) above the limit of detection but below the median value and 3) above the median value; µg/l serum.
[d] Integer categorization; µg/l serum.
[e] Subsequent dose–response modelling based on geometric means adjusted to µg/g lipid using conversion factor.
[f] Total DDT = *p*,*p*′-DDT + *p*,*p*′-DDE + *p*,*p*′-DDD + *o*,*p*′-DDT + *o*,*p*′-DDE; subsequent dose–response modelling based on geometric means adjusted to µg/g lipid using conversion factor.
[g] Association of total DDT with age at menarche, adjusted for birth year, BMI.

Table 16. Summary of dose–response analysis for non-cancer end-points from epidemiological studies[a]

Effect	Chemical associated with effect	Mean concentration data	BMD_{10} (µg/g serum lipid)	$BMDL_{10}$ (µg/g serum lipid)	Models applied	Study source
Oligozoospermia	DDE	DDT: 90 ± 102 µg/g serum lipid DDE: 216 ± 211 µg/g serum lipid	300	170	All eight[b]	Aneck-Hahn et al. (2007) [section 10.3.3.1(a)]
Asthenozoospermia	DDE	DDT: 90 ± 102 µg/g serum lipid DDE: 216 ± 211 µg/g serum lipid	290	170	All eight[b]	Aneck-Hahn et al. (2007) [section 10.3.3.1(a)]
Asthenozoospermia	DDE	DDE: 45 ± 31 µg/g plasma lipid	150	80	[c]	De Jager et al. (2006) [section 10.3.3.1(a)]
Sperm tail abnormalities	DDE	DDE: 45 ± 32 µg/g plasma lipid	140	76	[c]	De Jager et al. (2006) [section 10.3.3.1(a)]
Earlier menopause	DDT	DDT[d]: 4.24 µg/l serum	1.2	0.5	Linear, power, polynomial, Hill	Akkina et al. (2004) [section 10.3.3.2(d)]

Effect	Chemical associated with effect	Mean concentration data	BMD_{10} (µg/g serum lipid)	$BMDL_{10}$ (µg/g serum lipid)	Models applied	Study source
Spontaneous abortion	DDT	Total DDT[d]: 5.52–113.3 µg/l serum (range)	4.3	1.5	All eight[b]	Venners et al. (2005) [section 10.3.3.3(a)]
Preterm birth (< 37 weeks)	DDE	DDE[d]: 25 µg/l serum (median)	9.1	4.7	All eight[b]	Longnecker et al. (2001) [section 10.3.3.3(b)]
Early menarche	DDT + DDE	Total DDT[e]: 32 ± 17.8 µg/l serum	7.5	5.2	Linear, Hill, power	Ouyang et al. (2005) [section 10.3.3.3(e)]

[a] Only studies used in the risk characterization are presented.
[b] All eight = gamma, logistic, loglogistic, probit, logprobit, multistage, Weibull and quantal-linear.
[c] Calculated directly from the slope of the regression line response, not calculated using BMD software version 2.1.2.60.
[d] Subsequent dose–response modelling based on geometric means adjusted to µg/g lipid using conversion factor.
[e] Total DDT = *p*,*p*′-DDT + *p*,*p*′-DDE + *p*,*p*′-DDD + *o*,*p*′-DDT + *o*,*p*′-DDE; subsequent dose–response modelling based on geometric means adjusted to µg/g lipid using conversion factor.

11.2.2.1 Experimental animal studies

(a) Hepatotoxicity

Neither the Laug et al. (1950) nor the Fitzhugh & Nelson (1947) paper contains data in sufficient detail to permit BMD modelling. Dietary levels in the Fitzhugh & Nelson (1947) study were much higher than those in the Laug et al. (1950) study, so the Laug et al. (1950) study was used to derive a NOAEL for hepatocellular changes in rats, at 0.05 mg/kg bw per day. At this dose level, the concentration of DDT in the adipose tissue of the rats at 19–27 weeks was 17–33 µg/g (geometric mean concentration 25 µg/g) in males and 33–38 µg/g in females, analysed using a colorimetric method that was stated to measure the *p*,*p*′- and *o*,*p*′-isomers of DDT separately. The authors summed the results for the two isomers to provide the values cited (Laug et al., 1950).

(b) Neurotoxicity

The multigeneration mouse cancer study by Turusov et al. (1973) reported on serious neurological end-points that occurred at DDT dose levels of approximately 6.5 mg/kg bw per day and above. The NOAEL was 1.3 mg/kg bw per day, corresponding to an estimated lipid concentration of 1700 µg/g.

(c) Reproductive toxicity and hormonal alterations

A number of experimental animal studies assessed reproductive toxicity, including effects on hormone levels. The Ben Rhouma et al. (2001) study was used as a basis for BMD modelling of sperm counts, an important measure of male fertility. The BMD_{10} and $BMDL_{10}$ (i.e. the BMD and BMDL for a 10% response) for a 1 SD decrease in sperm counts corresponded to estimated adipose DDT concentrations of 1100 and 850 µg/g, respectively. BMDs for sperm motility were approximately 2 times higher.

The study by You et al. (1998) presents two end-points, reduced anogenital distance and nipple retention, for two rat strains, Long-Evans and Sprague-Dawley, that were amenable to a BMD approach for modelling the toxicity of DDE on the male reproductive system via estimation of numbers from a figure in the publication. For anogenital distance, the Long-Evans rat was

somewhat more sensitive than the Sprague-Dawley rat, with a BMD_{10} and $BMDL_{10}$ corresponding to measured adipose levels of 1900 and 1000 µg/g, respectively. The Long-Evans rats were also more sensitive for nipple retention; the BMD_{10} and $BMDL_{10}$ were 190 and 130 µg/g, respectively. In further investigations with the same rats (You et al., 1999a), various CYP enzymes and testosterone metabolites were investigated in response to dosing with DDE. For the Sprague-Dawley rat, the BMD_{10} and $BMDL_{10}$ for the level of hepatic testosterone-16α-hydroxylase activity corresponded to measured adipose tissue concentrations of 380 and 250 µg/g, respectively.

Other studies (Kelce et al., 1995; Krause, 1977) also identified LOAELs (but not NOAELs) for hormonal effects: Krause (1977) for decreased serum testosterone for DDT at 100 mg/kg bw per day and Kelce et al. (1995) for various anti-androgen effects for DDE at 100 mg/kg bw per day, corresponding to estimated adipose concentrations of 4500 µg/g for Krause (1977) and 2500 µg/g for Kelce et al. (1995).

(d) Cryptorchidism

The Veeramachaneni et al. (2007) rabbit study has one endpoint, cryptorchidism, that is amenable to BMD modelling; the BMD_{10} and $BMDL_{10}$ were 5000 and 2900 µg/g lipid, respectively.

(e) Puberty

The multigeneration study by Ottoboni et al. (1977) on puberty in Beagle dogs provides dose–response data for DDT and early puberty that can be modelled using a BMD approach. For lowering the age of puberty, the BMD_{10} and its lower bound ($BMDL_{10}$) were 510 and 110 µg/g, respectively.

(f) Fetal growth

Fabro et al. (1984) identified a LOAEL for DDT of 1 mg/kg bw per day for reduced fetal growth in rabbits and did not identify a NOAEL. This dosing corresponds to an estimated concentration of DDT in lipid of 17 µg/g.

(g) Developmental neurotoxicity

The series of studies by Eriksson and colleagues (1990a,b, 1992) using a single dose of DDT at 0.5 mg/kg bw identified both behavioural and neurochemical evidence for neurotoxicity. This LOAEL corresponds to an estimated concentration of DDT in lipid of 5.6 µg/g.

11.2.2.2 Human studies

(a) Hepatic toxicity

The Kreiss et al. (1981) study presented the relationship between DDT and elevated GGT as a regression slope, and it was not possible to carry out modelling given the data provided in this paper. However, it may be estimated that for an 89% change in the DDT concentration, there was a 10% increase in the serum GGT level within the exposure range studied (Kreiss et al., 1981).

(b) Sperm quality: adult exposures

Two studies (De Jager et al., 2006; Aneck-Hahn et al., 2007) in high-exposure areas had significant results. The Aneck-Hahn et al. (2007) study presented adjusted percentages of men with oligo-zoospermia and asthenozoospermia by quartile of DDE level (serum level lipid adjusted); the percentages were extrapolated from a figure, the excess number of cases was calculated and dichotomous BMD models were constructed (Table 16). The lower 95th percentiles of the BMD_{10} for both end-points are 170 µg/g serum lipid DDE. The De Jager et al. (2006) paper did not present results by categories of exposure. In the De Jager et al. (2006) study, a 1 SD decrease in sperm motility (15.3) was associated with a BMD of 150 µg/g serum lipid DDE and a BMDL of 80 µg/g serum lipid DDE. There was no difference in sperm counts, but a 1 SD increase in sperm with abnormal tail morphology had a BMD and BMDL of 140 and 76 µg/g serum lipid DDE, respectively.

(c) Menstrual cycle function

Several studies (Ouyang et al., 2005; Windham et al., 2005; Perry et al., 2006) showed positive responses for menstrual cycle function. For Ouyang et al. (2005), the finding of shortened

menstrual cycles could be fit to BMD models, as could the data from Windham et al. (2005) on shorter luteal phase for menstrual cycles. The Ouyang et al. (2005) paper reported its DDT results as total DDT; a separate analysis for *p*,*p*'-DDE was conducted but not reported, as the findings were stated to be very similar. Windham et al. (2005) presented results for DDT and DDE separately. For shorter menstrual cycles, the Ouyang et al. (2005) study $BMDL_{10}$ estimate was 5.3 µg/g serum lipid. For the Windham et al. (2005) study, the $BMDL_{10}$s for a 1 SD change in the luteal phase were 10 µg/g serum lipid for DDE and 0.9 µg/g serum lipid for DDT. The Perry et al. (2006) results were presented as regressions of DDT and DDT on the log of urinary PdG and E1C concentrations; SDs are not presented for PdG and E1C in the publication, and therefore BMDs could not be computed.

(d) Menopause

One large study (Akkina et al., 2004) suggests that DDT exposures are associated with earlier age of menopause. Results were presented in terms of three (below the limit of detection, above the limit of detection but below the median value and above the median value) and five (integer categorization = < 2, 2–2.99, 3–3.99, 4–5.99 and ≥ 6 µg/l) intervals of DDT exposure, and continuous BMD models were applied using the mean and SEM ages of women in the three/five DDT exposure groups. Only the Hill and power models were able to calculate a BMD (1.2 µg/g serum lipid); Hill, linear and power models gave a $BMDL_{10}$ of 0.8 µg/g, whereas the polynomial model indicated a $BMDL_{10}$ of 0.14 µg/g serum lipid.

(e) Fetal loss/spontaneous abortion

Several studies showed a relationship between DDT/DDE and spontaneous abortion, and two of them (Longnecker et al., 2005; Venners et al., 2005) gave ORs across categories of DDT/DDE exposure. The Longnecker et al. (2005) data, as presented categorically, did not follow a monotonic dose–response relationship and, as presented as a linear relationship, did not give an SD for the outcome variable (rate of fetal losses), so no BMDs were calculated for this study. In the Venners et al. (2005) study, BMD_{10} and $BMDL_{10}$ estimates were tightly clustered around a range between 1.5 and 5.5 µg/g serum lipid.

(f) Preterm birth

Longnecker et al. (2001) found an association between serum DDE and preterm birth (< 37 weeks' gestation). The $BMDL_{10}$ for increased incidence of preterm birth was estimated to be 4.7 μg/g serum lipid (the BMD_{10} was 9.1 μg/g).

(g) Menarche

One study (Ouyang et al., 2005) reported a linear dose–response model that was used to calculate a BMD and BMDL on the basis of a 1 SD decrease in age of menarche; these were 7.5 and 5.2 μg/g serum lipid, respectively.

(h) Fetal exposure and time to pregnancy

One study (Cohn et al., 2003) was evaluated. BMDs could not be computed because data were presented as linear regression slopes; the SD for the outcome (time to pregnancy) was not reported.

(i) Fetal exposure/growth

Two studies were evaluated for possible modelling. For Ribas-Fito et al. (2006a), the dose–response relationship was non-monotonic (for the nine data points for three low DDT exposure groups and three age groups, six showed a faster growth than the controls; only at the highest DDT exposure level was there a consistent decrease in growth with age), and BMD models could not be fit. For Karmaus et al. (2002), SDs for growth measures were not reported, and thus it was not possible to carry out BMD modelling.

(j) Developmental neurotoxicity

For Ribas-Fito et al. (2006b), the results for boys were non-monotonic dose–response relationships (improvement of performance at the low exposure level, marked decrease at the intermediate exposure level and a less marked depression at the high exposure level, none of the differences from the referents being significant), and models could not be fit. For girls, the power and linear BMD models could be fit for the memory score; the BMD_{10} and $BMDL_{10}$ for a lower McCarthy Memory Subscale among girls

age 4 were 0.29 and 0.14 µg/g serum lipid, respectively (both extrapolated outside the existing data). Eskenazi et al. (2006) looked at DDE and performance by infants and toddlers on the Bayley test subscores at 6, 12 and 24 months. The relationship between DDT and Bayley test scores was per $\log_{10}$ unit of DDT, and thus it was not possible to do a linear extrapolation of the BMDs. On the other hand, Torres-Sánchez et al. (2007) used a different technique for modelling DDE concentrations at several time points as well as Bayley subscore results at several intervals. Maternal DDE concentrations during the first trimester were associated with a decreased Psychomotor Developmental Index score overall over testing at 3, 6 and 12 months, with the BMD and BMDL equivalent to about 2.5 and 1.3 µg/g serum lipid, respectively.

11.2.3 Carcinogenicity

The results of the experimental animal carcinogenicity studies and the cancer epidemiological studies are summarized in Tables 17 and 18. The results of dose–response modelling from these cancer studies are presented in Tables 19 and 20.

11.2.3.1 Experimental animal studies

For DDT, there were a number of studies on mouse hepatomas and rat hepatomas that were usable (Terracini et al., 1973; Turusov et al., 1973; Cabral et al., 1982a). Mice formed hepatomas at lower dosage rates of DDT; however, once half-lives were accounted for in adipose tissue concentrations, BMDs were higher for mice than for rats. The BMD_{10} was 1600 µg/g in female rats (no significant increase was observed in male rats) and greater than 10 000 µg/g in mice (Table 19). The NCI study provided data on thyroid follicular carcinoma in rats that could be modelled; the BMD_{10} and $BMDL_{10}$ were 2000 and 1300 µg/g lipid, respectively.

For DDE, there were two studies that provided usable data: the NCI (1978) study, which includes results for hepatocellular cancer in mice and thyroid tumours in rats, and the Rossi et al. (1983) study on hepatoma in hamsters. For the hepatocellular cancers, mice recorded positive findings at lower dosage rates of DDE than hamsters, but once half-lives were accounted for in adipose tissue

Table 17. Summary of cancer results from experimental animal studies selected for dose–response assessment[a]

Chemical	Species, strain, sex	Dietary levels (mg/kg)	Doses (mg/kg bw per day)	Tumour	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	NOAEL (µg/g)[b]	LOAEL (µg/g)[b]	Study
DDT	Mouse BALB/c Male	0, 2, 20, 250	0, 0.26, 2.6, 33	Hepatoma	2.6	33	3438	42 980	Terracini et al. (1973) [multigeneration]
	Mouse BALB/c Female	0, 2, 20, 250	0, 0.26, 2.6, 33	Hepatoma	2.6	33	3438	42 980	Terracini et al. (1973) [multigeneration]
	Mouse CF-1 Male	0, 2, 10, 50, 250	0, 0.26, 1.3, 6.5, 33	Malignant hepatic tumours	0.26	1.3 (significant at 33)	344	1 719 (significant at 42 980)	Turusov et al. (1973) [multigeneration]
				Hepatoma	—	0.26	—	344	
	Mouse CF-1 Female	0, 2, 10, 50, 250	0, 0.26, 1.3, 6.5, 33	Hepatoma	0.26	1.3 (significant at 6.5)	344	1 719 (significant at 8 596)	Turusov et al. (1973) [multigeneration]

Chemical	Species, strain, sex	Dietary levels (mg/kg)	Doses (mg/kg bw per day)	Tumour	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	NOAEL (µg/g)[b]	LOAEL (µg/g)[b]	Study
	Rat Porton Female	0, 125, 250, 500	0, 6, 12, 24	Hepatoma	—	6	—	822	Cabral et al. (1982a)
DDE	Mouse B6C3F1 Male	0, 148, 261	0, 19, 34	Hepatocellular carcinoma	—	19	—	25 444	NCI (1978)
	Mouse B6C3F1 Female	0, 148, 261	0, 19, 34	Hepatocellular carcinoma	—	19	—	25 444	NCI (1978)
	Hamster Syrian Male	0, 500, 1000	0, 40, 80	Hepatoma	—	40	—	3 847	Rossi et al. (1983)

Chemical	Species, strain, sex	Dietary levels (mg/kg)	Doses (mg/kg bw per day)	Tumour	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	NOAEL (µg/g)[b]	LOAEL (µg/g)[b]	Study
	Hamster Syrian Female	0, 500, 1000	0, 40, 80	Hepatoma	—	40	—	3 847	Rossi et al. (1983)

[a] Only studies used in the risk characterization are presented.
[b] Calculated values; dose converted to body burden and then adjusted to adipose tissue concentrations by proportion of body fat.

Table 18. Summary of cancer results from epidemiological studies selected for dose–response assessment[a]

Chemical	Sex	End-point	Internal doses (not lipid adjusted)	Internal doses (lipid adjusted) (μg/g lipid)	Result	Study
DDT	Male/female	Liver cancer	—	Quintiles	OR (95% CI)	McGlynn et al. (2006)
				< 0.265	1.0	
				0.265–0.382	1.3 (0.7–2.5)	
				0.383–0.521	1.4 (0.7–2.6)	
				0.522–0.787	1.4 (0.7–2.7)	
				>0.787	2.0 (1.1–3.9)	
DDT (adjusted for DDE levels)	Male/female	Liver cancer	—	Quintiles	OR (95% CI)	McGlynn et al. (2006)
				< 0.265	1.0	
				0.265–0.382	1.5 (0.8–2.7)	
				0.383–0.521	1.7 (0.9–3.3)	
				0.522–0.787	2.1 (1.0–4.3)	
				> 0.787	3.8 (1.7–8.6)	

[a] Only studies used in the risk characterization are presented.

Table 19. Cancer benchmark dose analyses from experimental animal studies[a]

Chemical	Species	Sex	Tumour	BMD_{10} (µg/g lipid)[b]	$BMDL_{10}$ (µg/g lipid)[b]	Slope factor	Study
DDT	Mouse	Male	Hepatoma	17 100	9 960	10.3×10^{-6}	Terracini et al. (1973)
	Mouse	Female	Hepatoma	14 800	10 800	9.70×10^{-6}	Terracini et al. (1973)
	Mouse	Male	Malignant hepatic tumours	96 200	55 200	1.94×10^{-6}	Turusov et al. (1973)
	Mouse	Female	Hepatoma	10 100	8 260	16.2×10^{-6}	Turusov et al. (1973)
	Rat	Female	Hepatoma	1 590	1 190	103×10^{-6}	Cabral et al. (1982a)
DDE	Mouse	Male	Hepatocellular carcinoma	18 000	6 000	11.4×10^{-6}	NCI (1978)
	Mouse	Female	Hepatocellular carcinoma	9 400	4 400	27.5×10^{-6}	NCI (1978)
	Hamster	Male	Hepatoma	1 370	910	109×10^{-6}	Rossi et al. (1983)
	Hamster	Female	Hepatoma	3 550	2 300	55.1×10^{-6}	Rossi et al. (1983)

[a] Only studies used in the risk characterization are presented.

[b] Geometric means of modelling results from dichotomous models (gamma, logistic, log-logistic, multistage-cancer, probit, log-probit, Weibull and quantal-linear) are presented. BMD and BMDL are in units of µg/g lipid (adipose tissue), as calculated from body burden adjusted by proportion of body fat.

Table 20. Cancer benchmark dose analyses from epidemiological studies[a]

Chemical	Sex	End-point	Internal doses (not lipid adjusted)	Internal doses (lipid adjusted) (µg/g lipid)	Result	$BMD_{0.1}$	$BMDL_{0.1}$	Slope factor	Study
DDT	Male/female	Liver cancer	—	Quintiles	OR (95% CI)	[b]	[b]	3.6×10^{-6}	McGlynn et al. (2006)
				< 0.265	1.0				
				0.265–0.382	1.3 (0.7–2.5)				
				0.383–0.521	1.4 (0.7–2.6)				
				0.522–0.787	1.4 (0.7–2.7)				
				> 0.787	2.0 (1.1–3.9)				
DDT (adjusted for DDE levels)	Male/female	Liver cancer	—	Quintiles	OR (95% CI)	[b]	[b]	3.0×10^{-6}	McGlynn et al. (2006)
				< 0.265	1.0				
				0.265–0.382	1.5 (0.8–2.7)				
				0.383–0.521	1.7 (0.9–3.3)				
				0.522–0.787	2.1 (1.0–4.3)				
				> 0.787	3.8 (1.7–8.6)				

[a] Only studies used in the risk characterization are presented.
[b] Slope factors were selected for use in the risk characterization.

concentrations, BMDs were higher for mice than for hamsters. The BMD_{10} and its lower bound ($BMDL_{10}$) in hamsters were 1400 and 900 µg/g lipid for males and 3600 and 2300 µg/g lipid for females, respectively. A good fit was obtained for combined thyroid carcinomas and adenomas in female rats (NCI, 1978); the BMD_{10} and $BMDL_{10}$ were 1900 and 1100 µg/g lipid, respectively. For malignant thyroid tumours, the BMDs for DDE were higher in male rats than in females. The thyroid tumour findings in rats were not identified as positive in the NCI (1978) study reports.

11.2.3.2 Human studies

The Cohn et al. (2007) study presented breast cancer rates by category of earlier exposure to DDT. To calculate BMDs, the number of cases was estimated from the adjusted ORs, and data on prevalence of breast cancer in the Child Health and Development Studies cohort were obtained from an earlier paper by Cohn et al. (2001). Multistage cancer and Weibull models were examined and found to be similar, with a BMD_{10} for DDT of 2.7 µg/g serum lipid. It is acknowledged that there are a number of uncertainties around the input data for modelling from this study.

