United States Environmental Protection Agency

EPA/690/R-17/007
FINAL
09-26-2017

Provisional Peer-Reviewed Toxicity Values for

p,p′-Dichlorodiphenyldichloroethylene (*p,p′*-DDE)
(CASRN 72-55-9)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Lucina E. Lizarraga, PhD
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212

PRIMARY INTERNAL REVIEWERS

Paul Reinhart, PhD, DABT
National Center for Environmental Assessment, Research Triangle Park, NC

Jon Reid, PhD, DABT
National Center for Environmental Assessment, Cincinnati, OH

This document was externally peer reviewed under contract to

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

Questions regarding the content of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS AND ACRONYMS[1]

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

[1]Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *p,p′*-DICHLORODIPHENYLDICHLOROETHYLENE (*p,p′*-DDE) (CASRN 72-55-9)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by at least two National Center for Environment Assessment (NCEA) scientists and an independent external peer review by at least three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

PPRTV assessments are eligible to be updated on a 5-year cycle to incorporate new data or methodologies that might impact the toxicity values or characterization of potential for adverse human-health effects and are revised as appropriate. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. Environmental Protection Agency (EPA) Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. EPA programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the content of this PPRTV assessment should be directed to the EPA Office of Research and Development's (ORD's) NCEA, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

p,p′-Dichlorodiphenyldichloroethylene (*p,p′*-DDE), CASRN 72-55-9, belongs to the class of compounds known as aryl halides. It is a metabolite of the insecticide *p,p′*-dichlorodiphenyltrichloroethane (DDT), CASRN 50-29-3, and occurs as an impurity in DDT formulations. There are no commercial uses of *p,p′*-DDE (HSDB, 2010), but it can be produced by the dehydrochlorination of DDT in alkaline solution (NLM, 2010). *p,p′*-DDE is listed on U.S. EPA's Toxic Substances Control Act's public inventory (U.S. EPA, 2015a), but it is not registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2017). *p,p′*-DDE is listed as a Superfund hazardous substance by the EPA, is assigned a Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) reportable quantity of 1 lb (U.S. EPA, 2015b), and is listed on the 2015 CERCLA substance priority list (ATSDR, 2016). It is also included on The Proposition 65 list (Cal/EPA, 2017a).

The empirical formula for *p,p′*-DDE is $C_{14}H_8Cl_4$ (see Figure 1). Table 1 summarizes the physicochemical properties. *p,p′*-DDE is a white, crystalline solid at room temperature (NLM, 2010), with a vapor pressure that indicates it will exist in both the vapor and particulate phases in the atmosphere. The estimated half-life of vapor-phase *p,p′*-DDE in air by reaction with photochemically produced hydroxyl radicals is 1.4 days. It is also subject to direct photolysis by sunlight, showing 20% degradation after 7 days when adsorbed on silica gel, and half-lives ranging from 0.6−6.1 days in water. *p,p′*-DDE's Henry's law constant indicates that it may volatilize from moist surfaces, although volatilization is expected to be attenuated by adsorption to suspended solids and sediment in the water column. Its low vapor pressure indicates that *p,p′*-DDE is not expected to volatilize from dry soil surfaces. The low water solubility and high soil adsorption coefficient for *p,p′*-DDE indicate that it will be immobile in soil, and is therefore not expected to leach to groundwater or undergo runoff after a rain event. Hydrolysis is not expected to be an important fate process, as a measured half-life of 120 years has been reported at pH 3−5. Measured bioconcentration factor (BCF) values of 27,500−81,000 in fish suggest a high bioconcentration potential of *p,p′*-DDE in aquatic organisms, and no biodegradation has been observed in screening and lab tests (NLM, 2010). Based on physicochemical properties, the potential exposure routes for *p,p′*-DDE in humans occur primarily via ingestion of food, as well as inhalation of ambient air, drinking water, and dermal contact (NLM, 2010).

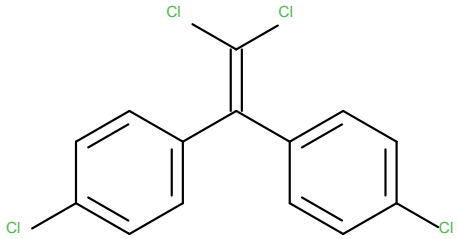

Figure 1. *p,p′*-DDE Structure

Table 1. Physicochemical Properties of *p,p′*-DDE (CASRN 72-55-9)

Property (unit)	Value
Physical state	Solid
Boiling point (°C)	336[a]
Melting point (°C)	89[a]
Density (g/cm^3 at 20°C)	NV
Vapor pressure (mm Hg at 25°C)	6×10^{-6} (extrapolated)[a]
pH (unitless)	NA
pKa (unitless)	NA
Solubility in water (mg/L at 25°C)	0.04[a]
Octanol-water partition coefficient (log K_{ow})	6.51[a]
Henry's law constant (atm-m^3/mol at 25°C)	4.16×10^{-5} [a]
Soil adsorption coefficient K_{oc} (L/kg)	2.63×10^4 and 7.586×10^4 [b]
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	7.4×10^{-12} (estimated)[a]
Atmospheric half-life (d)	1.4 (estimated)[a]
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	318[a]
Flash point (closed cup in °C)	NV

[a]U.S. EPA (2012c).
[b]HSDB (2010).

NA = not applicable; NV = not available; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

A summary of available toxicity values for *p,p′*-DDE from EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for *p,p′*-DDE (CASRN 72-55-9)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference
Noncancer			
IRIS	NV	NA	U.S. EPA (2017)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2017)
IPCS	NV	NA	IPCS (2017); WHO (2017)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2017a); Cal/EPA (2017b)
OSHA	NV	NA	OSHA (2006); OSHA (2011)
NIOSH	NV	NA	NIOSH (2016)
ACGIH	NV	NA	ACGIH (2016)
DOE (PAC)	PAC-1: 6.5 mg/m^3; PAC-2: 72 mg/m^3; PAC-3: 170 mg/m^3	Based on TEELs	DOE (2015)
USAPHC (air-MEG)	1-hr critical: 400 mg/m^3; 1-hr marginal: 75 mg/m^3; 1-hr negligible: 13 mg/m^3	Based on TEELs	U.S. APHC (2013)
USAPHC (water-MEG)	1-yr negligible: 0.29 mg/L	Based on hepatocellular carcinomas and hepatomas; 5 L intake rate	U.S. APHC (2013)
USAPHC (soil-MEG)	1-yr negligible: 3,640 mg/kg	Based on cancer	U.S. APHC (2013)
Cancer			
IRIS (WOE)	Classification, B2: Probable human carcinogen	Based on increased incidence of liver tumors including carcinomas in two strains of mice, and in hamsters.	U.S. EPA (1988a)
IRIS (OSF)	0.34 (mg/kg-d)$^{-1}$	Based on increased incidence of liver tumors including carcinomas in two strains of mice, and in hamsters.	U.S. EPA (1988a)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2014)
IARC	NV	NA	IARC (2017)

Table 2. Summary of Available Toxicity Values for *p,p′*-DDE (CASRN 72-55-9)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference
Cal/EPA (IUR)	0.000097 $(\mu g/m^3)^{-1}$	Based on U.S. EPA (1988a)	Cal/EPA (2017b)
Cal/EPA (ISF)	0.34 $(mg/kg\text{-}d)^{-1}$	Based on U.S. EPA (1988a)	Cal/EPA (2017b)
Cal/EPA (OSF)	0.34 $(mg/kg\text{-}d)^{-1}$	Based on U.S. EPA (1988a)	Cal/EPA (2017b)
ACGIH	NV	NA	ACGIH (2016)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; DOE = U.S. Department of Energy; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; USAPHC = U.S. Army Public Health Command.
[b]Parameters: IUR = inhalation unit risk; ISF = inhalation slope factor; MEG = military exposure guideline; OSF = oral slope factor; PAC = protective action criteria; WOE = weight of evidence.

NA = not applicable; NV = not available; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; TEEL = temporary emergency exposure limit.

Literature searches were conducted in December 2015 and updated in July 2017 for studies relevant to the derivation of provisional toxicity values for *p,p′*-DDE (CASRN 72-55-9). Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related data: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (Cal/EPA), U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST),U.S. EPA Office of Water (OW),U.S. EPA TSCATS2/TSCATS8e,U.S. EPA High Production Volume (HPV), U.S. EPA National Pesticide Information Retrieval System (NPIRS), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Japan Existing Chemical Data Base (JECDB), European Chemicals Agency (ECHA), Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), and Occupational Safety and Health Administration (OSHA).

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer databases, respectively, for *p,p′*-DDE and include all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies as well as reproductive and developmental toxicity studies. Principal studies are identified in bold. The terms "significance" or "significantly" used throughout the document, indicate a *p*-value of < 0.05 unless otherwise specified.

A carcinogenicity assessment for *p,p′*-DDE is available on IRIS (U.S. EPA, 1988b); therefore, cancer data are discussed below, but no cancer values are derived in this document.

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)							
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
Human							
1. Oral (mg/kg-d)							
ND[d]							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
Subchronic	5 M/5 F Osborne-Mendel rat, diet, 6 wk; reported doses: 0, 316, 562, 1,000, 1,780, 3,160 ppm	0, 27.6, 49.1, 87.5, 155.6, 276.3 (M); 0, 29.2 53.1, 94.5, 168.3, 298.7 (F)	Reduced survival in females	NDr	94.5 (FEL)	NCI (1978) (Study examined body weight and mortality only, precluding the determination of other effect levels)	PR
Subchronic	5 M/5 F B6C3F$_1$ mouse, diet, 6 wk; reported doses: 0, 139, 193, 269, 363, 519 ppm	0, 25.1, 34.8, 48.5, 65.5, 93.6 (M); 0, 27.1, 37.7, 52.5, 70.8, 101 (F)	Reduced survival in males	NDr	48.5 (FEL)	NCI (1978) (Study examined body weight and mortality only, precluding the determination of other effect levels)	PR
Subchronic	8−12 M Wistar rat, diet, 6 wk; reported doses: 0 or 200 ppm	0 or 18.4	Increased relative liver weight (17%) and potential immunotoxicity	NDr	18.4	Banerjee et al. (1996) (Study examined humoral and cell-mediated immune responses)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Chronic	60 M/60 F CF-1 mouse (100 M/90 F controls), diet, up to 123 wk; reported doses: 0, 250 ppm	0, 45.0 (M); 0, 46.0 (F)	Reduced survival in both sexes; early signs of intoxication in females (tremors, convulsions, and death); decreased body weight (~11%), and increased incidence of myocardial necrosis in males	NDr	45.0 (M) (FEL)	Tomatis et al. (1974) (Survival differences are confounded by the early appearance and high incidence of hepatomas)	PR
Chronic	50 M/50 F (20 M/20 F controls), Osborne-Mendel rat, diet, 78 wk; reported doses: 0, 437, 839 ppm (M); 0, 242, 462 ppm (F)	0, 30.6, 58.8 (M); 0, 18.7, 35.6 (F)	Reduced survival in both sexes and increased incidence of degenerative liver lesions in males	NDr	18.7 F (FEL)	NCI (1978) (Significant dose reductions during treatment, and long observation period between exposure and evaluation)	PR
Chronic	50 M/50 F (20 M/20 F controls), B6C3F$_1$ mouse, diet, 78 wk; reported doses: 0, 148, 261 ppm	0, 25.3, 44.8 (M); 0, 25.6, 45.1 (F)	Decreased body weight (10−15%) in females and clinical signs in males (hunched posture)	NDr	25.3 (M)	NCI (1978) (Low survival and high incidence of amyloidosis in male controls)	PR
Chronic	40 M/40 F, Syrian golden hamster, diet, up to 128 wk; reported doses: 0, 500, 1,000 ppm	0, 48.5, 97.0 (M); 0, 48.3, 96.6 (F)	Body-weight decrease (~23%) in males	48.5	97.0	Rossi et al. (1983) (High incidence of amyloidosis in control animals)	PR
R/D	51 F (54 F controls), S-D rat, gavage in corn oil, 5 d/wk, 5 wk premating through gestation and lactation, to PND 8 or 19; reported doses: 0, 10 mg/kg	0, 7.1	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: NDr	Kornbrust et al. (1986) (Inadequate data reporting preclude determination of critical effects and effect levels)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
R/D	8 F, Long-Evans rat, gavage in corn oil, GDs 14−18; reported doses: 0, 100 mg/kg-d	0, 100	Maternal: NR Offspring: Decreased AGD and retained nipples in males on PND 13	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: 100	Kelce et al. (1995) (Letter report with limited details)	PR
R/D	12 M, Long-Evans rat, gavage in corn oil, PNDs 21−57; reported doses: 0, 100 mg/kg-d	0, 100	Delayed preputial separation	NDr	100	Kelce et al. (1995) (Letter report with limited details)	PR
R/D	8−11 F, Long-Evans and S-D rat, gavage in corn oil, GDs 14−18; reported doses: 0, 10, 100 mg/kg-d	0, 10, 100	Maternal: NR Offspring (M): Nipple retention in male pups (S-D)	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: 10	You et al. (1998)	PR
R/D	F (number not reported), Long-Evans rat, gavage in corn oil, GDs 14−18; reported doses: 0, 10, 100 mg/kg-d	0, 10, 100	Maternal: NR Offspring: NDr	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: NDr	You et al. (1999a) (Limited endpoints and incomplete histopathological examinations preclude determination of critical effects and effect levels)	PR
R/D	6 F, Holtzman rat, gavage in a corn oil/acetone mixture, GDs 14−18; reported doses: 0, 1, 10, 50, 100, 200 mg/kg-d	0, 1, 10, 50, 100, 200	Maternal: Decreased body weight (9−17%) on GDs 17−21 (no other effects were reported) Offspring (M): Decreased AGD on PND 1 and 20% decreased relative ventral prostrate weight. At higher doses, larger decreases in relative prostate weight (ventral and dorsolateral) were observed, PND 13 nipple retention was increased, and onset of puberty was delayed.	Maternal: 100 Offspring: 10	Maternal: 200 Offspring: 50	Loeffler and Peterson (1999) (Study examined only male offspring for potential effects on the male reproductive tract)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
R/D	8−11 F, Long-Evans and S-D rat, gavage in corn oil, GDs 14−18; reported doses: 0, 100 mg/kg-d	0, 100	Maternal: Decreased body-weight gain Offspring (M): Nipple retention; decreased AGD; prostate atrophy; decreased weights of ventral prostate, glans penis, cauda epididymis and levator ani/bulbocavernosus muscles	Maternal: NDr Offspring: NDr	Maternal: 100 Offspring: 100	Gray et al. (1999) (Study examined only male offspring for potential effects on the male reproductive tract)	PR
R/D	6 F, Wistar rat, diet, GD 1−PND 21; reported doses: 0 or 10 mg/kg-d	0, 10	Maternal: No observed effects Offspring: No observed effects	Maternal: 10 Offspring: 10	Maternal: NDr Offspring: NDr	Makita (2008); Makita and Omura (2006)	PR
R/D	6 M, Wistar rat, diet, PNDs 42−84; reported doses: 0 or 10 mg/kg-d	0, 10	Maternal: No observed effects Offspring: No observed effects	Maternal: 10 Offspring: 10	Maternal: NDr Offspring: NDr	Makita et al. (2005)	PR
R/D	5−7 F, S-D rat, gavage in a dimethylsulfoxide/corn oil mixture, GDs 13.5−17.5; reported doses: 0, 50, 100 mg/kg-d	0, 50, 100	Maternal: No observed effects Offspring: NDr	Maternal: 100 Offspring: NDr	Maternal: NDr Offspring: NDr	Adamsson et al. (2009) (Inadequate reporting of histopathology data preclude the determination of developmental effects and effects levels)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)							
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
R/D	**10 F, Crl:CD (SD) rat, gavage in corn oil, GD 6−PND 20; reported doses: 0, 5, 15, 50 mg/kg-d**	**0, 5, 15, 50**	**Maternal: Increased relative liver weight (20%)** **Offspring: Increased relative liver weight in males at adulthood (≥10%). Delayed preputial separation in male pups, early vaginal opening in female pups, and decreased fertility in adult offspring at 50 mg/kg-d**	**Maternal: 15** **Offspring: NDr**	**Maternal: 50** **Offspring: 5**	**Yamasaki et al. (2009)**	**PR, PS**
R/D	20 F S-D rat, gavage in corn oil, GDs 8−15 (evaluations conducted for 3 generations; crossover mating for F3 generation); reported doses: 0, 100 mg/kg-d	0, 100	Maternal: NR Offspring (M): Sperm number and motility declined for three successive generations; apoptosis of spermatogonia and spermatocytes; small testes and decreased fertility in F3 males	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: 100	Song et al. (2014)	PR
R/D	20 F pregnant S-D rat, gavage for 14 d during gestation and continuing through PND 20, followed by direct exposure of male offspring from PNDs 21−90; reported doses: 0, 35 mg/kg-d	0, 35	Maternal: NR Offspring (M): Increased liver and testes weight (both absolute and relative); abnormal liver and testicular histology	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: 35	Patrick et al. (2016)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p,p′*-DDE (CASRN 72-55-9)							
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
R/D	4−6 F, Dutch-belted rabbit, oral (dosing method was not specified) in corn oil, GDs 15−30 (alternate days only); reported doses: 0, 100 mg/kg-d	0, 100	Maternal: NR Offspring: NDr	Maternal: NDr Offspring: NDr	Maternal: NDr Offspring: NDr	Veeramachaneni (2006) (Small number of animals examined and inadequate data reporting preclude determination of critical effects and effect levels)	PR
2. Inhalation (mg/m^3)							
ND							

[a]Duration categories are defined as follows: subchronic = repeated exposure for >30 days ≤10% lifespan for humans (>30 days up to approximately 90 days in typically used laboratory animal species); and chronic = repeated exposure for >10% lifespan for humans (>~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2012b).
[b]Dosimetry: Doses are presented as ADDs (mg/kg-day). In contrast to other repeated-exposure studies, values from animal gestational-exposure studies are not adjusted for exposure duration in calculation of the ADD.
[c]Notes: PR = peer reviewed; PS = principal study.
[d]Available information (primarily from epidemiology studies) is summarized in the "Human Studies" section. No exposure information is available for any of these studies.

ADD = adjusted daily dose; AGD = anogenital distance; F = female(s); FEL = frank effect level; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; M = male(s); NA = not available; ND = no data; NDr = not determined; NE = no effects; NOAEL = no-observed-adverse-effect level; NR = not reported; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; R/D = reproductive/developmental; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for *p,p′*-DDE (CASRN 72-55-9)					
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Dosimetry[a]**	**Critical Effects**	**Reference (comments)**	**Notes[b]**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
Carcinogenicity	60 M/60 F CF-1 mouse (100 M/90 F controls), diet, up to 123 wk; reported doses: 0, 250 ppm	0, 6.56 (M); 0, 6.55 (F)	Significant increase in the incidence of hepatomas in males and females	Tomatis et al. (1974)	PR, IRIS[c]
Carcinogenicity	50 M/50 F (20 M/20 F controls), Osborne-Mendel rat, diet, 78 wk; reported doses: 0, 437, 839 ppm (M); 0, 242, 462 ppm (F)	0, 8.96, 17.2 (M); 0, 5.11, 9.72 (F)	Significant dose-related trend in the incidence of thyroid tumors in females, but not significant at either dose by pairwise comparisons	NCI (1978)	PR
Carcinogenicity	50 M/50 F (20 M/20 F controls), B6C3F$_1$ mouse, diet, 78 wk; reported doses: 0, 148, 261 ppm	0, 3.86, 6.80 (M); 0, 3.84, 6.76 (F)	Significant increase in the incidence of hepatocellular carcinomas in males and females	NCI (1978)	PR, IRIS[c]
Carcinogenicity	40 M/40 F, Syrian golden hamster, diet, up to 128 wk; reported doses: 0, 500, 1,000 ppm	0, 10.1, 20.3 (M); 0, 10.3, 20.6 (F)	Significant increase in the incidence of liver and adrenal gland tumors in males and females	Rossi et al. (1983)	PR, IRIS[c]
2. Inhalation (mg/m^3)					
ND					

[a]Dosimetry: Oral exposures are expressed as HEDs (mg/kg-day). HEDs were calculated using species-specific DAFs recommended by U.S. EPA (2011b). The DAF is calculated as follows: DAF = ($BW_a^{1/4} \div BW_h^{1/4}$), where DAF = dosimetric adjustment factor, BW_a = animal body weight, and BW_h = human body weight. Reference body weights recommended by U.S. EPA (1988c) were used to calculate the DAFs: 70 kg for humans; 0.514 kg (M) and 0.389 kg (F) for Osborne-Mendel rats in a chronic-duration study; 0.0373 kg (M) and 0.0353 kg (F) for B6C3F$_1$ mice in a chronic-duration study; 0.134 kg (M) and 0.145 kg (F) for Syrian golden hamster in a chronic-duration study. No strain-specific reference body weights were available for CF-1 mice; instead, average mouse body weights in a chronic-duration study were used (0.0317 kg for M and 0.0288 kg for F).
[b]Notes: IRIS = used by IRIS (U.S. EPA, 1988c); PR = peer reviewed.
[c]The IRIS slope factor of 0.34 (mg/kg-day)$^{-1}$ is based on liver tumor data from these studies using a linearized multistage procedure (U.S. EPA, 1988c).

BW = body weight; F = female(s); HED = human equivalent dose; IRIS = Integrated Risk Information System; M = male(s); ND = no data; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

HUMAN STUDIES

The database of human epidemiological studies of *p,p′*-DDE is extensive. In the literature search update for papers published from 2008−2016, more than 350 epidemiological studies were identified; at least as many more were published before 2008. None of the studies provided estimates of oral or inhalation *p,p′*-DDE exposure but instead used concentrations of *p,p′*-DDE in blood serum, breast milk, or semen as measures of exposure. Some of the reviews did not distinguish studies of *p,p′*-DDE from 2,2-(2-chlorophenyl-4'-chlorophenyl)-1,1-dichloroethene (*o,p′*-DDE); however, as *p,p′*-DDE is the more prevalent DDE isomer produced from DDT, and most exposed humans were likely exposed to DDT, the reviews of DDE in general are expected to be applicable to *p,p′*-DDE. When the isomer was specified in the review, the text below reflects that specification. Because DDE is a metabolite of DDT and is relatively persistent in the body (ATSDR, 2002), it is not possible to determine whether biological measurements of DDE reflect exposure to DDE, or metabolism of DDT. In addition, the route(s) of exposure to DDE or DDT cannot be discerned in these studies, particularly in the more heavily exposed populations living in areas where DDT was used for mosquito control. Finally, the subjects in these studies generally had coexposure to other organochlorine pesticides, organophosphate pesticides, and/or polychlorinated biphenyls (PCBs); thus, the contribution of *p,p′*-DDE exposure to the observed effect(s) is unknown. For these reasons, none of these studies is considered adequate for the purpose of deriving provisional toxicity values. These studies, however, provide insight into potential hazards associated with DDE.

A number of reviews and meta-analyses summarizing the epidemiological literature on specific endpoints are available. Recent (2012−2016) reviews and meta-analyses examining epidemiological data on the following endpoints provide a state-of-the-science snapshot of the human data on associations between *p,p′*-DDE and breast, prostate, and testicular germ cell cancers; respiratory health and asthma; early puberty; male reproductive health; fecundability; birth weight; obesity and diabetes; and neurodevelopment. The results are briefly summarized below.

Meta-analyses of cohort and case-control studies have not demonstrated an association between DDE and breast or prostate cancer. Park et al. (2014) conducted a systematic review and meta-analysis of the relationship between breast cancer and DDE (isomer[s] not specified) in blood or adipose tissue. After searching for all cohort or case-control studies of this relationship, the study authors identified 35 studies (all case control) that were included in their meta-analysis. The meta-analysis indicated no association between DDE and breast cancer (summary odds ratio [OR] 1.03; 95% confidence interval [CI] = 0.95−1.12). Previous meta-analyses provided similar estimates (Ingber et al., 2013; López-Cervantes et al., 2004). *p,p′*-DDE was not significantly associated with prostate cancer in a meta-analysis of six cohort or case-control studies conducted by Lewis-Mikhael et al. (2015). The study authors calculated a pooled OR of 1.02 (95% CI = 0.69−1.35, *p*-value = 0.333) for prostate cancer in an analysis comparing high vs. low levels of *p,p′*-DDE in plasma or adipose tissue. A review of data on exposure to organochlorine pesticides and testicular germ cell tumors (TGCTs) examined four case-control studies of *p,p′*-DDE and TGCTs (Cook et al., 2011). Positive, but nonsignificant, associations between TGCT and higher serum *p,p′*-DDE were observed in two small studies ($n < 120$ cases plus controls), with a significant positive association in a third larger study ($n = 1{,}682$ cases plus controls) [McGlynn et al. (2008) as cited in Cook et al. (2011)]. The fourth study ($n = 918$ cases plus controls) [Biggs et al. (2008) as cited in Cook et al. (2011)] observed no association, but

studied a population with lower serum *p,p′*-DDE levels than McGlynn et al. (2008) as cited in Cook et al. (2011).

A meta-analysis examining the association between DDE (isomer[s] not specified) in cord blood and respiratory outcomes assessed data from 10 birth cohorts in 7 European countries, encompassing a total of 4,608 mother/child pairs (Gascon et al., 2014). The study authors estimated cord serum DDE concentrations from maternal serum, blood, or whole milk for populations that did not analyze cord serum. Respiratory outcomes included in the assessment were parent-reported bronchitis and wheezing in children up to 4 years of age. A borderline significant association was seen between bronchitis or wheeze occurring before 18 months of age and a doubling of DDE in cord serum (relative risk [RR] = 1.03; 95% CI = 1.00−1.07). These results were consistent with the results of a systematic review by the same study authors (Gascon et al., 2013), in which the available evidence on infections, allergic manifestations including asthma, and immune humoral and cell-mediated responses was synthesized. The study authors characterized the evidence associating prenatal levels of DDE or DDT with asthma symptoms and respiratory tract infections as limited, while evidence associating postnatal levels with all outcomes was characterized as inadequate. Furthermore, suggestive but inconclusive, evidence for an association between maternal blood DDE, and asthma or wheezing was reported in a systematic review of epidemiological studies of pesticides (including five studies of DDE) published by Mamane et al. (2015).