The testicular cancer results from the McGlynn et al. (2008) study could not be modelled, as the population at risk could not be calculated.

The McGlynn et al. (2006) liver cancer paper was used to construct dose–response models using both the multistage and Weibull techniques, using both DDT and DDT adjusted with DDE. The adjustment of DDT levels with DDE was justified given that this cohort includes people with both active exposures to DDT and people who are exposed only via more remote sources (e.g. dietary sources from past use) and thus includes people with non-homogeneous DDT to DDE ratios. The adjustment removes a source of non-homogeneity. Liver cancer is uncommon enough in the McGlynn et al. (2006) cohort that in order to construct a BMD_{10} (normally used in this text), it would have been necessary to extrapolate upward beyond the range of the data. In order to stay within the bounds in which the model could provide BMD estimates at or below the range of the data in the study, the McGlynn et al. (2006) study was assessed at the 0.1% benchmark response level.

Table 20 shows the slope factors that were derived from this study and used in the risk characterization.

12. EXPOSURE ASSESSMENT

12.1 Introduction

Current exposure to DDT is largely through food in developed countries and in developing countries where the insecticide is no longer used in agriculture or public health. Residues in adipose tissue (the major storage site of DDT metabolites) have been falling together with concentrations in blood serum. In countries still using DDT for IRS, concentrations remain stable or, in some cases, are rising.

Remaining use of DDT globally is restricted to public health application, mainly for control of endophilic vectors of malaria (mosquitoes carrying malarial parasites) and leishmaniasis (phlebotomine sand flies carrying protozoan parasites that cause leishmaniasis) through IRS; DDT use is not permitted for use against any other disease vectors. IRS involves application of pesticides to house walls as a preventive measure to kill mosquitoes alighting on indoor surfaces, mainly after feeding. Published guidance exists (Najera & Zaim, 2001, 2002; WHO, 2007b) describing the criteria that should be met to trigger the use of DDT in IRS. DDT also has a spatial repellent effect, preventing mosquitoes from entering sprayed dwellings. DDT is used for this purpose in houses with mud walls. The pesticide is also sprayed outside the houses under eaves and sometimes pens close to the houses.

This review concentrates on human exposure via IRS but puts this into the context of residues of both DDT and its metabolites in people exposed via previous agricultural and public health use of the insecticide. Commonly, DDT exposure is expressed as total DDT (the sum of various isomers of DDT and its major metabolites DDE and DDD). "Total DDT" is not, therefore, a consistent and absolute measure; studies simply add all of the components measured in their particular analytical method, and these components vary from study to study.

DDT use in malaria control is restricted to IRS. Other pesticides are recommended for use by WHO in other aspects of mosquito control (Najera & Zaim, 2002), such as use as a larvicide in shallow water bodies where mosquitoes might breed (largely in urban situations or over rice paddies rurally), for outdoor space spraying (e.g. in temporary camps for displaced populations) and in treatment

of bednets. Exposure is examined for those applying DDT for IRS, those living in treated dwellings, the general population not directly involved in IRS, infants who are exposed via breast milk and the fetus.

The literature has been searched up to November 2009. Since many of the studies on DDT are old, standard search methods failed to find all of them. All papers identified were used as a further search source; their reference lists completed the set of studies eventually used. Comprehensive coverage of studies on DDT use in IRS was attempted; other studies cited are illustrative. Some older studies from the use of DDT in agriculture, relevant to the areas where human epidemiological studies identified as key in the hazard assessment had been conducted, were included to put current public health use in context. However, much of the older literature used measures of DDT not consistent with current practice. Included papers were therefore restricted to those with measures comparable to those used in modern studies. Studies that included time series were used preferentially to illustrate trends over time in countries that had stopped all use of DDT for either agriculture or public health. All studies summarized used analysis by gas chromatography; the sensitivity of the analytical methods has increased over time.

12.2 DDT application by spray operators

IRS with DDT is almost always carried out using 75% wettable powder (WP). The 75% WP products have dominated IRS use for at least the last 20–30 years and probably since the 1960s (G. Matthews, personal communication, 2009). The application rate is 2 g/m^2 (India can be an exception, with 1 or 2 g/m^2 used). A specification covering the quality of DDT to be used is available (WHO, 2009). A manual covering application of residual sprays for vector control has also been published (WHO, 2007).

Most of the IRS operations have been organized by ministries of health on a national scale or at least to cover significant areas of a country where malaria transmission is a major problem. Spraying operations have been carried out by teams of labourers on a short-term basis, so their exposure will vary depending on the number of days on which they actually spray houses. Exposure will also

depend on the extent of training received, the maintenance of their equipment, their observance of the spraying technique and safety procedures and the number of houses treated per day (G. Matthews, personal communication, 2009).

The original recommendations for applying DDT with a compression sprayer meant that individual tank loads were applied with an 8002 nozzle operated over a range of pressures. In consequence, the initial spray was applied at 3.8 bar when the spray contained a high proportion of small droplets, which present an inhalation hazard. The decrease in tank pressure as spray continued resulted in changes in flow rate (dosage) and droplet size, so the operator was advised to repump the sprayer when pressure had decreased to 1.7 bar. Thus, while spraying some houses, the operator might have been exposed to more than one period with sprays applied at the high pressure (G. Matthews, personal communication, 2009).

Various attempts to add a control flow valve (CFV) were initially unsuccessful in the field, but recent development of a suitable valve has enabled WHO to recommend its use. The current recommendation is to use a flat fan nozzle with an 80 degree swathe and output of 550 ml/min at 1.5 bar pressure or 650 ml/min at 2 bar (WHO, 2007a). The spray equipment should be fitted with a CFV, which gives a constant output at the nozzle until the tank pressure is below the stated pressure of the CFV; when pressure falls below this, the spraying will stop, and the operator must repressurize the tank.

The tank mix for a 75% WP formulation is 712 g of the formulated product in 8 litres of suspension, giving an application rate of 30 ml/m^2 (with a CFV set at 1.5 bar) and coverage of 267 m^2 (WHO, 2007a).

IRS operators, being indoors, should not be exposed to direct spray, but when they spray ceilings or other high areas, they would be exposed to any spray falling down, especially if they do not wear a hat or other personal protective equipment (PPE). Leakage of spray from the trigger valve or other sprayer components would also change the amount of exposure to spray liquid (G. Matthews, personal communication, 2009).

Wearing overalls limits direct dermal exposure of most of the body, but reducing inhalation of small droplets requires a proper respirator-type face mask, and gloves are needed to protect hands. Face masks are seldom kept adequately clean with the appropriate filters, so their use can be worse than not having a respirator. DDT is formulated as a 75% WP, which has a very fine particle size to facilitate dispersion in water. Sprays were usually mixed separately and not directly in the tank. The formulation could also produce a lot of foam when large quantities are mixed. The packaging of the DDT in soluble sachets for individual tank loads would reduce the inhalation hazard during preparation of a spray (WHO, 2007a; G. Matthews, personal communication, 2009).

Wearing a face mask or gloves all day is not practical under tropical conditions, so engineering controls are essential to limit exposure. The use of a CFV set at 1.5 bar pressure reduces sprayer exposure during application, and suitable formulation and packaging reduce exposure to the insecticide during preparation of the spray. The use of a lower pressure also allows a longer spraying period before having to repressurize the tank (G. Matthews, personal communication, 2009).

Unfortunately, there have been no published reports of detailed operational field studies of exposure of spray operators during IRS. A study by Wolfe et al. (1959) was carried out in a building quite different from an African house. Nevertheless, it indicated that operators were exposed to 1755 mg DDT per person per hour while spraying with an average tank pressure of about 2.8 bar. However, operator exposure was less at pressures of about 1.4–2 bar. Wolfe et al. (1959) also found that the average concentration of DDT in air was 7.1 mg/m^3. The respiratory exposure was calculated at 3.4 mg/h or 0.39 mg/kg bw per day (although pads in a respirator suggested double this value). While operators were spraying outside houses, the respiratory exposure was 0.11 mg/h. By adopting an improved application technique and ensuring that appropriate PPE is used, the level of exposure of spray operators should be significantly less than indicated in 1959.

12.3 Generic model for occupational and residential exposure

A generic model has been developed to estimate exposure of both operators and residents in houses treated under IRS (WHO, 2010). A worked example for DDT is presented in Annex 5. Estimates are summarized in Table 21.

Table 21. Annual estimated exposure of spray operators and residents following IRS with DDT

Scenario	Estimated exposure (mg a.i./kg bw per day)	
Workers		
During mixing and loading of the insecticide formulation (dermal exposure)	Safest scenario	0.00255
	Realistic scenario	0.0255
	Worst-case scenario	0.609
Dermal exposure during application of the insecticide and cleaning and maintenance of spray equipment	Safest scenario	0.0138
	Realistic scenario	0.138
	Worst-case scenario	0.325
Total for workers	Safest scenario	0.016
	Realistic scenario	0.16
	Worst-case scenario	0.93
Residents[a]		
Ingestion due to contaminated foodstuffs, breast milk or hand-to-mouth behaviour	Adults	0.022
	Children	0.028
	Toddlers	0.068
	Breastfed infants	0.0988
	Toddlers hand-to-mouth	0.021
Dermal exposure from touching contaminated surfaces	Adults	0.028
	Children	0.042
	Toddlers	0.27
Totals for residents	Adults	0.05
	Children	0.07
	Toddlers	0.359
	Breastfed infants[b]	0.0988

[a] Exposure for residents given in milligrams of active ingredient (a.i.) per kilogram of body weight per day.

[b] Estimated on median exposure in IRS studies.

Source: WHO (2010)

12.4 Occupational exposure

12.4.1 Adipose tissue

Rivero-Rodriguez et al. (1997) took abdominal adipose tissue biopsies from a sample of workers involved in spraying DDT for malaria control in the state of Veracruz, Mexico. This is the only study of biopsied fat in workers. The total workforce of 375 people was approached for involvement in the study; 371 responded positively. The age of the participants ranged from 20 to 70 years, with a mean of 45 years. Most of the workers (78%) had been involved in DDT spraying for between 20 and 29 years. In addition to DDT, other pesticides (temephos, fenthion and malathion) had also been used. A subsample of 40 workers was chosen for biopsy; the subsample was randomly selected from each of 11 health jurisdictions to ensure full coverage of the state and excluded those with hypertension or glycaemia. The remaining workers, together with those biopsied, were interviewed by trained personnel who completed a questionnaire covering various aspects of exposure. All workers were allocated an exposure score based on a table of seven categories, scored between 0 and 10, representing those carrying out tasks ranging from full-time spraying (score 10) to office-based microscopists (score 0). All exposed workers (i.e. all except office-based staff) rotated tasks and had spent some time in both high- and low-exposure situations; the office workers acted as controls. The allocation of scores was based on the detail of the answers given in the questionnaires. The distribution of workers' scores was not stated. Scores were then used to generate an index of exposure, which used time, exposure intensity (score) weighting and number of different tasks undertaken during their occupational history. Other questions covered recalled ingestion or spraying with the chemical(s), time spent in the sprayed area, consumption of food and drink during work, smoking habits, changes in body weight, consumption of alcohol, etc. Residues of total DDT, *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD were quantified in the adipose tissue. The geometric mean total DDT concentration, at 104.48 µg/g fat (range 10.56–665.56 µg/g fat), was made up of *p*,*p*′-DDT at 31.00 µg/g fat (range 0.72–344.98 µg/g fat) and *p*,*p*′-DDE at 60.98 µg/g fat (range 9.57–298.42 µg/g fat), with minor amounts of *o*,*p*′-DDT and *p*,*p*′-DDD; DDT and DDE levels were positively correlated, and DDE was therefore preferred for further analyses of the data.

The geometric mean concentration of *p,p'*-DDE tended to be higher for the following workers: those not using any PPE, those who had stayed several days in the workplace, those who had smoked, those who had consumed alcohol and those who had recently gained weight or not recently lost weight. However, none of these comparisons were statistically significantly different (based on log-transformed data). Further multivariate analysis showed that the log concentration of *p,p'*-DDE in adipose tissue correlated positively with the index of exposure units by 0.0014 per unit ($P < 0.001$), whereas use of PPE ($P = 0.013$) and recent weight loss ($P = 0.03$) reduced it; the three variables accounted for 55% of the total variation. Similar analysis based on *p,p'*-DDT concentrations showed that 45% of the variation could be explained by these three factors. The statistical analysis of the measured levels was used to predict *p,p'*-DDE concentrations for the rest of the worker population at 67.4 µg/g fat (range 9.56–298 µg/g fat), based on estimates of their exposure from the questionnaire, suggesting that the subsample was representative of the overall worker population. Uncertainties and limitations of the methodology were identified as follows: 1) only 55% of the variation was accounted for (suggesting that other variables should have been included), 2) workers had difficulty remembering details of their exposure over many years, 3) there were changes in the intensity of spraying over time and 4) there were changes in personal hygiene and use of PPE over time. Concentrations in the body fat of this occupationally exposed population were stated to be 6 times higher than those in the general population of the state of Veracruz (Rivero-Rodriguez et al., 1997).

12.4.2 Blood

Carvalho (1991) sampled eight groups of workers and controls in Bahia State, Brazil, between 1983 and 1985. Groups 1–3 were spray operators of HCH but not currently DDT; it is not stated if or when they had also sprayed DDT in the past. Group 4 was agricultural technicians in cocoa farming (not spraying). Group 5 worked next to areas where DDT was sprayed. Group 6 was IRS spray operators of DDT for controlling malarial vectors who had worked in the programme for 1 year or less. Group 7 was DDT spray operators who had worked for more than 5 years, and Group 8 was controls with no known exposure to pesticides. Groups 1–3

showed mean blood serum levels of DDE between 20 and 90 µg/l. Results for Groups 4–8 are given in Table 22.

Table 22. Blood serum concentrations of DDT and its metabolites in spray operators and controls in Bahia State, Brazil

Group	*N*	Mean concentration in blood serum (µg/l not adjusted for lipid)[a,b]				
		p,*p*′-DDE	*p*,*p*′-DDD	*o*,*p*′-DDT	*p*,*p*′-DDT	Total DDT
4. Technicians	10	16.0 ± 11.1	ND	ND	2.1 ± 4.7	18.1 ± 14.5
5. Local workers not directly exposed	19	18.0 ± 19.4	ND	ND	ND	18.0 ± 19.4
6. Spray operators for < 1 year	15	47.4 ± 29.1	2.5 ± 2.5	10.7 ± 7.4	51.9 ± 25.7	112.8 ± 52.0
7. Spray operators for 5+ years	14	344.4 ± 362.8	26.3 ± 66.8	41.1 ± 64.1	290.9 ± 166.7	702.7 ± 614.1
8. No known exposure	50	8.3 ± 9.0	ND	ND	6.0 ± 9.1	14.3 ± 7.1

ND, not detected

[a] The ± values are probably standard deviation; methodology of statistical analysis not stated.

[b] All measured isomers tabulated.

Source: Carvalho (1991)

Minelli & Ribeiro (1996) sampled blood from 26 workers employed to spray DDT to control disease vectors in houses in the city of São José do Rio Preto, São Paulo State, Brazil; 16 unexposed workers were used as controls. Ages ranged from 50 to 65 years (mean 56 years) in the exposed group and from 42 to 69 years (mean 54 years) in the controls. Working years in the exposed group ranged from 22 to 35 (mean 30); however, it is unclear whether these are total years working or years working on the vector control programme, as working years are also given for unexposed workers. *p*,*p*′-DDE, *o*,*p*′-DDT and *p*,*p*′-DDT were quantified in blood serum; *p*,*p*′-DDD was detected in 11 samples but could not be quantified. Total DDT was calculated. Results are given in Table 23.

Table 23. Concentrations of DDT and its metabolites in blood serum of vector control spray operators in Brazil[a]

	Concentration in blood serum (µg/l; not adjusted for lipid)			
	p,*p*′-DDE	*o*,*p*′-DDT	*p*,*p*′-DDT	Total DDT
Exposed workers				
Mean	64.3	1.8	11.7	77.5
Maximum	405.9	4.7	62.9	473.5
Minimum	5.9	0.7	1.6	7.5
Median	46.4	1.4	8.0	56.5
Geometric mean	44.9	1.5	7.8	54.9
Unexposed workers				
Mean	14.3	ND	1.65	15.8
Maximum	31.6	ND	2.5	32.9
Minimum	7.0	ND	1.0	5.1
Median	13.8	ND	1.6	15.6
Geometric mean	13.1	ND	1.6	14.6

ND, not detected

[a] Values calculated from the raw data tabulated in Minelli & Ribeiro (1996).

Source: Minelli & Ribeiro (1996)

Dores et al. (2003) analysed *p*,*p*′-DDE and *p*,*p*′-DDT in blood from 41 spray operators and 20 drivers occupationally exposed to DDT through IRS for malaria control; 13 office workers working on the programme were used as unexposed controls. The workers were considered to be a representative sample of 144 spray operators, 15 drivers and 15 office workers in Sinop District and 74 spray operators, 13 drivers and 8 office workers in Cáceres District involved in the programme in Mato Grosso State, western Brazil. The authors believed that the exposed workers had not used PPE, based on previous studies in Brazil. Samples were collected in 1999; DDT was used here for malaria control until 1997. Drivers did not conduct spraying but were also considered to be exposed because they loaded and unloaded pesticide and accompanied spray operators into the houses. DDT had been applied more intensively in the Sinop District in the period immediately before sampling. *p*,*p*′-DDE and *p*,*p*′-DDT were measured in blood serum, and total DDT was calculated. The distribution of concentrations was described using 25% (quartile 1) and 75% (quartile 3) percentiles of the spread of residues (Table 24). Mann-Whitney non-parametric

variance analysis showed a significant difference ($P < 0.05$) between exposed and non-exposed workers; there was no significant difference between IRS workers and the drivers. These trends were consistent between the two districts. There was no statistically significant correlation between blood serum pesticide concentration and age of the workers or with time spent in the programme. Comparison of concentrations between workers in the two districts showed no significant difference in either DDE or total DDT but a highly significant ($P = 0.001$) difference for *p,p′*-DDT, reflecting more intensive recent use in Sinop. Five individuals had total DDT blood serum concentrations above 500 µg/l. The authors emphasized that exposure had ceased at the time of sampling, so concentrations could have been higher during use of DDT.

Radomski et al. (1971) measured whole blood concentrations of *p,p′*-DDT and *p,p′*-DDE in pesticide workers in two provinces of Argentina. Workers either currently sprayed DDT and had been doing so for at least 5 years on the malaria control programme or had previously sprayed DDT in the same programme but had not done so for at least 5 years. Results are presented in Table 25.

Mazzarri & Mazzarri de Lauschner (1989) took blood samples from 289 IRS operators applying DDT in Venezuela in 1984. Sampling covered all states within the country. Results were split between two areas; one was free of malaria, but DDT spraying continued prophylactically, whereas the other had active treatment of houses because malaria remained endemic or had recently been eradicated. *p,p′*-DDT and *p,p′*-DDE were measured in blood serum, and total DDT was calculated (the DDE measured concentration was "corrected" to DDT molecular mass, accounting for the loss of one hydrogen and one chlorine atom at metabolism; total DDT is therefore slightly higher than in other studies). DDE as a percentage of total DDT was higher (78%) in the area with less spraying than in the area with active spraying (60%), thought to reflect historical exposure in the areas with less or no malaria. The concentrations of both DDE and DDT increased with duration of exposure in both areas (Table 26).

Table 24. DDE and DDT concentrations in blood serum from workers in Mato Grosso State, Brazil, 2 years after cessation of DDT use

District	Workers	Chemical	*N*	Concentration in blood serum (µg/l; not adjusted for lipid)				
				Median	Maximum	Minimum	Q1	Q3
Sinop	Spray operators	*p*,*p*′-DDE	23	115.8	419.5	35.5	84.8	212.8
		p,*p*′-DDT		35.8	476.0	ND	18.8	87.3
		Total DDT		146.3	875.5	35.5	102.3	318.4
	Drivers	*p*,*p*′-DDE	12	102.4	374.0	41.3	83.5	213.5
		p,*p*′-DDT		66.0	204.8	ND	14.6	136.0
		Total DDT		186.6	562.3	41.3	106.3	376.5
	Unexposed	*p*,*p*′-DDE	6	12.6	24.0	ND	7.3	21.8
		p,*p*′-DDT		5.2	23.0	ND	ND	12.0
		Total DDT		18.6	47.0	ND	7.3	32.0
Cáceres	Spray operators	*p*,*p*′-DDE	18	104.2	518.5	7.5	50.0	131.5
		p,*p*′-DDT		16.4	209.3	ND	ND	23.0
		Total DDT		109.7	536.3	7.5	89.0	236.1
	Drivers	*p*,*p*′-DDE	8	110.5	265.0	34.5	73.6	142.1
		p,*p*′-DDT		ND	56.8	ND	ND	20.1
		Total DDT		110.5	351.8	34.5	73.6	162.3
	Unexposed	*p*,*p*′-DDE	7	29.0	94.8	6.0	8.0	53.5
		p,*p*′-DDT		ND	12.3	ND	ND	10.0
		Total DDT		29.0	94.8	6.0	14.5	65.8

ND, not detected; Q, quartile

Source: Dores et al. (2003)

Average annual application in litres of DDT, in suspension at 5%, by state also correlated with mean total DDT in blood serum (results for DDE separately were not reported).

Based on concentrations of total DDT in blood serum of 213 µg/l and 1067 µg/l for area 1 and area 2, respectively, the authors estimated concentrations of total DDT in adipose tissue to be 73 µg/kg and 367 µg/kg fat; the conversion factor used was 344, based on measured ratios in the literature. Estimations of total

absorption of DDT at 5 and 55 mg/person per day were also made for the two areas (Durham, 1969).

Table 25. DDT and DDE concentrations in whole blood of workers in two provinces of Argentina

Personnel	Sex	*N*	Concentration in whole blood (µg/l; ± SD)		
			p,*p*′-DDE	*p*,*p*′-DDT	Total DDT
Catamarca Province					
Administrative controls	M, F	10	15.78 ± 8.94	3.88 ± 1.64	19.66
Former DDT spray operators	M	10	86.11 ± 44.32	11.28 ± 5.41	97.39
Salta Province					
Administrative controls	M, F	10	13.30 ± 3.64	3.47 ± 1.70	16.77
Current DDT spray operators	M	9	363.00 ± 367.78	346.36 ± 348.39	709.36

F, female; M, male

Source: Radomski et al. (1971)

Table 26. Comparison between exposure duration and concentrations of DDT and DDE in blood of spray operators in Venezuela from Area 1 (free of malaria) and Area 2 (active IRS)

Exposure duration (years)	Compound	Mean concentration in blood (µg/l; not adjusted for lipid)[a]	
		Area 1 (*n* = 80)	Area 2 (*n* = 209)
1–5	DDT	36 ± 5	259 ± 23
	DDE	77 ± 12	362 ± 37
	Total DDT	122 ± 17	662 ± 60
> 5 – ≤ 10	DDT	52 ± 12	396 ± 72
	DDE	222 ± 52	496 ± 61
	Total DDT	299 ± 62	949 ± 135
> 10–15	DDT	—	614 ± 113
	DDE	—	859 ± 137
	Total DDT	—	1572 ± 204

[a] The ± values appear to be standard errors; no indication is given on how the statistical analysis was done.

Source: Mazzarri & Mazzarri de Lauschner (1989)

Yáñez et al. (2002) measured blood concentrations of DDT and its metabolites in four spray operators from the malarial vector control programme in Chiapas, Mexico, sampled in 1998 while DDT was still being used. Arithmetic mean concentrations of 165.5 (range 73.7–216.8) µg/l, 188.4 (range 114.3–279.9) µg/l and 8.9 (range 3.8–13.2) µg/l blood serum for *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD, respectively, were reported. This would equate to a "total DDT" concentration of 362.8 µg/l (not adjusted for lipid).

Bouwman et al. (1991a) sampled blood from 23 male spray operators applying DDT for IRS for malarial vector control in Natal, South Africa; this represented 72% of all spray operators in the region. The mean age of the spray operators was 34.8 ± 9.0 years, and the mean time spent in spraying was 8 ± 6.3 years. Samples were taken 1 month before annual resumption of spraying and therefore represent baseline DDT contamination before the season. No PPE was used by the spray operators. An age- and sex-matched group (n = 23) of non-spray operators who lived in sprayed dwellings was used as the "control" group (called residents hereafter); all had been resident in the area and had experienced spraying of their houses for more than 20 years. All dwellings of sampled residents were of mud construction. *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD were measured in blood serum, and total DDT was calculated. Only one applicator had no detectable residue of DDD; all were positive for DDT and DDE. Of the residents, only three had detectable DDD, but all were positive for DDT and DDE. Results are given in Table 27. DDT, DDD and total DDT levels were significantly higher in the occupationally exposed group than in the residents. Percentages of DDT in total DDT and DDE concentrations were higher in spray operators than in residents, but the difference was not statistically significant. No significant regression of DDT, DDD, DDE or total DDT was found in applicators on age or length of employment; this was true for original and log-transformed data. One significant regression was found of DDT as a percentage of total DDT against age (P = 0.0495). The authors suggested that the data support increased DDT metabolism to DDE with age at higher levels of exposure, consistent with induction of liver enzymes by DDT. However, they noted that other authors (Violante & Coltelli, 1986) did not find such a relationship. Significant regressions of DDE ($P < 0.001$) and total DDT against age of the residents were reported, but there was no

significant relationship for DDT, DDD or DDT as a percentage of total DDT.

Table 27. Levels of DDT and its metabolites in blood serum of spray operators in Natal, South Africa

	Concentration in blood serum (µg/l ± SD; not adjusted for lipid)				% DDT
	DDT[a]	DDE	DDD	Total DDT	
Applicators (*N* = 23)					
Mean	61.7 ± 29.4*	129 ± 29.0	11.0 ± 10.2*	202 ± 120.4**	33.4 ± 10.2
Median	55	84	8	155	34
Minimum	9	21	0	36	12.7
Maximum	130	358	42	453	55.6
Residents[b] (*N* = 23)					
Mean	28.9 ± 17.1*	105.2 ± 98.4	0.2 ± 0.7*	134.5 ± 114.6**	27.9 ± 12.9
Median	28	81	0	104	24
Minimum	5	6	0	11	8.4
Maximum	88	424	3	467	60.6

* $P < 0.0001$; ** $P < 0.05$ (using log-transformed data)

[a] Isomers of DDT, DDE and DDD not specified in the paper; personal communication to IPCS from the principal author indicates that they were *p*,*p*′-isomers.

[b] Living in sprayed houses.

Source: Bouwman et al. (1991a)

Dalvie et al. (2004c) conducted a cross-sectional study of 65 malarial vector control workers from Limpopo, South Africa. The workers completed a questionnaire with trained interviewers; questions related to years worked in vector control, job titles, which spraying and non-spraying activities to which they had been exposed in spraying and non-spraying seasons (in current and previous jobs) with estimates of duration, pesticide exposure previous to employment in vector control and exposure to industrial chemicals. "Spraying years" were calculated from the questionnaire results. However, whereas eight different job titles were identified, only three workers had neither spraying nor mixing jobs, thus limiting the variation in job description. Years working in vector control ("malaria years", which included surveillance work and

other duties outside the spraying season) gave greater range of exposure and correlated with *p*,*p*′-DDE concentration in blood serum; "malaria years" was therefore used as the measure of exposure. *o*,*p*′-DDT, *p*,*p*′-DDT, *o*,*p*′-DDE, *p*,*p*′-DDE, *o*,*p*′-DDD and *p*,*p*′-DDD were determined, and total DDT was calculated; blood serum from 47 workers was analysed. The mean age was 45 ± 9 years (range not stated), and participants had a low level of education (mean schooling years 6.9 ± 2.7). All workers reported alcohol consumption, and 58% had smoked at some time. Median work history with the control programme was 17 years (range 4–34 years). No PPE was used during spraying. Of all compounds measured, *p*,*p*′-isomers were substantially more abundant than *o*,*p*′-isomers; *p*,*p*′-DDE concentrations were the highest (Table 28), representing 60% of uncorrected (62% of lipid corrected) total DDT.

Table 28. Concentrations of DDT and its metabolites in blood serum of workers in Limpopo, South Africa

Compound	Median concentration (range) (*n* = 65)	
	Uncorrected DDT (µg/l)	DDT corrected for total lipids (µg/g lipid)
o,*p*′-DDT	13.1 (ND–72.9)	1.57 (ND–0.3)
p,*p*′-DDT	195.2 (2.5–828.3)	26.9 (0.32–63.33)
DDT (sum *o*,*p*′- and *p*,*p*′-)	206.5 (2.47–878.4)	28.2 (0.3–67.1)
DDD (sum *o*,*p*′- and *p*,*p*′-)	6.3 (0–33.75)	0.74 (0–3.1)
o,*p*′-DDE	0.46 (ND–2.47)	0.051 (ND–0.26)
p,*p*′-DDE	378.8 (8.46–4134.8)	52.3 (1.08–273.5)
DDE (sum *o*,*p*′- and *p*,*p*′-)	379.3 (8.46–4136.24)	52.4 (1.1–273.6)
Total DDT	642.8 (10.9–4761.4)	83.3 (1.40–315.0)

ND, not detected

Source: Dalvie et al. (2004c)

"Malaria years" correlated positively, linearly, significantly and strongly with *p*,*p*′-DDE, but not significantly, and mostly negatively, with DDT and other metabolites. "Malaria years" had a weaker association with uncorrected *p*,*p*′-DDE levels (R^2 = 0.53; P = 0.074) than with *p*,*p*′-DDE corrected for blood lipid (R^2= 0.56; P = 0.016). Age was a significant covariate, but BMI was not. In contrast to some other studies, *p*,*p*′-DDE and *p*,*p*′-DDT were not highly correlated (r = 0.41). The authors identified uncertainties and shortcomings in their study. The questionnaire did not adequately

inform exposure studies because it was insufficiently detailed on short-term to medium-term exposure history, necessitating the use of the cruder "malaria years" measure. Non-occupational exposure could have influenced correlations. Incorrect recall by workers of their spraying activities could have influenced the outcome. The study concluded that concentrations of DDT metabolites should be corrected to blood lipid to increase the likelihood of significant correlation with employment factors (Dalvie et al., 2004c).

Violante & Coltelli (1986) analysed blood samples from 64 male workers exposed during spray operations against mosquitoes and flies in Italy. DDT had been used in the spraying programme since the early 1950s, but its use was discontinued in 1978; limited use of a solid preparation continued until 1982. Organophosphorus compounds had also been used in the programme. Workers were sampled in 1982, at which time they had a mean age of 37.2 years (range 21–60 years) and a mean exposure period of 6.1 years (range 1–22 years) as spray operators. It was not stated whether any, or all, of the spray operators had used DDT in the period between 1978 and 1982, nor was the time of their last exposure given. *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD were measured; total DDT was calculated and not corrected for lipid. Arithmetic mean concentrations of total DDT and DDE were 24.2 and 16.4 µg/l, respectively. A subgroup of 27 workers within the age range of 40–60 years (mean not stated) had total DDT and DDE concentrations (± SD) of 28.8 ± 1.91 and 18.6 ± 2.14 µg/l, respectively. A control group of 27 males from the same geographical area had a mean age of 47.8 years (range 40–60 years) and had no known occupational exposure. Total DDT and DDE concentrations (± SD) in blood of the controls were 26.4 ± 2.04 and 21.4 ± 2.04 µg/l, respectively. Data from the 64 workers were divided into two groups: 33 who had more than 4 years of exposure and 31 with shorter and limited exposure to DDT. The more exposed personnel (mean age 43.5 years and average exposure 9.9 years) had mean total DDT and DDE concentrations (± SD) of 27.5 ± 1.82 and 18.2 ± 2.45 µg/l, respectively, compared with the less exposed (mean age 30.5 years and average exposure 2.1 years), with total DDT and DDE concentrations (± SD) of 12.6 ± 1.58 and 8.1 ± 1.7 µg/l, respectively. Differences are significant and could have been due to differences in age; analysis of covariance showed a significant difference ($P < 0.01$) due to exposure unrelated to age (values appear to have been log-transformed).

The fraction of total DDT present as metabolites (DDE plus DDD) did not correlate with age (overall 67%; SD ± 11%; range 35–90%); the authors concluded that the age effect was not caused by differences in metabolism of DDT with age.

Dua et al. (1996) collected whole blood by finger-prick and standard volume capillary tube and subsequently dried the samples on filter paper prior to analysis. Occupationally exposed individuals (n = 47), who were involved in spraying DDT and HCH for control of mosquitoes and house flies, and the general population were sampled at Hardwar, Uttar Pradesh, India. Residue levels were 16.13 ± 14.33 (range not detected [ND]–69.41) µg/l for *p*,*p*′-DDE and 20.79 ± 14.81 (range ND–69.41) µg/l (not adjusted for lipid) total DDT for the general population and 44.42 ± 48.02 (range 11.18–211.6) µg/l for *p*,*p*′-DDE and 58.43 ± 53.72 (range 11.18–238.3) µg/l for total DDT for the occupationally exposed.

12.5 Residents in sprayed areas

12.5.1 Known residents of sprayed houses

Bouwman et al. (1991b) conducted a cross-sectional study of two areas of South Africa. One, Ubombo (northern Natal), had received regular annual application of DDT as IRS for control of malarial vectors between 1977 and the sampling date (two yearly applications had occurred between 1957 and 1977). The other area, Port Shepstone, southern Natal, had no malaria and had never received IRS treatment. DDT had not been used since 1977 agriculturally in South Africa. Whole households were sampled, and only houses constructed from daub (mud) were included. The two groups were matched for age, but more females were included in the non-exposed group than in the exposed group because of demographic differences between the areas. Results are summarized in Table 29.

DDE was detected in close to 100% of both groups, whereas DDT was detectable in 100% of the exposed group but only 6.5% of the non-exposed group. DDE and DDT concentrations in blood serum of the exposed population were correlated; no correlation could be established in the non-exposed group, because such a high proportion of samples had no detectable DDT. The relationship

between age and total DDT was complex in the exposed group, with young people (aged between 3 and 20 years) showing decreasing concentrations with age and older people showing increasing concentrations with age beyond 20 years. In the non-exposed group, no significant trends with age were demonstrated. The authors suggested that transfer of DDT to infants via breast milk was responsible for the higher concentrations in infants; breastfeeding continued for 2 years in the area, in contrast to other studies. Falling levels throughout childhood and into early adulthood could be due to dilution effects of growth or enhanced metabolism associated with childhood (Bouwman et al., 1991b).

Table 29. DDT and DDE concentrations in blood serum from exposed and control populations in South Africa

Compound	Mean (± SD) concentration in blood serum (µg/l; not adjusted for lipid)	
	Exposed (*n* = 71)	Non-exposed (*n* = 77)
DDE[a]	103.4 ± 85.1[b]	5.95 ± 7.98
DDT[a]	37.3 ± 27.2[b]	0.077 ± 0.31
Total DDT	140 ± 108.3[b]	6.04 ± 8.19

[a] Isomers measured not stated; a personal communication to IPCS from the principal author indicates that they were *p,p'*-isomers.
[b] Significantly different between groups ($P < 0.05$).

Source: Bouwman et al. (1991b)

Bouwman & Schutte (1993) sampled blood from all siblings of eight families living in houses constructed of daub (mud) and treated regularly with DDT to control mosquitoes in Natal, South Africa. Siblings were between 3 and 20 years of age; households with a minimum residence of 10 years in the area were selected. Siblings aged 9 years and younger (n = 22; total DDT 162.5 µg/l) and older than 9 years (n = 18; total DDT 97.7 µg/l) had significantly different concentrations in blood serum ($P < 0.05$). The percentage of total DDT as DDT was significantly negatively correlated with age: 31.5% for siblings 9 years and younger and 27.6% for those older than 9 years. Reducing trends in DDT concentration over the sampling period were clear for six of the homesteads, with siblings showing similar downward trends. Results were consistent with previous findings by the same authors of higher exposure of young children in extended breastfeeding

typical of the area. They are also consistent with changes in metabolism of DDT with age.

Bouwman et al. (1994) conducted a longitudinal study of 71 people representing all members of 12 households with at least 10 years of continuous residence in an area of Natal, South Africa, where regular IRS for malarial vector control had taken place. All households lived in dwellings with mud walls; concrete dwellings were excluded from the study, because a different formulation of DDT was used in these. Blood samples were collected in November 1986, March 1987, June 1987 and November 1987; the March sampling took place 10 days after application of DDT to the houses. Not all participants could be sampled for all sampling dates, but those missed were not considered to have affected the overall results. There were 29 people at least 21 years of age and 49 younger; the separation into age groups was based on earlier observations that the younger people had a net loss of total DDT over a year, whereas the older people accumulated total DDT. Absolute levels of DDT and its metabolites are given in Table 30; DDD concentrations are included in total DDT but are not tabulated, as they represented a small proportion of the total. Blood serum levels of DDE, DDT and total DDT increased for both age groups following the single application of DDT to their houses in March. However, the increase in both DDT and DDE levels for the younger group was not significant; increases in the older group also resulted in increases for both groups combined. Older people showed falling DDT and DDE concentrations in blood in the period between March (application) and June, but levels rose again in the period between June and November, giving an increase for this group over the starting concentration. For younger people, levels over the year fell below the starting concentrations.

Rates of change in blood serum DDE, DDT and total DDT levels were compared between sampling points for each age group (Bouwman et al., 1994). Comparisons and their statistical significance are presented in Table 31. Two concurrent processes probably govern the increase and decrease in serum levels, and the relative contributions of each interchange as the individual becomes older. The results suggest that children in Natal experience conditions that differ from those of their parents, as well as from those that affect children in developed countries.

Table 30. Mean concentrations of DDT and DDE in blood serum of residents of Natal, South Africa, over a 1-year period (IRS conducted in March)

Date and age group	*N*	Mean (± SD) concentration in blood serum (µg/l; not adjusted for lipid)		
		DDE[a]	DDT[a]	Total DDT
November 1986				
≤ 20 years	42	103.9 ± 76.6	43.5 ± 31.0	147.5 ± 105.1
≥ 21 years	29	102.7 ± 97.6	28.2 ± 17.2	131.4 ± 113.9
Combined	71	103.4 ± 85.1	37.3 ± 27.2	140.9 ± 106.3
March 1987				
≤ 20 years	39	122.1 ± 93.0	53.0 ± 28.6	174.2 ± 110.6
≥ 21 years	27	134.3 ± 113.3	39.7 ± 23.6	175.3 ± 131.7
Combined	66	127.1 ± 101.1	47.5 ± 27.3	174.6 ± 118.8
June 1987				
≤ 20 years	36	111.0 ± 77.1	48.4 ± 30.9	160.7 ± 106.0
≥ 21 years	22	107.7 ± 93.3	32.3 ± 18.2	145.3 ± 111.8
Combined	58	109.7 ± 81.8	42.6 ± 27.9	155.4 ± 107.3
November 1987				
≤ 20 years	36	95.4 ± 65.6	31.1 ± 21.5	127.9 ± 86.5
≥ 21 years	27	124.7 ± 124.8	31.9 ± 21.8	161.1 ± 145.0
Combined	63	107.9 ± 95.8	31.4 ± 21.5	142.1 ± 115.4

[a] Isomers measured not stated; a personal communication from the principal author to IPCS indicated that they were *p*,*p*′-isomers.

Source: Bouwman et al. (1994)

Aneck-Hahn et al. (2007) sampled blood serum from 303 Venda men living in treated and non-treated houses in a malarial area of Limpopo Province, South Africa, as part of a study on semen quality. Blood serum concentrations and their lipid-adjusted equivalents are summarized in Table 32.

Table 31. Differences between rates of change of levels of DDT and DDE in serum for study periods and age groups over a 1-year sampling of residents of DDT-treated houses in Natal, South Africa

Period	Student's *t*-test for age group	
	≤ 20 years	≥ 21 years
November 1986 – March 1987	*0.0018*[a] (DDT, I)[b]	0.4432 (DDT, I)
March 1987 – June 1987	0.1545 (DDT, D)	*0.0418* (DDE, I)
June 1987 – November 1987	*0.0000* (DDT, D)	0.1319 (DDE, I)
March 1987 – November 1987	*0.0208* (DDT, D)	0.3385 (DDT, D)
November 1986 – November 1987	*0.0000* (DDT, D)	0.2076 (DDE, I)

[a] Significant differences in italics ($P < 0.05$).
[b] DDT or DDE level with largest *k* value; I = increase in blood serum level of compound with largest value, D = decrease in blood serum level of compound with largest value.

Source: Bouwman et al. (1994)

Table 32. Concentrations of *p*,*p*′-DDE and *p*,*p*′-DDT in blood serum from men living in Limpopo Province, South Africa

Compound	Mean (± SD) concentration				Maximum concentration	
	Non-treated houses (*n* = 48)		Treated houses (*n* = 249)		µg/l	µg/g lipid
	µg/l	µg/g lipid	µg/l	µg/g lipid		
p,*p*′-DDE	529.7 ± 658	99.5 ± 123	1409.8 ± 1339	239.0 ± 215	6621	997
p,*p*′-DDT	167.0 ± 339	30.5 ± 58	602.4 ± 630	101.9 ± 104	2644	519
Total DDT	696.7	—	2012.2	—	7265	—

Source: Aneck-Hahn et al. (2007)

Van Dyk et al. (2010) selected two villages in the Limpopo Province of South Africa: a "sprayed village" in an area with moderate to high incidence of malaria and a "control village" outside the malarial area. The two villages had homesteads selected for sampling, 12 and 9, respectively, which were matched for culture, construction and socioeconomic status. Both villages had round thatched huts, and the area between the huts was used for cooking on outdoor fires, as a play area for children and as an area where livestock (chickens, goats and domestic pets) roamed freely

during the day; vegetable gardens where leafy vegetables and maize were cultivated lay within 1–2 m of the huts. The sprayed village had been treated annually with DDT since 1945. Water for drinking and bathing, obtained from either boreholes or local natural springs, was stored inside the houses in large plastic containers. From each homestead, samples were taken of human blood serum, indoor air, floor dust, outdoor soil, leafy vegetables, potable water and chicken fat, liver and muscle tissue. Samples were taken 3 months following spraying. In the sprayed village, indoor air samples showed a median total DDT concentration of 2700 ng/m^3, with the majority of the content consisting of *p,p′*-DDT and *o,p′*-DDT, the components of the DDT WP applied. The median total DDT concentration in indoor air in the control village was much lower, at 13 ng/m^3, although it was still detectable. Floor dust, collected by suction over a standard area, in the sprayed village had a median total DDT level of 910 µg/m^2 compared with 1.3 µg/m^2 in the control village; these residues were also predominantly *p,p′*-DDT. The median total DDT concentrations in outdoor soil were 20.0 and 5.5 µg/kg for the sprayed and control villages, respectively, although *p,p′*-DDE predominated in soil, indicating metabolism of the applied spray. Concentrations in water were low but detectable in the sprayed village. Vegetables showed concentrations of 25.0 µg/kg (median) for total DDT with *p,p′*-DDT, DDD and DDE present, thought to derive from uptake from contaminated soil and reflecting the isomer profile of that medium. Chicken muscle showed identical median concentrations in the two villages, at 290 µg/kg total DDT, with most present as *p,p′*-DDE. Chicken fat was highly contaminated in the sprayed village, with a median concentration of 380 000 µg/kg total DDT compared with 170 µg/kg in the control village; again, the metabolites DDE and DDD represented the majority of the total. Chicken liver residues were at 1100 and 65 µg/kg total DDT for the sprayed and control villages, predominantly as DDE.