Associations of early puberty in boys and girls, and organohalogen exposures were examined in a systematic review by Poursafa et al. (2015). A total of six studies examining measures of puberty (first menarche, breast development, testicular development, Tanner stage signs, body size) in populations with data on DDE (isomer[s] not specified) exposure were identified; five studies examined growth or menarche in girls, and one examined puberty in both boys and girls. The study authors did not indicate the exposure metrics used in the studies. An association between DDE exposure and early puberty in girls was reported in a cohort study [Vasiliu et al. (2004) as cited in Poursafa et al. (2015)] and a case-control study [Ozen et al. (2012) as cited in Poursafa et al. (2015)], while a cross-sectional study [Denham et al. (2005) as cited in Poursafa et al. (2015)] reported no association with DDE. Another cohort study [Karmaus et al. (2002) as cited in Poursafa et al. (2015)] observed an association between reduced growth in girls up to 8 years old and background concentrations of DDE. A case-control study of early puberty in boys and girls [Deng et al. (2012) as cited in Poursafa et al. (2015)] reported a significant association with DDE in both sexes. Taken together, the data provide suggestive, but not conclusive, evidence for an increased risk of early puberty in girls with higher levels of DDE.

Govarts et al. (2012) conducted a meta-analysis of the relationship between birth weight and measurements of *p,p′*-DDE in biospecimens (maternal or cord blood, or breast milk) in 12 European birth cohorts. When cord serum levels were not reported, these values were estimated from measurements in maternal serum, blood, or breast milk. The meta-analysis indicated no association between cord serum levels of *p,p′*-DDE and birth weight (increase of 1 µg/L *p,p′*-DDE was associated with a 7 g decrease in birth weight [95% CI = −18−4]).

Reviews of the relationship between male reproductive tract malformations (cryptorchidism and hypospadias) in humans and *p,p′*-DDE in biological specimens (maternal serum, breast milk or colostrum, placenta, or cord blood) have reported that none of the available

studies (all case-control designs) observed a statistically significant association (Jeng, 2014; Cook et al., 2011). Two cross-sectional studies [Ayotte et al. (2001) and de Jager et al. (2006), both as cited in Jeng (2014)] reported diminished semen quality (decreased semen volume, sperm count, motility, or normal morphology) with increased levels of *p,p′*-DDE in serum or semen; however, no association was reported in two larger cross-sectional studies [Rignell-Hydbom et al. (2004) and Hauser et al. (2003), both as cited in Jeng (2014)] or in a case-control study [Charlier and Foidart (2005) as cited in Jeng (2014)].

Couple fecundity (as measured by time to pregnancy, and quantified as fecundability odds ratios [FORs]) and its association with environmental pollutants, including *p,p′*-DDE, was reviewed by Buck Louis (2014). Among the five cohort studies that examined associations with *p,p′*-DDE and were included in the review, three observed no statistically significant or unambiguous association with time to pregnancy [Axmon et al. (2006a, b), Gesink Law et al. (2005), and Harley et al. (2008), all as cited in Buck Louis (2014)], while two reported significantly lower FORs (indicating longer time to pregnancy) with higher *p,p′*-DDE in cord blood (Chevrier et al., 2013) or maternal serum when trying for pregnancy (Buck Louis et al., 2013). FORs were 0.60 (95% CI = 0.42−0.84) in 332 women in a French birth cohort (Chevrier et al., 2013) and 0.83 (95% CI = 0.70−0.97) in a cohort of 501 couples from various states within the United States (Buck Louis et al., 2013).

The relationship between biological measurements of DDE (isomer[s] not specified) and obesity has been intensively studied in humans, and the epidemiology was reviewed by Tang-Péronard et al. (2011). An update of longitudinal studies examining pre- and perinatal levels of *p,p′*-DDE and obesity in childhood was provided by Liu and Peterson (2015). Studies of obesity in adults included six that were cross-sectional in design and one case-control study [reviewed by Tang-Péronard et al. (2011)]. All of the cross-sectional studies, which examined populations with 42−749 participants, observed a significant positive association between serum DDE and body mass index (BMI), waist circumference, and/or body fat mass [reviewed by Tang-Péronard et al. (2011)]. In prospective cohort studies relating prenatal exposures to obesity in offspring, similar results were seen [reviewed by Liu and Peterson (2015) and Tang-Péronard et al. (2011)]. Higher DDE in maternal or cord serum was associated with risk of higher BMI or weight gain in puberty or adulthood in two of three cohort studies (n = 151 and 304 participants) examining these populations [Gladen et al. (2000) and Karmaus et al. (2009), both as cited in Tang-Péronard et al. (2011)], but no association was observed in the third study (n = 594) [Gladen et al. (2000) as cited in Tang-Péronard et al. (2011)]. In cohort studies that assessed measures of obesity during childhood (up to 7 years of age), *p,p′*-DDE in maternal or cord serum, or breast milk was positively associated with increased risk of obesity (assessed with a variety of metrics) in 8 of 12 studies [reviewed by Tang-Péronard et al. (2011) and Liu and Peterson (2015)]. The study authors of both reviews concluded that there was substantial evidence for an association between DDE and obesity. This conclusion was echoed by a multinational expert panel examining costs of obesity and diabetes associated with exposure to endocrine disrupting chemicals; the panel characterized the strength of epidemiological evidence for an association between DDE and childhood obesity as moderate (Legler et al., 2015).

An NTP workshop review of epidemiological data on the association between persistent organic pollutants (including *p,p′*-DDE) and Type II diabetes was published in 2013 (Taylor et al., 2013). Among 12 studies that included measurements of *p,p′*-DDE in serum and Type II diabetes, a positive and statistically significant association was reported in seven cross-sectional

studies (n = 80−3,049) and one case-control study (n = 749). The remaining three cross-sectional studies (n = 80−196) observed positive associations, but the results were not statistically significant. A single case-control study of 180 participants observed a negative association between Type II diabetes and *p,p′*-DDE in serum. The study authors concluded that there was a strong positive correlation between *p,p′*-DDE and diabetes, but that an assessment of causality could not be made due to potential confounding by obesity, as well as the lack of animal and mechanistic data (Taylor et al., 2013). The multinational expert panel examining costs of adult diabetes (Legler et al., 2015) characterized the strength of epidemiological evidence for an association between DDE and adult diabetes as low.

Epidemiological studies of neurodevelopmental outcomes associated with DDE have been the subject of two recent reviews: Berghuis et al. (2015) and Burns et al. (2013). Burns et al. (2013) reported isomer-specific data when available from the studies reviewed, while Berghuis et al. (2015) did not distinguish among isomers. The neurodevelopmental outcomes assessed in multiple studies, enabling a synthesis of findings, were head circumference at birth, Brazelton Neonatal Behavioral Assessment Scale (BNBAS), and Bayley Scales for Infant Development (BSID). Burns et al. (2013) summarized seven birth cohort studies that related DDE levels to head circumference; six of these, (with population sizes ranging from 41−930 subjects) reported no significant association. The seventh [Wolff et al. (2007) as cited in Burns et al. (2013)] reported a significant negative association between DDE and newborn head circumference; however, Burns et al. (2013) noted several confounding factors, including low maternal weight and older maternal age, that were also associated with smaller head circumference in the assessment by Wolff et al. (2007) as cited in Burns et al. (2013). Among five birth cohorts that tested infants in the BNBAS at various times, significant associations with DDE were reported in two; the others reported no relationship to DDE [reviewed by Berghuis et al. (2015) and Burns et al. (2013)]. Sagiv et al. (2008) observed a significant trend for increased irritability scores in infants ≤2 weeks of age with higher cord levels of *p,p′*-DDE, and Rogan et al. (1986) observed a dose-related increase in the incidence of hyperreflexia in infants (age at testing was not clear from the publication) with increasing concentration of DDE in breast milk fat.

Studies of BSID testing have not shown consistent associations between DDE levels and either the psychomotor or mental development indices (PDI or MDI) [reviewed by Berghuis et al. (2015) and Burns et al. (2013)]. Two studies [Torres-Sanches et al. (2007) and Eskenazi et al. (2006), both as cited in Berghuis et al. (2015)] suggested the possibility that higher DDE levels may be associated with a transient negative effect on the PDI, but not MDI in the first year of life, while a third study [Gladen et al. (1988) as cited in Burns et al. (2013)] suggested a transient negative effect on MDI, but not PDI. A fourth study of effects in the first year of life reported no significant associations with either PDI or MDI [Jusko et al. (2012) as cited in Berghuis et al. (2015)]. In studies testing infants older than 1 year, one [Ribos-Fito et al. (2003) as cited in Burns et al. (2013)] reported significant negative associations between *p,p′*-DDE and both MDI and PDI scores at 13 months of age, while others reported no association with either score [Torres-Sanchez et al. (2007) and Eskenazi et al. (2006), both as cited in Berghuis et al. (2015); Rogan and Gladen (1991) as cited in Burns et al. (2013)].

Based on the reviews and meta-analyses described above, the following conclusions can be drawn regarding the epidemiological data:

1. There is limited evidence for an association between *p,p′*-DDE in biological media and testicular germ cell cancers.
2. There is limited evidence for an association between DDE (isomer[s] not specified) in biological media and respiratory effects (asthma or respiratory tract infections).
3. There is moderate evidence for an association between prenatal DDE (isomer[s] not specified) in biological media and childhood obesity.
4. There is limited evidence for an association between *p,p′*-DDE in biological media and adult diabetes.

Evidence for other health outcomes are inconsistent, inadequate to draw conclusions, or suggest no association with DDE levels.

ANIMAL STUDIES

Oral Exposures

Subchronic-Duration Studies

NCI (1978)

In preparation for the chronic cancer bioassay, the National Cancer Institute (NCI, 1978) conducted a subchronic-duration dietary toxicity study of *p,p′*-DDE in rats and mice. *p,p′*-DDE (purity >95%) in corn oil was mixed with feed and administered ad libitum to groups of five male and five female Osborne-Mendel rats and B6C3F$_1$ mice per concentration for 6 weeks, followed by a 2-week observation period. Diets containing 0, 316, 562, 1,000, 1,780, or 3,160 ppm *p,p′*-DDE were given to rats (0, 27.6, 49.1, 87.5, 155.6, or 276.3 mg/kg-day in males, and 0, 29.9, 53.1, 94.5, 168.3, or 298.7 mg/kg-day in females[2]), while mice received diets containing 0, 139, 193, 269, 363, or 519 ppm (0, 25.1, 34.8, 48.5, 65.5, or 93.6 mg/kg-day in males, and 0, 27.1, 37.7, 52.5, 70.8, or 101 mg/kg-day in females[2]). Only mortality and body-weight changes were evaluated; no animals were necropsied.

One female rat treated at 1,000 ppm died and all of the female rats exposed to higher concentrations died by Week 6; in contrast, no male rats died at doses ≤1,780 ppm (no further details were provided) (NCI, 1978). Mean body weights were reduced in all dose groups among male rats (11% lower than controls at 1,000 ppm and 22% lower at 1,780 ppm), but were not affected among female rats (data were not provided). Among mice, one control male and one male exposed to 269 ppm *p,p′*-DDE died, as well as four males and two females receiving 363 ppm. *p,p′*-DDE did not affect body weights in the exposed mice (data were not provided). In rats, 1,000 ppm in the diet (94.5 mg/kg-day) represents a frank effect level (FEL) based on one female death (all female rats exposed to higher concentrations died). In mice, the FEL of 269 ppm (48.5 mg/kg-day) is based on a single male death. Although one death may not represent an increase over controls (one control mouse also died), the pronounced mortality (4/5 males and 2/5 females) at the next higher dose (363 ppm or 65.5 mg/kg-day in males)

[2]Dose estimates were calculated using reference values for food consumption and body weight (U.S. EPA, 1988c). Reference body weights for Osborne-Mendel rats in a subchronic-duration study: 0.263 kg (males) and 0.201 kg (females). Reference food consumption for Osborne-Mendel rats in a subchronic-duration study: 0.023 kg/day (males) and 0.019 kg/day (females). Reference body weights for B6C3F$_1$ mice in a subchronic-duration study: 0.0316 kg (males) and 0.0246 kg (females). Reference food consumption for B6C3F$_1$ mice in a subchronic-duration study: 0.0057 kg/day (males) and 0.0048 kg/day (females).

suggests that the death of the one male at 269 ppm could be a result of treatment; however, no mice exposed to 519 ppm were reported to have died. Individual variability in susceptibility to the lethal effects of *p,p′*-DDE may have contributed to the differences in survival rate, especially because only a small number of animals was tested. Due to the absence of gross and microscopic pathology examinations in the subchronic portion of the NCI study, other effect levels cannot be assigned.

Banerjee et al. (1996)

Banerjee et al. (1996) evaluated the effects of dietary *p,p′*-DDE exposure on humoral and cell-mediated immune response in Wistar rats. Male rats (n = 8−12) were given either the control diet or a diet containing 200 ppm *p,p′*-DDE (purity 99%) for 6 weeks (18.4 mg/kg-day[3]), during which general condition, food consumption, and body weights were recorded weekly. Three weeks before the end of the exposure period, half of each group was immunized by subcutaneous (s.c.) administration of 3 mg ovalbumin; the other half was left unstimulated. At the end of the exposure period, the rats were sacrificed and blood samples collected. The liver, spleen, and thymus from each animal were removed and weighed. The humoral immune response was quantified by measuring immunoglobulin levels (Immunoglobulin M [IgM] and Immunoglobulin G [IgG]), estimating the albumin:globulin (A:G) ratio, and measuring the ovalbumin antibody titer by enzyme-linked immunosorbent assay (ELISA). Cell-mediated response was assessed in vivo by quantifying the delayed type hypersensitivity (DTH) reaction (measuring footpad thickness after ovalbumin challenge) and in vitro by measuring leukocyte and macrophage migration inhibition. The latter tests assess whether chemical exposure results in suppression of lymphokine production.

Body weights did not differ between treated and control groups. *p,p′*-DDE-exposed rats had significantly ($p < 0.05$) higher relative liver weights (+17%) than control animals; spleen and thymus weights were not affected by treatment. *p,p′*-DDE treatment resulted in depression of both humoral and cell-mediated immune responses, based on significant ($p < 0.05$) reductions in all seven measures. Simultaneous studies with *p,p′*-DDT and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD) showed that *p,p′*-DDE was the most potent immunotoxin. This study establishes a lowest-observed-adverse-effect level (LOAEL) of 18.4 mg/kg-day for biologically significant increases in relative liver weight (+17%) and potential immunotoxicity in male rats fed *p,p′*-DDE for 6 weeks. Because 18.4 mg/kg-day is the only dose tested, a no-observed-adverse-effect level (NOAEL) could not be established.

Chronic-Duration/Carcinogenicity Studies

Tomatis et al. (1974)

Tomatis et al. (1974) evaluated the carcinogenicity of *p,p′*-DDE in CF-1 mice. The study authors administered *p,p′*-DDE in the diet (0 or 250 ppm) to 60 male and 60 female mice (6−7 weeks old) for up to 123 weeks; 100 males and 90 females were maintained on a control diet. A dietary concentration of 250 ppm corresponds to an estimated *p,p′*-DDE dose of 45.0 and 46.0 mg/kg-day for males and females, respectively.[4] The test compound was 99% pure and was

[3]Dose estimates were calculated using a reference body weight of 0.217 kg and a reference food consumption of 0.020 kg/day for male Wistar rats in a subchronic-duration study (U.S. EPA, 1988c).
[4]Dose estimates were calculated using reference values for food consumption and body weight (U.S. EPA, 1988c). Average reference body weight for mice in a chronic-duration study: 0.0317 kg (male) and 0.0288 kg (female). Average reference food consumption for mice in a chronic-duration study: 0.0057 kg/day (male) and 0.0053 kg/day (female).

dissolved in acetone prior to being mixed with powdered food and converted to pellets. Groups of four animals (sex not specified) were sacrificed either between Weeks 65−74 of treatment or between Weeks 94−118 of treatment for analysis of *p,p′*-DDE levels in the liver and interscapular fat (and sometimes in liver tumors and kidney). All animals dying spontaneously or killed humanely were necropsied; remaining animals were sacrificed at 130 weeks of age. Histopathology evaluation was restricted to the lungs, heart, thymus, liver, kidneys, spleen, brain, and any organs with gross abnormalities.

Survival was lower in the *p,p′*-DDE-treated group than in controls (see Table B-1), especially among males (Tomatis et al., 1974). Only 53% of males and 67% of females treated with *p,p′*-DDE survived to 70 weeks, compared with 88 and 87% of controls, respectively.. The study authors did not present a statistical analysis of mortality. However, the incidence of hepatomas was higher in *p,p′*-DDE-treated mice, and mice with hepatomas died earlier than others. Thus, the reduced survival time of treated mice likely resulted from the hepatomas. Clinical signs of toxicity (convulsions and tremors) were observed in three female mice treated with *p,p′*-DDE between the 15th−30th weeks of treatment. The symptoms preceded death in all three cases. Male mice treated with *p,p′*-DDE gained weight more slowly than controls. Terminal body weight was about 11% lower (based on graphical presentation of the data) in the *p,p′*-DDE-treated male mice than in control males. The study authors reported neither a statistical comparison of body weights nor the raw data. The only non-neoplastic lesion reported was an increased incidence in treated males of myocardial necrosis with diffuse hemorrhages, leukocytic infiltration, and fibroblastic reaction (1/98 control males vs. 22/53 treated males; $p < 0.001$; Fisher's exact test performed for this review). Myocardial effects also occurred in male rats in the NCI (1978) chronic-duration study (see below). The *p,p′*-DDE exposure level in this study (250 ppm or ~45 mg/kg-day) appears to be a FEL based on reduced survival in both sexes and early signs of intoxication in females (convulsions, tremors, and death in females). Other treatment-related effects include body-weight depression of ~11% and increased incidence of myocardial necrosis in male mice.

NCI (1978)

NCI (1978) conducted a carcinogenicity bioassay of *p,p′*-DDE in Osborne-Mendel rats and B6C3F$_1$ mice. *p,p′*-DDE (purity >95%) in corn oil was mixed with feed at varying concentrations and rats were fed ad libitum. Nominal concentrations, durations of exposure at these concentrations, and weighted average concentration and dose estimates over the 78-week exposure period are provided in Table B-2. As the table indicates, the exposure concentration was changed several times during the dosing period. In rats, the signs of toxicity during Week 24 prompted the investigators to decrease the exposure concentrations in all groups. A further reduction was made in the high-dose groups (beginning Week 56 in females and Week 60 in males) by suspending exposure for 1-week periods followed by 4 weeks of exposure at the previous concentration. This pattern continued until the exposure period was completed at 78 weeks. Rats were observed for up to 33 weeks after exposure termination and before sacrifice. Mice appeared to tolerate the initial concentrations well, so the investigators increased the exposure concentrations during Week 8. Beginning in Week 37, the dose-reduction pattern used in high-dose rats (1 week off, 4 weeks exposed) was applied to high-dose mice. The mice were observed for up to 15 weeks after the 78-week exposure period and before sacrifice. Weighted average *p,p′*-DDE concentrations given to rats were 0, 437, or 839 ppm (0, 30.6, or

58.8 mg/kg-day[5]) in males and 0, 242, or 462 ppm (0, 18.7, or 35.6 mg/kg-day[5]) in females. Mice received weighted average *p,p′*-DDE concentrations of 0, 148, or 261 ppm (0, 25.3, or 44.8 mg/kg-day in males and 0, 25.6, or 45.1 mg/kg-day in females[5]).

Body-weight and food-consumption measurements, clinical observations, and palpations for masses were conducted weekly for 10 weeks and monthly thereafter; daily mortality checks were performed (NCI, 1978). Necropsy was performed on all animals, but organ weights were not recorded. Histopathologic examination was initially limited to control animals, animals with visible tumors, and at least 10 grossly normal males and females from each group. Later in the study, the protocol was altered to include tissues from additional animals in the study; however, the study authors did not indicate how the other animals were selected, how many were included, or when the protocol change was initiated. Nearly 30 tissues were subjected to microscopic examination. The study authors noted that tissues were not examined from some animals that died early, and that some animals were missing, cannibalized, or in an advanced state of autolysis precluding histopathologic examination. Incidence of lesions was reported using the number of animals for which that specific tissue was examined as the number at risk, except where lesions were observed grossly or could appear at multiple sites (e.g., lymphoma), in which cases the number of animals necropsied was used.

In rats, clinical signs of toxicity began during Week 8 and included hunched or thin appearance, respiratory signs, urine staining, ocular signs, body sores, bloating, and alopecia (incidences and doses not reported), as well as isolated occurrences of tremors, ataxia, loss of equilibrium, hyperactivity, and vaginal discharge in one or two exposed rats (NCI, 1978). *p,p′*-DDE treatment produced significant dose-related trends for decreased survival in both sexes. Survival to 92 weeks (the time of largest difference from control) was 80, 68, and 52% for control, low- and high-dose males, and 100, 84, and 72% in females. A number of the high-dose deaths (including 9 of the 14 females that died prior to Week 92) occurred prior to the dose reduction during Week 24. Early deaths in the high-dose groups at the initial dietary concentrations were clearly treatment-related, but the relationship between mortality and exposure in the low-dose groups, which occurred primarily after the end of treatment at 78 weeks, is less clear. The 100% survival of control female rats was much higher than control male rat survival from the same study, which was 80% at 92 weeks. Other experiments published in the same NCI (1978) document showed 85 and 75% survival at 92 weeks in control female rats of the same strain (experiments for technical DDT and DDD, respectively), comparable to and lower than, respectively, the 92-week survival of 84% in the low-dose female rats in the *p,p′*-DDE experiment. The small number of control rats (20/sex/group) may have contributed to the considerable observed variability in mortality findings among controls. The study authors reported treatment-related reductions in body weight in both male and female rats, but did not present statistical comparisons of group mean body weights or raw data. Based on graphical presentation of the data, the body-weight decrements at the high dose appear to range

[5]Dose estimates were calculated using the reference values for food consumption and body weight (U.S. EPA, 1988c). Reference body weights for Osborne-Mendel rats in a chronic-duration study: 0.514 kg (males) and 0.389 kg (females). Reference food consumption for Osborne-Mendel rats in a chronic-duration study: 0.036 kg/day (males) and 0.030 kg/day (females). Reference body weights for $B6C3F_1$ mice in a chronic-duration study: 0.0373 kg (males) and 0.0353 kg (females). Reference food consumption for $B6C3F_1$ mice in a chronic-duration study: 0.0064 kg/day (males) and 0.0061 kg/day (females).

from 10−15% below controls over the course of the study; in general, lower reductions occurred in the low-dose group.

Histopathology evaluation showed evidence of hepatotoxicity in rats of both sexes, and pulmonary and heart lesions in males (NCI, 1978). Lesion incidences are shown in Table B-3. The study authors did not provide statistical analyses of the non-neoplastic lesions, but statistical tests conducted for this review found significant dose-related trends and pairwise increases in the high-dose group for centrilobular necrosis of the liver in female rats, and fatty metamorphosis of the liver, lung hemorrhage, and myocardial degeneration in male rats (see Table B-3). There was also a significant pairwise increase in the incidence of fatty metamorphosis in the liver of low-dose male rats. The only tumor-related finding was a dose-related trend in the incidence of thyroid tumors (combined incidence of follicular-cell adenomas and follicular-cell carcinomas) in female rats that was not, however, statistically significant at either dose in pairwise tests. Overall, the results of the rat bioassay were not considered by the NCI to provide convincing evidence for carcinogenicity.

A NOAEL is not established in this study. Effects at the high dose included increased early mortality, decreased body weight, and degenerative liver, lung, and heart lesions. At the low dose, degenerative liver lesions were increased in males and possible effects on survival were observed in both sexes. The lowest dose in this study (18.7 mg/kg-day) is classified as a FEL for reduced survival of female rats.

In mice, there were no clinical signs of toxicity until Week 22, when a majority (60−85%, dose not specified) of the male mice appeared hunched; this continued until the intermittent dosing period was instituted during Week 34 (NCI, 1978). Reduced survival of female mice was significantly ($p < 0.001$) associated with increasing *p,p′*-DDE exposure. Survival to 75 weeks was 95, 94, and 56% for control, low-, and high-dose females, respectively. Survival of male mice was higher in the treated groups than in the control group; however, survival of control male mice was low. Only 25% (5/20) of male controls survived at least 70 weeks, compared with 70% of low-dose and 62% of high-dose males. The high control male mortality can probably be attributed to intercurrent disease that also produced high incidences of amyloidosis of the spleen, kidneys, and liver in control males. The incidence of amyloidosis was lower in the exposed groups than in controls; one other study (Rossi et al., 1983) also suggested a protective effect of *p,p′*-DDE exposure on the incidence of amyloidosis. Body weights and weight gain of exposed male mice did not differ from controls. In contrast, body weights of female mice were reduced in both dose groups, with differences increasing throughout the exposure period. The study authors did not present statistical comparisons of group mean body weights or raw data. Based on graphical presentation of the data, the maximum decrement from control weights (near the end of the exposure period) was approximately 10−15% in the low dose females and 15−20% in the high-dose females. The body-weight differences in females persisted throughout the 15-week postexposure observation period.

The study authors reported that non-neoplastic lesions in the exposed mice were "similar in number and kind to those lesions naturally occurring in aged B6C3F$_1$ mice" (NCI, 1978). Examination of the summary data on incidence of non-neoplastic lesions suggested the possibility of a dose-related trend in chronic inflammation of the kidney (2/19, 11/41, and 16/45 at 0, 25.3, and 44.8 mg/kg-day, respectively) among male mice. Statistical tests conducted for this review indicated a significant dose-related trend ($p = 0.04$) and a significant increase over

controls at the high dose ($p = 0.04$). Historical control incidence data were not available to assess whether these incidences are within the normal range for this strain of mouse. The lowest dose tested constitutes the LOAEL for this study, based on body-weight reductions of 10−15% in female mice (25.3 mg/kg-day) and clinical signs of toxicity (hunched or thin appearance) in male mice (25.6 mg/kg-day); a NOAEL cannot be determined from these data. A dose-related increase in the incidence of hepatocellular carcinomas in mice of both sexes (see Table B-4) was observed (NCI, 1978). The incidence of other tumor types was not increased with exposure.

Rossi et al. (1983)

Rossi et al. (1983) treated Syrian golden hamsters with *p,p′*-DDE in the diet for up to 128 weeks. The test article was 99% pure and was dissolved in 3% olive oil before being mixed in the diet at concentrations of 0, 500, or 1,000 ppm (0, 48.5, or 97.0 mg/kg-day in males and 0, 48.3, or 96.6 mg/kg-day in females[6]). Groups of at least 40 male and female hamsters/concentration were given the diet ad libitum beginning at 8 weeks of age. Measurements of body weight and food consumption were made weekly through the first 20 weeks and biweekly thereafter. Animal health observations were recorded with the same frequency. Animals found dead or moribund were necropsied, and any animals surviving to 128 weeks of age were sacrificed and necropsied at that time. A gross examination of all organs and histological examination of the liver, spleen, kidneys, adrenal glands, urinary bladder, thyroid, lungs, testes, ovaries, and organs with gross lesions were performed.

Although statistical analysis did not indicate a difference in mortality among the groups, the animals treated with *p,p′*-DDE lived longer, on average, than controls (Rossi et al., 1983). The study authors attributed this to a protective effect of *p,p′*-DDE against amyloidosis of the liver, kidney, and adrenal glands (amyloidosis occurred with greater incidence in controls than in treated animals). Another study (NCI, 1978) supported a protective effect of *p,p′*-DDE exposure on amyloidosis incidence. Body-weight gain was reduced in a dose-related fashion among the *p,p′*-DDE-treated groups. The study authors did not present a statistical comparison of body weights, nor provide the raw data to permit statistical analysis. Based on graphical presentation of the body-weight data, the terminal body weight was approximately 23% lower than controls among males in the high-dose group and 16% lower than controls among females in the high-dose group. In addition, terminal body weight was about 9% lower than controls among males treated at the low dose, but was not different from controls among females at the low dose. Body-weight differences began about 10 weeks after study commencement among males, but not until 30 weeks of treatment among females. Food consumption was not affected by treatment. The study authors suggested that the lower weight gain may have resulted from liver necrosis, which was more severe in the 1,000-ppm groups than in those treated at 500 ppm (incidence and severity not reported for either group). The incidence of hyperplastic foci of the liver was increased in hamsters treated at 1,000 ppm *p,p′*-DDE (see Table B-5); these lesions are assumed to be preneoplastic. The incidences of liver and adrenal gland tumors were increased in males and females treated with *p,p′*-DDE.

[6]Dose estimates were calculated using the U.S. EPA (1988c) reference values for body weight and food consumption. Reference body weight for Syrian golden hamsters in a chronic-duration study: 0.134 kg (male) and 0.145 kg (female). Average reference food consumption for Syrian golden hamsters in a chronic-duration study: 0.013 kg/day (male) and 0.014 kg/day (female).

The primary aim of this study was to evaluate the carcinogenic potential of *p,p′*-DDE (Rossi et al., 1983). Little information was given regarding noncancer effects; for example, liver necrosis was noted in *p,p′*-DDE treated animals, but the incidence and severity were not reported. Nevertheless, there was a clear effect of treatment on body weight, and mice treated at the high dose (1,000 ppm *p,p′*-DDE) had ≥10% terminal body-weight reductions, which is considered biologically significant. Importantly, the body-weight differences in males appeared long before the first tumor appeared (55 weeks in males), so the body-weight decrements were not secondary to effects of tumors. Thus, the high dose of this study (1,000 ppm or 97.0 mg/kg-day) is considered a LOAEL for body-weight reductions in males. The low treatment dose of 500 ppm (48.5 mg/kg-day) is identified as a NOAEL.

Reproductive/Developmental Studies

Kornbrust et al. (1986)

In a reproductive/developmental (R/D) toxicity study, Kornbrust et al. (1986) treated female Sprague-Dawley (S-D) rats with *p,p′*-DDE (purity >99%) in corn oil by gavage. Groups of 54 and 51 rats were given vehicle or 10 mg/kg (respectively) 5 days/week, for 5 weeks before mating (with untreated males), during gestation, and during lactation through either Postnatal Day (PND) 8 or 19. The 10 mg/kg *p,p′*-DDE dose corresponds to an adjusted daily dose (ADD) of 7.1 mg/kg-day. Body weights were recorded weekly until mating. Reproductive parameters were assessed after parturition, including percent sperm positive, percent pregnant, gestation duration, litter size, and sex ratio. Litter weights were measured on PND 0, 2, 8, 14, and 19. Litters designated for lactation studies were normalized to six male and six female pups. Lactation parameters were assessed just before sacrifice on PND 9 or 20 and included milk production (decrease in body weight after nursing) and milk composition. Milk and blood were collected for *p,p′*-DDE analysis. Upon sacrifice, left-side mammary glands were removed for histologic examination, while right-side glands were used for determining deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content. Liver, kidneys, thymus, ovaries, and uterus of the dams were removed, weighed, and examined microscopically. Four pups per litter were also examined histologically.

Treatment with *p,p′*-DDE had no significant effect on the percentage of females that were sperm positive or pregnant, or on litter size, length of gestation, sex distribution of offspring, or growth of litters (Kornbrust et al., 1986). In the first 8 days following parturition, mortality was slightly higher in the pups from treated dams (3.8%) than in pups from control dams (2.2%), but was within the range of historical controls. Most pup deaths occurred on the day of birth, with no more than three deaths in any litter. The investigators concluded that pup mortality was unrelated to treatment. No further details were provided; therefore, the significance of the slight increase in pup mortality is unknown. In the dams, treatment with *p,p′*-DDE had no effects on organ weights or on indices of lactation capacity (including mammary gland weight). No gross lesions were seen during necropsy of the dams. Histopathological examination revealed hepatocellular changes in the *p,p′*-DDE-treated dams, including swelling, inflammation, increased eosinophilia, increased mitoses, and occasional focal necrosis and hemorrhage. The hepatic lesions were considered to be mild by the investigators. Due to the lack of reporting of incidence data for control and treated animals, the relevance of the observed liver lesions could not be independently reviewed. Treatment-related histological changes were not seen in other maternal organs or in the pups. The concentrations of *p,p′*-DDE in (whole) milk from the *p,p′*-DDE-treated dams were 24.4 ppm on PND 9 and 21.9 ppm on PND 20. Inadequate

reporting of pup mortality incidence and liver histopathology in dams preclude the determination of maternal and developmental effect levels.

Kelce et al. (1995)

The antiandrogenic effects of *p,p′*-DDE on fetal, pubertal, and adult rats were investigated by Kelce et al. (1995). A series of experiments was reported in a brief letter, with few details of each individual experiment. In one experiment, groups of eight pregnant Long-Evans hooded rats were given vehicle or 100 mg/kg *p,p′*-DDE (purity not specified) in corn oil via gavage on Gestation Days (GDs) 14−18. The anogenital distance (AGD) in male pups of treated dams was significantly reduced ($p < 0.04$ by analysis of litter means) and these pups retained thoracic nipples to PND 13. The incidence of the latter effect was not reported; however, the study authors did not observe this effect in control offspring. This experiment provides a LOAEL of 100 mg/kg-day based on demasculinization of male offspring (reduced AGD and presence of thoracic nipples). Because 100 mg/kg-day is the lowest dose tested, a NOAEL cannot be identified.

In a study on effects in pubertal rats, weanling male Long-Evans rats (12/group) were given vehicle or 100 mg/kg-day *p,p′*-DDE (exposure route assumed to be gavage as with the other experiments) from PND 21 until after puberty (to Day 57; exposure duration of 36 days) (Kelce et al., 1995). Serum testosterone levels in exposed rats were not different from controls (timing of samples not reported). However, the onset of puberty (defined as the age at preputial separation) was significantly delayed (5 days later than controls, $p < 0.005$) in treated rats (see Table B-6). The study authors noted that because treated rats had higher body weights than controls, the pubertal delay was not confounded by growth retardation. This experiment suggests a LOAEL of 100 mg/kg-day for delayed puberty; no NOAEL can be identified, as only one dose was tested.

You et al. (1998)

Antiandrogenic effects of gestational *p,p′*-DDE exposure in Long-Evans hooded and S-D rats were assessed by You et al. (1998). Groups of 8−11 pregnant Long-Evans and S-D rats were given *p,p′*-DDE (purity not specified, administered in corn oil) via gavage in doses of 0, 10, or 100 mg/kg on GDs 14−18. A separate group of rats was given flutamide as a positive control. Three pregnant rats from each of the *p,p′*-DDE-treated groups were sacrificed on GD 20 for analysis of *p,p′*-DDE in maternal serum, brain, liver, fat, placenta, and in fetal liver. Remaining dams were allowed to give birth. Pup weights and AGDs were measured on PND 2. External examination of male offspring for thoracic nipple retention was conducted on PND 14. Both male and female pups were examined for sex organ development (preputial separation and vaginal opening).

On PND 21, *p,p′*-DDE-treated dams, as well as two male and one female pups from each litter were sacrificed (You et al., 1998). *p,p′*-DDE content was measured in blood, liver, and fat of both dams and pups, and in brains of the dams. Testes, prostate, and epididymis were removed from one of the two male pups from each litter. One testis was used for northern blot analysis of androgen receptor (AR) messenger RNA (mRNA), while the other testis and the remaining organs were subjected to immunohistochemistry for the AR. On PND 57, two males from each treated litter were sacrificed for analysis of testosterone levels in blood and *p,p′*-DDE content of blood, liver, and fat.

Pup body weights did not differ among the groups in either strain of rat (You et al., 1998). Long-Evans male pups showed statistically significant reductions in AGD (~14%; data presented graphically) and increased thoracic nipple retention at the high treatment dose. Based on graphical representation of the data, the mean number of nipples per pup was approximately 0.5, 0.6, and 2.9 in control, low-dose, and high-dose Long-Evans rats, respectively. In S-D rats, AGD was decreased by 8% in the high-treatment group, and the difference was statistically marginal ($p = 0.065$). The mean number of nipples per pup was significantly increased in the low-dose (1.1) and high-dose (3.8) S-D rats compared to controls (0.3). *p,p′*-DDE treatment did not affect vaginal opening or preputial separation time, and hypospadias were not observed in any *p,p′*-DDE treatment group. There was no significant difference in weights of the testis, epididymis, seminal vesicles, or ventral prostate between the *p,p′*-DDE-treated groups and control groups. *p,p′*-DDE treatment did not result in statistically significant differences in serum testosterone levels measured on PND 57.

Immunohistochemistry for the AR in S-D rats showed decreased staining intensity in the testicular tissues of male offspring of high-dose *p,p′*-DDE treated rats, but no observable difference in low-dose offspring (You et al., 1998). Similarly, staining was reduced in the epithelial cells of the epididymal duct and the glandular acini of the prostate; however, quantitative measures of staining intensity were not provided and there was no mention of the statistical significance. The study authors also indicated that the number of Sertoli cells showing staining for the receptor was lower in the high-*p,p′*-DDE-dose group than in controls (no other details were provided). In Long-Evans rats, there were no observable differences in staining intensity between the *p,p′*-DDE-treated groups and the control groups. AR mRNA was increased in Long-Evans rats in the high-*p,p′*-DDE-dose group, but not in any of the S-D rats. Analysis of *p,p′*-DDE levels in organs and blood showed higher levels in both dams and offspring of the Long-Evans strain compared with S-D rats, especially in blood concentrations measured in dams on GD 20.

This study identifies a developmental LOAEL of 10 mg/kg-day for impaired sexual development (demasculinization represented by retention of thoracic nipples) in male S-D pups. Because 10 mg/kg-day is the lowest dose tested, a NOAEL cannot be identified from this study.

You et al. (1999a)

You et al. (1999a) conducted a follow-up study to evaluate whether in utero exposure to *p,p′*-DDE modified the effect of adult exposure to *p,p′*-DDE on male reproductive organs. Groups (number not reported) of pregnant Long-Evans rats given gavage doses of 0, 10, or 100 mg/kg *p,p′*-DDE (purity >99%, in corn oil) on GDs 14−18 were allowed to give birth. Male pups from all groups were weaned on PND 21 and body weights were measured twice a week from weaning until PND 80. At approximately PND 80, the male pups were divided into two subgroups (5−8 per subgroup) and treated either with corn oil alone or with 70 mg/kg-day *p,p′*-DDE by gavage for 4 days. One day after the final treatment, the rats were sacrificed and *p,p′*-DDE content of liver and perirenal fat was analyzed. Trunk blood was collected and analyzed for serum testosterone and luteinizing hormone (LH) by radioimmunoassay. The testes, epididymides, seminal vesicles, ventral prostates, and kidneys were weighed. Finally, levels of mRNA for two androgen-regulated genes (the C3 subunit of the prostatic secretory prostatein and transient receptor potential cation channel, subfamily M, member 2 [TRPM-2]) and a housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase) were measured in ventral prostate tissues via northern blot analysis. TRPM-2 is an androgen-repressed gene with an

expression that has been associated with cell death during prostatic involution (You et al., 1999a).

Adult body weights were not affected by either in utero or adult treatment with *p,p′*-DDE (You et al., 1999a). Ventral prostate weights were reduced by 18 and 31% in the low- and high-dose groups, respectively, treated in utero alone, when compared with controls not treated in utero (data shown graphically). Neither change was statistically significant ($p = 0.076$ for the high-dose group). As such, the toxicological relevance of the dose-related decreases in ventral prostate weight is uncertain. In rats treated with 70 mg/kg-day as adults and not treated in utero, there was a statistically significant ($p < 0.05$) reduction (31%) in ventral prostate weight when compared with controls untreated at either time. Weights of seminal vesicles and epididymides were also significantly ($p < 0.05$) reduced (32 and 11%, respectively) in rats treated as adults but not treated in utero (see Table B-7). Adult treatment did not affect prostate, seminal vesicle, or epididymis weight in rats previously exposed to *p,p′*-DDE in utero. One rat treated at the high dose in utero and not treated as an adult had suppurative inflammation of the prostate. The study authors noted that only rats demonstrating gross abnormalities were examined histologically; therefore, the true incidence of prostatitis associated with *p,p′*-DDE treatment is unknown.

In utero treatment with *p,p′*-DDE resulted in expression of TRPM-2 but adult treatment with *p,p′*-DDE did not (You et al., 1999a). C3 mRNA was abundant in all treatment groups; therefore, group-related differences were difficult to discern. Serum testosterone levels were higher in rats treated with *p,p′*-DDE as adults, but the increases were not statistically significant at either dose.

The study examined few endpoints and, although potential effects of in utero *p,p'*-DDE treatment on the prostate were reported, the biological significance of such findings is unclear (dose-related decreases in ventral prostate weight were not statistically significant and prostatitis was observed in a single treated rat, but only a few animals were examined histologically). Therefore, the identification of critical effects and associated effect levels from these data is precluded. Acute effects in adult rats treated for 4 days at 70 mg/kg-day are considered outside of the scope of this assessment given the very brief exposure duration.

Loeffler and Peterson (1999)

Loeffler and Peterson (1999) administered *p,p′*-DDE (purity 99%, in 95% corn oil/5% acetone vehicle) by daily gavage to groups of six pregnant Holtzman rats between GDs 14−18. Administered doses were 0, 1, 10, 50, 100, or 200 mg/kg-day. Body weights of dams were measured daily until parturition. After parturition, litters were weighed, and sex ratio and number of live pups were recorded. Litters were then culled to 10 pups, maximizing the number of male pups. On PNDs 1 and 4, crown-rump length and AGDs were measured. Thoracic and abdominal nipple retention was evaluated on PND 13. Body weights were recorded on PNDs 1, 4, 7, 14, 21, 32, 49, and 63. After weaning, dams were sacrificed and the number of uterine implant sites recorded. Daily examination for preputial separation was conducted beginning on PND 38. On PNDs 21, 32, 49, and 63, one or two male rats/litter were sacrificed (3–6 litters per treatment group), at which times, ventral prostate, dorsolateral prostate, seminal vesicles, epididymides, and testes were weighed.

Body-weight data were not reported (Loeffler and Peterson, 1999). The study authors indicated that dams exposed to the highest dose of *p,p′*-DDE had significantly lower body

weights (9−17%) on GDs 17−21, but weights returned to normal by PND 1. *p,p′*-DDE treatment significantly ($p \leq 0.05$) reduced AGD (as a ratio of crown-rump length) in male pups of rats exposed to doses of ≥50 mg/kg-day (see Table B-8); however, this difference persisted to PND 4 only in the 200-mg/kg-day group. In addition, there was a dose-related increase in the number of nipples per male pup on PND 13 that was significant at ≥100 mg/kg-day. Preputial separation was significantly delayed only among offspring of the highest dose group. Prostate-weight data were reported graphically. On PND 21 (weaning), relative weight of the ventral prostate was significantly ($p \leq 0.05$) lower than the control in offspring of dams exposed to ≥50 mg/kg-day (~20−40%, estimated visually), and relative weight of the dorsolateral prostate was significantly decreased at 200 mg/kg-day (~40%). On PND 32 (prepuberty), mean relative ventral prostate weight appeared to decrease (~10−30%) with dose, but differences from control were not statistically significant at any dose. PND 32 relative dorsolateral prostate weight was reported to be significantly ($p < 0.05$) decreased in all treated groups, but based on graphical representation of the data, there was no dose-response relationship between 1−100 mg/kg-day (all ~10% less than control), and a sizeable effect was present only in the 200-mg/kg-day group (~40% less than control). No differences in ventral or dorsolateral prostate weights were observed on PND 49 (puberty) or PND 63 (postpuberty). Weights of seminal vesicles, testes, and epididymides did not differ from controls at any time.

Based on this study, a developmental LOAEL of 50 mg/kg-day is identified for effects on male reproductive development, with a NOAEL of 10 mg/kg-day. Significant effects in male pups at 50 mg/kg-day were a decreased ratio of AGD to crown-rump length on PND 1 and a 20% decreased relative ventral prostate weight on PND 21. At higher doses, larger decreases in relative prostate weight (ventral and dorsolateral) were observed, PND 13 nipple retention was increased, and onset of puberty was delayed. Although statistically significant reductions in relative dorsolateral prostate weight on PND 32 were reported at doses below 200 mg/kg-day, the biological significance of these findings is uncertain due to the small nature of the response, the fact that these changes were transient and did not occur at other PND measurements (PNDs 21, 49, and 63), and the lack of similar results from other male reproductive endpoints (e.g., relative weight of ventral prostrate). Ultimately, the developmental LOAEL is established based on the weight of evidence (WOE), which suggests treatment-related effects occurred at *p,p′*-DDE doses ≥50 mg/kg-day. A maternal LOAEL of 100 mg/kg-day and a NOAEL of 200 mg/kg-day is identified for decreased body weight on GDs 17−21.

Gray et al. (1999)

Gray et al. (1999) also assessed the antiandrogenic effects of *p,p′*-DDE, using both Long-Evans and S-D rats treated during gestation. Pregnant rats were given *p,p′*-DDE (purity 99%, in corn oil) at gavage doses of 0 or 100 mg/kg-day on GDs 14−18 and allowed to give birth. Group sizes were 8 control and treated Long-Evans rats, and 9 control and 11 treated S-D rats. Maternal-weight gain was monitored throughout pregnancy, but the frequency of weight measurements was not reported. The examination of offspring and timing of sacrifice are not clearly described in the publication. Based on the results, it appears that the male offspring were examined for reproductive organ development, including external abnormalities (number and location of retained nipples, cleft phallus, vaginal pouch, and hypospadias) and internal abnormalities (ectopic or atrophic testes, agenesis of the gubernaculums, epididymides, sex accessory glands, and ventral prostate; epididymal granulomas, hydronephrosis, and enlarged bladder with stones). Male offspring were sacrificed for necropsy at either 5 or 15 months of age (it is not clear from the report). Based on the description for a parallel study, it appears that body

weight and the following organ weights were recorded: pituitary, adrenal, kidneys, liver, ventral prostate, seminal vesicles, testes, and epididymides. Histologic examination appears to have been limited to the ventral prostate and seminal vesicles.

The study authors reported that maternal-weight gain was reduced by 35 g (compared with controls) during treatment with *p,p′*-DDE, but weight gain returned to normal after treatment ended (Gray et al., 1999). There were no effects of treatment on pup weight measured at PND 2. There were clear antiandrogenic effects of *p,p′*-DDE treatment on development of male sex organs. Table B-9 shows the parameters affected by *p,p′*-DDE treatment. In Long-Evans rats, maternal exposure to *p,p′*-DDE resulted in significant increases in the percentage of male offspring with areolas, the mean number of retained nipples, and the incidence of prostate atrophy, along with decreasing mean weight of the ventral prostate. In S-D rats, the effects were more pronounced; *p,p′*-DDE exposure resulted in a significant decrease in AGD and decreased weights of the glans penis, cauda epididymis, ventral prostate, and levator ani/bulbocavernosus muscles. In addition, a significantly increased percentage of male S-D offspring had areolas and the mean number of retained nipples was increased. Finally, 7.8% of male S-D offspring of treated dams had hypospadias, while no control animals displayed this effect. Despite the reporting limitations, this study shows a clear effect on the development of male reproductive organs; thus, the dose used (100 mg/kg-day) is a LOAEL both for maternal toxicity (decreased weight gain) and for antiandrogenic effects on male reproductive organ development. A NOAEL cannot be determined from these data because only a single dose was used.

Makita (2008); Makita and Omura (2006)

Two studies investigated the developmental effects of *p,p′*-DDE administration during the prenatal and/or early postnatal periods in rats (Makita, 2008; Makita and Omura, 2006). Pregnant Wistar rats (six/group) were fed a diet containing 0 or 10 mg/kg-day *p,p′*-DDE on GD 1 to PND 21. Doses were selected based on a preliminary study (data not provided) in which administration of *p,p′*-DDE at 50 mg/kg-day induced decreases in maternal body-weight gain of dams during gestation, smaller litter sizes, and increased abortions. On PND 1, pups were counted, gender recorded, and pups examined for gross malformations. Litter parameters measured included number of pups per litter, average litter size, sex ratio, and number of live pups on PND 1. On PND 2, pups were culled (four/sex/litter) and were allowed to nurse until weaned on PND 22 after which time the pups were housed separately by gender and litter. Pup weights were recorded on PNDs 4, 7, 14, and 21, and thereafter every 7 days. AGD was measured on PND 4 and eye opening was determined on PNDs 14, 15, and 16. Pups were sacrificed at 13 weeks of age. Weights were determined for liver, kidneys, spleen, thymus, testes, epididymides, prostate, seminal vesicles, uterus, and ovaries and these tissues were subjected to histopathological examinations. Blood samples collected from pups at sacrifice were analyzed for LH, follicle-stimulating hormone (FSH), testosterone (males only), 17*beta*-estradiol, and thyroxine (females only). Estrous cyclicity, vaginal opening, sperm count, and motility were also determined. Statistical analyses included one-way analysis of variance (ANOVA), followed by Fisher's least significant difference test. Data were analyzed using the litter as the experimental unit.

No maternal deaths or overt signs of toxicity were observed during the study. Litter size, sex ratio, pup weights, AGD, and time to eye opening were similar among the control and treated groups. No test substance-related effects on pup serum hormone levels, organ weights,

histopathology, estrous cyclicity, vaginal opening, or sperm count were observed. The maternal and developmental NOAEL values were 10 mg/kg-day, the only dose tested.

Makita et al. (2005)

Makita et al. (2005) investigated the effects *p,p′*-DDE on pubertal male rats. In this study, *p,p′*-DDE was administered in the diet of male Wistar rats (six/group) at 0 or 10 mg/kg-day during PNDs 42−84. Body weights were recorded every 7 days during the study. At the end of the exposure, all rats were sacrificed. Blood was collected for determination of testosterone, LH, FSH, and biochemical measures of liver and kidney function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and blood urea nitrogen (BUN). Weights were determined for liver, kidneys, spleen, thymus, testes, epididymides, seminal vesicles, and prostate, and these tissues were microscopically examined. Epididymal sperm counts were determined. Statistical analyses included one-way ANOVA, followed by Fisher's least significant difference test. No test substance-related effects on body-weight gain, hormone levels, serum biochemical parameters, sperm count, organ weights, or histopathology were observed. The developmental NOAEL value for pubertal male rats was 10 mg/kg-day, based on no effects at the only dose tested.

Adamsson et al. (2009)

In a developmental toxicity study, pregnant S-D rats (five to six/group) were administered 50 or 100 mg/kg-day *p,p′*-DDE by gavage in dimethylsulfoxide (DMSO)/corn oil on GDs 13.5−17.5 (Adamsson et al., 2009). Control animals (seven/group) were administered vehicle only. Dams were sacrificed on GD 19.5, and the number of fetuses per dam, gender, and fetal body weights were recorded. Blood was collected from male fetuses and pooled for each litter to determine plasma corticosterone and LH levels. Testosterone level and expression of selected steroidogenic enzymes, regulatory factors, and AR were determined in testes and/or adrenal tissue. Fetal testicular and adrenal tissues were examined for histopathological and ultrastructural changes using light and electron microscopy. Statistical analyses included one-way ANOVA, followed by Dunnett's or Dunn's pairwise multiple comparison *t*-test. Data were analyzed using the litter as the experimental unit.