Multivariate analysis was conducted to examine the associations between the sampled components in the homesteads and the isomers of DDT. The strongest association for indoor air and floor dust was with DDT, whereas for outdoor soil it was with DDD and DDE. The authors suggested that prolonged DDT in the air of treated homesteads contaminates stored water supplies and food. Air and floor dust regularly swept out of the houses contaminates surrounding soil, which leads to residues in vegetables. Contam-

inants in both floor dust and soil are bioaccumulated into the chickens, which roam freely both indoors and outdoors. Some wider contamination of the environment may occur from IRS, but it could also result from illegal use of DDT in agriculture. Human blood serum collected from the exposed village only showed mean total DDT and *p,p′*-DDE concentrations of 7.3 and 5.9 µg/g lipid, respectively (median concentrations 5.1 and 4.7 µg/g lipid, respectively) (Van Dyk et al., 2010).

12.5.2 General population living in areas using indoor residual spraying

Waliszewski et al. (1996b) reported concentrations of DDE and DDT in human adipose tissue collected during autopsy from 31 cadavers in 1988 and 56 in 1991. Abdominal adipose tissue was sampled; sampling was random from residents dying in the state of Veracruz, Mexico, where it was legal to take tissue. Two samples from each period were classified as residing "out of state" (Mexico City or other Mexican states). Age, sex, area of residence and cause of death were recorded against each sample. No significant difference (analysis of variance) was found in concentrations of either DDT or its metabolites between the sampling periods except for *p,p′*-DDT, which declined significantly between the two sampling dates. It is not stated whether log transformation was conducted. All adipose tissues sampled had both *p,p′*-DDE and *p,p′*-DDT present, but less than half had detectable *o,p′*-DDT. Concentrations are presented in Table 33.

Total DDT concentrations were highest in samples from suburban Veracruz (mean 38.05 ± 29.54 µg/g adipose tissue in 1988 and 28.64 ± 29.76 µg/g adipose tissue in 1991), where extensive use of DDT for malaria control occurred. Urban, elsewhere in Veracruz state and out-of-state mean concentrations ranged from 2.09 to 16.80 µg/g fat. Levels in fat differed between males and females, although the number of samples was biased in favour of males; concentrations between sampling dates were the same for males, but fell significantly for females in the second sampling period (although this is based on arithmetic means). There was a tendency for residues to increase with age. Mean total DDT was higher in those dying of cardiovascular disease compared with those dying from other causes (Waliszewski et al., 1996b).

Table 33. Concentrations of DDT and DDE in human adipose tissue from Veracruz, Mexico

Compound	Concentration (µg/g)	
	Mean ± SD	Range
1988		
p,p'-DDE	9.97 ± 8.54	0.55–33.62
o,p'-DDT	1.79 ± 3.02	0.20–9.13
p,p'-DDT	6.67 ± 13.53	0.20–22.17
Total DDT	17.45 ± 21.03	0.99–86.95
1991		
p,p'-DDE	10.00 ± 10.46	0.70–44.58
o,p'-DDT	1.35 ± 1.34	0.20–5.54
p,p'-DDT	4.02 ± 7.18	0.20–46.43
Total DDT	14.06 ± 15.98	0.90–90.38

Source: Waliszewski et al. (1996b)

Waliszewski et al. (1998) extended the sampling period to 1997, covering the time when DDT use for malaria control ceased in Mexico (substitution of DDT by malathion and pyrethroids in this area from 1996). Mean concentrations over 6 sampling years, representing 287 tissue samples, are summarized in Table 34. Median concentrations are not given in the paper (Waliszewski et al., 1998). Adipose tissue concentrations (not separated between sub-urban and urban populations) remained unchanged between 1997 and 1998 (Waliszewski et al., 2001).

Table 34. Concentrations of total DDT in human adipose tissue from Veracruz, Mexico, from 1988 to 1997

Origin	Mean concentration of total DDT (µg/g)					
	1988	1991	1992	1994	1995	1997
Suburban	38.05	28.61	38.55	18.42	23.23	5.13[a]
Urban	8.53	16.80	18.77	6.02	4.08	4.20[a]
Ratio of urban to suburban	4.5	1.7	2.1	3.1	5.7	1.2

[a] No statistical difference between suburban and urban groups in 1997. Not stated whether log transformation of the data was conducted.

Source: Waliszewski et al. (1998)

Waliszewski et al. (2001) sampled abdominal adipose tissue, blood serum and umbilical blood serum from 60 volunteer mothers in Veracruz state, Mexico; mothers had a mean age of 28 years (range 18–44 years) and mean deliveries of 2.2 (range 1–3). Colostrum and mature milk were sampled from the mothers on the 5th and 13th days postpartum. Comparisons were made, on a fat basis throughout, between concentrations of DDT and its metabolites in the various tissues/sera. Minor amounts of *p,p′*-DDD and *o,p′*-DDT were measured. There was good correlation between concentrations in adipose tissue and mature milk (Table 35). Sampling coincided with cessation of the use of DDT for malaria control in Mexico. Levels of *p,p′*-DDT and total DDT in adipose tissue fell significantly over a 2-year period, but concentrations of DDE were not significantly reduced.

Table 35. Comparison between concentrations of DDT and its metabolites in adipose tissue (at birth) and milk (13 days postpartum) of mothers in Veracruz, Mexico

Compound	Concentration (µg/g fat)				Correlation coefficient
	Adipose tissue		Mature milk		
	Mean ± SD	Range	Mean ± SD	Range	
p,p′-DDE	4.36 ± 3.46	0.31–16.04	4.00 ± 5.16	0.18–34.28	0.89
p,p′-DDT	1.22 ± 2.12	0.01–9.03	0.65 ± 0.94	ND–4.27	0.95
Total DDT	5.66 ± 5.02	0.34–24.98	4.70 ± 5.87	0.32–38.70	0.91

ND, not detected

Source: Waliszewski et al. (2001)

A similar comparison between colostrum and mature milk showed a high correlation (0.97 for DDE and 0.98 for total DDT), but lower levels in mature milk than in colostrum (geometric mean concentrations [95% CIs] were 2.41 [2.73–4.53] and 3.27 [3.51–5.37] µg/g fat, respectively). There was also a good correlation between concentrations in maternal blood serum and umbilical blood serum (0.87 for DDE and 0.90 for total DDT), but lower levels in umbilical blood serum (arithmetic mean concentrations 14.5 ± 28.0 µg/l and 6.0 ± 8.7 µg/l for DDE in maternal and umbilical blood serum, respectively). Total DDT levels in cord blood were reported to be 16.4 ± 30.8 µg/l. These concentrations appear not to be adjusted for blood lipid content; the authors explained the lower concentration in umbilical blood serum as

reflecting lower fat content (1.5–4 times lower) compared with maternal blood (Waliszewski et al., 2001).

Waliszewski et al. (2004) compared concentrations of DDT and DDE on a fat basis between postmortem abdominal adipose tissue and blood serum collected during autopsy of 126 cadavers in Veracruz, Mexico. Co-partition coefficients were calculated. Results are given in Table 36.

Table 36. Comparison of concentrations of DDT and its metabolites in abdominal adipose tissue and blood serum lipid[a]

Compound	Mean concentration (µg/g)		Two-tailed *P*-value	Co-partition coefficient
	Adipose tissue	Blood lipids		
p,p′-DDE	1.06 ± 0.54	2.79 ± 1.71	0.0001	0.38 ± 0.21
o,p ′-DDT	0.06 ± 0.08	0.13 ± 0.17	0.0001	0.57 ± 0.33
p,p′-DDT	0.59 ± 0.57	0.34 ± 0.40	0.0001	2.16 ± 0.97
Total DDT	1.71 ± 1.03	3.26 ± 2.00	0.0001	0.65 ± 0.41

[a] ± standard deviation; no indication given as to whether log transformation was used.

Source: Waliszewski et al. (2004)

There was a preferential distribution to blood lipids for all compounds other than *p,p′*-DDT. The authors concluded that blood lipid concentration does not predict adipose tissue concentration. Further analysis comparing co-partition coefficients with the data split according to blood lipid levels (< 500, 500–800 and > 800 mg/l, representing hypolipidaemic, normal and hyperlipidaemic individuals) showed no significant differences between groups. Comparison of concentrations of DDT and its metabolites between abdominal and breast adipose tissue showed no significant differences (Waliszewski et al., 2003a).

Waliszewski et al. (2010) sampled abdominal adipose tissue during autopsy, taking 80 samples each from the Veracruz and Puebla areas of Mexico in 2008, 9 years following cessation of the use of DDT in vector control. The Veracruz area had endemic malaria and had received extensive treatment with DDT in the past; the inland, cooler Puebla area had not had problems with malaria, and use of DDT was less. Both *p,p′*-DDE and *p,p′*-DDT were detected in all samples. Median concentrations of *p,p′*-DDE were

1.83 and 0.35 mg/kg lipid for the two areas, respectively; median concentrations of *p,p′*-DDT were 0.114 and 0.056 mg/kg lipid, respectively, reflecting the cessation in DDT use. The values for Puebla were significantly lower than those for Veracruz in both cases.

Dale et al. (1965) reported an average total DDT content of 24.3 mg/kg in the fat of 104 human samples from India; DDE accounted for 40% of the total DDT. Ramachandran et al. (1973) conducted a follow-up survey in body fat of 94 people, 45 male and 49 female, including 8 children below the age of 5 years. Total DDT was present at a mean concentration (± SE) of 21.3 ± 3.9 µg/g for males (range 0.17–99.35 µg/g) and 22.3 ± 4.2 µg/g for females (range 13.58–96.13 µg/g). Krawinkel et al. (1989) collected blood and fat samples (details not stated) from in-patients in Quetta, Baluchistan Province, Pakistan; the patients all underwent surgical operations for different conditions, mostly laparotomies. Median reported concentrations were 0.61 and 8.58 µg/l whole blood (maxima 4.83 and 32.61 µg/l, respectively) and 0.87 and 4.76 µg/g fat (maxima 10.10 and 81.83 µg/g, respectively) for *p,p′*-DDT and *p,p′*-DDE, respectively.

Waliszewski et al. (1999) reported mean blood serum concentrations of 14.5 ± 28.0 (SD) µg/l for *p,p′*-DDE and 16.4 ± 30.8 µg/l for total DDT in 64 mothers from Veracruz state, Mexico. The mothers were sampled during their hospital stay for delivery, and all had lived in Veracruz state for at least 1 year.

Ayotte et al. (2001) reported arithmetic mean concentrations of *p,p′*-DDE in blood serum lipid at 77.9 µg/g fat (range 17.0–177.2 µg/g fat) in non-occupationally exposed young men (mean age 21 years; range 16–28 years) from Chiapas state, Mexico, where DDT was used in houses to control malarial mosquitoes.

Yáñez et al. (2002) reported whole blood concentrations of DDT and its metabolites in children (age range 3–14 years) and adults (age range 24–70 years) sampled in two different states of Mexico: Chiapas and Oaxaca. Chiapas was sampled during 1998 when active IRS of DDT for malaria control was still under way. Oaxaca was sampled in 2000, 2 years after cessation of the IRS programme there. Results are given in Table 37. The proportion of

DDE in the total DDT concentration in whole blood was greater for both children and adults in Oaxaca, reflecting the later sampling period and cessation of DDT use.

Table 37. Whole blood concentrations of DDT and its metabolites in children and adults living in malarial areas treated with DDT

Compound	Group	*N*	Concentration in whole blood (µg/l)	
			Mean ± SD	Range
Chiapas state[a]				
p,p′-DDT	Children	9	67.8 ± 31.6	21.8–113.1
	Adults	11	27.1 ± 16.1	11.2–57.1
p,p′-DDE	Children	9	86.7 ± 37.4	50.3–167.2
	Adults	11	60.8 ± 31.5	24.9–138.8
Oaxaca state[b]				
p,p′-DDT	Children	28	20.4 ± 13.7	7.5–53.3
	Adults	10	13.2 ± 7.7	3.1–29.6
p,p′-DDE	Children	28	74.5 ± 33.6	34.9–179.6
	Adults	10	41.6 ± 27.4	5.7–97.7

[a] Current IRS treatment.
[b] IRS treatment stopped 2 years previously.

Source: Yáñez et al. (2002)

Women were also sampled from the same areas and from two further regions: San Luis Potosi City, with no exposure to DDT spraying, and Huasteca, an area with substantially lower application of DDT. Results are given in Table 38. The authors considered that the higher levels in children in sprayed areas were due either to ingestion of contaminated milk or to exposure via household dust or surface soil adjacent to their homes, which were considered recreational areas for infants. They had measured high concentrations of DDT, DDE and, to a lesser extent, DDD in both indoor and outdoor surface sediments (Yáñez et al., 2002).

Rugama et al. (1993) measured *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT in human plasma sampled from three different populations in Nicaragua: Danish residents who had been in the country for 3 years or less, residents of the capital city, Managua, and residents of rural areas where DDT had been used for malarial vector control until 1989; the sampling date was not stated. Results are given only as total DDT. Means concentrations (± SD) and ranges were 2.49 ±

1.55 (0.48–6.05) µg/l for the Danes, 12.46 ± 6.07 (5.43–27.16) µg/l for the residents of Managua and 35.23 ± 17.84 (13.91–82.81) µg/l for the rural residents.

Table 38. Whole blood concentrations in women living in malarial and non-malarial areas of Mexico

Compound	Group	*N*	Concentration in whole blood (µg/l)	
			Mean ± SD	Range
p,p′-DDT	San Luis Potosi City	10	1.88 ± 1.0	0.02–3.0
	Huasteca	44	5.19 ± 3.7	1.23–20.7
	Chiapas & Oazaca	12	19.01 ± 12.0	3.1–46.6
p,p′-DDE	San Luis Potosi City	10	1.85 ± 1.0	0.04–2.9
	Huasteca	44	7.21 ± 8.6	0.97–39.2
	Chiapas & Oazaca	12	42.45 ± 25.4	5.7–97.7

Source: Yáñez et al. (2002)

Radomski et al. (1971) measured concentrations of *p,p′*-DDE and *p,p′*-DDT in whole blood of non-occupationally exposed adults and children in Argentina. From the province of Catamarca, 10 people were sampled, consisting of 1 male and 9 female administrative workers who had no known exposure to pesticides; ages ranged from 25 to 58, and body weights from 58.5 to 88 kg. From another province, Salta, 10 people were sampled, consisting of 3 female and 7 male administrative workers, maintenance workers, laboratory technicians and office workers who had no known exposure to pesticides; ages ranged from 21 to 35, and body weights from 51 to 70 kg. Mean *p,p′*-DDE concentrations across all 20 sampled personnel were 14.653 ± 6.76 µg/l whole blood, and mean *p,p′*-DDT concentrations were 3.18 ± 1.70 µg/l. Children between 1 and 10 years of age were also sampled; they were equally divided as to sex. Mean DDE concentrations in whole blood were 8.13 ± 4.06 and 5.56 ± 4.83 µg/l for children 5–10 years and 1–5 years, respectively (n = 18 and 19, respectively). Mean DDT concentrations were 4.21 ± 1.41 and 2.49 ± 2.26 µg/l for the two age groups, respectively. Thirteen mothers and their newborn infants (province not stated) were also sampled; results are presented in Table 39. The ratios between DDE and DDT in mothers and newborns were identical. Newborn infants had blood concentrations approximately 40% of those in maternal whole blood for both DDE and DDT.

Table 39. Concentrations of DDT and DDE in whole blood from mothers and their newborn infants in Argentina

Subjects	Mean concentration (± SD) in whole blood (µg/l; not adjusted for lipid)			Ratio of *p,p′*-DDE to *p,p′*-DDT
	p,p′-DDE	*o,p ′*-DDT	*p,p′*-DDT	
Mother	13.43 ± 7.69	0.27 ± 0.32	6.85 ± 2.07	1.93 ± 0.84
Newborn	4.72 ± 2.54	ND	2.54 ± 1.46	2.13 ± 1.46
Ratio of newborn to mother	0.39 ± 0.14	—	0.41 ± 0.16	—

ND, not detected

Source: Radomski et al. (1971)

Kanja et al. (1992) collected maternal subcutaneous adipose tissue during caesarean delivery in a hospital in Nairobi, Kenya, maternal and umbilical blood serum immediately after delivery and milk 6 days postpartum. Mothers were giving birth to their first or second child. Concentrations of DDT and its metabolites, corrected for fat content of the tissues, were compared using paired data for samples. Results are presented in Table 40. There was significant correlation between levels of total DDT in maternal subcutaneous fat and milk fat of the mothers (r = 0.97), between maternal blood serum levels and milk levels (r = 0.87) and between maternal fat and maternal blood serum (r = 0.84). No correlation was found between total DDT in adipose tissue and cord blood or between maternal blood and cord blood.

Dorea et al. (2001) reported *p,p′*-DDE concentrations in umbilical cord blood in Nicaragua with a mean value at 6.39 (ND–9.35) µg/l and a median at 5.10 µg/l. Total DDT was not reported.

To put IRS studies in context, results from 31 papers on DDT/DDE levels in adipose tissue have been plotted (Figs. 3 and 4). In almost all cases, the adipose tissue sampled was abdominal fat. Some studies are plotted in the figures but not expanded upon in the text; these gave values only or covered exposures other than from mosquito control. Results are categorized according to the use of DDT at the time of sampling, geography, exposure population, etc., as detailed in the notes to the figures. The date of sampling is plotted, not the date of publication of the report. More than one plot is possible from each study (e.g. different geographical regions or

areas; non-exposed and exposed populations in a single paper). Some studies report both total DDT (Fig. 3) and DDE (Fig. 4), but some report one or the other. Some studies report multiple populations in similar geographical areas (e.g. different regions in the same country); for these, all individual determinations are plotted.

Table 40. Levels of DDT and its metabolites in paired samples from Kenyan mothers and their babies delivered by caesarean section

Compound	Mean concentration (range) (µg/g fat)			
	Subcutaneous fat (*n* = 11)	Maternal blood serum (*n* = 11)	Umbilical cord blood serum (*n* = 11)	Milk (*n* = 8)
p,*p*′-DDT	2.49 (0.75–9.88)	0.81 (0.31–2.63)	0.60 (0.15–1.77)	2.14 (0.54–7.18)
p,*p*′-DDE	3.26 (1.47–6.07)	1.52 (0.63–2.52)	1.26 (0.28–2.63)	2.31 (1.06–6.04)
Total DDT[a]	5.91[b] (2.27–16.3)	2.75[b] (1.10–5.56)	1.87 (0.46–4.66)	4.86[b] (1.79–14.22)
Ratio of DDT to DDE	0.72 (0.39–1.65)	0.67 (0.38–1.04)	0.54 (0.28–0.90)	0.87 (0.48–1.32)

[a] Total DDT includes *o*,*p*′-DDT, which is not tabulated and occurred in few samples of maternal blood serum and no samples of umbilical blood.

[b] Significant differences between maternal fat and milk fat, maternal fat and maternal blood serum, and maternal blood serum and milk fat. Not stated as to whether data were log-transformed prior to statistical analysis.

Source: Kanja et al. (1992)

Studies have been allocated into four categories representing usage patterns of DDT. Those from countries having endemic malaria have been grouped (sector B of both figures); reported concentrations in adipose tissue have come from IRS, agricultural use or both. Authors have not necessarily indicated whether their samples could be attributed to either source. Sector D of both figures represents studies of adipose tissue after usage of DDT stopped in the countries, whereas sector A covers studies while DDT was still being used; in both cases, allocation of studies to sectors was largely based on countries' statements to WHO on DDT use (WHO/UNEP DDT Information System, http://www.chem.unep.ch/ddt/ProfileCriteria.html). Allocation to sectors has uncertainties. Cessation of DDT use did not occur on a

single date for most countries; many countries banned use in agriculture but retained use in public health until a later date.

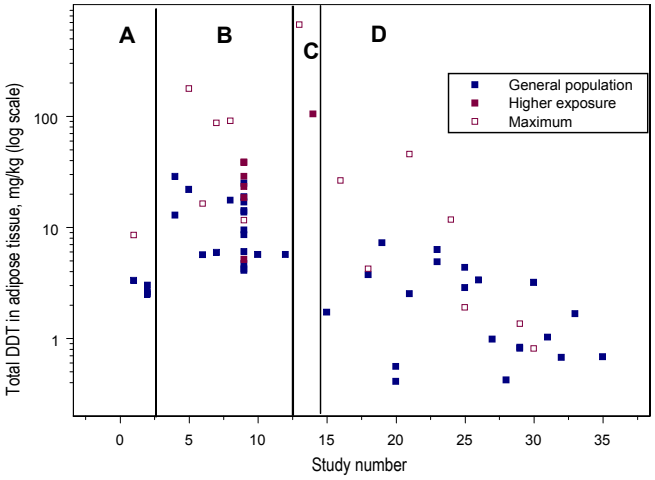

Fig. 3. Total DDT (sum of all compounds measured) as mean, median or geometric mean and maximum concentration, as mg/kg fat, reported in 31 studies on adipose tissue.

Notes:
A: Studies from countries using DDT, but results not related to IRS; studies are arranged in date order of sampling from left to right; dates are 1962 and 1972, respectively.
B: Studies from countries with endemic malaria, using DDT for IRS, although use in agriculture cannot be excluded; studies are arranged in date order of sampling from left to right; dates are between 1965 and 1998.
C: Study from a country where samples were taken immediately following cessation of use of DDT; date of sampling is 1996.
D: Studies from countries not using DDT for either agriculture or IRS; studies are arranged in date order of sampling from left to right; dates are between 1982 and 2000.

Study number: References for all studies are tabulated in Table 41.

Table 41. Study numbers and references for Figures 3, 4 and 5

No.	Reference	No.	Reference
1	Egan et al. (1965)	20	Teufel et al. (1990)
2	Abbott et al. (1972)	21	Lordo et al. (1996)
4	Dale et al. (1965)	22	Sasaki et al. (1991)

No.	Reference	No.	Reference
5	Ramachandran et al. (1973)	23	Ludwicki & Góralczyk (1994)
6	Krawinkel et al. (1989)	24	van't Veer et al. (1997)
7	Kanja et al. (1992)	25	Gómez-Catalán et al. (1995)
8	Waliszewski et al. (1996b)	26	Dewailly et al. (1999)
9	Waliszewski et al. (1998)	27	van der Ven et al. (1992)
10	van der Ven et al. (1992)	28	Stellman et al. (2000)
11	López-Carrillo et al. (1996)	29	Zheng et al. (1999)
12	Waliszewski et al. (2001)	30	Alawi et al. (1992)
14	Rivero-Rodriguez et al. (1997)	31	Bagga et al. (2000)
15	Waliszewski et al. (2004)	32	Aronson et al. (2000)
17	Skaare & Polder (1990)	33	Minh et al. (2001)
18	Ahmad et al. (1988)	34	Sanz-Gallardo et al. (1999)
19	Gómez-Catalán et al. (1993)	35	Smeds & Saukko (2001)

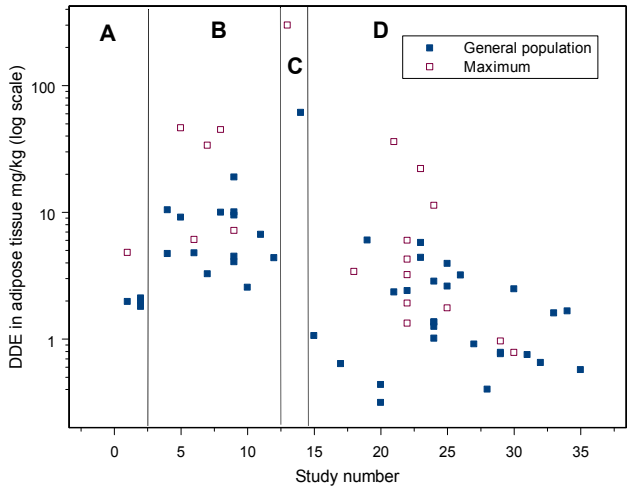

Fig. 4. *p,p'*-DDE as mean, median or geometric mean and maximum concentration, as mg/kg fat, reported in 31 papers on adipose tissue. Notes as for Figure 3.

Although concentration in fat has been seen as the preferred measure of exposure to the lipid-soluble, persistent DDT metabolites, it is measured less frequently than blood serum because of practical difficulties in obtaining samples and cost. Many measurements of concentrations in fat have been conducted on adipose tissue obtained at autopsy, some sampled during caesarean delivery and a few obtained by biopsy (the last exclusively from those spraying DDT for IRS). Both allocation to sector in the figures and the different sources of adipose tissue samples could influence how representative individual samples are of population exposure. One independent check can be made; a lower ratio of DDE to total DDT is generally held to be indicative of recent exposure to DDT (Fig. 5). This ratio is not absolute; "total DDT" is the sum of all compounds measured rather than all possible compounds and thus varies from study to study. However, measurements of most populations with no recent exposure suggest that the ratio is 0.8 plus.

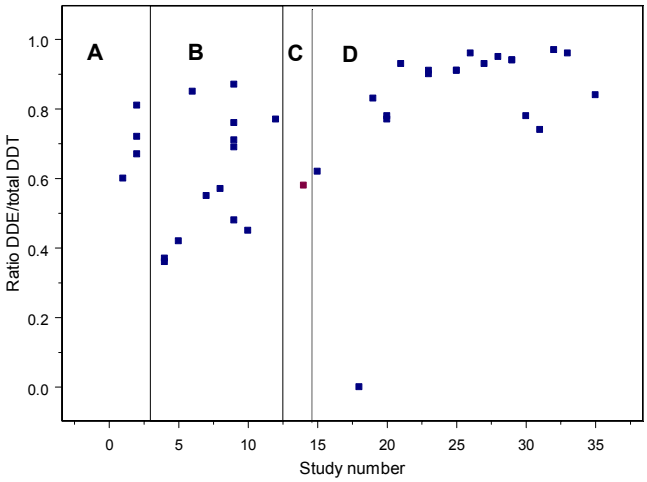

Fig. 5. Ratio of DDE to total DDT in studies on adipose tissue. Notes as for Figure 3.

The majority of points in sector D have high DDE to total DDT ratios. The few points below 0.8 may represent some continued use of DDT. Similarly, the higher points in sectors A and B will represent uneven exposure to DDT, even with widespread use in countries. Use in either IRS or agriculture does not expose all of the

population. Figure 5 suggests that allocation to sectors is reasonable. Comparison of Figure 5 with Figures 3 and 4 suggests that much of the DDT stored in fat is converted to DDE within 2 years following cessation of DDT usage (at least 2 years after banning was the criterion for designating a country as not using DDT). Probable downward trends in sector D of both Figures 3 and 4 could reflect loss of stored DDT/DDE over time following stoppage of DDT use. Few time-series studies are available on DDT/DDE concentrations in fat, and the x-axes of Figures 3 and 4 are in date order of sampling, but do not represent true time. There are too few data points in sector B, representing controlled studies on spray operators using DDT for IRS, to allow firm conclusions to be drawn. The general downward trend in this sector is likely to reflect decreasing use of DDT in agriculture rather than use in IRS. The one controlled study on spray operators clearly shows increased concentrations in adipose tissue following occupational exposure to DDT compared with non-exposed (the red squares in Figure 3 compared with blue squares in the same study).

The mean concentration of total DDT in adipose tissue in sector B studies from Figure 3 (general population living in malarial countries using DDT for vector control) is 15.7 µg/g fat (range 4.1–38.6 µg/g fat), with the median value at 13.7 µg/g fat and the highest maximum reported as 176.5 µg/g fat. In the single occupational study measuring DDT in abdominal adipose tissue of spray operators, the geometric mean concentration was 104.5 µg/g (Rivero-Rodriguez et al., 1997).

More usually, DDT exposure is monitored using concentration in blood rather than adipose tissue. A number of studies examined the relationship between blood DDT and adipose tissue DDT to justify the simpler procedure as a reasonable measure.

Concentrations of total DDT and *p,p′*-DDE in blood are plotted in Figures 6 and 7. Some studies are plotted in the figures but not expanded upon in the text; these gave values only or covered exposures other than for mosquito control. Results are categorized according to the use of DDT at the time of sampling, geography, exposure population, etc., as detailed in the notes to the figures. More than one plot is possible from each study (e.g. different geographical regions or areas; non-exposed and exposed populations in

a single paper). Some studies report both total DDT (Fig. 6) and DDE (Fig. 7), but some report one or the other. Some studies report multiple populations in similar geographical areas (e.g. different regions in the same country); for these, all individual determinations are plotted.

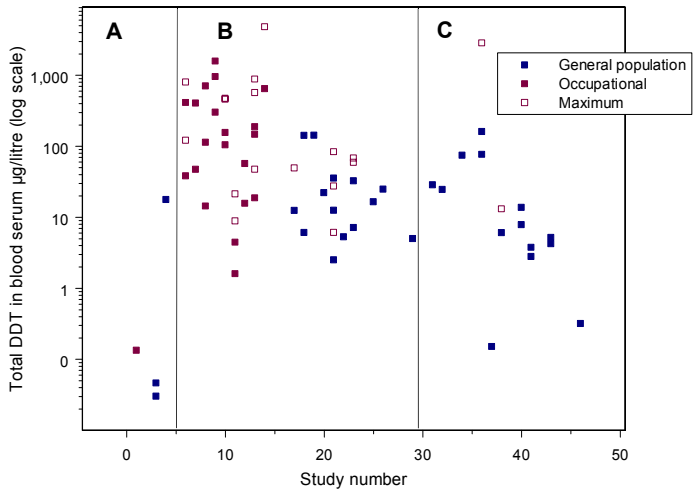

Fig. 6. Total DDT (sum of all compounds measured) as mean, median or geometric mean and maximum concentration, as µg/l blood serum, reported in 41 studies.

Notes:
A: Studies from countries using DDT, but results not related to IRS; studies are arranged in date order of sampling from left to right; dates are 1968, 1968 and 1998, respectively.
B: Studies from countries with endemic malaria using DDT for IRS, although use in agriculture cannot be excluded. Occupational exposure is plotted to the left of sector B with red squares; general population exposure is plotted to the right with blue squares. Studies are arranged in date order of sampling from left to right; dates are between 1979 and 2004 for occupational exposure and between 1986 and 2001 for general population exposure.
C: Studies from countries not using DDT for either agriculture or IRS; studies are arranged in date order of sampling from left to right; dates are between 1975 and 1996.

Study number: References for all studies are tabulated in Table 42.

Table 42. Study numbers and references for Figures 6, 7, 8, 9 and 10

No.	Reference	No.	Reference
1	Perron & Barrentine (1970)	28	López-Carrillo et al. (2001)
3	Perron & Barrentine (1970)	29	Delgado et al. (2002)
4	Korrick et al. (2001)	30	Ayotte et al. (2001)
7	Misra et al. (1984)	27	Waliszewski et al. (1999)
8	Carvalho (1991)	31	Ataniyazova et al. (2001)
9	Mazzarri & Mazzarri de Lauschner (1989)	32	Dorea et al. (2001)
10	Bouwman et al. (1991a)	33	Waliszewski et al. (2003b)
11	Chandra et al. (1992)	34	Waliszewski et al. (2004)
12	Minelli & Ribeiro (1996)	35	Polishuk et al. (1977)
13	Dores et al. (2003)	36	Krauthacker et al. (1980)
14	Dalvie et al. (2004c)	37	Kreiss et al. (1981)
15	Subramaniam & Solomon (2006)	38	Skaare et al. (1988)
18	Yáñez et al. (2002)	39	Krauthacker (1991)
19	Kanja et al. (1992)	40	Laden et al. (2001)
20	Bouwman et al. (1991b)	41	Porta et al. (1999)
21	Bouwman et al. (1994)	42	Demers et al. (2000)
22	Romieu et al. (2000)	43	Hoppin et al. (2000)
23	Rugama et al. (1993)	44	Weiderpass et al. (2000)
24	López-Carrillo et al. (1997)	45	Shen et al. (2008)
25	Schecter et al. (1997)	46	Beard et al. (2000)
26	Mendonça et al. (1999)	47	Soliman et al. (1997)

It is clear from the plotted data that studies reported a wide range of exposures covering over 4 orders of magnitude. Although the occupationally exposed tend overall to have higher concentrations of both total DDT and DDE compared with the general population, there are many individual results from the general population that are even higher than the median value for the occupationally exposed. For the few studies reporting raw data (exclusively blood serum concentrations), there is a highly skewed distribution between individuals in the sampled population, with a few having very high concentrations. Median or geometric mean is

therefore a better indication of population exposure than arithmetic mean.

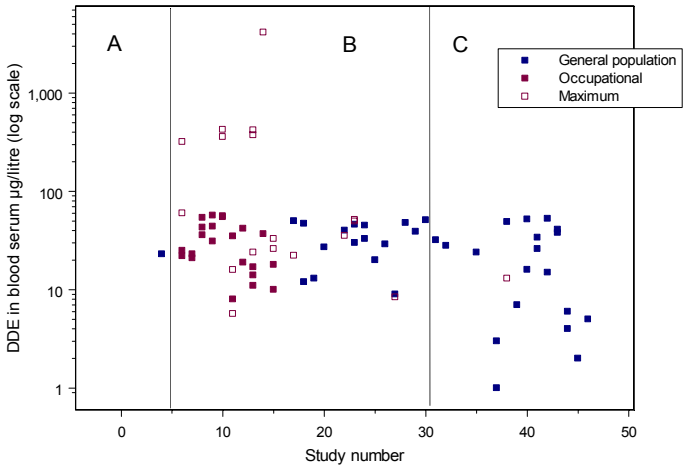

Fig. 7. *p,p'*-DDE as mean, median or geometric mean and maximum concentration, as µg/l blood serum, reported in 41 studies. Notes as for Figure 6.

Blood concentrations have been expressed differently by different study authors. A few studies report concentrations in whole blood, particularly those done early (in the 1960s and early 1970s); these have not been included in the figures. A very few report concentrations in plasma; these have been plotted but not used in calculating means. The rest report concentrations in blood serum or concentrations in blood lipids or both. It has been argued that blood lipid concentration is a better measure, but it has also been argued that correcting for blood lipid masks some effects (see discussion in section 3.2). Many more studies report concentrations in blood serum without lipid adjustment than with lipid adjustment. To avoid too many conversions and to include as much of the total data set as possible, Figures 6 and 7 plot blood serum concentrations without lipid conversion. A few conversions from data reported as micrograms per gram lipid were made; for most of these, blood lipid measurements were not reported in the papers, and an average conversion ratio from those studies that reported both corrected and uncorrected data was used for the conversion. There are clear

uncertainties in the conversion process, but results are unlikely to affect overall conclusions.

As for adipose tissue, studies have been allocated to sectors in the figures representing usage patterns of DDT. Sector B plots results from countries having endemic malaria; reported concentrations could have come from IRS, agriculture or both. In contrast to adipose tissue, larger numbers of studies are available for occupational exposure, although this is sometimes IRS and sometimes agricultural. For blood concentrations, sector B has occupational exposure plotted to the left (red squares) and general population exposure to the right (blue squares); each has studies plotted in date order (date of sampling, not date of publication of the report) from left to right. As with adipose tissue, allocation to sectors was checked by plotting the ratio of DDE to total DDT for those studies that reported it (or it could be calculated). Results are presented in Figure 8.

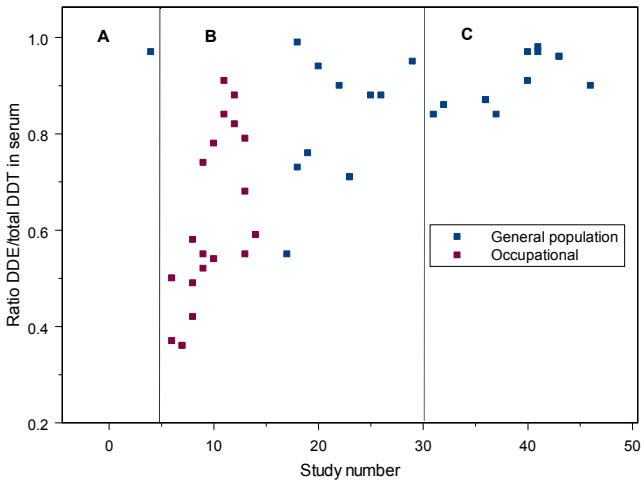

Fig. 8. Ratio of DDE to total DDT in studies on blood serum for the occupationally exposed and general populations. Notes as for Figure 6.

Sector B can be further categorized. Both occupational and general population controlled studies looked at different populations comparing higher and lower exposed subpopulations. For occupational studies, those directly spraying were classified as highest

exposure groups, whereas others involved in spraying operations were classified as lower exposure groups; "controls" were generally administrative workers from the IRS operations. In general population studies, the highest exposure class would live in treated houses in intensively sprayed areas, lower exposure would be less intensively sprayed and non-exposed would live in non-malarial areas. A few general population studies were less controlled. Results for sector B studies are further classified in Figures 9 and 10.

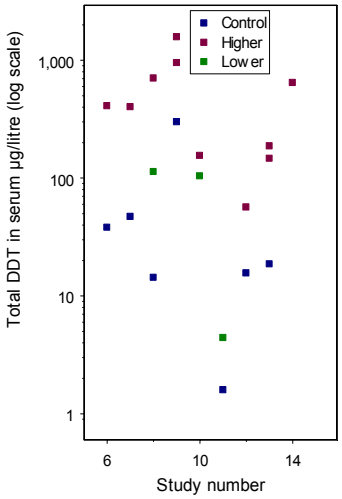

Fig. 9. Total DDT (mean, median or geometric mean) in blood serum for the occupationally exposed (spray operators) and non-exposed from the same studies

Study number: References for all studies are tabulated in Table 42.

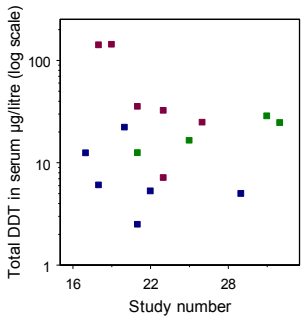

Fig. 10. Total DDT (mean, median or geometric mean) in blood serum for general population exposed via IRS or agriculture

Study number: References for all studies are tabulated in Table 42.

12.6 Summary of adult occupational and residential exposure

As expected, all studies comparing occupationally highly exposed groups with others showed elevated concentrations in both blood serum and adipose tissues. A higher proportion of DDT in both blood and adipose tissue is indicative of recent exposure to the pesticide. Ratios of DDE to total DDT typically rise with time following exposure to DDT, stabilizing at around 0.8.

Studies attempting to show relationships between duration of occupational exposure and concentrations of DDT or DDE did not always succeed. Most studies showed increased residues of DDT and/or DDE in body tissues with longer time periods working with the insecticide for IRS for malaria control. This relationship is not straightforward. Correlation with years worked in spraying operations has been reported in some studies (e.g. Mazzarri & Mazzarri de Lauschner, 1989; Carvalho, 1991). More complex indices of exposure, scoring not only years worked but other measures of exposure (relative time on different jobs, etc.), have correlated well with measured concentrations in other studies (Rivero-Rodriguez et al., 1997). One major study (Dalvie et al., 2004c) failed to demonstrate a relationship and attributed this to poor information on short-term and medium-term exposure. Detailed exposure information is needed to establish correlations, and this has seldom been available. Jobs performed by personnel involved in IRS tend to rotate, and this is true from studies in many different parts of the world (although

not all); job rotation is a major factor contributing to difficulties in establishing relative exposure. In one study (Mazzarri & Mazzarri de Lauschner, 1989), clear correlation was established with tonnage used in different regions of a country and concentrations in spray operators' tissues.

All occupational studies indicated that referents (often administrative workers from the IRS programmes) showed lower exposure. "Controls" from agriculture, spraying other pesticides, also showed very low residues of DDT or its metabolites.

In the single occupational study measuring DDT in abdominal adipose tissue of spray operators, the geometric mean concentration was 104.5 µg/g (Rivero-Rodriguez et al., 1997). The mean concentration of total DDT in adipose tissue in residents of areas where spraying of DDT for IRS occurs is 25.33 µg/g fat (range 5.1–38.6 µg/g fat), with the median value at 25.92 µg/g fat and the highest maximum reported as 176.5 µg/g fat. Much of the DDT stored in fat is converted to DDE within 2 years, as evidenced by measurements made following cessation of the use of DDT in countries.

For occupational exposure, both high exposure and "control" categories show a highly skewed distribution of total DDT concentrations in blood serum. "Controls" in these studies are spray programme personnel who are not directly exposed through IRS (technicians in laboratories, administration workers). The arithmetic mean value of the study estimates of central tendency (median or geometric means) is 517.5 µg/l (median 521.9 µg/l; range 56.5–1572 µg/l) for the highest exposure group. The highest exposure groups had all been exposed through the use of DDT for disease vector control; all but one had definitely used DDT in IRS.

Exposure of residents is via the immediate environment of treated houses, most likely through skin contact with contaminated surfaces and ingestion via contamination of food in homes, food produced in the homesteads and hand-to-mouth activity (house walls, floor and soil outside the dwelling).

Those living in houses treated with DDT by IRS showed elevated DDT and DDE concentrations in blood serum compared

with residents in untreated houses, although concentrations were lower than those of spray operators. The relationship between application of DDT sprays for IRS and subsequent accumulation of residues by exposed populations may not be a simple one; a longitudinal study showed changes over a year, but no direct correlation between spraying time and concentrations in those living in sprayed houses. Elevated residues in blood and adipose tissue, where measured, are restricted to those intimately associated with sprayed housing. Even residents in adjacent communities can show markedly different concentrations of DDT and DDE.

For the general population living in malarial countries but not directly exposed through IRS, the mean concentration of total DDT in blood serum is 31.9 µg/l (not adjusted for lipid content; median 18.8 µg/l; range 2.49–170 µg/l). For residents of IRS-treated homes, the mean concentration of total DDT in blood serum is 63.7 µg/l (not adjusted for lipid content; median 82.8 µg/l; range 7.1–142 µg/l). Newborn infants had blood concentrations approximately 40% of those in maternal blood for both DDT and DDE. Only three studies reported concentrations of DDE in umbilical cord blood in areas likely to be exposed via IRS, too few to give reliable mean estimates for risk assessment. However, the mean DDE level in cord blood relative to maternal blood is 47% (median 42%; range 32–62%) across eight studies globally where paired samples are reported. Umbilical cord blood values for the risk characterization have therefore been estimated as 47% of the values in serum.

A single study, Aneck-Hahn et al. (2007), reported much higher concentrations of DDT in blood serum for men living in treated houses in Limpopo Province, South Africa, with a mean total DDT concentration of 2012 µg/l blood serum (maximum 7265 µg/l). Men living in non-treated houses in the same study had mean blood serum concentrations of 697 µg/l. These values are noted, but were not included in the calculations of means.

Concentrations of DDE tend to increase with age; this has particularly been noted in highly exposed populations. In young children, blood levels can fall throughout childhood from a high attained during breastfeeding. However, this has been studied systematically only in one area, South Africa, with extended periods of breastfeeding (up to 2 years).

Mean concentrations in different body tissues for different populations are summarized in Table 43.

Table 43. Exposures of different populations, measured as total DDT or DDE concentrations[a]

Tissue	Occupational exposure (IRS spray operators)		Residents in IRS-treated homes[b]		General population[c]	
	Total DDT	DDE	Total DDT	DDE	Total DDT	DDE
Blood serum (lipid adjusted)	Mean: 77.8 (8.7–241.1) µg/g lipid	Mean: 41.8 (7.1–131.8) µg/g lipid	Mean: 9.8 (1.09–21.8) µg/g lipid	Mean: 19.7 (0.8–77.9) µg/g lipid	Mean: 5.0 (0.38–26.1) µg/g lipid	Mean: 1.0 (0.2–3.18) µg/g lipid
	Median: 58.6 µg/g lipid	Median: 25.6 µg/g lipid	Median: 5.18 µg/g lipid	Median: 9.7 µg/g lipid	Median: 0.93 µg/g lipid	Median: 0.77 µg/g lipid
Umbilical cord blood	—	—	Mean: 29.9 µg/l	Mean: 60.3 µg/l	Mean: 15.0 µg/l	Mean: 3.1 µg/l
	—	—	Mean: 4.6 µg/g lipid	Mean: 9.3 µg/g lipid	Mean: 0.44 µg/g lipid	Mean: 0.36 µg/g lipid

[a] Mean is the arithmetic mean of the set of individual study estimates of central tendency values (median or geometric mean). The range is from the lowest to highest estimate of central tendency in each data set. Medians are also given for the same data sets. Details of the methods used to convert the data to a common metric are presented in section 11.2.1.