All dams survived. No overt clinical signs and no test substance-related effects on maternal body-weight gain, litter size, number of male fetuses per litter, or fetal body weight were observed. Administration of *p,p′*-DDE to dams at 100 mg/kg-day induced histological and ultrastructural changes in steroidogenic cells of fetal rat testes and adrenals. Changes observed in fetal testicular and adrenal cortex tissue at 100 mg/kg-day included a reduction in the number of lipid droplets and increased vacuolation of lipid droplets. Degeneration of smooth endoplasmic reticulum and mitochondria was also observed in cells of the adrenal cortex at this dose. Incidences of histopathological and ultrastructural changes in fetal testes and adrenals were not provided; therefore, the degree and severity of these lesions is unclear. No other test substance-related toxicological effects were reported. This study identified a maternal NOAEL of 100 mg/kg-day, based on no observed adverse effects. Developmental effect levels were not established due to the lack of reporting of incidence data for histopathology in rat fetal testes and adrenals.

Yamasaki et al. (2009)

The study by Yamasaki et al. (2009) is selected as the principal study for the derivation of the subchronic and screening chronic provisional reference doses (p-RfDs). This study examined the reproductive and developmental effects of *p,p′*-DDE in rats. Pregnant Crl:CD (SD) rats (10/group) were administered 0, 5, 15, or 50 mg/kg-day *p,p′*-DDE by gavage in corn oil from GD 6 to PND 20. Control animals received the vehicle only. Concentration and stability of the test item in the vehicle were confirmed. Doses were selected based on the results of a preliminary study in which administration at 75 mg/kg-day via gavage from GD 6 to PND 20 increased liver weight in both dams and their offspring and decreased the offspring viability index (results not provided). Dams were observed daily throughout the dosing period and their body weights were recorded on GDs 0, 6, 13, and 20, and PNDs 4, 7, 14, and 21. Dams were allowed to deliver and nurse their pups (F1 generation) through PND 21. At birth, pups were counted and examined for anomalies, and sex ratios were recorded. Following weaning of their pups, dams were sacrificed and necropsied, and weights were determined for the liver, ovaries, and uterus. Reproductive and offspring parameters included the following: number of litters; number of pups born; gestation length; gestation, delivery, birth, and live birth indices; sex ratio on PND 0; numbers of live pups on PNDs 4 and 21; viability index on PND 4; and weaning index on PND 21. During the postnatal period, pups were monitored daily for general condition and their body weights were recorded on PNDs 0, 4, 7, 14, and 21 (weaning), and weekly thereafter until sacrifice. AGD was measured on PND 4. On PND 13, offspring were examined for retention of thoracic and abdominal nipples. Weanlings were also examined for vaginal opening beginning on PND 21, or preputial separation beginning on PND 35.

Prior to weaning, F1 animals were randomly assigned to two groups: a group of 15−23 animals (from 10 litters)/sex/dose sacrificed at 12 weeks of age (Group 1) and a group of 18−20 animals/sex/dose evaluated for reproductive performance (Group 2). For Group 1, individual body weights were recorded weekly during the study and immediately prior to necropsy. Group 1 animals were additionally examined for abnormalities, including number and location of retained nipples, cleft phallus, vaginal pouch, and hypospadias. Vaginal cytology examinations were performed on Group 1 females beginning at 8 weeks of age for evaluation of estrous cyclicity. At 12 weeks of age, Group 1 animals were sacrificed, necropsied, and examined internally for ectopic or atrophic testes; agenesis of the gubernacula, epididymides, and sex accessory glands; and epididymal granulomas. Group 2 animals were mated at 12 weeks of age (sibling matings were avoided) and sacrificed on GD 12. At the time of sacrifice, Group 2 dams were examined by cesarean section for numbers of corpora lutea and implantations. Reproductive and offspring parameters for Group 2 animals included number of copulated females, pairing days until copulation, copulation and fertility indices, number of pregnant females, numbers of corpora lutea, implantations, and intrauterine fetal deaths, implantation index, implantation loss, and number of live fetuses. Group 2 males were sacrificed and necropsied at the same time as Group 2 females. Organs weighed for animals in Groups 1 and 2 included the following: uterus, ovaries, testes, epididymides, ventral prostate, seminal vesicles with coagulation gland, levator ani/bulbocavernosus muscles, brain, liver, adrenals, kidneys, thyroids, and pituitary. Histopathology was performed on the following tissues for Group 1 and 2 animals: liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, and thyroids. Statistical analyses included χ^2 tests for copulation, fertility, and gestation indices and histopathology. Offspring data collected prior to weaning were analyzed using the litter as the experimental unit. Birth indices, incidence of external malformations, and offspring viability were analyzed using the Kruskal-Wallis rank sum test, and statistical differences in rank means

among the groups were analyzed by Dunnett's multiple comparison test. Other parameters were analyzed by Dunnett's test, preceded by Bartlett's test for homogeneity of variance. When variance was homogenous, one-way ANOVA was performed; when variance was not homogenous, Kruskal-Wallis rank sum test was performed (Yamasaki et al., 2009).

No mortalities, treatment-related clinical signs, or changes in body weight were observed in parental generation dams (Yamasaki et al., 2009). A statistically significant increase in relative liver weights of dams was observed at 50 mg/kg-day, compared to controls (+20%); however, tabular results were not provided for all groups. Reproductive parameters are shown in Table B-10. A slight, statistically significant increase (+3%) in live birth index ([number of live pups on PND 0 ÷ number of pups born] × 100) was observed in mid- and high-dose animals. On PND 21, the number of pups alive was statistically significantly decreased at the high-dose level (−4%). The reduced number of pups at the high dose was accompanied by a statistically significant decrease in weaning index ([number of live pups on PND 21 ÷ number of live pups after culling on PND 4] × 100) on PND 21 (−4%). No other test substance-related effects on reproductive parameters were reported. Male pups showed significantly delayed preputial separation in the 50-mg/kg-day group (43.0 ± 2.5 vs. 41.8 ± 1.8 days in control), while female pups in this group had completed vaginal openings significantly earlier than controls (28.6 ± 1.8 vs. 30.7 ± 1.9 days). No other effects on pup parameters, including body weight, AGD, and nipple retention were reported.

Reproductive parameters for the F1 generation Group 2 animals are shown in Table B-11. Decreases in the copulation index ([number of copulated females ÷ number of mated females] × 100) (−35%) and fertility index ([number of pregnant females ÷ number of copulated females] × 100) (−35%) were observed at all exposure doses, but statistical significance was only achieved at the highest dose group (50 mg/kg-day). No other test substance-related effects on reproductive parameters and no effects on pup parameters, including body weight, were reported for the F1 generation (Yamasaki et al., 2009).

Body- and organ-weight parameters for F1 generation adults (Group 1) are shown in Table B-12. No effects on terminal body weight or estrous cyclicity (data not reported) were observed in the Group 1 animals sacrificed at 12 weeks of age (Yamasaki et al., 2009). Relative liver weights were statistically significantly increased in low-, mid-, and high-dose males (+9, +10, and +11%, respectively). Relative seminal vesicle weight was statistically significantly increased in high-dose males (+18%). In females, statistically significant increases in relative adrenal weight and relative liver weight were observed at the high dose (+14 and +9%, respectively). No histopathological changes or other test substance-related toxicity effects were reported.

Maternal NOAEL and LOAEL values of 15 and 50 mg/kg-day are identified based on increased relative liver weight (≥10%) in dams. The increases in relative liver weight (9−11%) in adult male offspring are considered developmental effects, given that the animals were exposed to *p,p′*-DDE during gestation and via lactation. Therefore, a developmental LOAEL of 5 mg/kg-day is identified for increased relative liver weight in males (statistically significant and ≥5% increase relative to controls, which is considered biologically significant for developmental liver-weight changes). A developmental NOAEL cannot be identified from this study. Evidence of reproductive and antiandrogenic effects was seen at 50 mg/kg-day.

Song et al. (2014)

Song et al. (2014) investigated fertility and transgenerational inheritance of an epigenetic change in the *Igf2* gene, which is associated with impaired male fertility in male offspring over three generations. Timed-pregnant S-D rats (20/group) were administered 0 or 100 mg/kg-day *p,p′*-DDE by gavage in corn oil on GDs 8−15. Control animals received the vehicle only. When the F1 generation reached maturity, males and females (20/sex/group) were mated to produce F2 progeny. To determine whether test substance-related epigenetic changes in *Igf2* were carried by males or females, F3 progeny were generated from the following four pairings (20−22/sex/group) of F2 controls (C) or *p,p′*-DDE-exposed (*p,p′*-DDE) male (M) or female (F) animals: (1) C-M × C-F, (2) *p,p′*-DDE-M × *p,p′*-DDE-F, (3) *p,p′*-DDE-M × C-F, or (4) *p,p′*-DDE-F × C-M. No inbreeding or sibling crosses were performed. Litter sizes were recorded for each generation. All adult F1−F3 generation males were sacrificed on PND 120. Following sacrifice, collections of blood and sperm were made for determining serum testosterone level, sperm count, and sperm motility. Testes were weighed and microscopically examined. Testicular samples were also evaluated for spermatogenic cell apoptosis, expression of paternally (*Igf2*) and maternally (*H19*) expressed genes, methylation status of *Igf2*, and global methylation status of sperm DNA. Statistical analyses included independent-samples *t*-test, Pearson's χ^2 test, or ANOVA.

No mortalities or clinical signs were reported. Mean litter size and testosterone levels were similar for all generations (F1−F3). Small testes and impaired fertility were observed in F3 generation males exposed to *p,p′*-DDE at incidences (percent affected) of 0/13 (0%), 3/13 (23%), 4/20 (20%), and 0/20 (0%) for the following respective pairings: C-M × C-F; *p,p′*-DDE-M × *p,p′*-DDE-F; *p,p′*-DDE-M × C-F; and *p,p′*-DDE-F × C-M. Microscopic examination of testes revealed histopathological changes in the testes of animals exposed to *p,p′*-DDE, including abnormal seminiferous tubule morphology without elongated spermatids; however, incidences among groups were not provided. Sperm parameters are shown in Table B-13. Statistically significant decreases in sperm number and motility were observed in *p,p′*-DDE-exposed animals of all three generations (F1−F3) relative to controls. Sperm abnormalities were not present in F3 generation animals of the C-M × DDE-F pairing, indicating that the male germline carried the transgenerational sperm quality defects. Significant increases in apoptosis of spermatogenic cells, including the spermatogonia and primary spermatocytes, were observed in all three generations exposed to *p,p′*-DDE. Exposure to *p,p′*-DDE also induced significant changes in mRNA expression in sperm of *Igf2* (decrease) and *H19* (increase). In addition, significant decreases in methylation status were observed at several CpG-methylation sites in *Igf2*, indicating that *p,p′*-DDE exposure induces heritable changes in *Igf2*-methylation status. No other test substance-related toxicological effects were reported. Maternal NOAEL and LOAEL values are not identified due to the absence of any data on treated dams. A developmental LOAEL value of 100 mg/kg-day is identified for male offspring, based on decreases in sperm number and sperm motility, increases in apoptosis of spermatogonia and primary spermatocytes, and small testis. Because 100 mg/kg-day is the only dose tested, a NOAEL is not identified (Song et al., 2014).

Patrick et al. (2016)

Patrick et al. (2016) investigated effects in male reproductive development of rats exposed in utero, during lactation and directly to *p,p′*-DDE. Pregnant S-D rats (six/group) were given 0 or 35 mg/kg-day *p,p′*-DDE for 14 days during gestation and continuing through PND 20. Subsequently, *p,p′*-DDE was administered by gavage directly to male F1 rats (24−27/group) for

70 days (PNDs 21−90) at 0 or 35 mg/kg-day. The control group was treated with cottonseed oil. At PND 90, male F1 rats were euthanized. The animals were examined for ADG, body weight, and blood testosterone levels. Weights were determined for liver, testes, epididymis, prostrate, and seminal vesicles. Histopathological examinations were conducted for testes, epididymis, seminal vesicles, and liver. No maternal endpoints were measured. Statistical analyses involved survey linear regression to adjust for the dependence of data within litters. Differences among exposure groups were analyzed using adjusted Wald test at a 0.05 significance level.

Developmental parameters for F1 male rats reported in the study are shown in Table B-14. No statistically significant changes were observed in terminal body weights or AGD with *p,p′*-DDE exposure. Significant organ-weight changes were restricted to increases in absolute (+18%) and relative (+23%) liver weight, as well as, increases in absolute (+7%) and relative (+12%) testis weight in *p,p′*-DDE-exposed male offspring compared to controls. Histological examinations revealed the presence of irregular hepatocellular organization and lipid droplet formation in liver samples from the *p,p′*-DDE-exposed group. No abnormalities were noted in the liver tissue of the control animals. Incidence data was not provided but histological images (100 µm) for each of the treatment groups were presented. Testicular lesions including, seminiferous tubules containing dilated tubular lumens, marked detachment of the seminiferous tubule, necrosis in the interstitium, marked disorganization of the seminiferous epithelium, absent seminiferous tubules, and decreased cellularity of the seminiferous epithelium were associated with *p,p′*-DDE treatment. Furthermore, seminiferous tubule diameter, epithelium thickness, and lumen diameter were significantly reduced in the *p,p′*-DDE-exposed group. Testosterone levels were elevated with *p,p′*-DDE exposure. No other treatment-related effects were reported. The single dose tested is a LOAEL (35 mg/kg-day) for increases in liver and testis weights, and abnormal liver and testicular histology. A NOAEL is not identified.

Veeramachaneni (2006)

In a developmental toxicity study in rabbits, Veeramachaneni (2006) evaluated the effects of gestational exposure to *p,p′*-DDE on the development of reproductive tissues in male offspring. Pregnant Dutch-belted rabbits (4−6/group) were orally (dosing method was not specified) administered 0 or 100 mg/kg-day *p,p′*-DDE in corn oil on alternate days during GDs 15−30. This window of exposure was selected because it encompasses sexual differentiation of rabbits, including differentiation of Leydig cells and Sertoli cells. The dose level was selected based on the results of a preliminary study in which no effects on maternal or offspring survival were observed. Dams were allowed to deliver and nurse their pups until the pups were weaned at 6 weeks of age. Upon weaning, male pups were individually caged and monitored weekly for testicular descent. Pups were sacrificed between 24−26 weeks of age and their testes were collected. Testes were examined for histopathological and ultrastructural changes using light and electron microscopy, with emphasis on normalcy of differentiating germ cells and the presence of atypical germ cells resembling testicular carcinoma in situ (CIS).

Effects on maternal animals were not reported. Unilateral cryptorchidism was observed at an increased incidence (4/12) in pups exposed to *p,p′*-DDE during gestation relative to controls (0/4); however, only a small subset of pups (i.e., 4 or 12/group) was examined for testicular descent and statistical analyses were not discussed. Undescended testes of pups exposed to *p,p′*-DDE contained atypical germ cells, which exhibited morphological hallmarks of CIS, including large nuclei with irregular contours and abnormal chromatin patterns and clumps, unusual cytoplasmic inclusions, occasional mitotic figures, unusual membranous profiles,

irregular nuclear contours, and swollen mitochondria. Incidences of histopathological and ultrastructural changes in pup testes were not provided. No other test substance-related toxicological effects were reported. Maternal NOAEL and LOAEL values are not identified based on the lack of information for maternal effects. Although the study noted an increased incidence of unilateral cryptorchidism and presence of atypical germ cells in undescended testes in male pups exposed in utero, the small number of animals examined and the lack of incidence data for testicular histopathology, preclude the determination of effect levels.

Inhalation Exposures

No studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Genotoxicity Studies

Genotoxicity data for *p,p′*-DDE have been summarized by WHO (2011), ATSDR (2002), and IARC (1991). *p,p′*-DDE was negative in multiple tests for mutagenicity in bacteria with or without activation, but was positive in a test for sex-linked recessive lethal mutations in *Drosophila*, and assays for mutagenicity in Chinese hamster ovary (CHO) cells and mouse lymphoma L5178Y cells. Assays for DNA damage in bacteria and unscheduled DNA synthesis in primary rat, mouse, and hamster hepatocytes were negative, but a comet assay for DNA damage in peripheral blood mononuclear cells collected from healthy human donors and exposed in vitro was positive. *p,p′*-DDE did not produce chromosomal aberrations (CAs) in CHO cells, but did give weak positive results in Chinese hamster V79 and B14F28 cells. A test for sister chromatid exchanges (SCEs) in CHO cells gave weak positive results with activation. Studies of exposed human populations in vivo found positive associations between blood levels of *p,p′*-DDE and DNA damage, but not peripheral blood lymphocyte micronuclei (MN) counts.

Recent studies evaluating the potential genotoxicity of *p,p′*-DDE are summarized below (see Table 4A for more details). These studies provide additional support for DNA damage and micronucleus formation associated with *p,p′*-DDE exposure.

In nonmammalian eukaryotic cells, DNA damage was reported in *Dreissena polymorpha* (zebra mussel) hemocytes in the single-cell gel electrophoresis (SCGE) assay (i.e., the comet assay) following 48−168 hours of exposure to *p,p′*-DDE at concentrations of 0.1, 2.0, or 10 µg/L (Binelli et al., 2008a). In a MN test, exposure to *p,p′*-DDE at concentrations ≥2 µg/L increased the frequency of MN formation in *D. polymorpha* hemocytes after 48−96 hours of treatment (Binelli et al., 2008b).

Mixed results were produced in mammalian cell genotoxicity assays. Gerić et al. (2012), in a cytokinesis-block micronucleus assay, reported that exposure to *p,p′*-DDE at 4.1 µg/mL for 1−24 hours significantly increased the frequency of micronucleated cells, numbers of nucleoplasmic bridges, and nuclear buds in human peripheral blood lymphocytes. This concentration was cytotoxic after 6 hours. The same concentration of *p,p′*-DDE (4.1 µg/mL) was positive for induction of DNA damage in vitro in human peripheral blood lymphocytes in the comet assay (measures DNA strand breaks, DNA-DNA or DNA-protein crosslinks, and alkali-labile sites), but was negative for induction of oxidative DNA damage, as measured by 8-OHdG formation, in the formamido-pyrimidine DNA glycosylase (FPG)-modified comet assay (Gerić et al., 2012). Ennaceur et al. (2008) reported a significantly increased frequency of binucleated cells with MN in primary human peripheral lymphocytes exposed to *p,p′*-DDE in a

cytokinesis-block micronucleus assay, but only at the highest concentration tested (25,400 µg/mL). Significant cytotoxicity was also reported at this concentration. MN were not induced in human HepaRG cells following single or repeated in vitro exposure to *p,p′*-DDE (Jossé et al., 2012) at substantially lower concentrations (up to 31.8 µg/mL), followed by a 72-hour mitogenic stimulation with epidermal growth factor (EGF).

Genotoxicity tests in vivo have also produced mixed results for induction of DNA damage following exposures to environmental pollutants, including DDT. The comet assay was performed on blood from children exposed to low or high concentrations of DDT within their communities (and thus, with low or high total DDT blood concentrations) (Jasso-Pineda et al., 2015). DNA damage, based on tail moment, was significantly increased in children from communities with high DDT exposure; however, these children were also found to have high exposure to polycyclic aromatic hydrocarbons (PAHs). There was no attempt to quantify *p,p′*-DDE concentrations in the blood in this study. Scheirs et al. (2006) did not find a relationship between muscle tissue concentrations of *p,p′*-DDE in wood mice and DNA damage, as analyzed in a comet assay.

Table 4A. Recent *p,p′*-DDE (CASRN 72-55-9) Genotoxicity Studies						
Endpoint	**Test System**	**Doses/ Concentrations Tested[a]**	**Results without Activation[b]**	**Results with Activation[b]**	**Comments**	**References**
Genotoxicity studies in prokaryotic organisms						
ND						
Genotoxicity studies in nonmammalian eukaryotic organisms						
DNA damage (SCGE; alkaline comet assay)	*Dreissena polymorpha* (zebra mussel); mussels were exposed under semistatic conditions for up to 168 hr. Hemolymph (100 µL) was extracted from the posterior adductor muscle of 10 mussels/concentration at 48, 96, and 168 hr after exposure initiation, and hemocytes were evaluated for DNA damage.	0, 0.1, 2, 10 µg/L in DMSO for 48, 96, and 168 hr	+	NDr	DNA damage was significantly increased over control in a dose- and time-related manner following exposure to *p,p′*-DDE, as indicated by increases in length of migration:comet head diameter ratio and in the percentage of DNA in the comet tail. By 48 hr of exposure, between 59−92% of the hemocytes examined fell into the three highest DNA damage classes. Cytotoxicity was not reported.	Binelli et al. (2008a)
MN test	*D. polymorpha* (zebra mussel) hemocytes; mussels were exposed under semistatic conditions for up to 168 hr. Hemolymph (100 µL) was extracted from the posterior adductor muscle of 10 mussels/concentration at 48, 96, and 168 hr after exposure initiation and hemocytes were examined for MN formation.	0, 0.1, 2, 10 µg/L in DMSO for 48, 96, and 168 hr	+	NDr	MN frequencies were observed to increase above controls with length of exposure to *p,p′*-DDE and were significantly increased at concentrations ≥2 µg/L after 48 hr. Cytotoxicity was not reported.	Binelli et al. (2008b)

Table 4A. Recent *p,p′*-DDE (CASRN 72-55-9) Genotoxicity Studies

Endpoint	Test System	Doses/ Concentrations Tested[a]	Results without Activation[b]	Results with Activation[b]	Comments	References
Genotoxicity studies in mammalian cells—in vitro						
DNA damage (alkaline comet assay)	Human peripheral blood lymphocytes	0, 4.1 µg/mL in water for 1, 6, and 24 hr	+	NDr	Tail intensity (percent of DNA in comet tail) was significantly increased after 6 and 24 hr of exposure to *p,p′*-DDE. After 24 hr, the percentage of DNA in the tail was 11.21%, compared to 1.81% for the control. In a concurrent cytotoxicity test, the percentage of viable cells decreased after 6 and 24 hr (~25−50%); necrosis was the primary observed effect.	Gerić et al. (2012)
DNA damage (FPG-modified comet assay; 8-OhdG formation)	Human peripheral blood lymphocytes	0, 4.1 µg/mL in water for 1, 6, and 24 hr	−	NDr	Tail intensity (percent of DNA in comet tail) was not significantly increased following exposure to *p,p′*-DDE for up to 24 hr. In a concurrent cytotoxicity test, the percentage of viable cells decreased after 6 and 24 hr (~25−50%); necrosis was the primary observed effect.	Gerić et al. (2012)

Table 4A. Recent *p,p′*-DDE (CASRN 72-55-9) Genotoxicity Studies

Endpoint	Test System	Doses/ Concentrations Tested[a]	Results without Activation[b]	Results with Activation[b]	Comments	References
Cytokinesis-block MN assay	Human peripheral blood lymphocytes	0, 4.1 µg/mL in water for 1, 6, and 24 hr	+	NDr	*p,p′*-DDE induced significant increases in the number of micronucleated cells (~fourfold), as well as in the total numbers of MN and nuclear buds after all exposure periods. The total number of nucleoplasmic bridges was significantly increased after 6 and 24 hr. Cytotoxicity was observed only after 24 hr of exposure, based on a significant decrease (−11%) in the cytokinesis-block proliferation index. However, in a concurrent cytotoxicity test, the percentage of viable cells decreased after 6 and 24 hr (~25−50%); necrosis was the primary observed effect.	Gerić et al. (2012)
MN test (with modifications)	Human HepaRG cells	0, 1, 10, 50, 100 µM in DMSO (0, 0.318, 3.18, 15.9, 31.8 µg/mL, respectively)[c] for 24 hr; or 0, 10, 50 µM in DMSO, multiple treatments (3) over 7 d	−	NDr	Modifications of the standard MN test included: increased cell seeding density; in situ exposure followed by a 72-hr mitogenic stimulation with EGF. *p,p′*-DDE did not induce an increase in the frequency of micronucleated cells after 24 hr or after repeated exposures over 7 d. Cytotoxicity was not reported.	Jossé et al. (2012)
Cytokinesis-block MN assay	Primary human peripheral blood lymphocytes (three donors)	0, 10, 20, 40, 80 mM in DMSO (0, 3,180, 6,360, 12,700, 25,400 µg/mL, respectively)[c]	+	NDr	The frequency of binucleated cells with MN was significantly increased (1- to 18-fold) only at the highest concentration (80 mM). Cytotoxicity was observed based on a significant decrease (−35−66%) in the cytokinesis block proliferation index at this concentration.	Ennaceur et al. (2008)

Table 4A. Recent *p,p′*-DDE (CASRN 72-55-9) Genotoxicity Studies						
Endpoint	**Test System**	**Doses/ Concentrations Tested[a]**	**Results without Activation[b]**	**Results with Activation[b]**	**Comments**	**References**
Genotoxicity studies—in vivo						
DNA damage (comet assay)	Peripheral blood cells; 256 children representing 11 communities with varied DDT exposure resulting in varied DDT/DDE blood concentrations. Blood samples were drawn from the cubital vein and total DDT (DDT + DDE) were quantified using extraction, concentration, and GC coupled with MS. Mean total DDT blood concentrations were determined per community and compared to the National Geometric Mean total DDT blood concentration (2,050 ng/g lipid). DNA damage was then assessed using the comet assay.	Communities A−G: Mean blood concentrations of total DDT: 12.5−285 ng/g lipid (low-exposure group); Communities H−K: 8,500−21,500 ng/g lipid (high-exposure group)	+	NA	DNA damage (measured as tail moment) was evaluated according to exposure level: (1) low exposure: total DDT blood concentration <2,050 ng/g lipid (National Geometric Mean total DDT blood concentration); (2) high exposure: majority had total DDT blood concentration ≥2,050 ng/g lipid. Tail moment was significantly greater (~50%) in children with high total DDT blood concentrations than in those with low total DDT blood concentrations; however, children with high DDT exposure also had high concurrent exposure to PAHs. There was no attempt to quantify *p,p′*-DDE concentrations in the blood.	Jasso-Pineda et al. (2015)

Table 4A. Recent *p,p′*-DDE (CASRN 72-55-9) Genotoxicity Studies

Endpoint	Test System	Doses/ Concentrations Tested[a]	Results without Activation[b]	Results with Activation[b]	Comments	References
DNA damage (comet assay)	*Apodemus sylvaticus* (wood mouse) (*n* = 10, 10, 11, and 15) blood. Mice were collected from two heavily contaminated areas (Forts 8 and 7) at a primary pollution source (a nonferrous smelter), and two control areas (Forts 5 and 4) located farther away. Retro-orbital blood samples were drawn for analysis in the standard comet assay. Mice were sacrificed and muscle tissue was harvested to determine *p,p′*-DDE (and PCB) concentrations.	Concentrations of *p,p′*-DDE in mouse tissue ranged between 0.15−25.1 ng/g wet weight (6.2−556.5 ng/g lipid weight)	−	NA	No relationship was found between *p,p′*-DDE levels in mice and DNA damage. Mice from Fort 5 had the highest mean *p,p′*-DDE concentration (161.4 ng/g lipid). Concentrations of *p,p′*-DDE in mice from Forts 4, 7, and 8 were lower: 55.2, 72.5, and 56.4 ng/g lipid, respectively. Based on increasing tail moments as a measure of DNA damage, mice from Fort 5 (highest *p,p′*-DDE concentration) had the least amount of DNA damage, relative to mice from the other forts.	Scheirs et al. (2006)

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive; − = negative.
[c]Molarity conversion based on molecular weight of 318.0292.

DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; DDT = *p,p′*-dichlorodiphenyltrichloroethane; EGF = epidermal growth factor; FPG = formamidopyrimidine-DNA glycosylase; GC = gas chromatography; MN = micronuclei; MS = mass spectrometry; NA = not applicable; ND = no data; NDr = not determined; PAH = polycyclic aromatic hydrocarbon; PCB = polychlorinated biphenyl; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SCGE = single cell gel electrophoresis.

Supporting Animal Toxicity Studies

Several short-term duration screening studies via the oral route (summarized in Table 4B) designed to investigate the androgenic and antiandrogenic effects of *p,p′*-DDE provide supportive evidence that the liver and reproductive organs are potential targets of *p,p′*-DDE toxicity. In four Hershberger assays (Moon et al., 2009; Freyberger et al., 2007; Shin et al., 2007; Freyberger et al., 2005), *p,p′*-DDE was administered to groups of castrated, young adult (49−52 days old) male Wistar or S-D rats (six/group) at gavage doses of 5−160 mg/kg-day for 10 days. All rats received supplementary testosterone propionate (TP) via s.c. injection. In all of these studies, administration of *p,p′*-DDE at 16 mg/kg-day for 10 days induced statistically significant changes in absolute and/or relative tissue weights, including significant increases in liver weights and significant decreases in seminal vesicles, glans penis, levator ani/bulbocavernosus muscles, ventral prostate, and Cowper's glands weights, relative to controls (TP only). One of the studies also reported an increased incidence of histopathological changes in the liver at 16 mg/kg-day, including hepatocellular hypertrophy and cytoplasmic changes (Freyberger et al., 2005).

In two additional Hershberger assays (Freyberger and Schladt, 2009; Tinwell et al., 2007), intact weanling (22−23 days old) male Wistar or S-D rats (six/group) received the same *p,p′*-DDE doses as above (5−160 mg/kg-day) via gavage for 10 days. All rats received supplementary TP via s.c. injection. Effects observed in intact weanlings were similar to those observed in castrated young adult rats (effects reported above) and were as follows: significant increases in liver weights and significant decreases in the weights of several reproductive tissues, including the epididymides, levator ani/bulbocavernosus muscles, seminal vesicles, and prostate after 10 days of exposure to *p,p′*-DDE.

In another study, adult (120 days old) male rats (six/group) were castrated and implanted with testosterone-containing capsules (to provide constant serum androgen levels) and then treated by gavage with doses of 0 or 200 mg/kg-day for 4 days (Kelce et al., 1995). Seminal vesicle and ventral prostate weights in treated rats were significantly lower than controls. Serum testosterone levels were not affected by treatment.

Table 4B. Short-Term-Duration Screening Studies of the Androgenic and Antiandrogenic Effects of *p,p′*-DDE

Test	Materials and Methods	Results	References
Hershberger assay (oral); young adult; stimulated with TP (0.4 mg/kg, s.c.)	Groups of castrated male Wistar rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 5, 16, 50, and 160 mg/kg-d in corn oil via gavage for 10 d.	Increased absolute liver weight; decreased Cowper's gland weight; hepatocellular hypertrophy, and cytoplasmic change	Freyberger et al. (2005)
Hershberger assay (oral); young adult; stimulated with TP (0.4 mg/kg, s.c.)	Groups of castrated male Wistar rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 16, or 160 mg/kg-d in corn oil via gavage for 10 d.	Increased relative liver weight	Freyberger et al. (2007)
Hershberger assay (oral); young adult; stimulated with TP (0.4 mg/kg, s.c.)	Groups of castrated male S-D rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 16, or 160 mg/kg-d in corn oil via gavage for 10 d.	Increased relative liver weight; decreased relative weights of ventral prostate, seminal vesicles, levator ani/bulbocavernosus muscles, glans penis, and Cowper's glands	Moon et al. (2009)
Hershberger assay (oral); young adult; stimulated with TP (0.4 mg/kg, s.c.)	Groups of castrated male S-D rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 5, 16, 50, or 160 mg/kg-d in corn oil via gavage for 10 d.	Increased relative liver weight; decreased relative weights of ventral prostate and seminal vesicles	Shin et al. (2007)
Hershberger assay (oral); weanling rats; stimulated with TP (0.4 mg/kg, s.c.)	Groups of intact male Wistar rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 16, or 160 mg/kg-d in corn oil via gavage for 10 d.	Increased relative liver weight; decreased absolute and relative seminal vesicle weights	Freyberger and Schladt (2009)
Hershberger assay (oral); weanling rats; stimulated with TP (1 mg/kg, s.c.)	Groups of intact male S-D rats (6/group) were exposed to *p,p′*-DDE at doses of 0, 5, 16, 50, or 160 mg/kg-d in corn oil via gavage for 10 d.	Increased absolute weight of liver; decreased absolute weights of epididymides, levator ani/bulbocavernosus muscles, seminal vesicles, and prostate	Tinwell et al. (2007)

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene; s.c. = subcutaneous; S-D = Sprague-Dawley; TP = testosterone propionate.

Metabolism/Toxicokinetic Studies

In a study of the toxicokinetics of DDT and its metabolites (including *p,p′*-DDE), an adult male volunteer ingested 5 mg/day of *p,p′*-DDE for 92 days (Morgan and Roan, 1974, 1971). The pesticide was mixed with vegetable oil, emulsified with gum arabic and water, and taken with meals (no further detail on dosing was provided). Serum and adipose levels of *p,p′*-DDE rose steadily during the exposure period, peaking at exposure termination at approximately 240 ppb in serum and 45 ppm in adipose tissue (based on visual examination of data presented graphically). Ninety-one percent of the ingested dose was stored in adipose tissue over the exposure period. After exposure was withdrawn, serum and adipose levels of *p,p′*-DDE remained elevated. *p,p′*-DDE concentrations measured 260 days after exposure termination were approximately 150 ppb in serum and 40 ppm in adipose tissue. No urinary excretion was measured for up to 1 year after the first dose (urine concentrations were measured monthly). *p,p′*-DDE was detected in adipose tissue, breast milk, and placenta from many study populations of environmental exposure (Xu et al., 2015; Hernik et al., 2014; Man et al., 2014; Bedi et al., 2013; Song et al., 2013; Bergkvist et al., 2012; Cok et al., 2012; Wang et al., 2011; Azeredo et al., 2008; Shen et al., 2007; Bouwman et al., 2006). Storage in adipose tissue was also observed in rats given a single intravenous (i.v.) dose of *p,p′*-DDE (Mühlebach et al., 1991). Placental and lactational transfer was demonstrated in rats after oral dosing with *p,p′*-DDE from GDs 14–18, although lactational transfer accounted for a greater accumulation of *p,p′*-DDE in the rat offspring (You et al., 1999b).

The metabolism and excretion of *p,p′*-DDE has been studied in experimental animals (ATSDR, 2002). *p,p′*-DDE is an intermediate metabolite of the organochlorine pesticide, *p,p′*-DDT, resulting from dehydrodechlorination of the parent compound. In rats, *p,p′*-DDE is slowly converted to 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethene (DDMU), 1,1-*bis*(*p*-chlorophenyl)ethene (DDNU), and eventually to 2,2-*bis*(*p*-chlorophenyl) acetic acid (DDA), which is conjugated and excreted in the urine (ATSDR, 2002; Datta, 1970). Metabolism occurs in both the liver and kidney, and DDMU-epoxide is postulated as a possible reactive intermediate of *p,p′*-DDE in rats (ATSDR, 2002). Methylsulfonyl metabolites of *p,p′*-DDE have also been found in several mammalian species, including humans (ATSDR, 2002). Formation of 2- and 3-methylsulfonyl-DDE follows cytochrome (CYP) oxidation to an arene oxide, glutathione (GSH) conjugation, excretion into bile, cleavage by microbial C-S lyase, methylation of thiols, reabsorption in the gut, and oxidation to form methyl sulfones that are distributed in the blood (ATSDR, 2002). 3-Methylsulfonyl-DDE produces adrenal cortical toxicity in mice (Jönsson et al., 1992; Jönsson et al., 1991; Lund et al., 1988). In rats given a single i.v. dose of *p,p′*-DDE, 28−34% of the administered dose was excreted in feces and 0.2−1% in urine (14 days after dosing). Approximately 10% of the excreted radioactivity was unchanged *p,p′*-DDE in the feces; no unchanged *p,p′*-DDE was detected in the urine (ATSDR, 2002; Mühlebach et al., 1991). The total body burden half-life from this study was 120 days (Mühlebach et al., 1991).

Mode-of-Action/Mechanistic Studies

Toxicological studies of *p,p′*-DDE have identified the liver and developing male reproductive tract as target organs in animals (see Table 3A for summary); thus, mechanistic data pertinent to these endpoints were reviewed. Hepatic effects reported after *p,p′*-DDE exposure include increased liver weight (Yamasaki et al., 2009), liver necrosis (Kornbrust et al., 1986; Rossi et al., 1983), hepatocyte swelling and inflammation (Kornbrust et al., 1986), and fatty metamorphosis (NCI, 1978). However, very little mechanistic information is available; the available data were reviewed by ATSDR (2002) and WHO (2011). In vivo and in vitro studies

reviewed by ATSDR (2002) and WHO (2011) indicate that *p,p′*-DDE induces hepatic enzymes in rats, including CYP2B1, CYP3A1, CYP2A1, CYP2C11, and aromatase (CYP19). Wyde et al. (2003) as cited in WHO (2011) provided a possible mechanism for the induction of CYPs 3A1 and 2B1, as they observed increased transcriptional activities of constitutive androstane receptor (CAR) and pregnane X receptor (PXR), nuclear receptors that regulate these CYPs, in rats exposed to *p,p′*-DDE. In mechanistic studies published since 2011 and identified in the literature search, only one (Mota et al., 2011) reported data pertinent to liver toxicity. Measuring effects of *p,p′*-DDE on hepatic mitochondrial function in vitro, Mota et al. (2011) observed significant decreases in maximum electrical potential, repolarization potential, succinate CYPc reductase activity, and oxygen consumption, as well as increased lag phase. These findings indicate that *p,p′*-DDE exposure may reduce the energy level in hepatocytes via altered mitochondrial function.

Effects of *p,p′*-DDE on male reproductive tract development and function have been observed in several studies of gestational or postnatal exposure (see Table 3A). In these studies, effects included cryptorchidism (Veeramachaneni, 2006), decreased AGD and/or nipple retention (Gray et al., 1999; Loeffler and Peterson, 1999; You et al., 1998; Kelce et al., 1995), delayed preputial separation (Kelce et al., 1995), decreased weights of male reproductive organs (Gray et al., 1999; Loeffler and Peterson, 1999), testicular histopathology changes (Adamsson et al., 2009), and changes in sperm parameters and reduced fertility (Song et al., 2014). Many of these effects may be attributable to inhibition of androgen-mediated functions. *p,p′*-DDE is a well-established antagonist of the AR (WHO, 2011; ATSDR, 2002). *p,p′*-DDE has been shown to bind the AR receptor in vitro, inhibiting binding of endogenous androgens and androgen-mediated transcriptional activity, and induce effects consistent with inhibition of AR activity in exposed animals (Kelce et al., 1995). Effects on AR-dependent physiological organs and functions were observed in rats exposed in utero (reduced AGD and thoracic nipple retention), prepubertally (delayed preputial separation), and as adults (reduced seminal vesicle and ventral prostate weights) (Kelce et al., 1995). Kelce et al. (1997) and Kelce et al. (1995) demonstrated that the effects of *p,p′*-DDE were mediated via inhibition of the AR rather than through effects on testosterone levels.

In studies published since 2011, additional mechanisms of action on the male reproductive tract have been identified, with studies demonstrating that *p,p′*-DDE induces apoptosis in testicular cells, alters testicular levels of proteins responsible for maintaining seminiferous epithelium integrity and cell-cell interactions, and induces spontaneous acrosomal reaction in sperm. *p,p′*-DDE exposure induced apoptosis via oxidative stress in Sertoli cells in vitro (Song et al., 2011) and in rat testes in vivo (Shi et al., 2013). The in vitro studies showed that exposure to *p,p′*-DDE increased measures of reactive oxygen species (ROS) and apoptosis in Sertoli cells, and that pretreatment with an ROS inhibitor (*N*-acetyl-L-cysteine) completely abolished the *p,p′*-DDE-induced apoptosis (Song et al., 2011). In prepubertal rats exposed to *p,p′*-DDE by intraperitoneal (i.p.) injection, an increase in apoptosis was observed in the testes, along with increased measures of oxidative stress (decreased superoxide dismutase [SOD] and glutathione peroxidase [GSH-Px], as well as increased malondialdehyde) (Shi et al., 2013). Shi et al. (2013) also provided evidence for a potential role of endoplasmic reticulum stress in the induction of apoptosis. Endoplasmic reticulum stress can initiate apoptosis through calcium signaling and unfolded protein response pathways that activate calpain and caspase-12. mRNA levels of both calpain and capsase-12 were significantly increased in prepubertal rats exposed to *p,p′*-DDE (Shi et al., 2013).

Mota et al. (2011) examined the effects of *p,p′*-DDE on testicular mitochondrial function in vitro and provided a possible mechanism for increased ROS production. These experiments showed that *p,p′*-DDE induced a hyperpolarization of the testicular mitochondrial membrane that could trigger ROS production. In addition, Mota et al. (2011) observed decreases in oxidative phosphorylation in *p,p′*-DDE-treated testicular mitochondria, which could reduce the availability of adenosine triphosphate (ATP), especially in metabolically active cells such as meiotic spermatocytes and spermatids. The study authors postulated that these effects could play a role in the effects of *p,p′*-DDE on spermatogenesis and spermiogenesis.

p,p′-DDE has also been shown to significantly reduce protein levels of vimentin, *N*-cadherin, and FSH receptor (FSHR) in testes of exposed rats and in Sertoli cells in vitro (Yan et al., 2013). Vimentin is an integral part of the Sertoli cell cytoskeleton, and *N*-cadherin is involved in the regulation of cell-cell interactions in the seminiferous epithelium (Yan et al., 2013). FSHR expression is a primary determinant of FSH action on targets of FSH in Sertoli cells (Yan et al., 2013). Alterations in these protein levels may play a role in *p,p′*-DDE effects on the structural integrity and function of the seminiferous epithelium.

Direct effects of *p,p′*-DDE on human sperm in vitro were studied by Tavares et al. (2013), who showed that exposure resulted in an increase in the intracellular influx of calcium that was prevented by blockage of the CatSper plasma membrane calcium channel. Decreased acrosomal integrity was also observed in treated sperm. The study authors noted that the acrosomal reaction is strongly dependent on calcium and that the increase in calcium induced by *p,p′*-DDE may be responsible for triggering premature acrosomal reactions, adversely affecting sperm viability and potentially compromising sperm fertilizing ability (Tavares et al., 2013).

More recent studies have examined the underlying mechanisms contributing to the effects of *p,p′*-DDE on metabolism, given the epidemiological evidence that suggest a potential association between *p,p′*-DDE and metabolic disorders in humans such as diabetes and obesity (see "Human Studies" section for more details). Liu et al. (2017b) showed hepatocellular changes involving cytoplasmic vacuolation and substantial mitochondrial damage in mice gavaged with *p,p′*-DDE at a dose of 1 mg/kg-day for 8 weeks. Liver histopathology was accompanied by gene expression changes in enzymes involved in fatty acid synthesis and by changes in liver metabolomics indicative of disturbances in phospholipid, fatty acid, and amino acid metabolism (Liu et al., 2017a). In vitro results in cultured hepatocytes confirmed the effects of *p,p′*-DDE on mitochondrial function, including reductions in ATP levels, mitochondrial membrane potential, oxygen consumption rate, and expression of enzymes responsible for fatty acid *β*-oxidation (Liu et al., 2017a). In a related experiment, Liu et al. (2017a) demonstrated alterations in the relative abundance and composition of gut bacteria, bile acid composition and hydrophobicity, and expression of genes related to intestinal bile acid resorption and bile acid synthesis in mice with subchronic *p,p′*-DDE administration via gavage (1 mg/kg-day for 8 weeks). Finally, Pestana et al. (2017) reported on the enhanced effects of repeated-dose *p,p′*-DDE exposure via drinking water (0.1 mg/kg-day for 12 weeks) on dyslipidemia, glucose intolerance, and hypertension associated with a high-fat diet.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present summaries of noncancer and cancer references values, respectively.

Table 5. Summary of Noncancer Reference Values for *p,p′*-DDE (CASRN 72-55-9)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Increased relative liver weight in adult male offspring exposed during gestation and via lactation	3×10^{-4}	LOAEL	1	3,000	Yamasaki et al. (2009)
Screening Chronic p-RfD (mg/kg-d)	Rat/M	Increased relative liver weight in adult male offspring exposed during gestation and via lactation	3×10^{-4}	LOAEL	1	3,000	Yamasaki et al. (2009)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; M = male(s); NDr = not determined; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

Table 6. Summary of Cancer Reference Values for *p,p′*-DDE (CASRN 72-55-9)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	An OSF value is available on IRIS (U.S. EPA, 1988a)			
p-IUR $(mg/m^3)^{-1}$	NDr			

IRIS = Integrated Risk Information System; NDr = not determined; OSF = oral slope factor; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

DERIVATION OF ORAL REFERENCE DOSES

Human data provide suggestive evidence for an effect of *p,p′*-DDE on a number of health outcomes, most notably testicular germ cell cancers, respiratory effects, childhood obesity, and adult diabetes. However, epidemiological studies are not considered adequate for deriving subchronic and chronic p-RfDs for *p,p′*-DDE due to lack of exposure information, potential confounding factors such as coexposure to related organochlorine pesticides, and other study design issues (see "Human Studies" section).

The oral toxicity database of *p,p′*-DDE in animals consists of 6-week dose range-finding studies in rats and mice (NCI, 1978), a 6-week immunotoxicity study in rats (Banerjee et al., 1996), and three chronic-duration studies in multiple species (Rossi et al., 1983; NCI, 1978;

Tomatis et al., 1974). Additionally, there are 13 R/D toxicity studies in rats (Patrick et al., 2016; Song et al., 2014; Adamsson et al., 2009; Yamasaki et al., 2009; Makita, 2008; Makita and Omura, 2006; Makita et al., 2005; Gray et al., 1999; Loeffler and Peterson, 1999; You et al., 1999a; You et al., 1998; Kelce et al., 1995; Kornbrust et al., 1986) and one in rabbits (Veeramachaneni, 2006) available for consideration in the derivation of the subchronic and chronic p-RfDs.

NCI (1978) conducted dose range-finding studies in rats and mice exposed to *p,p′*-DDE for 6 weeks via the diet and reported mortality at FELs of 94.5 mg/kg-day in female rats and 48.5 mg/kg-day in male mice. No mortality was observed at doses ≤53.1 mg/kg-day in rats or at doses ≤34.8 mg/kg-day in mice. Because only body weight and mortality were recorded, the study is of limited use for qualitative and quantitative risk assessment. The 6-week immunotoxicity study found increases in relative liver weight (+17%) and depression of humoral and cell-mediated responses in male rats at a dietary *p,p′*-DDE dose level of 18.4 mg/kg-day (Banerjee et al., 1996). The study included relevant immune function assays (i.e., DTH reaction and ovalbumin-specific IgG and IgM measurements) that indicated an immunosuppressive effect, as well as more general immune system tests (i.e., macrophage and lymphocyte migration, and A:G ratio) that provide equivocal evidence of immunotoxicity; however, the study suffers from methodological issues, such as the use of one treatment dose, one species, and one sex (males). In the absence of additional supporting information, the findings of immunotoxicity with *p,p′*-DDE exposure are not considered further for the derivation of oral toxicity values.

Chronic-duration animal toxicity studies of *p,p′*-DDE are available in rats, mice, and hamsters. These studies were primarily designed as cancer bioassays, but provide some information on non-neoplastic endpoints (i.e., clinical toxicity observations, body weight, mortality, and tissue histopathology). In the NCI (1978) study in Osborne-Mendel rats and $B6C3F_1$ mice, the usefulness of the data is somewhat compromised, however, by the long postexposure observation period, which may have allowed for recovery from effects and by the substantial adjustments in dietary levels during the course of treatment. For rats, the lowest time-weighted average (TWA) *p,p′*-DDE dose, 18.7 mg/kg-day, was classified as a FEL for decreased survival in females. Hepatotoxicity in the form of liver necrosis or fatty metamorphosis was observed in male and female rats at doses ≥30.6 mg/kg-day; males also showed pulmonary and heart lesions at the highest exposure dose (58.8 mg/kg-day). The findings in mice are further compromised by low survival and a high incidence of amyloidosis in male controls. The lowest TWA dose tested in this study, ~25 mg/kg-day, was a LOAEL for suppression of body weight in female mice (10−15%) and clinical signs in male mice. Increased incidence of chronic kidney inflammation was found in male mice at the high-dose group (44.8 mg/kg-day). In the chronic-duration feeding study in Syrian golden hamsters (Rossi et al., 1983), which also reported high incidence of amyloidosis in control animals, a NOAEL of 48.5 mg/kg-day and a LOAEL of 97.0 mg/kg-day were established for body-weight reductions in males (~23%). In the chronic-duration feeding study in CF-1 mice (Tomatis et al., 1974), the only *p,p′*-DDE dose tested, ~45.0 mg/kg-day, appeared to be a FEL for reduced survival in both sexes and early signs of intoxication in females (i.e., tremors, convulsions and death), although the observed mortality may have been largely a result of the carcinogenic response. Body-weight depression (~11%) and myocardial effects (necrosis) were also noted in males in this study. Taken together, the chronic-duration studies fail to define a reliable LOAEL or NOAEL on which a p-RfD could be based.

Seven of the developmental toxicity studies in rats (Patrick et al., 2016; Song et al., 2014; Makita, 2008; Makita and Omura, 2006; Makita et al., 2005; Gray et al., 1999; Kelce et al., 1995) administered only one dose level of *p,p′*-DDE, either 10, 35, or 100 mg/kg-day. These studies are of limited use for quantitative assessment but were of sufficient quality to establish potential target organ effects for *p,p′*-DDE. No significant effects on reproductive development were observed at a dietary dose level of 10 mg/kg-day *p,p′*-DDE in male and female offspring of dams exposed during gestation and lactation (GD 1−PND 21) (Makita, 2008; Makita and Omura, 2006) or in pubertal male rats with direct *p,p′*-DDE exposure from PNDs 42−84 (Makita et al., 2005). Male rat offspring exposed to 35 mg/kg-day *p,p′*-DDE in utero for 14 days, via lactation for 20 days (PNDs 1−20), and directly from PNDs 21−90 exhibited increases in absolute and relative liver weight (≥18%), absolute and relative testis weight, and histopathology of the liver and testes (Patrick et al., 2016). At 100 mg/kg-day, Kelce et al. (1995) reported decreased AGD and retained nipples on PND 13 in the male offspring of dams administered *p,p′*-DDE during gestation (GDs 14−18) and delayed preputial separation in pubertal male rats administered *p,p′*-DDE via gavage during the postnatal period (PNDs 21−57). Gray et al. (1999) reported nipple retention, decreased AGD, prostate atrophy and decreased weights of ventral prostate, glans penis, cauda epididymis, and levator ani/bulbocavernosus muscles in male offspring of dams exposed to 100 mg/kg-day via gavage during gestation (GDs 14−18). Song et al. (2014) reported decreased sperm number and motility, and apoptosis of spermatogonia and spermatocytes in three successive generations of male offspring, as well as small testes and decreased fertility in F3 generation male offspring, of rats administered *p,p′*-DDE at 100 mg/kg-day during GDs 8−15. Limitations in study design (e.g., small number of animals and limited toxicity endpoints) and/or inadequate data reporting of treatment-related effects precluded the determination of effect levels from developmental studies by Kornbrust et al. (1986), You et al. (1999a), Veeramachaneni (2006), and Adamsson et al. (2009).