[b] This population lives in areas where IRS is used extensively; they have been sampled at local clinics, and many, but not all, studies specifically indicate that they were selected because they lived in IRS-treated homes.

[c] The "general population" is people living in malarial countries where IRS is used, but they are identified in the studies as having no direct exposure through IRS; they live in unsprayed areas and will be subject to general environmental exposure.

Models attempting to link concentrations in exposed populations with factors outlined above have failed to explain more than around 40–50% of the variation, so other factors must be involved. Many study authors assume that the remaining DDT exposure is via food, often citing studies from developed countries as evidence. It seems reasonable to suppose that exposure through food is a factor. However, no direct evidence is available to prove that food exposure is a significant factor in overall DDT/DDE residues, as studies have not concurrently measured both concentrations in body tissues and concentrations in total diet. Although

food should be removed from houses prior to IRS treatment, food introduced into houses after IRS may become contaminated. As well, food produced adjacent to treated houses in gardens, including free-ranging chickens foraging there, becomes significantly contaminated with DDT and its metabolites.

12.7 Breast milk

Infant exposure can be directly related to intake through breast milk. Although many studies have been conducted on levels of DDT and its metabolites in breast milk, few have systematically looked at human milk related to IRS spraying for malarial vector control. The latter are reviewed first.

12.7.1 Known residents of sprayed houses

Bouwman et al. (1990a) conducted four cross-sectional studies sampling breast milk from mothers visiting baby clinics in two areas of South Africa. One area (Mseleni) had regular IRS for control of malarial vectors, and the other (Port Shepstone) had no use of DDT either for vector control or for agriculture. Sampling took place in 1986–1987; DDT use in agriculture had been banned in South Africa since 1976. One hundred and thirty-two samples were taken in the malarial area, and 88 in the non-malarial area. There were no differences in parity, maternal age, infant age or percentage of milk fat between the two groups. The exposed group was sampled 4 times: November 1986 and March (just prior to IRS), June and November 1987. The non-malarial area group was sampled similarly, except that the final sampling was not conducted (clear differences were seen between the two groups after the third sampling period). Results are presented in Table 44. Log-transformed data were used in statistical analyses. Levels of DDE were significantly lower in the non-exposed group than in the exposed group, although DDE was detected in all samples of both groups. Per cent DDT (i.e. DDT/total DDT) was significantly higher in the exposed group; only 19 of the non-exposed samples had detectable DDT. The highest value for per cent DDT occurred immediately after spraying in March, as expected. During the following months, DDT was metabolized or excreted (milk production is a highly significant route of elimination of DDT in women), such that in June 1987, the per cent DDT was not different

from either the starting or finishing values (November 1986 and 1987).

Table 44. Levels of DDT and DDE in milk fat from an exposed and non-exposed area of South Africa

	Concentration in milk fat (µg/g)		
	Mean ± SD	Median	Range
November 1986			
Exposed n = 39			
DDE[a]	6.86 ± 4.95	5.90	0.60–18.5
DDT[a]	4.89 ± 2.47**	4.70	0.42–11.00
Total DDT	12.21 ± 7.38	12.63	1.05–30.00
% DDT	42.57 ± 8.37*	42.54	25.00–67.20
Non-exposed n = 25			
DDE	0.61 ± 0.38	0.54	0.10–1.56
DDT	0.05 ± 0.07	0.04	ND–0.36
Total DDT	0.66 ± 0.41	0.55	0.12–1.56
% DDT	7.5 ± 8.9	7.3	ND–24.0
March 1987			
Exposed n = 35			
DDE	7.06 ± 5.95	5.15	0.30–30.80
DDT	6.59 ± 3.57	5.30	0.70–18.50
Total DDT	13.79 ± 9.08	14.11	1.00–44.20
% DDT	50.87 ± 8.60**	50.30	27.60–66.60
Non-exposed n = 29			
DDE	0.92 ± 0.95	0.59	ND–4.73
DDT	0.04 ± 0.05	0.02	ND–0.23
Total DDT	0.96 ± 0.97	0.80	ND–4.79
% DDT	4.2 ± 5.2	2.8	ND–4.8
June 1987			
Exposed n = 28			
DDE	10.95 ± 10.96	5.50	0.50–46.90
DDT	7.93 ± 5.80	6.40	1.40–28.80
Total DDT	19.45 ± 16.10	19.45	2.00–59.30
% DDT	45.85 ± 9.84	45.79	19.20–72.60
Non-exposed n = 34			
DDE	0.50 ± 0.46	0.38	0.03–2.08

	Concentration in milk fat (µg/g)		
	Mean ± SD	Median	Range
DDT	0.03 ± 0.03	0.02	ND–0.23
Total DDT	0.52 ± 0.47	0.41	0.03–2.10
% DDT	5.3 ± 5.6	4.07	ND–21.70
November 1987[b]			
Exposed n = 30			
DDE	9.98 ± 7.99	7.70	1.30–36.90
DDT	7.85 ± 4.80**	6.50	1.22–20.30
Total DDT	18.34 ± 12.60	18.34	2.70–57.60
% DDT	43.7 ± 10.04**	44.35	3.90–57.50

ND, not detected; * $P < 0.001$ and ** $P < 0.01$ in two-tailed *t*-test of log-transformed data for differences between surveys

[a] Isomers measured not stated; a personal communication to IPCS from the principal author indicated that they were *p*,*p*′-isomers.

[b] The non-malarial area (non-exposed) was not sampled in November 1987 because clear differences had been identified at the previous sampling period.

Source: Bouwman et al. (1990a)

Bouwman et al. (1992) collected milk samples from mothers ($n = 23$) attending well-baby clinics at Mseleni hospital, northern Natal, South Africa, during 1987. Blood samples were taken from their babies aged 0–2 years. All mothers and offspring lived in mud-constructed dwellings and were resident in the area, which was treated annually by IRS to control malarial vectors. Total DDT levels in infant blood increased with infant age.

There was good correlation between the per cent DDT in breast milk and infant blood, but the proportion was higher (by 5.4%) in infant blood. Multiple regression was used to model the levels of total DDT, DDT and DDE in infant blood. The best models showed a relationship with infant age and parity; inclusion of maternal age reduced the accuracy of the models.

In a follow-up to their earlier studies (Bouwman et al., 1990a, 1992), Bouwman et al. (2006) collected milk samples in 2000, 1 year after resumption of the use of DDT in malaria control in South Africa (DDT was not used between 1995 and 2000). Again, study areas were selected with high and low exposure from IRS: Jozini and Mkuzi (high) and Kwaliweni (low). Highly significant differences in concentrations of DDT and its metabolites were found

between primiparous and multiparous mothers in the high-exposure towns, but there was no significant difference in the low-exposure town. Results are summarized in Table 45.

Table 45. Selected results of concentrations of DDT and its metabolites in breast milk from mothers in South Africa

	Mean (± SD) concentration in breast milk (range) (μg/g milk fat)			
	p,*p*′-DDE	*p*,*p*′-DDD	*p*,*p*′-DDT	Total DDT
Jozini Primiparous *n* = 33	4.17 ± 3.83 (0.16–13.78)	0.09 ± 0.086 (0.013–0.44)	2.00 ± 2.04 (0.08–9.69)	6.24 ± 5.6 (0.27–22.24)
Jozini Multiparous *n* = 52	1.87 ± 1.51 (0.12–6.64)	0.1 ± 0.14 (0.015–0.96)	1.40 ± 0.91 (0.14–4.18)	3.36 ± 2.22 (0.45–10.12)
Mkuzi Primiparous *n* = 4	2.12 ± 3.33 (0.11–7.09)	0.056 ± 0.016 (0.045–0.067)	1.64 ± 2.16 (0.11–3.17)	2.96 ± 4.91 (0.22–10.32)
Mkuzi Multiparous *n* = 22	0.83 ± 0.97 (0.03–3.71)	0.016 ± 0.015 (0.005–0.055)	0.31 ± 0.35 (0.024–1.49)	1.15 ± 1.24 (0.11–4.46)
Kwaliweni Primiparous *n* = 16	0.41 ± 0.39 (0.08–1.51)	0.05 ± 0.07 (0.002–0.27)	0.27 ± 0.34 (0.04–1.31)	0.73 ± 0.69 (0.14–2.15)
Kwaliweni Multiparous *n* = 25	0.57 ± 0.41 (0.03–1.66)	0.06 ± 0.07 (0.014–0.29)	0.25 ± 0.27 (0.004–1.24)	0.87 ± 0.60 (0.11–2.17)

Source: Bouwman et al. (2006)

Higher mean *p*,*p*′-DDT levels in the exposed population coupled with a higher proportion of DDT in the total indicated recent exposure, which was deemed to be largely via IRS. Further, although DDE levels were 4 times higher in the exposed towns than in the non-exposed town, DDT levels were 7 times higher, again suggesting recent exposure via vector control. Of note also was the much higher concentration in milk taken by first-born infants in areas of high exposure compared with all other groups, indicating an elevated risk for these children. Although no straightforward comparison can be made between earlier and later studies, a general comparison suggested that concentrations of DDE, DDD and DDT were lower in the 2006 study by 66%, 75–80% and 60%,

respectively, over a 14-year period. A cautious conclusion was made that not enough time had elapsed for DDT concentrations to stabilize in milk following its reintroduction.

Sereda et al. (2009) compared water, bovine milk and IRS for malarial vector control as a possible source of DDT and its metabolites in human breast milk in KwaZulu-Natal (formerly known as Natal), South Africa. Two sampling areas were studied; one (Makatini Flats) was treated regularly with DDT as IRS, and participating mothers confirmed minimal residence of 5 years, whereas the second (Lebombo Mountains) was not treated regularly with DDT. Food consumption was comparable between the two groups, which both used the same market town to buy food (with some supplement from home gardens). All mothers had detectable levels of both *p*,*p*′-DDT and *p*,*p*′-DDE in their breast milk. From Makatini Flats, all samples from primiparous mothers had higher levels of DDT compounds compared with multiparous mothers, with results for *p*,*p*′-DDE, *p*,*p*′-DDT and *o*,*p*′-DDE being statistically significant. Mean DDT residues in bovine milk were significantly lower than those in human milk by 1–2 orders of magnitude. All mean concentrations in water were at least 4 orders of magnitude lower than mean concentrations in bovine milk and up to 6 orders of magnitude lower than those in breast milk. Non-metric multidimensional scaling was used to plot similarities and differences in three-dimensional space; this avoids assumptions on linearity of relationships between variables. The DDT compounds, presumably derived primarily from IRS, showed a close relationship with human breast milk, but little relationship with bovine milk sampled in the same area. Multiple routes of exposure were indicated, but most uptake and transfer to breast milk were judged to be via chronic skin contact (both within the houses and in the immediate vicinity) and inhalation.

12.7.2 General population living in areas using indoor residual spraying

Studies reported in section 12.7.1 sampled milk from mothers who were definitely resident in homes treated with DDT as IRS. In this section, sampling occurred in areas where DDT IRS was taking place, but there is no direct evidence that sampled mothers were resident in treated homes.

Elvia et al. (2000) sampled milk from mothers attending baby clinics at state medical facilities in Mexico City, Cuernavaca City and Morelos (representative of rural areas surrounding Cuernavaca City). All mothers represented the poorer sector of society. Sampling took place while DDT was still being used actively in IRS spraying for vector control in Mexico (samples collected 1989–1990). Homes treated with pesticides were 21%, 42% and 73% of the total for the three sampling areas, respectively (it is unclear whether all of this "treatment" is IRS with DDT). Median and maximum concentrations are given in Table 46.

Table 46. Concentrations of DDE and DDT in breast milk in urban and rural Mexico

Compound	Concentration in milk (µg/g fat)					
	Mexico City (*n* = 79)		Cuernavaca (*n* = 26)		Rural Morelos (*n* = 42)	
	Median	Maximum	Median	Maximum	Median	Maximum
p,*p*′-DDE	2.49	50.83	4.28	22.09	13.32	49.71
p,*p*′-DDT	1.83	3.16	0.66	7.93	3.13	23.75

Source: Elvia et al. (2000)

Although DDE, representing longer-term exposure to DDT, was present in all samples from all areas, *p*,*p*′-DDT, representing more recent exposure, was detected in most people from the three regions of Mexico (96%, 92.3% and 99%, respectively); even urban populations showed recent exposure to DDT from some source. Factors determining geometric mean concentrations of *p*,*p*′-DDE and *p*,*p*′-DDT in milk were examined independently. DDE concentrations in milk were statistically significantly lower when mothers had two children rather than one child. Both DDT and DDE levels were higher in milk of mothers resident in homes treated with insecticides. The presence of a family member who worked with insecticides (details not provided) also consistently increased DDT and DDE levels in milk, but none of the differences were statistically significant. Correction of geometric means for these three factors (homes treated/untreated, family member working with pesticides, number of children) generated geometric means for the three sampling areas that explained the variation in DDE and DDT levels by only 40% and 37%, respectively (Table 47). Other factors must therefore explain the remaining variability. Although the authors suggested food as the other major source of DDT and its metabolites, no data supporting this view were presented.

Table 47. Concentrations of DDT and DDE in mothers' milk in different areas of Mexico adjusted to account for number of children,whether the house was treated with pesticides and whether a family member worked with pesticides

Location	Geometric mean concentrations (95% CI) in mothers' milk (µg/g fat)	
	p,*p*′-DDE	*p*,*p*′-DDT
Mexico City	2.97 (2.26–3.90)	0.39 (0.20–0.55)
Cuernavaca	5.30 (0.27–3.50)	0.99 (0.50–1.60)
Rural Morelos	15.78 (10.43–23.87)	3.55 (2.14–5.89)

Source: Elvia et al. (2000)

Minh et al. (2004) sampled milk from city-dwelling mothers in Viet Nam (Hanoi, $n = 42$; Ho Chi Minh City, $n = 44$). The route of exposure, via IRS or agriculture, is unclear, although the authors indicated that both were possible until recently in Viet Nam. Mean ages were 29 and 27 years. The mean number of children was 1.6 for both cities; however, the numbers of children ranged from 1 to 7 and from 1 to 5, respectively. Concentrations of total DDT in milk declined with number of children, although only significantly over three children in Hanoi and two in Ho Chi Minh City.

The authors compared their data with those from a previous survey in Viet Nam (Schecter et al., 1989); mean concentrations of *p*,*p*′-DDT and *p*,*p*′-DDE declined from 4.7 to 0.3 µg/g milk fat and from 6.7 to 2.0 µg/g milk fat, respectively, between 1989 and 2001. Mean daily intake of total DDT for a 5 kg infant was calculated at 7.0 (range 0.8–27) µg/kg bw per day and 11.0 (range 1.3–110) µg/kg bw per day for Hanoi and Ho Chi Minh City, respectively.

Devanathan et al. (2009) reported concentrations of DDT and its metabolites in human breast milk from four different cities in India. For *p*,*p*′-DDE, New Delhi ($n = 21$) had a mean concentration of 2.1 (range 68–10 000) µg/kg milk lipid; Mumbai ($n = 26$) had a mean of 380 (39–1300) µg/kg milk lipid; Kolkata ($n = 17$) had a mean of 920 (110–2300) µg/kg milk lipid; and Chennai ($n = 12$) had a mean of 1100 (640–2800) µg/kg milk lipid. Mean values for total DDT were 1500, 450, 1100 and 1200 µg/kg milk lipid for the four cities, respectively. A more detailed study of the Chennai area (Subramanian et al., 2007) compared concentrations in breast milk obtained from the central city area, a waste site within the city limits,

a farming town in the area and a remote fishing village with high consumption of fish. *p,p′*-DDE concentrations in milk from primiparous mothers were 1100 ± 770 (SD) (range 640–2800) µg/kg milk lipid, 520 ± 130 (220–650) µg/kg milk lipid, 280 ± 120 (190–450) µg/kg milk lipid and 210 ± 150 (45–410) µg/kg milk lipid, respectively. Both studies remarked on the higher concentrations in urban areas compared with rural ones. The authors suggested use of DDT in urban areas for disease vector control and greater consumption of animal products in the more prosperous urban areas as possible explanations.

To put the IRS studies in context, results from 144 studies on DDT and DDE levels in breast milk have been plotted (Figs. 12 and 13). Some studies are plotted in the figures but not expanded upon in the text; these gave values only or covered exposures other than for mosquito control. Results are categorized according to the use of DDT at the time of sampling, geography, exposure population, etc., as detailed in the notes to the figures. More than one plot is possible from each study (e.g. different geographical regions or areas; non-exposed and exposed populations in a single paper). Some studies report both total DDT (Fig. 12) and DDE (Fig. 13), but some report one or the other. Some studies report multiple populations in similar geographical areas (e.g. different regions in the same country); for these, all individual determinations are plotted.

Studies have been allocated into four categories representing usage patterns of DDT. Those from countries having endemic malaria have been grouped (sector B of both figures); reported concentrations should have come from IRS, but possible agricultural use cannot be excluded. Authors regularly state that DDT is used for either or both purposes but do not indicate if their samples can be attributed to either source. Sector C covers studies done immediately following cessation of the use of DDT in IRS. Sector D of the figures represents studies done on breast milk after DDT use for agriculture or vector control had stopped in the countries for at least 3 years, whereas sector A covers studies conducted while DDT was still used, but for agriculture, not for vector control; in both cases, allocation of studies to sectors was largely based on countries' statements to WHO on DDT use (WHO/UNEP DDT Information System, http://www.chem.unep.ch/ddt/ProfileCriteria.html). Allocation to sectors has uncertainties. Cessation of DDT use did

not occur on a single date for most countries; many countries banned use in agriculture but retained use for public health until a later date.

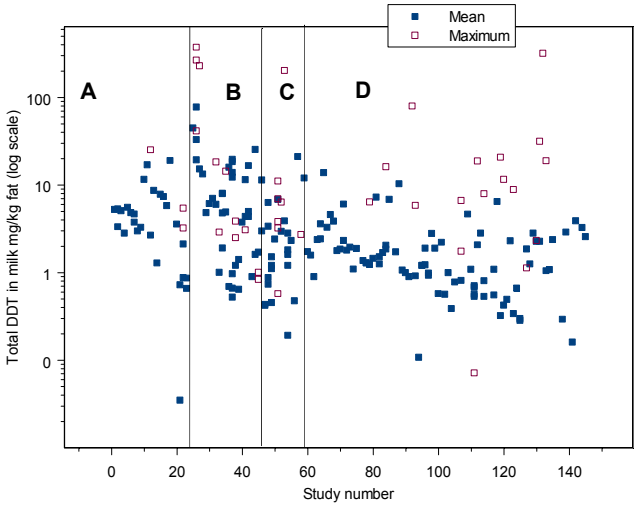

Fig. 12. Total DDT (sum of all compounds measured) as mean, median or geometric mean and maximum concentration, as µg/g milk fat, reported in 144 studies on breast milk.
Notes:
A: Studies from countries using DDT, but results not related to IRS; studies are arranged in date order of sampling from left to right; dates are between 1950 and 2006.
B: Studies from countries with endemic malaria using DDT for IRS, although agricultural use cannot be excluded; studies are arranged in date order of sampling from left to right; dates are between 1970 and 2000.
C: Studies from countries where samples were taken immediately following cessation of use of DDT; dates of sampling are between 1970 and 2000.
D: Studies from countries not using DDT for either agriculture or IRS; studies are arranged in date order of sampling from left to right; dates are between 1973 and 2004
Study number: References for all studies are tabulated in Table 48.

Table 48. Study numbers and references for Figures 12, 13, 14, 15 and 16

No.	Reference	No.	Reference
1	Laug et al. (1951)	74	Dillon et al. (1981)
2	Quinby et al. (1965)	75	Mes & Davies (1979)
3	Egan et al. (1965)	76	Rogan et al. (1986b)

No.	Reference	No.	Reference
4	Curley & Kimbrough (1969)	77	Krauthacker et al. (1980)
5	Ritcey et al. (1972)	78	Stacey et al. (1985)
6	Siyali (1973)	79	Eckenhausen et al. (1981)
7	Newton & Greene (1972)	80	Skaare (1981)
8	Mes et al. (1993)	81	Hernández et al. (1993)
9	Brevik & Bjerk (1978)	82	Hofvander et al. (1981)
10	Sikorski et al. (1990)	83	Stacey & Tatum (1985)
11	Miller & Fox (1973)	84	Somogyi & Beck (1993)
12	Stacey & Thomas (1975)	85	Sikorski et al. (1990)
13	Miller & Fox (1973)	87	Collins et al. (1982)
14	Siyali (1973)	88	Baluja et al. (1982)
15	Graca et al. (1974)	89	Vaz et al. (1993)
16	Prachar et al. (1993)	90	Mes et al. (1986)
17	Polishuk et al. (1977)	91	Wickström et al. (1983)
18	Ip (1983)	92	Skaare & Polder (1990)
19	Ip & Phillips (1989)	93	Skaare et al. (1988)
20	Wong et al. (2002)	94	Skaare & Polder (1990)
21	Yao et al. (2005)	95	Mes et al. (1984)
22	Kunisue et al. (2004)	96	Mes et al. (1986)
23	Leng et al. (2009)	97	Fürst et al. (1994)
25	Olszyna-Marzys et al. (1973)	98	Weisenberg et al. (1985)
26	de Campos & Olszyna-Marzys (1979)	99	Dommarco et al. (1987)
27	Winter et al. (1976)	100	Mussalo-Rauhamaa et al. (1988)
28	Albert et al. (1981)	101	Di Muccio et al. (1990)
29	Siddiqui et al. (1981a)	102	Norén et al. (1996)
30	Jani et al. (1988)	103	Mattison et al. (1992)
31	Kanja et al. (1986)	104	Mes et al. (1993)
32	Matuo et al. (1992)	105	Fürst et al. (1994)
33	Matuo et al. (1992)	106	Krauthacker (1991)
34	Ramakrishnan et al. (1985)	107	Fürst et al. (1994)
35	Kanja et al. (1992)	108	Larsen et al. (1994)
36	Bouwman et al. (1990b)	109	Sikorski et al. (1990)
37	Bouwman et al. (1990a)	110	Krauthacker (1991)
38	Tanabe et al. (1990)	111	Fürst et al. (1994)
39	Sant'Ana et al. (1989)	112	Bates et al. (1994)

No.	Reference	No.	Reference
40	Nair & Pillai (1992)	113	Üstünbas et al. (1994)
41	Schecter et al. (1989)	114	Schecter et al. (1989)
42	Elvia et al. (2000)	115	Dewailly et al. (1996)
43	Spicer & Kereu (1993)	116	Dwarka et al. (1995)
44	Chikuni et al. (1997)	117	Krauthacker (1991)
45	Paumgartten et al. (2000)	118	Alawi et al. (1992)
46	Waliszewski et al. (1996a)	119	Vaz et al. (1993)
47	Kunisue et al. (2002)	120	Norén et al. (1996)
48	Bouwman et al. (2006)	121	Duarte-Davidson et al. (1994)
49	Devanathan et al. (2009)	122	Bordet et al. (1993)
50	Kroger (1972)	123	Johansen et al. (1994)
51	Wilson et al. (1973)	124	Hernández et al. (1993)
52	Norén et al. (1996)	125	Norén et al. (1996)
53	Krauthacker et al. (1980)	126	Newsome & Ryan (1999)
54	Kunisue et al. (2002)	127	Prachar et al. (1993)
55	Minh et al. (2004)	128	Polder et al. (1998)
56	Kinyamu et al. (1998)	129	Gladen et al. (1999)
57	Stuetz et al. (2001)	130	Hooper et al. (1997)
59	Hagyard et al. (1973)	131	Hooper et al. (1997)
60	Jonsson et al. (1977)	132	Quinsey et al. (1995)
61	Vuori et al. (1977)	133	Schoula et al. (1996)
62	Wickström et al. (1983)	134	Czaja et al. (1997)
63	Barnett et al. (1979)	135	Çok et al. (1997)
64	Musial et al. (1974)	136	Shen et al. (2008)
65	Strassman & Kutz (1977)	137	Norén & Meironyté (2000)
66	Bakken & Seip (1976)	138	Konishi et al. (2001)
67	Bradt & Herrenkohl (1976)	139	Wong et al. (2002)
68	Dommarco et al. (1987)	140	Saeed et al. (2000)
69	Brevik & Bjerk (1978)	141	Kalantzi et al. (2004)
70	Norén et al. (1996)	142	Ennaceur et al. (2007)
71	Savage et al. (1982)	143	Erdoğrul et al. (2004)
72	Krauthacker et al. (1980)	144	Ejobi et al. (1996)
73	Nakayama & Aoki (1977)		

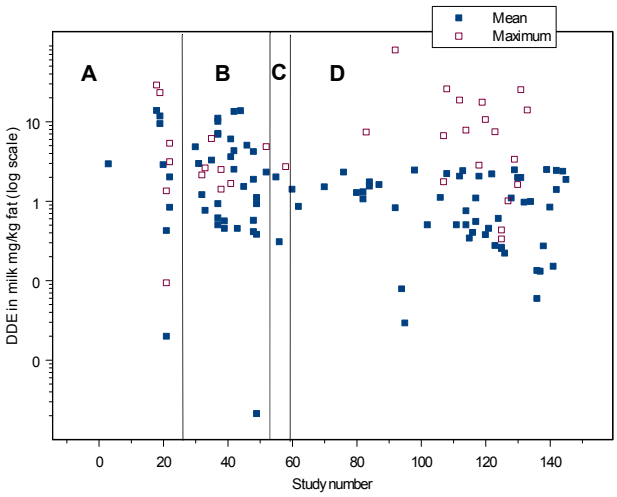

Fig. 13. *p,p'*-DDE as mean, median or geometric mean and maximum concentration, as µg/g milk fat, reported in 144 studies on breast milk. Notes as for Figure 9.

One independent check can be made; the ratio of DDE to total DDT is generally held to be indicative of recent exposure to DDT. Although this ratio could not be calculated for all studies, it could for many (Fig. 14). This ratio is not absolute; "total DDT" is the sum of all compounds measured, rather than the sum of all possible compounds, and thus varies from study to study. However, measurements of most populations with no recent exposure suggest that the ratio is 0.8 plus. A few populations in countries actively using DDT (sectors A and B) have ratios equal to or higher than this, reflecting unevenness in exposure across countries. A few populations in countries no longer using DDT have ratios that are lower than 0.8, suggesting perhaps some continued usage. Many of the studies in sector B of Figures 12 and 13 represent snapshot measurements of DDT in milk in developing countries in comparative studies with developed ones. These measurements tend to be taken in cities. Controlled studies looking at the effect of IRS tend to choose cities as non-exposed areas, with lower exposure than rural ones, where IRS tends to take place. In Figures 15 and 16, total DDT and DDE are plotted according to designation as higher,

lower or non-exposed areas; all snapshot measurements are classified as "lower". As with previous figures, points are plotted in date order of sampling from left to right. There is a general downward trend with time for total DDT, possibly reflecting gradually reduced DDT usage over the period. This is not likely to relate to use in IRS. Specific studies on IRS usage have their "higher" exposure points above the average, suggesting that the large number of snapshot measurements do reflect non-exposure.

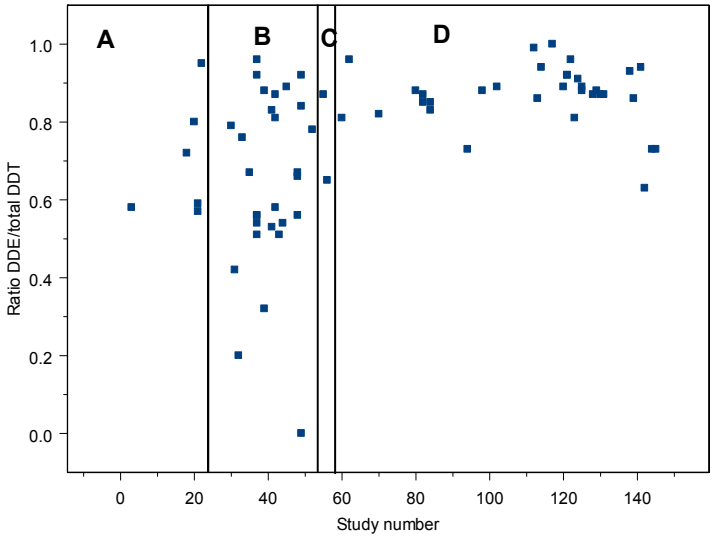

Fig. 14. Ratio of DDE to total DDT in studies on breast milk. Notes as for Figure 12.

For all sector B studies representing the general population in countries using DDT for IRS, there is a mean concentration of *p,p'*-DDE of 3.4 mg/kg milk fat (range 0.0021–13.3 mg/kg milk fat; n = 34) and a mean concentration of total DDT of 7.93 mg/kg milk fat (range 0.19–76.8 mg/kg milk fat; n = 62 in 32 studies). The mean of maximum reported total DDT concentrations is 47.29 (range 0.57–370.4) mg/kg milk fat. Controlled IRS studies discussed in the text reported average total DDT concentrations of 14.7 mg/kg milk fat in their higher exposure groups. In controlled studies, DDT exposure in first-born children is higher than that for subsequent children. Although actual values for total DDT in milk received by first-born children, in the few specific studies, were lower than these means, it

is suggested that assessment of risk globally considers adding a further factor to the calculated means to protect first-born children. Means represent population exposure. The considerable skew in all data sets relating to DDT leads to much higher exposure for a small proportion of the population. This also needs to be taken into account in risk characterization.

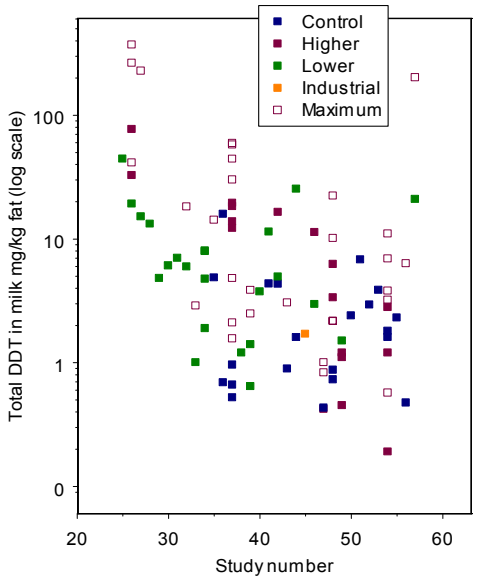

Fig. 15. Total DDT in breast milk from malarial countries actively using DDT for IRS; some agricultural use cannot be excluded. Studies from Sector B in Figure 12. Notes for Figure 12 apply.

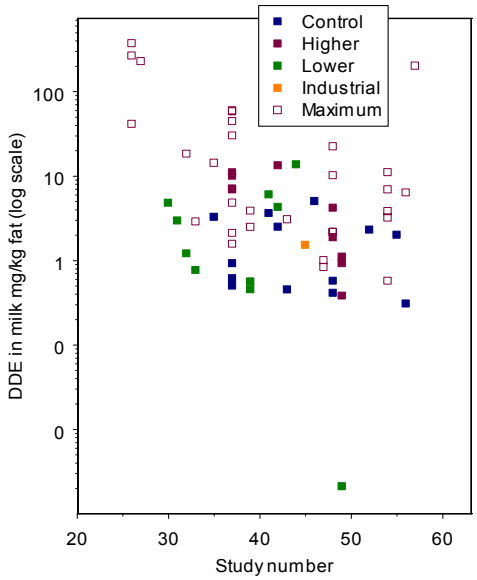

Fig. 16. DDE in breast milk from malarial countries actively using DDT for IRS; some agricultural use cannot be excluded. Studies from Sector B in Figure 12. Notes for Figure 12 apply.

12.8 Summary of infant exposure

Where studies have been done on residents of treated homes, IRS is the major source of the DDT in breast milk. Infants are exposed to DDT and its metabolites through breast milk. Lactation is a means for excretion of the DDT body burden of mothers. The concentration of DDT and its metabolites tends to be greater in breast milk produced by primiparous mothers, especially at high exposure levels, exposing the first-born infants to higher levels compared with the following siblings. In one study, the concentrations of total DDT and DDE were twice as high for milk from primiparous mothers as for milk produced for subsequent infants. Although having more children does further reduce the DDT concentration in milk, the effect is substantially lower than for first-borns. Concentrations of total DDT and of DDE in infants can be

reasonably accurately predicted solely from concentrations in breast milk, whether or not a child is first-born and infant age.

For highly exposed mothers in controlled IRS studies, an average total DDT concentration of 12.8 (range 0.2–76.8; median 6.24) mg/kg milk fat is found (equivalent to 640 µg/l whole milk, assuming a value of 5% for fat content of the milk; range 10–3840 µg/l; median 312 µg/l). For the general population in malarial countries without specific exposure to IRS, a mean total DDT concentration is 2.8 (range 0.47–15.8; median 1.6) mg/kg milk fat (equivalent to 140 µg/l whole milk, assuming a value of 5% for fat content of the milk; range 23.5–790 µg/l). The mean of reported maxima is 72.5 (range 0.57–370.4; median 26.12) mg/kg milk fat (equivalent to 3625 µg/l whole milk; range 28.5–18 520 µg/l) for total DDT.

Mean concentrations in milk for different populations are summarized in Table 49.

Table 49. Concentrations of total DDT in breast milk from different populations

	Occupational exposure (IRS spray operators)	Occupational not exposed in spraying	Residents in IRS-treated homes	General population
Milk	Not studied	Not studied	Mean: 12.8 (0.2–76.8) mg/kg milk fat Median: 6.24 mg/kg milk fat	Mean: 2.8 (0.47–15.8) mg/kg milk fat Median: 1.6 mg/kg milk fat

12.9 Uncertainties

There are few controlled studies with known exposure for either the occupationally exposed or the general population. Sample sizes are, generally, small. Diversity of exposure occurs in different areas of the world, depending on patterns of application of DDT for IRS (e.g. high urban use is common in India, but rare in Mexico). Populations sampled across geographical areas may therefore represent a wide range of exposures. Timing of sampling also varies in relation to application, although the long half-life of DDT metabolites in tissues will tend to maintain residues over long

periods. Patterns of different DDT and metabolite isomers require greater study.

Patterns of child-bearing and culture of child-rearing vary across the world. Effects of pregnancy and breastfeeding on DDT residues warrant further attention.

All studies report a highly skewed distribution in data sets, with a small proportion of the population having much higher residues. True "controls" having no exposure to DDT do not exist globally; all humans have some exposure to DDT.

Equipment for application of DDT has varied over time, with personal exposure likely to have fallen with improved technology. Use of PPE, in reality, is probably small to non-existent in tropical climates.

Exposure through food is difficult to quantify, as no studies have systematically measured DDT or its metabolites in the total diet.

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ANNEX 1: PARTICIPANTS IN THE WHO CONSULTATION ON DDT HAZARD ASSESSMENT

WHO Consultation on DDT Hazard Assessment
2–4 June 2009
WHO Headquarters, Geneva, Switzerland

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ANNEX 2: PARTICIPANTS IN THE WHO CONSULTATION ON DDT HUMAN EXPOSURE ASSESSMENT

WHO Consultation on DDT Human Exposure Assessment
15–16 December 2009
Bradford, United Kingdom

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ANNEX 3: PARTICIPANTS IN THE WHO CONSULTATION ON DDT RISK CHARACTERIZATION

WHO Consultation on DDT Risk Characterization
29–30 November 2010
WHO Headquarters, Geneva, Switzerland

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ANNEX 4: ESTIMATION OF CONVERSION FACTOR TO CALCULATE LIPID-ADJUSTED SERUM LEVELS OF DDT

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: February 4, 2010

SUBJECT: Estimation of Conversion Factor to Calculate Lipid-Adjusted Serum Levels of DDT Using 2003-04 NHANES Biomonitoring Data

PC Code: 029201
MRID No.: NA
Petition No.: NA
Assessment Type: NA
TXR No.: NA

DP Barcode: NA (D000000)
Registration No.: NA
Regulatory Action: NA
Reregistration Case No.: NA
CAS No.: 50-29-3

FROM: Aaron Niman, MPH
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Office of Pesticide Programs
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THROUGH: David Miller, Branch Chief
Health Effects Division
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TO: Antero Aitio
International Programme on Chemical Safety
World Health Organization

SUMMARY

WHO/IPCS has proposed a generic conversion factor of 160 to convert reported serum volume concentrations of DDT to lipid-adjusted concentrations. While WHO/IPCS has proposed this value, it also sought guidance during its 2009 Expert Group Meeting in Bradford, UK. As follow-up to the Expert Group Meeting, the attached memorandum provides an analysis which was used to calculate the generic conversion data using the U.S. Centers for Disease Control and Prevention's NHANES 2003-04 biomonitoring data. [1] Overall, the values derived from analysis of relevant NHANES biomonitoring data are in agreement with those presented at the Expert Group Meeting.

Overview

WHO/IPCS has proposed a generic conversion factor of 160 to convert reported serum volume concentrations of DDT (and its lipophilic metabolites) to lipid-adjusted concentrations. While WHO/IPCS has proposed this value, it also sought guidance during its Expert Group Meeting because there is uncertainty related to the: (1) range of conversion factor values reported in the literature; (2) use of different methods to estimate blood lipid levels; and (3) limited number of studies that can be used to derive the conversion factor (i.e. studies that report concentration in terms of both serum volume and lipid-adjusted concentration). Based on discussion during the Expert Group Meeting, I suggested that it may be helpful to compare the proposed conversion factor of 160 with organochlorine biomonitoring data collected through CDC's National Health and Nutrition Examination Survey (NHANES).[2]

NHANES Organochlorine Biomonitoring Data

NHANES is a continuous two-year cycle health survey that is statistically representative of the United States population. As part of NHANES, organochlorine biomonitoring data are measured in a one-third sub-sample of survey participants aged 12-years or older (roughly 2,000 individuals per two-year survey cycle). Organochlorine blood serum concentrations are reported by NHANES in terms of both serum volume and lipid-adjusted concentration.

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[1] SAS code used to derive summary statistics presented memorandum is provided in Attachment A of the document.

[2] http://www.cdc.gov/nchs/nhanes.htm.

NHANES lipid-adjusted concentrations are determined using each study participant's blood chemistry data on serum lipids. The specific method used to derive each individual's lipid adjusted concentration is not completely clear, although the NHANES organochlorine biomonitoring documentation indicates, "*The total lipid content of each specimen is estimated from its total cholesterol and triglycerides values using a "summation" method*" and provides the following citations:[1]

- Akins J.R., Waldrep K., and Bernert J.T. Jr. The Estimation of Total Serum Lipids by a Completely Enzymatic 'Summation' Method. Clin. Chim. Acta. 184: 219-226 (1989).

- Phillips, D.L., Pirkle, J.L., Burse V.W., Bernert, J.T., Henderson, L.O., and Needham, L.L. Chlorinated Hydrocarbon Levels in Humans Serum: Effects of Fasting and Feeding. Arch. Environ. Contam. Toxicol. 18: 495-500 (1989).

Both of these citations appear to be referenced in Appendix II of the draft DDT Human Exposure Assessment, suggesting that the lipid-adjusted approach used by NHANES is comparable to the biomonitoring studies considered by WHO/IPCS. On this basis, it seems reasonable to use NHANES organochlorine biomonitoring data on DDE, p,p'-DDE, and o,p'-DDT to estimate the conversion factor that is of interest to WHO/IPCS.

Estimation of Conversion Factor Using NHANES

NHANES 2003-04 biomonitoring data were used to estimate the conversion factor to interconvert between serum volume concentrations and lipid-adjusted concentrations. 2003-04 Demographic and Organochlorine datasets (i.e.,"Lab 28 Organochlorine Pesticides") were downloaded from CDC's NHANES website and analyzed using SAS 9.2 (See Appendix A for detailed SAS code).[2] Demographic and Organochlorine datasets were then merged in SAS using the field *SEQN* which is a respondent sequence number that uniquely identifies each study

[1] http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l28ocp_c.pdf.

[2] http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/nhanes03_04.htm.

participant. This was done so that the descriptive statistics could be stratified by demographic characteristics, including age, gender, and race/ethnicity. Next, data fields corresponding to the conversion factors for DDE, p,p'-DDE, and o,p'-DDT were created using a SAS DATA step. The general equation used to determine the conversion factors for DDE, p,p'-DDE, and o,p'-DDT is provided below:

$$\text{Conversion Factor}\left[\frac{\text{mL serum}}{\text{g serum lipids}}\right] = \frac{\text{Lipid Adjusted Concentration}\left[\frac{\text{ng Chemical}}{\text{g serum lipids}}\right]}{\text{Serum Concentration}\left[\frac{\text{ng Chemical}}{\text{g serum}}\right] * \text{Density}\left[\frac{\text{1.025 g serum}}{\text{mL serum}}\right]}$$

Serum density was included in the equation above because CDC reports blood serum concentration in terms of total serum mass, rather than serum volume. The assumed density value was 1.025 g/mL, based on the density of blood plasma. It should also be noted that the units of the conversion factors are in terms of mL serum per g serum lipids. When using the conversion factor values presented in this document to convert a serum concentration to a lipid-adjusted concentration, the units of the serum concentration must be in terms of mass chemical per mL serum to correctly calculate the lipid-adjusted concentration in terms of mass chemical per g serum lipid.

After the conversion factors data fields were created, various descriptive statistics were calculated to characterize the distribution of conversion factors for DDE, p,p'-DDE, and o,p'-DDT. Because NHANES uses a complex multi-stage sample design and also deliberatively over-samples some demographic groups, analysis of NHANES data requires incorporating survey weights to estimate mean and percentiles, and additionally strata and primary sampling units (PSU) data to calculate variance and standard error estimates. To simplify the analysis, only mean and percentile estimates were calculated using the survey weights from the Organochlorine dataset (i.e.,"Lab 28 Organochlorine Pesticides"). Due to the additional complexity associated with variance estimation procedures that incorporate the strata and PSU information, standard errors and confidence intervals have not been provided.

Additionally, observations below the limit of detection (LOD) are also typically taken into account when analyzing NHANES biomonitoring data. However, concentrations of DDT and its

metabolites that are below the LOD do not provide information on the conversion factors (i.e. the conversion factor can only be estimated using data on known concentrations of DDT metabolites), so observations less than the LOD were excluded from the analysis. Since the conversion factor is not anticipated to materially differ between those individuals with non-detectable DDT/metabolite concentrations in their serum and those with detectable concentrations, the exclusion of these individuals is considered to be appropriate and is not expected to affect the results.

Results

Descriptive statistics for the conversion factor (expressed in mL serum /g serum lipids) are shown in Tables 1 and 2 below for the General Population and by demographic category, respectively. In sum, the mean values for the General Population for this conversion factor are 172-, 164, and 158 - mL serum /g serum lipids for DDE, p,p'-DDT, and o,p'-DDT, respectively. These values are variable among individuals as shown by the percentile values in these two tables as well as in Figures 1 through 3.

Overall, the values found by analysis of the relevant NHANES biomonitoring data in are in agreement with those presented at the 2009 WHO workshop in Bradford, UK and anticipated for use. The above analysis confirms that a conversion factor in the range of 160 to 170 mL serum /g serum lipids is reasonable for use for adults >19 years of age, for both sexes, and for all racial categories.

Table 1: Descriptive statistics for DDE, p,p'-DDT, and o,p'-DDT conversion factors for general population (expressed in mL serum /g serum lipids).

Conversion Factor	n	Mean	Percentile						
			5th	10th	25th	50th	75th	90th	95th
DDE	1,951	172	111	122	143	168	199	227	242
p,p'-DDT	1,400	164	107	117	137	160	187	215	232
o,p'-DDT	115	158	99	116	129	155	177	213	231

Table 2: Descriptive statistics for DDE, p,p'-DDT, and o,p'-DDT conversion factors, by demographic category (expressed in mL serum /g serum lipids).