The remaining developmental studies (Yamasaki et al., 2009; Loeffler and Peterson, 1999; You et al., 1998) tested multiple *p,p′*-DDE exposure doses. Yamasaki et al. (2009) reported statistically significant increases in relative liver weight in adult male rats exposed during gestation and via lactation (GD 6−PND 20) at all treatment doses (LOAEL of 5 mg/kg-day) in the absence of body-weight changes. Effects on reproductive development (delayed preputial separation and early vaginal opening) and performance (decreased fertility index) in adult offspring were noted at the highest exposure dose (50 mg/kg-day). A NOAEL was not identified in this study. Data on absolute organ weight was not provided. Although no histopathology occurred in the liver of these animals, the observed liver enlargement in developing rats is considered biologically significant (≥5% increase over controls). Incremental liver-weight changes were also found in rat dams (+20%) and in adult female offspring (+9%) at 50 mg/kg-day in this study. You et al. (1998) observed nipple retention in the male offspring of rats administered *p,p′*-DDE at a LOAEL of 10 mg/kg-day during gestation (GDs 14−18) with no NOAEL identified. Loeffler and Peterson (1999) identified a NOAEL of 10 and LOAEL of 50 mg/kg-day for decreased AGD and 20% decreased relative ventral prostate weight in the male offspring of rats administered *p,p′*-DDE from GDs 14−18. Importantly, maternal toxicity reported in the Yamasaki et al. (2009) and Loeffler and Peterson (1999) studies occurred at higher exposure doses (LOAELs of 50 and 200 mg/kg-day, respectively) than those associated with developmental effects.

In summary, animal toxicity studies suggest the liver and male reproductive tract as primary target organs associated with repeat-dose exposure to *p,p′*-DDE via the oral route.

Developmental toxicity studies by Yamasaki et al. (2009), Loeffler and Peterson (1999), and You et al. (1998) tested more than one dose, identified sensitive endpoints and established NOAEL and/or LOAEL values. Therefore, these studies were considered further for the derivation of p-RfDs.

Derivation of a Subchronic Provisional Reference Dose

The potential *p,p′*-DDE-induced effects observed in developmental toxicity studies conducted by You et al. (1998), Yamasaki et al. (2009), and Loeffler and Peterson (1999) were evaluated using Benchmark Dose Software (BMDS, Version 2.6) to determine the most sensitive response. As previously discussed, these studies were considered most adequate in design and scope for assessing the dose-response relationship of potential liver and adverse male reproductive effects in rats after gestational and postnatal exposure to *p,p′*-DDE. Systemic toxicity studies provide insufficient information for quantitative risk assessment (see "Derivation of Oral Reference Doses" section). The most sensitive effects in offspring of rats exposed during gestation and/or via lactation from each of the selected developmental studies (LOAELs ranging from 5−50 mg/kg-day) were considered for benchmark dose (BMD) modeling, including increases in relative liver weight in adult males, reductions in fertility index in adult animals (Yamasaki et al., 2009), nipple retention in males pups on PND 14 (You et al., 1998), and decreases in AGD on PND 1 and in prostate weight on PND 21 in males pups (Loeffler and Peterson, 1999). Infertility effects were modeled as dichotomous data by estimating the incidence of nonpregnant F1 females from the fertility indexes (%) reported in the original study ([number of pregnant females ÷ number of copulated females] × 100) (see Table 7). Incomplete data reporting prevented BMD modeling of other reproductive effects in offspring (i.e., delayed preputial separation and early vaginal opening) from Yamasaki et al. (2009). The results for deceased AGD in Loeffler and Peterson (1999) were excluded from BMD modeling due to the absence of a dose-response relationship for these effects (see Table B-8). Similarly, ventral prostate-weight data were unamendable for modeling due to reporting deficiencies (results presented graphically with no error bars on the high-dose group) (Loeffler and Peterson, 1999). Instead, nipple retention on PND 13, which showed a nonsignificant increase at 50 mg/kg-day but was significantly increased in a dose-related manner at higher doses, was modeled from this study.

Prior to BMD modeling, exposure doses for the selected developmental studies were converted to human equivalent doses (HEDs). In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for deriving an RfD from effects that are not portal-of-entry effects or effects resulting from direct exposure of neonatal or juvenile animals. In the Yamasaki et al. (2009) study, the observed increases in relative liver weight in adult male offspring resulted from exposure of the dams; there was no direct exposure of neonates in this study. The same is true for the You et al. (1998) and Loeffler and Peterson (1999) studies, which involved only gestational exposure. Incidence data for the selected endpoints are shown in Table 7. Infertility in adult offspring was the only candidate endpoint successfully modeled using BMDS (see Appendix C).

Table 7. Data for Sensitive Developmental Endpoints in Offspring of Rats Exposed to *p,p′*-DDE during Gestation and/or Lactation via Gavage

Reference/Endpoint	Dose, mg/kg-d (HED)[a]					
***Yamasaki et al. (2009)*; Crl-CD (S-D) rats, GD 6−PND 20**						
	0	**5 (1)**	**15 (3.5)**	**50 (12)**		
Relative liver weight (% of BW)[b, c]	3.659 ± 0.291	3.978 ± 0.338* (+9%)	4.031 ± 0.366* (+10%)	4.066 ± 0.412* (+11%)		
Animals (*n*)	23	20	21	21		
Infertility[c, d]	0/19	2/17	1/16	5/13*		
***You et al. (1998)*; S-D rats, GDs 14−18**						
	0		**10 (2.3)**		**100 (23.0)**	
Number of nipples per pup[b, c, e]	0.3 ± 0.1		1.1 ± 0.4*		3.8 ± 0.5*	
Litters (*n*)	9		8		9	
***Loeffler and Peterson (1999)*; Holtzman rats, GDs 14−18**						
	0	**1 (0.2)**	**10 (2.2)**	**50 (11)**	**100 (22.0)**	**200 (44.0)**
Number of nipples per pup[b, c]	0	0	0.125 ± 0.25	0.28 ± 0.51	1.76 ± 1.25*	4.83 ± 0.74*
Animals (*n*)	4	5	4	6	5	3

[a]Dosimetry: Oral exposures are expressed in mg/kg-day as reported by the study authors. In parenthesis, doses as expressed in HEDs (mg/kg-day); HEDs were calculated using species-specific DAFs recommended by U.S. EPA (2011b). The DAF is calculated as follows: DAF = ($BW_a^{1/4} \div BW_h^{1/4}$), where DAF = dosimetric adjustment factor, BW_a = animal body weight, and BW_h = human body weight. Reference body weights recommended by U.S. EPA (1988c) were used to calculate the DAFs: 70 kg for humans and 0.204 kg (F) for S-D rats in a subchronic-duration study. No strain-specific reference body weights were available for Holtzman rats; instead, an average female rat body weight in a subchronic-duration study was used (0.173 kg).
[b]Values expressed as mean ± SD.
[c]Modeling was conducted using U.S. EPA BMDS (Version 2.6). BMD analysis details are available in Appendix C.
[d]Values denote number of nonpreganant females ÷ total number of copulated females. Incidence data was extracted from fertility indexes reported in the original study (Fertility index [%] = [number of pregnant females ÷ number of copulated females] × 100). See Table B-11 for more details on the data obtained from the study report.
[e]Data was digitally extracted using GrabIt! software.
*Significantly different from control ($p \leq 0.05$), as reported by the study authors.

BMD = benchmark dose; BMDS = Benchmark Dose Software; BW = body weight; DAF = dosimetric adjustment factor; F = female(s); GD = gestation day; HED = human equivalent dose; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; S-D = Sprague-Dawley; SD = standard deviation.

Table 8 provides candidate points of departure (PODs) from rat developmental studies by Yamasaki et al. (2009), Loeffler and Peterson (1999), and You et al. (1998). Candidate PODs that were not successfully evaluated via BMDS analysis are presented as NOAELs and LOAELs. The POD (HED) values from these studies calculated using $BW^{3/4}$ are shown in Table 8.

Table 8. Candidate PODs in Offspring of Rats Exposed to *p,p′*-DDE Orally during Gestation and/or Lactation for Derivation of the Subchronic p-RfD

Endpoint	NOAEL[a] mg/kg-d	LOAEL[a] mg/kg-d	BMDL (HED)[b, c] mg/kg-d	Selected POD	POD (HED)[c] mg/kg-d	Reference
Increased relative liver weight in adult males	**NDr**	**5**	**DUB (No model provided a reliable BMDL)**	**LOAEL**	**1**	**Yamasaki et al. (2009)**
Infertility in adult animals	15	50	0.6	BMDL	0.6	Yamasaki et al. (2009)
Retained nipples in male pups	NDr	10	DUB (No models provided adequate fit to data)	LOAEL	2.3	You et al. (1998)
Decreased AGD and decreased ventral prostate weight in male pups	10	50	DUB (Data reported in a manner unsuitable for modeling)	NOAEL	2.2	Loeffler and Peterson (1999)
Retained nipples in male pups	50	100	DUB (No models provided adequate fit to data)	NOAEL	11	Loeffler and Peterson (1999)

[a]Doses expressed as ADDs (mg/kg-day).
[b]Modeling was conducted using U.S. EPA BMDS (Version 2.6). BMD analysis details are available in Appendix C.
[c]Following U.S. EPA (2011b) guidance, the potential PODs for the subchronic p-RfD were converted to HEDs through the application of a DAF. The DAF is calculated as follows: DAF = ($BW_a^{1/4} \div BW_h^{1/4}$), where DAF = dosimetric adjustment factor, BW_a = animal body weight, and BW_h = human body weight. Reference body weights recommended by U.S. EPA (1988c) were used to calculate the DAFs: 70 kg for humans and 0.204 kg (F) for S-D rats in a subchronic-duration study. No strain-specific reference body weights were available for Holtzman rats; instead, an average female rat body weight in a subchronic-duration study was used (0.173 kg).

ADD = adjusted daily dose; AGD = anogenital distance; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit; BMDS = Benchmark Dose Software; BW = body weight; DAF = dosimetric adjustment factor; DUB = data unamenable to BMDS; F = female(s); HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; p-RfD = provisional reference dose; S-D = Sprague-Dawley.

The benchmark dose lower confidence limit (BMDL) (HED) of 0.6 mg/kg-day for infertility in adult rats exposed in utero and as neonates in the Yamasaki et al. (2009) study is the lowest candidate POD in the database. However, potential POD values for increased relative liver weight (Yamasaki et al., 2009) and nipple retention (You et al., 1998) in male rat offspring (LOAEL [HED] of 1 and 2.3 mg/kg-day, respectively) are within two- to fourfold of the BMDL identified for infertility. Because no NOAEL (or BMDL) values were identified for effects on the liver or nipple retention, it is unclear whether the POD for infertility would be sufficiently protective for these endpoints. Furthermore, there is support for the increased sensitivity of liver toxicity over outcomes on reproductive development and performance from gestational and postnatal exposure studies. Statistically and biologically significant increases in relative liver weight (≥5%) occurred in adult male offspring at a LOAEL of 5 mg/kg-day in the Yamasaki et al. (2009) study, while adverse effects in fertility reached statistical significance only at the

highest exposure dose (50 mg/kg-day). Nipple retention in male offspring was not observed by Yamasaki et al. (2009) up to treatment doses of 50 mg/kg-day, and was associated with higher exposure doses (≥100 mg/kg-day) in other rat developmental studies (Gray et al., 1999; Loeffler and Peterson, 1999; Kelce et al., 1995). Lastly, the LOAEL for increased liver weight is approximately an order of magnitude below the FELs identified from 6-week dietary studies in rats and mice (94.5 and 48.5 mg/kg-day, respectively); therefore, it is expected to be protective of mortality effects associated with subchronic exposure.

Consistent results of potential liver toxicity (increased liver weight and/or degenerative liver lesions) from other rat developmental studies (Patrick et al., 2016; Kornbrust et al., 1986) and from chronic studies in rats and hamsters (Rossi et al., 1983; NCI, 1978) occurring at similar or higher *p,p′*-DDE doses (7.1−48.5 mg/kg-day) further validate the significance of the liver-weight changes in male offspring (Yamasaki et al., 2009) as the critical effect for derivation of the subchronic p-RfD. *p,p′*-DDE is a liver carcinogen, and the IRIS oral slope factor (OSF) for this chemical is based on liver tumors in mice and hamsters (U.S. EPA, 1988a). A mode of action (MOA) for the liver tumors has not been established and it is unclear how the observed non-neoplastic liver effects may be related to development of tumors. *p,p′*-DDE is also a major intermediate metabolite of *p,p′*-DDT, which is a well-known hepatotoxicant (U.S. EPA, 1988a). Altogether, the WOE indicates that the liver is a primary target organ of toxicity for *p,p′*-DDE. **The LOAEL (HED) of 1 mg/kg-day for increased relative liver weight in adult male offspring exposed during gestation and via lactation (Yamasaki et al., 2009) is identified as the most sensitive endpoint and is selected as the POD for derivation of the subchronic p-RfD.**

The subchronic p-RfD is derived by applying a composite uncertainty factor (UF_C) of 3,000 to the selected POD from the Yamasaki et al. (2009) study.

Subchronic p-RfD	=	POD (HED) ÷ UF_C
	=	1 mg/kg-day ÷ 3,000
	=	**3×10^{-4} mg/kg-day**

Table 9 summarizes the uncertainty factors for the subchronic p-RfD for *p,p′*-DDE.

Table 9. Uncertainty Factors for the Subchronic p-RfD for *p,p′*-DDE

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between animals and humans following oral *p,p′*-DDE exposure. The toxicokinetic uncertainty is accounted for by calculation of an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database. The oral database for *p,p′*-DDE includes 6-wk dose range-finding studies in rats and mice that reported body weight and mortality incidence only (NCI, 1978) and a 6-wk immunotoxicity study in rats (Banerjee et al., 1996). Chronic-duration studies are available in three species (Rossi et al., 1983; NCI, 1978), but were primarily conducted as cancer bioassays with limited reporting of noncancer endpoints, although the NCI (1978) study performed histopathology on nearly 30 tissues and presented detailed results for non-neoplastic lesions. Several R/D studies that tested a single dose (Song et al., 2014; Makita, 2008; Makita et al., 2005; Gray et al., 1999; You et al., 1998; Kelce et al., 1995) or multiple doses (Yamasaki et al., 2009; Loeffler and Peterson, 1999; You et al., 1998) are available in rats. These studies identified sensitive targets of *p,p′*-DDE following oral exposure and were of sufficient quality to determine NOAEL and/or LOAEL values. Nevertheless, the database lacks comprehensive subchronic- and chronic-duration studies (with hematology, serum chemistry, and uranalysis measurements), multi-generational reproductive studies, and teratogenicity studies (with examination of fetuses for skeletal and visceral abnormalities).
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of *p,p′*-DDE in humans.
UF_L	10	A UF_L of 10 is applied for a LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 is applied because the POD is based on a developmental endpoint (i.e., increased relative liver weight) in which offspring were exposed to *p,p′*-DDE during gestation and via lactation, which is considered a sensitive life stage; therefore, the application of a duration adjustment UF is precluded.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; p-RfD = provisional reference dose; R/D = reproductive/developmental; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Confidence in the subchronic p-RfD for *p,p′*-DDE is low as described in Table 10.

Table 10. Confidence Descriptors for the Subchronic p-RfD for *p,p′*-DDE

Confidence Categories	Designation	Discussion
Confidence in study	M	Confidence in the principal study Yamasaki et al. (2009) is medium. The principal study did not report the use of GLP procedures; however, the study is a peer-reviewed article that examined the effects of *p,p′*-DDE in rats with in utero and lactational exposure (GD 6−PND 20). The study was conducted with an adequate number of dose groups and dose spacing, group sizes, and quantitation of results to describe dose-response relationships of critical developmental and reproductive effects of *p,p′*-DDE. Indeed, a number of reproductive and offspring parameters were analyzed, including organ-weight measurements and histopathology of reproductive sex organs and other selected tissues in males and females. The study reported sensitive developmental effects and identified a LOAEL value. Nevertheless, confidence in the principal study is reduced to medium because a NOAEL was not identified.
Confidence in database	L	There is low confidence in the database. The database consists of 6-wk dose range-finding studies in rats and mice, a 6-wk immunotoxicity study in rats and chronic cancer bioassays in three species with limited reporting of noncancer endpoints. Additionally, several pup R/D studies with only one dose, and three pup development toxicity studies that tested several doses during gestation (Loeffler and Peterson, 1999; You et al., 1998) or gestation and lactation (Yamasaki et al., 2009) are available. No comprehensive subchronic- and chronic-duration, two-generation reproduction, or teratogenicity studies are available.
Confidence in subchronic p-RfD[a]	L	The overall confidence in the subchronic p-RfD is low.

[a]The overall confidence cannot be greater than the lowest entry in the table (low).

GD = gestation day; GLP = Good Laboratory Practice; L = low; LOAEL = lowest-observed-adverse-effect level; M = medium; NOAEL = no-observed-adverse-effect level; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; p-RfD = provisional reference dose; R/D = reproductive/developmental.

Derivation of a Chronic Provisional Reference Dose

As previously discussed, chronic-duration toxicity studies in rats, mice, and hamsters exposed to *p,p′*-DDE via the diet failed to established reliable LOAEL or NOAEL values on which an RfD could be based (Rossi et al., 1983; NCI, 1978; Tomatis et al., 1974). Among the selected R/D studies considered for the derivation of the subchronic p-RfD, the most sensitive LOAELs were 5−10 mg/kg-day for effects on offspring (delayed reproductive development and performance, and increased liver weight) exposed in utero and/or by lactation. These LOAEL values are close to the lowest effect level identified for reduced survival in female rats with chronic exposure, a FEL of 18.6 mg/kg-day (NCI, 1978). Therefore, it is uncertain whether the LOAELs based on perinatal exposure are sufficiently protective for chronic exposure. For this reason, a chronic p-RfD is not derived. Instead, a screening p-RfD value is derived in Appendix A.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Provisional reference concentrations (p-RfCs) cannot be derived for *p,p′*-DDE because no studies of inhalation exposure in humans or animals have been identified.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

IRIS lists a cancer classification of Group B2 (probable human carcinogen) for *p,p′*-DDE based on increased incidence of liver tumors including carcinomas in two strains of mice and in hamsters and of thyroid tumors in female rats by diet (U.S. EPA, 1988a).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of a Provisional Oral Slope Factor

IRIS (U.S. EPA, 1988a) lists an OSF of 0.34 $(mg/kg\text{-}day)^{-1}$ for *p,p′*-DDE based on liver tumors in two strains of mice and hamsters. Therefore, OSF values are not derived in this document.

Derivation of a Provisional Inhalation Unit Risk

IRIS (U.S. EPA, 1988a) does not include an inhalation unit risk (IUR) for *p,p′*-DDE. Derivation of quantitative estimates of cancer risk following inhalation exposure to *p,p′*-DDD is precluded by the absence of inhalation data for this compound.

APPENDIX A. SCREENING PROVISIONAL VALUES

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive a chronic provisional reference dose (p-RfD) for *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE). However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

DERIVATION OF A SCREENING CHRONIC PROVISIONAL REFERENCE DOSE

As discussed in the main body of the report, there is considerable uncertainty regarding the appropriateness of using the results of perinatal exposure studies to derive an assessment for chronic exposure to *p,p′*-DDE. Chronic-duration studies suggest that the chemical affects survival in rats at doses (frank effect level [FEL] of 18.6 mg/kg-day) close to those associated with developmental toxicity (lowest-observed-adverse-effect levels [LOAELs] of 5−50 mg/kg-day).

To account for this extra uncertainty, the chronic assessment is considered to be a screening-level assessment. The screening chronic p-RfD for *p,p′*-DDE is derived using the same point of departure (human equivalent dose) (POD [HED]) as the subchronic p-RfD (1 mg/kg-day) and the same composite uncertainty factor (UF_C) of 3,000 (reflecting an interspecies uncertainty factor [UF_A] of 3, an intraspecies uncertainty factor [UF_H] of 10, a database uncertainty factor [UF_D] of 10, and a LOAEL-to-no-observed-adverse-effect level (NOAEL) uncertainty factor [UF_L] of 10). A subchronic-to-chronic uncertainty factor (UF_S) is not applied because the POD is based on increased relative liver weight in adult male offspring from a rat developmental study with exposure to *p,p′*-DDE via gestation and lactation (Yamasaki et al., 2009).

Screening Chronic p-RfD = POD (HED) ÷ UF_C
= 1 mg/kg-day ÷ 3,000
= **3×10^{-4} mg/kg-day**

Table A-1 summarizes the uncertainty factors for the screening chronic p-RfD for *p,p′*-DDE.

Table A-1. Uncertainty Factors for the Screening Chronic p-RfD for *p,p′*-DDE

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between animals and humans following oral *p,p′*-DDE exposure. The toxicokinetic uncertainty is accounted for by calculation of an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database. The oral database for *p,p′*-DDE includes 6-wk dose range-finding studies in rats and mice that reported body weight and mortality incidence only (NCI, 1978) and a 6-wk immunotoxicity study in rats (Banerjee et al., 1996). Chronic-duration studies are available in three species (Rossi et al., 1983; NCI, 1978), but were primarily conducted as cancer bioassays with limited reporting of noncancer endpoints, although the NCI (1978) study performed histopathology on nearly 30 tissues and presented detailed results for non-neoplastic lesions. Several R/D studies that tested a single dose (Song et al., 2014; Makita, 2008; Makita et al., 2005; Gray et al., 1999; You et al., 1998; Kelce et al., 1995) or multiple doses (Yamasaki et al., 2009; Loeffler and Peterson, 1999; You et al., 1998) are available in rats. These studies identified sensitive targets of *p,p′*-DDE following oral exposure and were of sufficient quality to determine NOAEL and/or LOAEL values. The database lacks comprehensive subchronic- and chronic-duration studies (with hematology, serum chemistry, and uranalysis measurements), multi-generational reproductive studies, and teratogenicity studies (with examination of fetuses for skeletal and visceral abnormalities).
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of *p,p′*-DDE in humans.
UF_L	10	A UF_L of 10 is applied for a LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 is applied because the POD is based on a developmental endpoint (i.e., increased relative liver weight) in which offspring were exposed to *p,p′*-DDE during gestation and via lactation, which is considered a sensitive life stage; therefore, the application of a duration adjustment UF is precluded.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; p-RfD = provisional reference dose; R/D = reproductive/developmental; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

APPENDIX B. DATA TABLES

Table B-1. Survival and Incidence of Tumors and Myocardial Necrosis in CF-1 Mice Administered *p,p′*-DDE in the Diet for up to 123 Weeks[a]

Parameter[b]	Dose Group, ppm (mg/kg-d)	
Male	**0**	**250 (45.0)**
Survival: Wk 70 Wk 100 Wk 130	 89/101 (88%) 52/101 (51%) 12/101 (12%)	 32/60 (53%) 1/60 (2%) 0/60 (0%)
Hepatomas (number with tumors/effective number)	33/98 (34%)	39/53 (74%)
Myocardial necrosis	1/98 (1%)	22/53 (42%)
Female	**0**	**250 (46.0)**
Survival: Wk 70 Wk 100 Wk 130	 84/97 (87%) 55/97 (57%) 13/97 (13%)	 40/60 (67%) 1/60 (2%) 0/60 (0%)
Hepatomas (number with tumors/effective number)	1/90 (1%)	54/55 (98%)
Myocardial necrosis	0/90 (0%)	1/55 (2%)

[a]Tomatis et al. (1974).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence).

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

Table B-2. Group Sizes, Dietary Concentrations, and Dose Estimates for Cancer Bioassays in Osborne-Mendel Rats and B6C3F$_1$ Mice Exposed to *p,p′*-DDE[a]

Group	Group Size	Nominal Concentration, ppm	Duration at this Concentration, wk	Untreated Duration, wk	Weighted Average Concentration[b], ppm	Weighted Average Daily Dose[c], mg/kg-d
Male rat						
Control	20	0		111	0	0
Low dose	50	675 338 0	23 55	 33	437	30.6
High dose	50	1,350 675 675[d] 0	23 36 15	 4 33	839	58.8
Female rat						
Control	20	0		111	0	0
Low dose	50	375 187 0	23 55	 34	242	18.7
High dose	50	750 375 375[d] 0	23 32 18	 5 34	462	35.6
Male mouse						
Control	20	0		92	0	0
Low dose	50	125 150 0	7 71	 14	148	25.3
High dose	50	250 300 300[d] 0	7 29 33	 9 14	261	44.8
Female mouse						
Control	20	0		92	0	0
Low dose	50	125 150 0	7 71	 15	148	25.6
High dose	50	250 300 300[d] 0	7 29 33	 9 15	261	45.1

[a]NCI (1978).
[b]Calculated by the study authors as the sum of concentration × time, averaged over 78 weeks.
[c]Calculated using weighted average concentration and reference values for body weight and food consumption from U.S. EPA (1988c).
[d]Administered as 1 dose-free week followed by 4 weeks at this level.

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

Table B-3. Incidence of Selected Non-Neoplastic Lesions in Osborne-Mendel Rats Exposed to *p,p′*-DDE in the Diet for up to 78 Weeks[a]

Parameter[b]	Dose Group (weighted average), ppm (mg/kg-d)		
Male	**0**	**437 (30.6)**	**839 (58.8)**
Liver: Centrilobular necrosis Fatty metamorphosis	 0/20 (0%) 2/20* (10%)	 2/40 (5%) 25/40** (63%)	 3/40 (8%) 20/40** (50%)
Lung hemorrhage	0/20* (0%)	3/21 (14%)	6/23** (26%)
Myocardial degeneration	10/20* (50%)	18/24 (75%)	21/25** (84%)
Female	**0**	**242 (18.7)**	**462 (35.6)**
Liver: Centrilobular necrosis Fatty metamorphosis	 1/20* (5%) 11/20 (55%)	 7/34 (21%) 3/34 (9%)	 10/33** (30%) 10/33 (30%)
Lung hemorrhage	5/20 (25%)	11/29 (38%)	5/28 (18%)
Myocardial degeneration	11/20 (55%)	12/29 (41%)	8/22 (36%)

[a]NCI (1978).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence).
*Significant trend by Cochran-Armitage test ($p < 0.05$) conducted for this review.
**Significantly different from control by Fisher's exact test (one-sided $p < 0.05$) conducted for this review.

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

Table B-4. Incidence of Hepatocellular Carcinomas in B6C3F$_1$ Mice Exposed to *p,p′*-DDE in the Diet for up to 78 Weeks[a]

Parameter[b]	Dose Group (weighted average), ppm (mg/kg-d)		
Male	**0**	**148 (25.3)**	**261 (44.8)**
Hepatocellular carcinomas	0/19* (0%)	7/41 (17%)	17/47** (36%)
Female	**0**	**148 (25.6)**	**261 (45.1)**
Hepatocellular carcinomas	0/19* (0%)	19/47** (40%)	34/48** (71%)

[a]NCI (1978).
[b]Values denote number of animals affected ÷ total number of animals examined (% incidence).
*Significant trend by Cochran-Armitage test ($p < 0.001$), as reported by the study authors.
**Significantly different from control by Fisher's exact test ($p < 0.001$), as reported by the study authors.

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

Table B-5. Incidence of Hepatic Hyperplastic Foci, Liver Cell Tumors, and Adrenal Tumors in Male and Female Syrian Golden Hamsters Exposed to *p,p′*-DDE for up to 128 Weeks[a]

Parameter	Dose Group (weighted average), ppm (mg/kg-d)		
Male	**0**	**500 (48.5)**	**1,000 (97.0)**
Adrenal tumors[b]	8/31 (26%)	5/30 (17%)	17/39 (44%)
Liver cell tumors[c]	0/10 (0%)	7/15 (47%)	8/24 (33%)
Hyperplastic foci in the liver[c]	0/10 (0%)	0/15 (0%)	5/24 (21%)
Female	**0**	**500 (48.3)**	**1,000 (96.6)**
Adrenal tumors[b]	2/42 (5%)	7/39 (18%)	8/39 (21%)
Liver cell tumors[c]	0/31 (0%)	4/26 (15%)	5/24 (21%)
Hyperplastic foci in the liver[c]	0/31 (0%)	0/26 (0%)	3/24 (13%)

[a]Rossi et al. (1983).
[b]Values denote number of animals with adrenal gland tumors ÷ number of survivors at time of first tumor observation (% incidence).
[c]Number of animals with liver cell tumors or foci/survivors at time the first liver cell tumor was seen.

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

Table B-6. Age and Weight of Prepubertal Male Long-Evans Rats at Preputial Separation and Selected Organ Weights of Adult Males after Exposure to *p,p′*-DDE by Gavage from Weaning (21 Days of Age) until after Puberty (Day 57)[a]

Parameter[b]	Dose, mg/kg-d	
	0	**100**
Pubertal effects		
Age at preputial separation (d)	43.3 ± 0.08	48 ± 0.08* (+11%)
Weight at preputial separation (g)	230 ± 8.2	273 ± 10.4* (+19%)

[a]Kelce et al. (1995).
[b]Values are means ± SD (% change relative to control).
*Significantly different from control ($p < 0.005$), as reported by the study authors.

p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

Table B-7. Changes in Body, Epididymis, and Seminal Vesicle Weights in PND 85 Male Long-Evans Rats Exposed In Utero to *p,p′*-DDE (GDs 14−18), with or without an Adult *p,p′*-DDE Exposure from PND 80−84[a]

Parameter[b]	Dose, mg/kg-d		
In utero only	**Control**	**10**	**100**
BW (g)	436.5 ± 19.3	413.3 ± 22.1 (−5%)	403.8 ± 35.7 (−8%)
Epididymis (g)	0.62 ± 0.06	0.57 ± 0.08 (−8%)	0.58 ± 0.07 (−7%)
Seminal vesicle (g)	1.50 ± 0.23	1.09 ± 0.26 (−27%)	1.05 ± 0.24 (−30%)
Adult only	**Control**	**70**	**NA**
BW (g)	436.5 ± 19.3	412.5 ± 27.0 (−6%)	NA
Epididymis (g)	0.62 ± 0.06	0.55 ± 0.05* (−11%)	NA
Seminal vesicle (g)	1.50 ± 0.23	1.02 ± 0.27* (−32%)	NA
In utero and adult	**Control**	**10/70**	**100/70**
BW (g)	436.5 ± 19.3	413.5 ± 58.5 (−5%)	398.2 ± 54.1 (−9%)
Epididymis (g)	0.62 ± 0.06	0.53 ± 0.16 (−15%)	0.52 ± 0.05 (−2%)
Seminal vesicle (g)	1.50 ± 0.23	1.17 ± 0.5 (−22%)	1.27 ± 0.15 (−15%)

[a]You et al. (1999a).
[b]Values are means ± SD (% change relative to control); n = 5−8; average of the right and left sides was used for paired organs.
*Significantly different from its corresponding in utero control-adult control group ($p < 0.05$, t-test), as reported by the study authors.

BW = body weight; GD = gestation day; NA = not applicable; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

Table B-8. Effects of Maternal Exposure to *p,p′*-DDE on GDs 14−18 on Selected Reproductive Developmental Endpoints in Male Holtzman Rats[a]

Parameter[b]	Dose, mg/kg-d					
	0	**1**	**10**	**50**	**100**	**200**
Number of animals/group	4	5	4	6	5	3
AGD/crown-rump length PND 1	0.09 ± 0.003	0.09 ± 0.002 (0%)	0.09 ± 0.002 (0%)	0.08 ± 0.001* (−11%)	0.08 ± 0.002* (−11%)	0.08 ± 0.005* (−11%)
AGD/crown-rump length PND 4	0.10 ± 0.004	0.11 ± 0.002 (+10%)	0.10 ± 0.002 (0%)	0.10 ± 0.001 (0%)	0.10 ± 0.001 (0%)	0.09 ± 0.004* (−10%)
Nipple retention[c]	0	0	0.125 ± 0.125	0.28 ± 0.21	1.76 ± 0.56*	4.83 ± 0.43*
Onset of puberty (PND of preputial separation)	42.48 ± 0.29	42.27 ± 0.52 (−0.5%)	42.83 ± 0.29 (+0.8%)	42.33 ± 0.27 (−0.4%)	42.65 ± 0.44 (+0.40%)	44.22 ± 0.62* (+4%)

[a]Loeffler and Peterson (1999).
[b]Values are mean ± SE (% change relative to control).
[c]Number of nipples retained on PND 13.
*Significantly different from control ($p \leq 0.05$), as reported by the study authors.

AGD = anogenital distance; GD = gestation day; PND = postnatal day;
p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SE = standard error.

Table B-9. Effects of Maternal Exposure to *p,p′*-DDE from GDs 14−18 on Male Reproductive Organ Development[a]

Parameter[b]	Dose, mg/kg-d	
	0	**100**
Long-Evans hooded rat		
Number of litters	8	8
Number of males examined externally	61	42
Number of males necropsied	26	27
AGD (mm)	2.85 ± 0.04	2.69 ± 0.06 (−6%)
Percent with areolas	0	21 ± 10*
Mean number of retained nipples	0	0.74 ± 0.15*
Weight glans penis (mg)	ND	ND
Weight cauda epididymis (mg)	ND	ND
Weight testes (g)	3.79 ± 0.10	3.86 ± 0.08 (+2%)
Weight ventral prostate (mg)	529 ± 25	417 ± 23* (−21%)
Weight levator ani/bulbocavernosus muscles (mg)	ND	ND
Percent with hypospadias	0	0
Incidence prostate atrophy	0/26	8/27* (+30%)
S-D rat		
Number of litters	9	11
Number of males examined externally	49	83
Number of males necropsied	44	70
AGD (mm)	2.76 ± 0.10	2.51 ± 0.08* (−9%)
Percent with areolas	0	71 ± 9*
Mean number of retained nipples	0	3.13 ± 0.5*
Weight glans penis (mg)	112 ± 2.9	102 ± 1.5* (−9%)
Weight cauda epididymis (mg)	331 ± 9.6	305 ± 6.2* (−8%)
Weight testes (g)	3.40 ± 0.08	3.42 ± 0.07 (+1%)
Weight ventral prostate (mg)	747 ± 36	575 ± 29* (−23%)
Weight levator ani/bulbocavernosus muscles (mg)	1,400 ± 40	1,204 ± 23* (−14%)
Percent with hypospadias	0	7.8 ± 7.8*
Incidence prostate atrophy	ND	ND

[a]Gray et al. (1999).
[b]Values are incidence (%) or mean ± SD or SE (not specified) (% change relative to control).
*Significantly different from control ($p < 0.05$), as reported by the study authors.

AGD = anogenital distance; GD = gestation day; ND = no data; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; S-D = Sprague-Dawley; SD = standard deviation; SE = standard error.

Table B-10. Reproductive Parameters in Female Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (GD 6−PND 20)[a]

Endpoint[b]	Exposure Group, mg/kg-d			
	0	**5**	**15**	**50**
Number of dams	10	10	10	10
Number of litters	10	10	9	10
Gestation index (%)[c]	100	100 (0%)	100 (0%)	100 (0%)
Gestational length (d)[c, d]	22.1 ± 0.3	22.0 ± 0.0 (0%)	22.0 ± 0.0 (0%)	22.3 ± 0.5 (+1%)
Number of pups born[c, d]	14.7 ± 2.6	14.4 ± 1.3 (−2%)	13.8 ± 1.3 (−6%)	14.0 ± 2.4 (−5%)
Delivery index (%)[c, d]	92.1 ± 10.5	95.5 ± 7.8 (+4%)	94.0 ± 7.3 (+2%)	89.6 ± 9.0 (−3%)
Birth index (%)[c, d]	89.4 ± 10.2	94.8 ± 7.7 (+6%)	94.0 ± 7.3 (+5%)	89.6 ± 9.0 (0%)
Live birth index (%)[c, d]	97.2 ± 3.6	99.2 ± 2.4 (+2%)	100.0 ± 0.0* (+3%)	100.0 ± 0.0* (+3%)
Sex ratio on PND 0[c, d]	0.47 ± 0.19	0.54 ± 0.10 (+15%)	0.59 ± 0.17 (+26%)	0.50 ± 0.14 (+6%)
Number of live pups on PND 4[c, d]	14.2 ± 2.6	14.3 ± 1.5 (+1%)	13.8 ± 1.3 (−3%)	13.2 ± 2.7 (−7%)
Viability index on PND 4 (%)[c, d]	99.4 ± 2.0	100.0 ± 0.0 (+1%)	100.0 ± 0.0 (+1%)	94.9 ± 13.8 (−5%)
Number of live pups on PND 21[c, d]	8.0 ± 0.0	8.0 ± 0.0 (0%)	8.0 ± 0.0 (0%)	7.7 ± 0.5* (−4%)
Weaning index on PND 21 (%)[c, d]	100.0 ± 0.0	100.0 ± 0.0 (0%)	100.0 ± 0.0 (0%)	96.3 ± 6.0* (−4%)

[a]Yamasaki et al. (2009).
[b]Gestation index = (number of pregnant females with live pups ÷ number of pregnant females) × 100. Delivery index = (number of pups born ÷ number of implantations) × 100. Birth index = (number of live pups on PND 0 ÷ number of implantations) × 100. Live birth index = (number of live pups on PND 0 ÷ number of pups born) × 100. Sex ratio on PND 0 = number of male live pups on PND 0 ÷ number of live pups on PND 0. Viability index on PND 4 = (number of live pups on PND 4 ÷ number of live pups on PND 0) × 100. Weaning index = (number of live pups at on PND 21 ÷ number of live pups after culling on PND 4) × 100.
[c]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100.
[d]Values are mean ± SD.
*Significantly different from controls ($p < 0.05$), as reported by the study authors.

GD = gestation day; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

Table B-11. Reproductive Parameters in Offspring of Female Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (GD 6−PND 20)[a]

Endpoint[b]	Exposure Group, mg/kg-d			
	0	5	15	50
Number of mated females	19	20	18	20
Number of copulated females	19	17	16	13
Copulation index (%)[c]	100.0	85.0 (−15%)	88.9 (−11%)	65.0* (−35%)
Pairing days until copulation[c, d]	4.0 ± 2.2	3.3 ± 1.3 (−18%)	2.9 ± 1.4 (−28%)	2.8 ± 2.2 (−30%)
Number of pregnant females[c]	19	15 (−21%)	15 (−21%)	8 (−58%)
Fertility index (%)[c]	100.0	88.2 (−12%)	93.8 (−6%)	65.1* (−35%)
Number of corpora lutea[c, d]	15.1 ± 2.2	15.9 ± 2.3 (+5%)	15.6 ± 2.7 (+3%)	16.9 ± 3.1 (+12%)
Number of implantations[c, d]	13.6 ± 2.7	14.0 ± 2.2 (+3%)	12.2 ± 4.9 (−10%)	13.4 ± 1.3 (−1%)
Implantation index (%)[c, d]	91.1 ± 15.0	89.7 ± 14.1 (−2%)	77.5 ± 29.4 (−15%)	81.4 ± 15.8 (−11%)
Number of intrauterine deaths[c, d]	0.6 ± 0.9	1.1 ± 1.0 (+83%)	0.9 ± 1.5 (+50%)	1.4 ± 1.3 (+133%)
Implantation loss (%)[c, d]	4.7 ± 6.2	7.8 ± 6.5 (+66%)	13.6 ± 27.1 (+189%)	10.2 ± 9.9 (+117%)
Number of live fetuses[c, d]	13.0 ± 2.6	12.9 ± 2.4 (−1%)	11.3 ± 5.2 (−13%)	12.0 ± 1.7 (−8%)

[a]Yamasaki et al. (2009).
[b]Copulation index (%) = (number of copulated females ÷ number of mated females) × 100. Fertility index (%) = (number of pregnant females ÷ number of copulated females) × 100. Implantation index (%) = (number of implantations ÷ number of corpora lutea) × 100. Implantation loss (%) = (number of intrauterine deaths ÷ number of implantations) × 100.
[c]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100.
[d]Values are mean ± SD.
*Significantly different from controls ($p < 0.05$), as reported by the study authors.

GD = gestation day; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

Table B-12. Body Weights and Relative Organ Weights in Offspring (12 Weeks Old) of Female Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (GD 6−PND 20)[a, b]

Endpoint	Exposure Group, mg/kg-d			
	0	5	15	50
Male				
Number of animals	23	20	21	21
BW (g)[c, d]	523.6 ± 39.8	541.7 ± 43.6 (+3%)	540.4 ± 33.4 (+3%)	545.3 ± 61.9 (+4%)
Testis (% BW)[c, d]	0.686 ± 0.076	0.638 ± 0.057 (−8%)	0.659 ± 0.034 (−4%)	0.641 ± 0.134 (−7%)
Epididymis (% BW)[c, d]	0.225 ± 0.026	0.218 ± 0.023 (−3%)	0.218 ± 0.020 (−3%)	0.215 ± 0.045 (−4%)
Prostate (% BW)[c, d]	0.115 ± 0.029	0.125 ± 0.034 (+8%)	0.120 ± 0.026 (+4%)	0.123 ± 0.033 (+7%)
Seminal vesicle (% BW)[c, d, e]	0.250 ± 0.059	0.248 ± 0.037 (−1%)	0.260 ± 0.037 (+4%)	0.296 ± 0.055* (+18%)
Muscle (% BW)[c, d, f]	0.219 ± 0.017	0.202 ± 0.024 (−8%)	0.205 ± 0.022 (−6%)	0.206 ± 0.038 (−6%)
Brain (% BW)[c, d]	0.404 ± 0.029	0.392 ± 0.030 (−3%)	0.392 ± 0.025 (−3%)	0.383 ± 0.035 (−5%)
Pituitary (% BW)[c, d]	0.003 ± 0.000	0.003 ± 0.001 (0%)	0.003 ± 0.000 (0%)	0.002 ± 0.000 (−33%)
Thyroid (% BW)[c, d]	0.005 ± 0.001	0.005 ± 0.001 (0%)	0.006 ± 0.001 (+20%)	0.006 ± 0.002 (+20%)
Adrenal (% BW)[c, d]	0.012 ± 0.002	0.012 ± 0.002 (0%)	0.011 ± 0.002 (−8%)	0.011 ± 0.002 (−8%)
Kidney (% BW)[c, d]	0.621 ± 0.046	0.642 ± 0.042 (+3%)	0.647 ± 0.044 (+4%)	0.644 ± 0.047 (+4%)
Liver (% BW)[c, d]	3.659 ± 0.291	3.978 ± 0.338* (+9%)	4.031 ± 0.366* (+10%)	4.066 ± 0.412* (+11%)

Table B-12. Body Weights and Relative Organ Weights in Offspring (12 Weeks Old) of Female Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (GD 6−PND 20)[a, b]

Endpoint	Exposure Group, mg/kg-d			
	0	5	15	50
Female				
Number of animals	18	20	15	16
BW (g)[c, d]	289.0 ± 30.1	301.6 ± 31.2 (+4%)	300.0 ± 34.4 (+4%)	303.8 ± 44.3 (+5%)
Ovary (% BW)[c, d]	0.031 ± 0.007	0.027 ± 0.005 (−15%)	0.031 ± 0.006 (0%)	0.028 ± 0.007 (−10%)
Uterus (% BW)[c, d]	0.159 ± 0.026	0.149 ± 0.023 (−7%)	0.201 ± 0.169 (+26%)	0.154 ± 0.023 (−3%)
Brain (% BW)[c, d]	0.669 ± 0.074	0.651 ± 0.060 (−3%)	0.644 ± 0.074 (−4%)	0.643 ± 0.083 (−4%)
Pituitary (% BW)[c, d]	0.005 ± 0.001	0.005 ± 0.001 (0%)	0.005 ± 0.001 (0%)	0.005 ± 0.001 (0%)
Thyroid (% BW)[c, d]	0.008 ± 0.002	0.008 ± 0.002 (0%)	0.008 ± 0.002 (0%)	0.009 ± 0.002 (+13%)
Adrenal (% BW)[c, d]	0.022 ± 0.003	0.021 ± 0.003 (−5%)	0.021 ± 0.005 (−5%)	0.025 ± 0.004* (+14%)
Kidney (% BW)[c, d]	0.667 ± 0.047	0.668 ± 0.067 (0%)	0.664 ± 0.044 (0%)	0.693 ± 0.100 (+4%)
Liver (% BW)[c, d]	3.495 ± 0.179	3.578 ± 0.220 (+2%)	3.647 ± 0.223 (+4%)	3.795 ± 0.310* (+9%)

[a]Yamasaki et al. (2009).
[b]Absolute weights were not provided.
[c]Values are mean ± SD.
[d]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100.
[e]Seminal vesicle with coagulation gland.
[f]Levator ani/bulbocavernosus muscles.
*Significantly different from controls ($p < 0.05$), as reported by the study authors.

BW = body weight; GD = gestation day; PND = postnatal day; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

Table B-13. Sperm Parameters in Three Generations of Offspring of Female S-D Rats Exposed to *p,p′*-DDE via Gavage during Gestation (GDs 8–15)[a]

Generation/Exposure	*n*	Endpoint[b, c]						
		Total Sperm, million/mL	Motility, %	VAP	VSL	VCL	ALH	LIN%
F1 generation								
Control	8	70.63 ± 4.17	76.75 ± 2.68	216.40 ± 13.19	158.71 ± 11.27	368.19 ± 13.92	14.05 ± 0.52	44.50 ± 1.05
p,p′-DDE	8	50.00 ± 4.62* (−29%)	62.42 ± 3.32* (−19%)	153.27 ± 12.88* (−29%)	101.52 ± 5.82* (−36%)	297.86 ± 28.16* (−19%)	11.44 ± 0.84* (−19%)	38.57 ± 1.64* (−13%)
F2 generation								
Control	7	82.86 ± 14.59	82.14 ± 2.43	248.21 ± 12.49	162.07 ± 6.52	519.23 ± 35.40	17.51 ± 0.98	32.14 ± 1.53
p,p′-DDE	8	43.75 ± 8.85[#] (−47%)	60.88 ± 8.85[#] (−26%)	179.23 ± 26.44[#] (−28%)	116.80 ± 17.36[#] (−28%)	389.41 ± 58.46 (−25%)	14.14 ± 2.13 (−19%)	28.38 ± 4.18 (−12%)
F3 generation								
C-M × C-F	9	68.89 ± 13.79	68.56 ± 3.56	203.57 ± 11.93	135.40 ± 6.89	411.17 ± 21.04	16.54 ± 0.88	35.44 ± 0.41
p,p′-DDE-M × *p,p′*-DDE-F	6	21.66 ± 10.14[†] (−69%)	29.33 ± 13.35[†] (−57%)	95.93 ± 43.13[†] (−53%)	63.37 ± 29.18[†] (−53%)	152.53 ± 78.28[†] (−63%)	10.07 ± 5.08 (−39%)	15.83 ± 7.38[†] (−55%)
p,p′-DDE-M × C-F	5	28.00 ± 11.90[†] (−59%)	34.6 ± 14.13[†] (−50%)	149.90 ± 40.78[†] (−26%)	98.46 ± 26.51[†] (−27%)	301.9 ± 80.77[†] (−27%)	14.16 ± 3.71 (−14%)	27.80 ± 7.02 (−22%)
p,p′-DDE-F × C-M	11	55.50 ± 13.32 (−19%)	67.20 ± 4.68 (−2%)	178.73 ± 21.97 (−12%)	119.28 ± 15.47 (−12%)	358.34 ± 44.14 (−13%)	14.98 ± 1.79 (−9%)	32.40 ± 4.08 (−9%)

[a]Song et al. (2014).
[b]Data are means ± SE.
[c]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100.
*, [#], [†]Significantly different from respective (F1, F2, F3) controls ($p < 0.05$), as reported by the study authors.

ALH = amplitude of lateral head displacement; C = control; F = female(s); GD = gestation day; LIN% = linearity percent; M = male(s); *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; S-D = Sprague-Dawley; SE = standard error; VAP = average path velocity; VCL = curvilinear velocity; VSL = straight-line velocity.

Table B-14. Developmental Effects in Male F1 S-D Rats Exposed to *p,p′*-DDE In Utero, during Lactation, and Directly from PNDs 21−90[a]

Parameter[b]	Dose, mg/kg-d	
	0	35
Number of rats	24	27
AGD (mm)[c]	17.54 ± 0.65	17.33 ± 0.41 (−1%)
Body weight (g)	430.34 ± 34.92	414.91 ± 32.15 (−4%)
Absolute liver weight (g)	17.36 ± 2.16	20.65 ± 5.06* (+18%)
Relative liver weight (% BW)	4.028 ± 0.31	4.962 ± 1.01* (+23)
Absolute prostrate weight (g)	0.83 ± 0.24	0.82 ± 0.23 (−1%)
Absolute seminal vesicle weight (g)	1.46 ± 0.37	1.57 ± 0.47 (+8%)
Absolute epididymis weight (g)	1.47 ± 0.26	1.42 ± 0.30 (−3%)
Absolute testis weight (g)	3.68 ± 0.22	3.95 ± 0.32* (+7%)
Relative testis weight (% BW)	0.86 ± 0.08	0.96 ± 0.08* (+12%)
Seminiferous tubule diameter (µm)	295.42 ± 19.25	260.00 ± 14.53* (−12%)
Seminiferous epithelium thickness (µm)	100.40 ± 8.58	86.33 ± 4.10* (−14%)
Lumen diameter (µm)	106.84 ± 20.38	80.15 ± 8.08* (−25%)
Total sperm count (× 10^6/mL)	48.46 ± 14.36	50.69 ± 16.47 (+5%)
Testosterone (nmol/L)	21.33 ± 1.74	28.12 ± 3.53* (+32%)

[a]Patrick et al. (2016).
[b]Values are incidence (%) or mean ± SD (% change relative to control).
[c]Corrected for body weight.
*Significantly different from control ($p < 0.05$), as reported by the study authors.

AGD = anogenital distance; BW = body weight; PND = postnatal day;
p,p′-DDE = *p,p′*-dichlorodiphenyldichloroethylene; S-D = Sprague-Dawley; SD = standard deviation.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

Benchmark dose (BMD) modeling is conducted with EPA's Benchmark Dose Software (BMDS, Version 2.6). All continuous models available within the software are fit using a default benchmark response (BMR) of 1 standard deviation (SD) relative risk (RR) unless a biologically determined BMR is available (e.g., BMR 10% relative deviation [RD] for body weight based on a biologically significant weight loss of 10%), as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). For the liver-weight data in male offspring, a BMR of 5% RD was attempted based on ≥5% increases in relative liver weight, which are considered biologically significant for developmental effects. For other continuous developmental endpoints (i.e., nipple retention), a BMR of 0.5 SD RR was used. All available dichotomous-variable models in the BMDS were fit to the incidence data on infertility. The BMR typically used for dichotomous datasets is 10% extra risk (ER). However, infertility effects were observed in adult offspring rats exposed during gestation and via lactation, which is considered a sensitive life stage; therefore, these responses were modeled with a BMR of 5% ER.

An adequate fit is judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected (*p*-value < 0.1), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set is considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit/benchmark concentration lower confidence limit (BMDL/BMCL) is selected if the BMDL/BMCL estimates from different models vary >threefold; otherwise, the BMDL/BMCL from the model with the lowest Akaike's information criterion (AIC) is selected as a potential point of departure (POD) from which to derive the reference dose/reference concentration (RfD/RfC).

In addition, in the absence of a mechanistic understanding of the biological response to a toxic agent, data from exposures much higher than the study lowest-observed-adverse-effect level (LOAEL) do not provide reliable information regarding the shape of the response at low doses. Such exposures, however, can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve. Thus, if lack of fit is due to characteristics of the dose-response data for high doses, then the *Benchmark Dose Technical Guidance* document allows for data to be adjusted by eliminating the high-dose group (U.S. EPA, 2012b). Because the focus of BMD analysis is on the low-dose regions of the response curve, elimination of the high-dose group is deemed reasonable.

BMD Modeling to Identify Potential Points of Departure for the Derivation of a Provisional Reference Dose

The data sets for sensitive pup development endpoints observed in the studies of rats exposed orally to *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) during gestation and/or

lactation (Yamasaki et al., 2009; Loeffler and Peterson, 1999; You et al., 1998), were selected to determine potential PODs for the provisional reference dose (p-RfD), using BMD analysis. Table 7 shows the data that were modeled. Summaries of modeling approaches and results (see Tables C-1 to C-4 and Figures C-1 to C-2) for each data set follow.