Demographic Category	Conversion Factor	n	Mean	Percentile						
				5th	10th	25th	50th	75th	90th	95th
Gender										
Female	DDE	999	166	109	121	141	164	190	214	230
	p,p'-DDT	698	160	105	116	136	158	181	207	223
	o,p'-DDT	63	156	99	102	122	156	176	207	230
Male	DDE	952	163	104	115	135	158	185	219	236

Demographic Category	Conversion Factor	n	Mean	Percentile						
				5th	10th	25th	50th	75th	90th	95th
	p,p'-DDT	702	156	96	111	128	154	180	207	226
	o,p'-DDT	52	152	104	110	123	144	169	219	230
Age										
	DDE	585	201	143	155	178	200	224	244	263
12-19 Years	p,p'-DDT	280	195	138	146	173	196	220	243	258
	o,p'-DDT	13	220	177	178	212	230	230	231	263
	DDE	1,366	160	104	116	135	157	181	206	224
>19 Years	p,p'-DDT	1,120	155	101	111	130	153	177	202	217
	o,p'-DDT	102	152	99	110	122	149	169	195	219
Race/Ethnicity										
Mexican American	DDE	457	166	109	119	138	164	191	221	235
	p,p'-DDT	347	160	104	114	132	159	186	213	227

Demographic Category	Conversion Factor	n	Mean	Percentile						
				5th	10th	25th	50th	75th	90th	95th
	o,p'-DDT	32	152	102	120	139	144	163	202	213
	DDE	476	179	124	135	154	177	202	228	255
Non-Hispanic Black	p,p'-DDT	309	174	123	130	149	170	193	224	249
	o,p'-DDT	35	163	116	126	142	156	178	218	246
	DDE	875	162	105	116	136	158	185	211	232
Non-Hispanic White	p,p'-DDT	629	155	100	111	129	152	177	199	219
	o,p'-DDT	29	145	98	99	116	142	159	219	230
	DDE	65	163	104	112	141	156	195	217	225
Other Hispanic	p,p'-DDT	45	157	95	104	141	155	173	212	221
	o,p'-DDT	1	139	-	-	-	-	-	-	-
	DDE	78	167	111	121	139	166	189	215	223
Other Race - Including Multi-	p,p'-DDT	70	166	111	121	131	165	188	217	223
	o,p'-DDT	18	166	104	121	138	165	181	195	299

Figure 1: DDE conversion factor boxplots, by demographic category (Box length indicates 25th and 75th percentiles; central line indicates median; and whiskers indicate 5th and 95th percentiles).

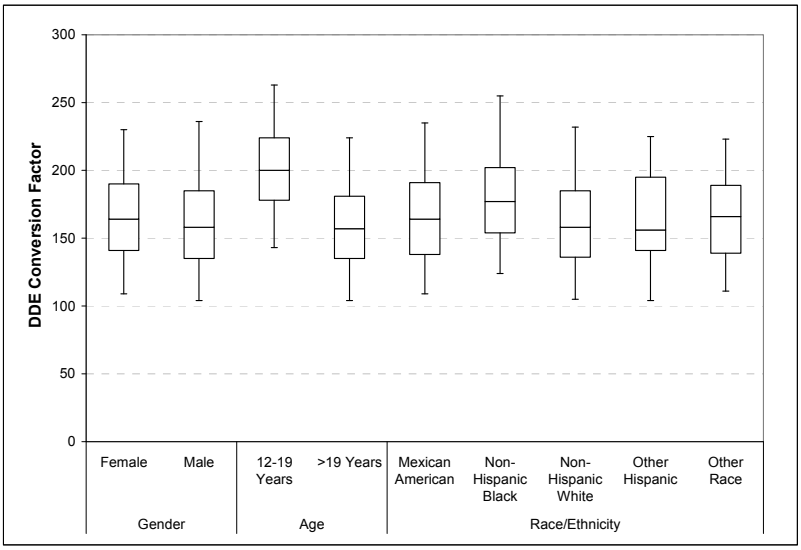


Figure 2: p,p'-DDT conversion factor boxplots, by demographic category (Box length indicates 25th and 75th percentiles; central line indicates median; and whiskers indicate 5th and 95th percentiles).

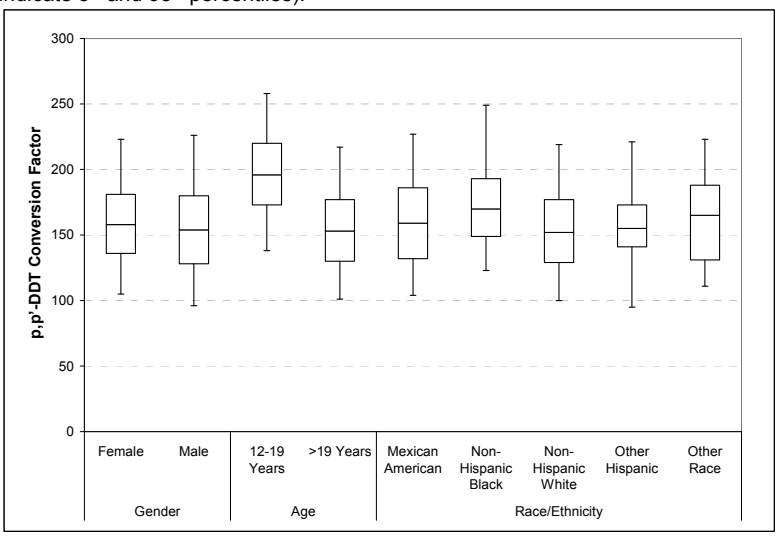
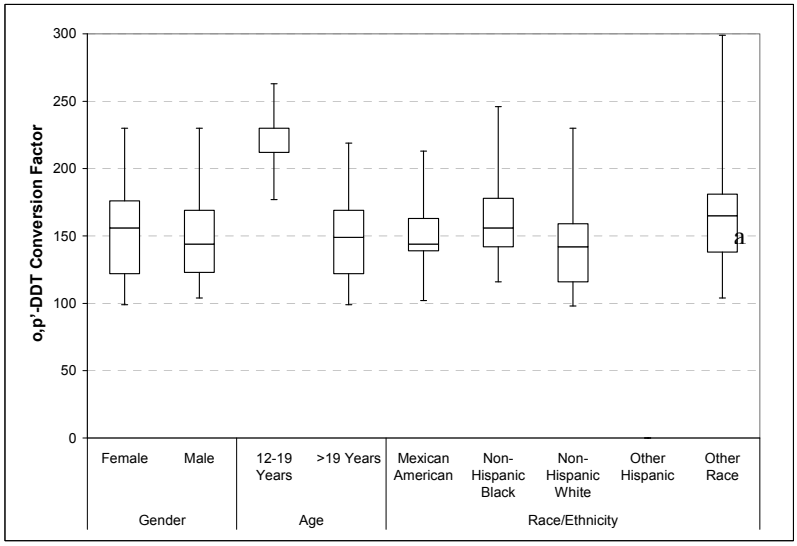


Figure 3: o,p'-DDT conversion factor boxplots, by demographic category (Box length indicates 25th and 75th percentiles; central line indicates median; and whiskers indicate 5th and 95th percentiles). [a] Median value of 230 for 12-19 year olds cannot be visually discerned from the 75th percentile.



Appendix A: SAS Code

```
libname a "[INSERT LIBRARY NAME]";

*****************************************************
Step 1: Merge demographic and Organochlorine Datasets
*****************************************************;

proc sort data = a.Demo_c;
        by SEQN;

proc sort data = a.L28ocp_c;
        by SEQN;

data a.demo_pops;
        merge a.Demo_c a.L28ocp_c;
        by SEQN;

*Review merge contents;
proc contents data= a.demo_pops varnum;
run;

*****************************************************
Step 2: Calculate [DDE Lipid Adjusted value]: [DDE Serum Value]
conversion
Create Demographic Class Variables
*****************************************************;

data a.demo_pops_conversion;      set a.demo_pops;

        *Calculate DDT Metabolite Conversion Factors- Blood
        Density = 1.06 [g serum]/ [mL serum];
        if LBDPDELC= 0 then DDE_Conversion = LBXPDELA /
        (LBXPDE * 1.025); *DDE;
        if LBDPDTLC= 0 then ppDDT_Conversion =
        LBXPDTLA / (LBXPDT * 1.025); *p,p'-DDT;
        if LBDODTLC= 0 then opDDT_Conversion =
        LBXODTLA / (LBXODT * 1.025);          *o,p'-DDT;

        *Code Gender;
```

```
        if RIAGENDR    = 1 then sex = "Male ";
        else if RIAGENDR = 2 then sex = "Female";
        else if RIAGENDR = . then sex = .;

        *Code Race/Ethnicity;
        if RIDRETH1 = 1          then race = "Mexican
        American";
        else if RIDRETH1 = 2 then race = "Other Hispanic";
        else if RIDRETH1 = 3 then race = "Non-Hispanic White";
        else if RIDRETH1 = 4 then race = "Non-Hispanic Black";
        else if RIDRETH1 = 5 then race = "Other Race - Including
        Multi-Racial";
        else if RIDRETH1 = . then race = .;

        *Code Race/Ethnicity;
        if (RIDAGEYR >= 12 and RIDAGEYR <=19) then age =
        "12-19 Years";
        else if RIDAGEYR > 19 then age = ">19 Years";

run;

*Review Results;
proc print data= a.demo_pops_conversion;
        var LBXPDELA LBXPDE DDE_conversion sex race age;
run;

*******************************************************
Step 3a: Generate general descriptive statistics
*******************************************************;
proc means data = a.demo_pops_conversion n mean p5 p10 p25
p50 p75 p90 p95;
        var DDE_conversion ppDDT_conversion
        opDDT_conversion;
        weight WTSB2YR;
ods html file = 'summary.html';
run;
ods html close;

*******************************************************
Step 3b: Generate general descriptive statistics, by class variables
*******************************************************;
proc means data = a.demo_pops_conversion n mean p5 p10 p25
p50 p75 p90 p95;
```

```
        var DDE_conversion ppDDT_conversion
        opDDT_conversion;
        class age;
        weight WTSB2YR;
ods html file = 'Age.html';
run;
ods html close;

proc means data = a.demo_pops_conversion n mean p5 p10 p25
p50 p75 p90 p95;
        var DDE_conversion ppDDT_conversion
        opDDT_conversion;
        class sex;
        weight WTSB2YR;
ods html file = 'sex.html';
run;
ods html close;

proc means data = a.demo_pops_conversion n mean p5 p10 p25
p50 p75 p90 p95;
        var DDE_conversion ppDDT_conversion
        opDDT_conversion;
        class race;
        weight WTSB2YR;
ods html file = 'race.html';
run;
ods html close;
```

ANNEX 5: WORKED EXAMPLE FOR DDT OF THE WHO (2010) GENERIC MODEL FOR EXPOSURE DURING INDOOR RESIDUAL SPRAYING

In this worked example, a 75% WP formulation of DDT is used.

Generic risk assessment model	Worked example
Covered in hazard assessment	**1. Toxicity data** *Covered in hazard assessment* **2. Exposure estimation** In this worked example, a 75% WP formulation of DDT is assumed. The a.i. content of the formulation is 750 g/kg. The target concentration on the wall is 2 g DDT/m^2, and the spray application rate is assumed to be 40 ml/m^2. The vapour pressure of DDT is approximately 21 × 10^{-6} Pa (ATSDR, 2002).
2.1 Worker dermal exposure during mixing and loading and application Mixing and loading and application exposures are estimated by using unit exposures from suitable databases or other defaults. In realistic-case estimations, it is assumed that there is no use of PPE (gloves); in the safest-case estimations, gloves are used. In the realistic worst-case scenario, poor maintenance and condition of the equipment are assumed to cause contamination to larger areas of skin. The skin on the operator's back is used as an example, with an area of	**2.1 a) Estimate dermal exposure of workers during mixing and loading of the insecticide formulation** The concentration needed for the spray liquid (2 g/m^2 applied at 40 ml/m^2) is 50 g/l. Therefore, one tank (10 litres) will require 500 g DDT dissolved in 10 litres of water to give a spray concentration of 50 g/l. As 12 tank loads are assumed to be prepared and used daily, the amount of DDT handled is 12 × 500 g = 6000 g/day. The product contains 750 g of DDT per kilogram of product; therefore, the amount of product used (6000 g ÷ 75%) is 8000 g product per day (ML). The dermal exposure is 9.7 mg/kg of product handled (UE_{dermal}); therefore, 9.7 mg × 8 kg = 77.6 mg/day. In the realistic worst-case scenario, there is exposure from contamination of a larger area of skin = 35.5 ml × 50 mg/ml = 1775 mg/day. The exposure duration (ED) (assumes 6 days/week, 6 weeks per treatment campaign, 2 treatments per year) is divided by the averaging

Generic risk assessment model	Worked example
3550 cm^2 and a film thickness of 0.01 cm, leading to a volume of spray on the skin of 0.01 × 3550 = 35.5 ml. It has been assumed that during mixing and loading, the inhalation exposure is negligible (in line with the WHOPES 2010 generic model). Inhalation exposure during application is also assumed to be negligible following the vapour pressure–based recommendation of WHO (2010). Therefore, only the dermal route has been taken into account in the calculations.	time (AT) (1 year): 72 days/365 days = 0.197. Body weight (BW) is 60 kg, and dermal absorption (A) is assumed to be 10%. *Predicted systemic dose =* $UE_{dermal} \times ML \times ED \times A / BW \times AT$ **Realistic scenario:** The systemic exposure during mixing and loading (no PPE, 10% dermal absorption) is 9.7 × 8 × 72 × 0.1 / 60 × 365 = **0.0255 mg a.i./kg bw per day**. **Safest scenario:** The systemic exposure during mixing and loading with full PPE (giving 90% cover) is predicted to be 9.7 × 8 × 72 × 0.1 × 0.1 / 60 × 365 = **0.00255 mg a.i./kg bw per day**. **Realistic worst-case scenario:** The systemic exposure during mixing and loading with no PPE (10% dermal absorption) and with additional contamination of the skin is predicted to be (9.7 × 8 × 72 × 0.1) + (1775 × 72 × 0.1) / 60 × 365 = **0.609 mg a.i./kg bw per day**. **2.1 b) Estimate dermal exposure during application of the insecticide formulation and cleaning and maintenance of spraying equipment** It is assumed that the hands are immersed in the spray liquid, which represents the realistic case. In the realistic worst-case, the forearms are also exposed. In the safest case, hands only are exposed, and the gloves are assumed to give 90% protection. The liquid layer (VS_{dermal}) covering the hands is assumed at 8.4 ml, and that of forearms at 11.4 ml of the spray liquid. The spray liquid contains 50 mg a.i./ml. Therefore, the exposure via hands (no PPE) is 420 mg, and that via hands + forearms, 990 mg. CS = concentration of the a.i. in the final spray; A, ED and AT as above.

Generic risk assessment model	Worked example
	Predicted dose = VS_{dermal} × *CS* × *ED* × *A* / *BW* × *AT* When the exposure duration, the averaging time and the body weight are taken into account, the systemic dose from the dermal exposure in the **realistic scenario** is 8.4 × 50 × 72 × 0.1 / 60 × 365 = **0.138 mg a.i./kg bw per day**. In the **safest scenario**, the gloves give 90% protection, and the predicted dose is 8.4 × 0.1 × 50 × 72 × 0.1 / 60 × 365 = **0.0138 mg a.i./kg bw per day**. In the **realistic worst-case scenario**, the dermal exposure is (8.4+11.4) × 50 × 72 × 0.1 / 60 × 365 = **0.325 mg/kg bw per day.**
2.2 Residential post-application ingestion exposure due to contaminated foodstuff, breast milk or hand-to-mouth behaviour Adults weigh 60 kg, older children 40 kg, and toddlers (who also get ingestion exposure due to hand-to-mouth behaviour) 13 kg. Newborn babies consuming only breast milk are assumed to weigh 3 kg. The amount available for transfer from contaminated surfaces is 50% of the a.i. on the contact surfaces, which are assumed to be covered with 30% of the target concentration of the treated walls. The concentration decreases with a default half-life of 60 days. The average concentration, therefore, is 0.42 × the original	**2.2 a) Residential post-application ingestion exposure due to contaminated foodstuff** The application rate is at 2000 mg/m^2; it is assumed that the concentration on surfaces that might be transferred to food is 30% of this = 600 mg/m^2. By default, it is estimated to decrease exponentially with a half-time of 60 days. Thus, the average concentration during the half year between applications is 0.42 × the original concentration. Using this average, the exposure time is 365 days/year, and so is the averaging time. Fifty per cent of this is available to contaminate food, so the translodgeable residue = 600 × 0.42 × 0.5 = 126 mg/m^2. *Daily volume of food eaten = Daily food consumption rate / (Energy value × $Density_{water}$)* The daily volume of the food eaten per day is 1.1 dm^3 for adults (60 kg), 0.85 dm^3 for children (40 kg) and 0.55 dm^3 for toddlers (13 kg). *Surface area of food = [(Volume of food eaten)^{⅔}]^2* The surface area available for contamination is 1.07 dm^2, for adults, 0.9 dm^2 for children and

Generic risk assessment model	Worked example
concentration over the half-year interval between sprayings. The predicted dose depends on the amount applied to the house, the transferable amount of the insecticide, the surface area of the food that may be contaminated and the volume of the food eaten. The volume depends on the daily consumption rate and energy value. According to FAO, the daily food consumption rate for adults is 2200 kcal/day, and the energy value of the food is 2000 kcal/day. The default density is the density of water. The calorie intake of the 40 kg child is assumed to be 1700 kcal/day, and that of a toddler (13 kg), 1100 kcal/day. The breast milk exposure calculation presented in the generic model is a very rough estimation. It is highly recommended that chemical-specific data be used.	0.7 dm^2 for toddlers. *Predicted dose = (Translodgeable residue × Surface area of food) / BW* For a 60 kg adult, the predicted daily average intake during the 12-month period following the spraying is 1.26 mg/dm^2 × 1.07 dm^2 / 60 = **0.022 mg/kg bw** For children and toddlers, the corresponding figures are **0.028** and **0.068** mg/kg bw. **2.2 b) Residential post-application ingestion exposure via breast milk** The generic model uses exposure of the mother, her body burden in equilibrium and fat content of breast milk as the basis for the calculation of the concentration of the insecticides in breast milk. For DDT, actual analytical data are available and are therefore used in lieu of the default procedure. The median concentration of total DDT in milk from direct IRS exposure, 312 µg/l, or 0.000 000 312 kg/l, is multiplied by the ingestion rate of milk (0.95 kg/day). Predicted median dose is 1 × 0.000 000 312 kg/l × 0.95 kg/day divided by 3 kg (body weight of a newborn) = **0.0988 mg a.i./kg bw per day**. **2.2 c) Residential post-application ingestion exposure of toddlers via hand-to-mouth behaviour (ingestion of house dust)** Toddlers eat house dust, which, after IRS, may be contaminated with the insecticide. Limited data indicate that the concentration of DDT in house dust was approximately 1 mg/kg (1 ng/mg) after IRS presumably at the WHO recommended dose rate of 2 g/m^2. The 95th percentile of dust eaten is 587 mg/day (USEPA, 1997); thus, the daily dose for a 13 kg child would be 1 ng/mg × 587 mg/day / 13 kg = **45 ng/kg bw per day**.

Generic risk assessment model	Worked example
	Insecticide from the surfaces in contact with hands—see dermal exposure calculation.
2.3 Residential post-application dermal exposure due to touching of contaminated surfaces Amount deposited onto skin: 50% of the amount actually on the sprayed surface transfers onto the skin from the surfaces. The target amount sprayed per square metre is 40 ml of the spray liquid. Exposed skin areas: 0.198 m^2 for adults and older children (hands and forearms) 0.42 m^2 for toddlers The hand area needed for calculating the hand-to-mouth exposure for toddlers is 0.032 m^2. For the transfer from hands to mouth, a default of 10% can be used. Dermal absorption is assumed to be 10%, if no other (chemical-specific) data can be found. Body weight, 60 kg for adults, 40 kg for older children and 13 kg for toddlers. Exposure duration = averaging time = 365 days/year.	**2.3 Residential post-application dermal exposure due to touching of contaminated surfaces (walls, floors, furniture), adults, children, toddlers** The target amount sprayed to the house wall surfaces is 2000 mg/m^2. The concentration on the floor immediately adjacent to the walls is assumed to be equal to that on the walls and then rapidly decreases with increasing distance from the wall to reach zero at 50 cm. The average on this 50 cm strip is 30% of that on the wall, the average on the floor and all in-house surfaces, except the sprayed floors is 10% of that of the walls. Of this, a 20% default value for the overall average concentration on the contact surfaces is assumed based on 10% of skin contact with walls and 90% skin contact with floors and furniture. DDT is assumed to decay with a half-time of 60 days, and thus the sprayed surfaces are assumed to have an average concentration of 0.42 × 2000 × 20% = 168 mg/m^2 over the year following the biannual spraying. Exposed skin areas (ESA) are 0.198 m^2 for adultsand older children and 0.42 m^2 for toddlers. A is the fraction absorbed, which is defaulted as 0.1 (10%). AV = average proportion of the sprayed DDT of the original concentration = 0.42. P = proportion translodged onto the skin, 50%. Conc = Target concentration of DDT on the wall = 2 g/m^2. *Predicted dose = (0.2 × AV × Conc. × P × ESA × A) / BW*

Generic risk assessment model	Worked example
	Predicted doses: Adults: **0.028 mg a.i./kg bw per day** (0.2 × 0.42 × 2000 × 0.5 × 0.198 × 0.1 / 60) Children: **0.042 mg a.i./kg bw per day** (0.2 × 0.42 × 2000 × 0.5 × 0.198 × 0.1 / 40) Toddlers: **0.27 mg a.i./kg bw per day** (0.2 × 0.42 × 2000 × 0.5 × 0.42 × 0.1 / 13) For toddlers, hand-to-mouth transfer (THM) is assumed to be 10% of the amount on the hands (surface area, 0.032 m^2), and the gastrointestinal absorption, 100%. *Absorbed dose = (0.2 × AV × Conc. × ESA × P × THM) / BW* which equals **0.021 mg a.i./kg bw per day** (0.2 × 0.42 × 2000 × 0.032 × 0.5 × 0.1 / 13).