Increased Relative Liver Weight in Adult Male Offspring of Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (Yamasaki et al., 2009)

The procedure outlined above was applied to the data for increased relative liver weight in the adult male offspring (12 weeks old) of Crl:CD (SD) rats exposed to *p,p′*-DDE during Gestation Day (GD) 6 to Postnatal Day (PND) 20 (Yamasaki et al., 2009) (see Table 7). The constant variance model provided adequate fit to the variance data. With the constant variance model applied, the only models that provided adequate fit to the means were the Exponential 4 and 5 models and the Hill model. None of these models, however, generated reliable BMDL values (the BMDLs were three to five orders of magnitude lower than the corresponding BMDs) in part due to limited information in the low-dose region (see Figure C-1). Thus, modeling of this data point was unsuccessful. Table C-1 summarizes the BMD modeling results.

Table C-1. Modeling Results for Relative Liver-Weight Data in Male Offspring of Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation[a]

Model	Variance *p*-Value[b]	Means *p*-Value[b]	Scaled Residual	AIC	BMD_{05} (HED) mg/kg-d	$BMDL_{05}$ (HED) mg/kg-d
Constant variance						
Exponential (model 2)[c]	0.44	0.0041	−0.53	−82.63	8.50	5.36
Exponential (model 3)[c]	0.44	0.0041	−0.53	−82.63	8.50	5.36
Exponential (model 4)[c]	0.44	0.74	−0.0015	−91.51	0.38	6.77×10^{-4}
Exponential (model 5)[c]	0.44	0.74	−0.0015	−91.51	0.38	9.13×10^{-4}
Hill[c]	0.44	0.90	−0.0011	−91.60	0.24	2.38×10^{-6}
Linear[d]	0.44	0.0043	−0.56	−83	8.29	10.25
Polynomial (2-degree)[d]	0.44	0.0043	−0.56	−83	8.29	10.25
Polynomial (3-degree)[d]	0.44	0.0043	−0.56	−83	8.29	10.25
Power[c]	0.44	0.0043	−0.56	−83	8.29	10.25

[a]Yamasaki et al. (2009).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Coefficients restricted to be positive.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% ER); ER = extra risk; HED = human equivalent dose; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

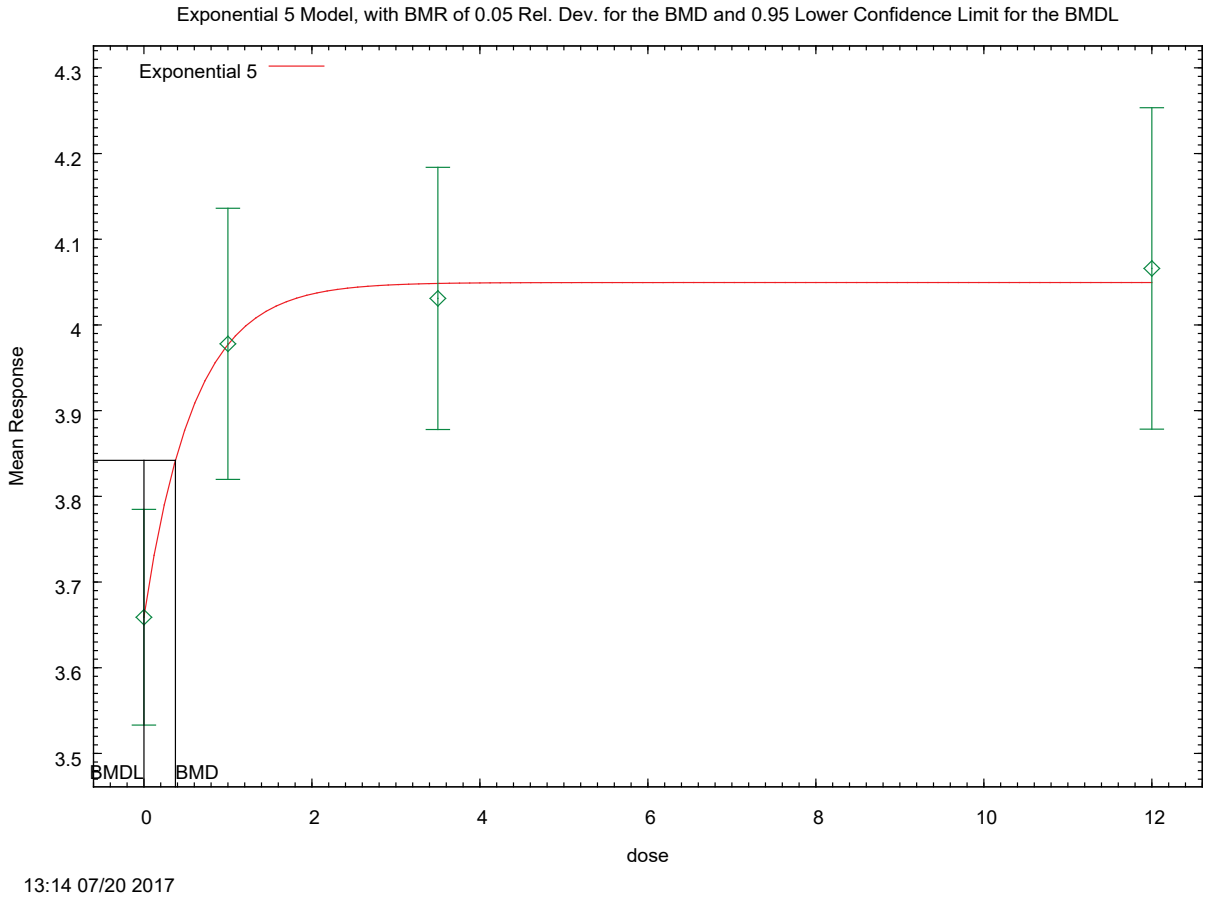

Figure C-1. Exponential Model 5 for Relative Liver-Weight Data in Male Offspring of Crl:CD (SD) Rats Exposed to *p,p'*-DDE via Gavage during Gestation and Lactation (Yamasaki et al., 2009)

Text Output for Figure C-1:

```
 ================================================================================
        Exponential Model. (Version: 1.10;  Date: 01/12/2015)
        Input Data File: C:/Users/llizarra/Desktop/BMDS2601/Data/exp_Yamasaki
2009_pup liver weight 7'20'17_Opt.(d)
        Gnuplot Plotting File:
                                                Thu Jul 20 13:14:31 2017
 ================================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.
```

```
      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.17193	-2.17193	-2.17193	-2.17193
rho	0 *	0 *	0 *	0 *
a	3.82904	3.82904	3.47605	3.47605
b	0.00595914	0.00595914	0.137033	0.137033
c	0 *	0 *	1.2282	1.2282
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.04272	-2.04272	-2.17068	-2.17068
rho	0 *	0 *	0 *	0 *
a	3.83457	3.83457	3.65911	3.65911
b	0.00573984	0.00573984	1.68517	1.68517
c	--	--	1.10667	1.10667
d	--	1	--	1

-- Indicates that this parameter does not appear in model
* Indicates that this parameter has been specified

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	8.19559e-153	0.0198914	0.0175022	0.0175022
rho	NA	NA	NA	NA
a	0.0510083	0.0510083	0.0704697	0.0704697
b	0.00204191	0.00204191	1.2652	1.26519
c	NA	NA	0.025776	0.025776
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form)
or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	23	3.659	0.291
1	20	3.978	0.338
3.5	21	4.031	0.336
12	21	4.066	0.412

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	3.835	0.3601	-2.338
	1	3.857	0.3601	1.507
	3.5	3.912	0.3601	1.51
	12	4.108	0.3601	-0.5344
3	0	3.835	0.3601	-2.338
	1	3.857	0.3601	1.507
	3.5	3.912	0.3601	1.51
	12	4.108	0.3601	-0.5344
4	0	3.659	0.3378	-0.001543
	1	3.977	0.3378	0.0125
	3.5	4.048	0.3378	-0.2354
	12	4.049	0.3378	0.2249
5	0	3.659	0.3378	-0.001544
	1	3.977	0.3378	0.0125
	3.5	4.048	0.3378	-0.2354
	12	4.049	0.3378	0.2249

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

```
  Model  R:        Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	49.80688	5	-89.61376
A2	51.15974	8	-86.31949
A3	49.80688	5	-89.61376
R	40.62189	2	-77.24378
2	44.3155	3	-82.631
3	44.3155	3	-82.631
4	49.75377	4	-91.50754
5	49.75377	4	-91.50754

Additive constant for all log-likelihoods = -78.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	21.08	6	0.001778
Test 2	2.706	3	0.4393
Test 3	2.706	3	0.4393
Test 4	10.98	2	0.004122
Test 5a	10.98	2	0.004122
Test 5b	8.669e-013	0	N/A
Test 6a	0.1062	1	0.7445
Test 6b	10.88	1	0.0009739
Test 7a	0.1062	1	0.7445
Test 7b	10.88	1	0.0009739
Test 7c	3.126e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous

variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

Degrees of freedom for Test 7c are less than or equal to 0. The Chi-Square test for fit is not valid.

Benchmark Dose Computations:

Specified Effect = 0.050000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	8.50027	5.35931
3	8.50026	5.35931
4	0.37533	0.000677215
5	0.37533	0.000913241

Infertility in Adult Offspring of Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation (Yamasaki et al., 2009)

The procedure outlined above was applied to the data for infertility in adult offspring of Crl:CD (SD) rats exposed to *p,p′*-DDE during GD 6 to PND 20 (Yamasaki et al., 2009) (see Table 7). With the constant variance model applied, all models except the Multistage (M3) model provided an adequate fit to the data. BMDLs for models providing an adequate fit differed by >threefold, thus the model with the lowest BMDL was selected (LogLogistic). Table and Figure C-2 summarize the BMD modeling results.

Table C-2. Modeling Results for Infertility Data in Offspring of Crl:CD (SD) Rats Exposed to *p,p′*-DDE via Gavage during Gestation and Lactation[a]

Model	*p*-Value[b]	Scaled Residual	AIC	BMD_{05} (HED) mg/kg-d	$BMDL_{05}$ (HED) mg/kg-d
Constant variance					
Gamma	0.23	1.44	43.71	1.31	0.73
Logistic	0.29	−0.33	44.00	3.70	2.38
LogLogistic[c]	**0.42**	**1.37**	**41.62**	**1.06**	**0.56**
LogProbit	0.12	−0.002	46.25	5.63	2.10
Multistage (M2)	0.23	1.44	43.71	1.31	0.73
Multistage (M3)	0.088	1.44	45.71	1.34	0.73
Probit	0.28	−0.39	43.99	3.31	2.14
Weibull	0.23	1.44	43.71	1.31	0.73
Quantal-Linear	0.23	1.44	43.71	1.31	0.73

[a]Yamasaki et al. (2009).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Selected model
AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% ER); ER = extra risk; HED = human equivalent dose; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene.

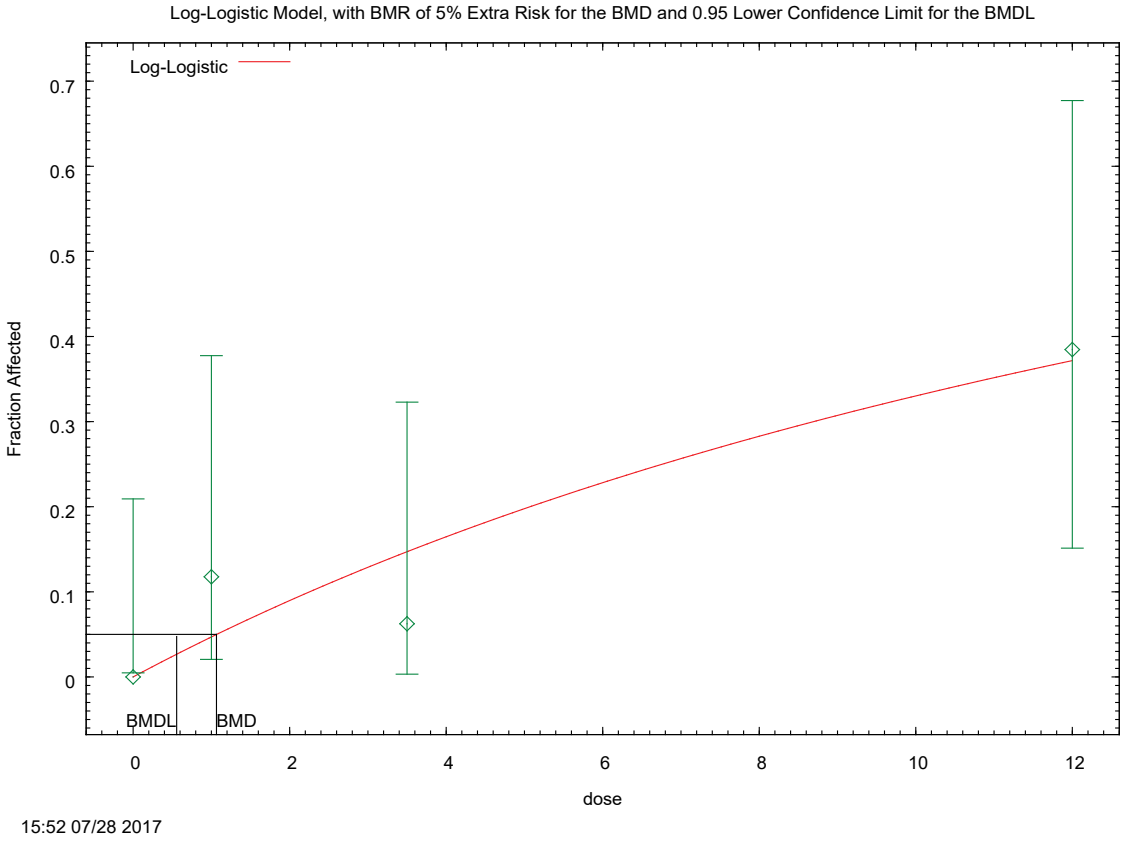

Figure C-2. Selected Model (LogLogistic) for Infertility Data in Offspring of Crl:CD (SD) Rats Exposed to *p,p'*-DDE via Gavage during Gestation and Lactation (Yamasaki et al., 2009)

Text Output for Figure C-2:

```
 ================================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
        Input Data File: C:/Users/llizarra/Desktop/BMDS2601/Data/lnl_Yamasaki
2009_fertility index_Opt.(d)
        Gnuplot Plotting File:  C:/Users/llizarra/Desktop/BMDS2601/Data/lnl_Yamasaki
2009_fertility index_Opt.plt
                                                Fri Jul 28 15:52:06 2017
 ================================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
```

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -3.18516
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-3.00731	0.41478	-3.82027	-2.19436
slope	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.5599	4			
Fitted model	-19.8079	1	2.49615	3	0.476
Reduced model	-24.2457	1	11.3717	3	0.009877

AIC: 41.6159

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	19.000	0.000
1.0000	0.0471	0.801	2.000	17.000	1.373
3.5000	0.1475	2.360	1.000	16.000	-0.959
12.0000	0.3723	4.840	5.000	13.000	0.092

```
 Chi^2 = 2.81      d.f. = 3        P-value = 0.4214

   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.06489

            BMDL =       0.556456
```

Increased Nipple Retention in Male Offspring of S-D Rats Exposed to *p,p′*-DDE via Gavage during Gestation (You et al., 1998)

The procedure outlined above was applied to the data for mean number of nipples retained per pup (PND 13) in Sprague-Dawley (S-D) rats exposed to *p,p′*-DDE during GDs 14−18 (You et al., 1998) (see Table 7). Neither the constant or nonconstant variance models provided adequate fit to the data on nipple retention reported by You et al. (1998). Table C-2 summarizes the BMD modeling results.

Table C-2. Modeling Results for Nipple Retention Data in Male Offspring of S-D Rats Exposed to *p,p′*-DDE via Gavage during Gestation[a]

Model	Variance *p*-Value[b]	Means *p*-Value[b]	Scaled Residual	AIC	$BMD_{0.5SD}$ (HED) mg/kg-d	$BMDL_{0.5SD}$ (HED) mg/kg-d
Nonconstant variance						
Exponential (model 2)[c]	0.060	<0.0001	2.32	−8.34	3.70	2.87
Exponential (model 3)[c]	0.060	<0.0001	2.32	−8.34	3.70	2.87
Exponential (model 4)[c]	0.060	NV	0.26	−32.25	0.14	0.088
Linear[d]	0.060	<0.0001	−1.66	−16.11	1.00	0.64
Polynomial (2-degree)[d]	0.060	<0.0001	−1.66	−16.11	1.00	0.64
Polynomial (3-degree)[d]	0.060	<0.0001	−2.8	55.14	−9,999	0.034
Power[c]	0.060	<0.0001	−1.66	−16.11	1.00	0.64

[a]You et al. (1998).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Coefficients restricted to be positive.

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; NV = not available; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; S-D = Sprague-Dawley; SD = standard deviation.

Increased Nipple Retention in Male Offspring of Holtzman Rats Exposed to *p,p′*-DDE via Gavage during Gestation (Loeffler and Peterson, 1999)

The procedure outlined above was applied to the data for mean number of nipples retained per pup (PND 13) in Holtzman rats exposed to *p,p′*-DDE during GDs 14−18 (Loeffler and Peterson, 1999) (see Table 7). Neither the constant or nonconstant variance models provided adequate fit to the data. Thus, the full data set was not considered appropriate for BMD modeling. Elimination of the highest dose group did not result in a data set for which the data could be properly modeled. Elimination of additional dose groups was not considered, as it would result in the elimination of all doses that resulted in a statistically significant increase in mean number of nipples retained per pup. In summary, the nipple retention data reported by Loeffler and Peterson (1999) were not suitable for BMD modeling. Table C-3 summarizes the BMD modeling results.

Table C-3. Modeling Results for Nipple Retention Data in Male Offspring of Holtzman Rats Exposed to *p,p′*-DDE via Gavage during Gestation[a]

Model	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residual	AIC	$BMD_{0.5SD}$ (HED) mg/kg-d	$BMDL_{0.5SD}$ (HED) mg/kg-d
Nonconstant variance—highest dose dropped						
Exponential (model 2)[e]	0.0078	<0.0001	0	26.06	NV	NV
Exponential (model 3)[e]	0.0078	<0.0001	7.61×10^{130}	8.88×10^{261}	8.71×10^{7}	NV
Exponential (model 4)[e]	0.0078	NV	0	NV	NV	NV
Exponential (model 5)[e]	0.0078	NV	0	NV	NV	NV
Hill[e]	<0.0001	<0.0001	3.3	30.06	164.26	1.83×10^{-4}
Linear[d]	<0.0001	0.33	1.39	−159.51	−9,999	NV
Polynomial (2-degree)[d]	<0.0001	<0.0001	1.84	−174.61	−9,999	NV
Polynomial (3-degree)[d]	<0.0001	<0.0001	−7.06	−134.65	0	NV
Power[e]	<0.0001	<0.0001	0	−9.40	NV	NV

[a]Loeffler and Peterson (1999).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; NV = not available; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; SD = standard deviation.

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2016). 2016 TLVs and BEIs: Based on documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH. https://www.acgih.org/forms/store/ProductFormPublic/2016-tlvs-and-beis

Adamsson, A; Salonen, V; Paranko, J; Toppari, J. (2009). Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland. Reprod Toxicol 28: 66-74. http://dx.doi.org/10.1016/j.reprotox.2009.03.002

ATSDR (Agency for Toxic Substances and Disease Registry). (2002). Toxicological profile for DDT, DDE, and DDD [ATSDR Tox Profile]. (RISKLINE/2003010002). Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/tp35.pdf

ATSDR (Agency for Toxic Substances and Disease Registry). (2016). The priority list of hazardous substances that will be the subject of toxicological profiles [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/spl/

ATSDR (Agency for Toxic Substances and Disease Registry). (2017). Minimal risk levels (MRLs). June 2017. Atlanta, GA: Agency for Toxic Substances and Disease Registry (ATSDR). Retrieved from http://www.atsdr.cdc.gov/mrls/index.asp

Azeredo, A; Torres, JP; de Freitas Fonseca, M; Britto, JL; Bastos, WR; Azevedo E Silva, CE; Cavalcanti, G; Meire, RO; Sarcinelli, PN; Claudio, L; Markowitz, S; Malm, O. (2008). DDT and its metabolites in breast milk from the Madeira River basin in the Amazon, Brazil. Chemosphere 73: S246-S251. http://dx.doi.org/10.1016/j.chemosphere.2007.04.090

Banerjee, BD; Ray, A; Pasha, ST. (1996). A comparative evaluation of immunotoxicity of DDT and its metabolites in rats. Indian J Exp Biol 34: 517-522.

Bedi, JS; Gill, JP; Aulakh, RS; Kaur, P; Sharma, A; Pooni, PA. (2013). Pesticide residues in human breast milk: Risk assessment for infants from Punjab, India. Sci Total Environ 463: 720-726. http://dx.doi.org/10.1016/j.scitotenv.2013.06.066

Berghuis, SA; Bos, AF; Sauer, PJ; Roze, E. (2015). Developmental neurotoxicity of persistent organic pollutants: an update on childhood outcome [Review]. Arch Toxicol 89: 687-709. http://dx.doi.org/10.1007/s00204-015-1463-3

Bergkvist, C; Aune, M; Nilsson, I; Sandanger, TM; Hamadani, JD; Tofail, F; Oyvind-Odland, J; Kabir, I; Vahter, M. (2012). Occurrence and levels of organochlorine compounds in human breast milk in Bangladesh. Chemosphere 88: 784-790. http://dx.doi.org/10.1016/j.chemosphere.2012.03.083

Binelli, A; Riva, C; Cogni, D; Provini, A. (2008a). Assessment of the genotoxic potential of benzo(a)pyrene and pp '-dichlorodiphenyldichloroethylene in Zebra mussel (Dreissena polymorpha). Mutat Res Genet Toxicol Environ Mutagen 649: 135-145. http://dx.doi.org/10.1016/j.mrgentox.2007.08.011

Binelli, A; Riva, C; Cogni, D; Provini, A. (2008b). Genotoxic effects of p,p'-DDT (1,1,1-trichloro-2,2-bis-(chlorophenyl)ethane) and its metabolites in Zebra mussel (D. polymorpha) by SCGE assay and micronucleus test. Environ Mol Mutagen 49: 406-415. http://dx.doi.org/10.1002/em.20400

Bouwman, H; Sereda, B; Meinhardt, HM. (2006). Simultaneous presence of DDT and pyrethroid residues in human breast milk from a malaria endemic area in South Africa. Environ Pollut 144: 902-917. http://dx.doi.org/10.1016/j.envpol.2006.02.002

Buck Louis, GM. (2014). Persistent environmental pollutants and couple fecundity: an overview [Review]. Reproduction 147: R97-R104. http://dx.doi.org/10.1530/REP-13-0472

Buck Louis, GM; Sundaram, R; Schisterman, EF; Sweeney, AM; Lynch, CD; Gore-Langton, RE; Maisog, J; Kim, S; Chen, Z; Barr, DB. (2013). Persistent Environmental Pollutants and Couple Fecundity: The LIFE Study. Environ Health Perspect 121: 231-236. http://dx.doi.org/10.1289/ehp.1205301

Burns, CJ; McIntosh, LJ; Mink, PJ; Jurek, AM; Li, AA. (2013). Pesticide exposure and neurodevelopmental outcomes: Review of the epidemiologic and animal studies [Review]. J Toxicol Environ Health B Crit Rev 16: 127-283. http://dx.doi.org/10.1080/10937404.2013.783383

Cal/EPA (California Environmental Protection Agency). (2014). All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as of June 2014. Sacramento, CA: Office of Health Hazard Assessment. http://www.oehha.ca.gov/air/allrels.html

Cal/EPA (California Environmental Protection Agency). (2017a). Chemicals known to the state to cause cancer or reproductive toxicity January 27, 2017. (Proposition 65 list). Sacramento, CA: Office of Environmental Health Hazard Assessment. http://oehha.ca.gov/proposition-65/proposition-65-list

Cal/EPA (California Environmental Protection Agency). (2017b). OEHHA toxicity criteria database [Database]: Office of Environmental Health Assessment. Retrieved from http://www.oehha.ca.gov/tcdb/index.asp

Chevrier, C; Warembourg, C; Gaudreau, E; Monfort, C; Le Blanc, A; Guldner, L; Cordier, S. (2013). Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. Epidemiology 24: 251-260. http://dx.doi.org/10.1097/EDE.0b013e31827f53ec

Cok, I; Mazmanci, B; Mazmanci, MA; Turgut, C; Henkelmann, B; Schramm, KW. (2012). Analysis of human milk to assess exposure to PAHs, PCBs and organochlorine pesticides in the vicinity Mediterranean city Mersin, Turkey. Environ Int 40: 63-69. http://dx.doi.org/10.1016/j.envint.2011.11.012

Cook, MB; Trabert, B; McGlynn, KA. (2011). Organochlorine compounds and testicular dysgenesis syndrome: Human data [Review]. Int J Androl 34: e68-84; discussion e84-65. http://dx.doi.org/10.1111/j.1365-2605.2011.01171.x

Datta, PR. (1970). In vivo detoxication of p,p'-DDT via p,p'-DDE to p,p' -DDA in rats. IMS Ind Med Surg 39: 190-194.

DOE (U.S. Department of Energy). (2015). Table 3: Protective Action Criteria (PAC) Rev. 28 based on applicable 60-minute AEGLs, ERPGs, or TEELs. The chemicals are listed by CASRN. December 2015. Retrieved from http://www.atlintl.com/DOE/teels/teel/Revision_28_Table3.pdf

ECHA (European Chemicals Agency). (2017). Registered substances [Database]. Helsinki, Finland. Retrieved from https://echa.europa.eu/information-on-chemicals/registered-substances

Ennaceur, S; Ridha, D; Marcos, R. (2008). Genotoxicity of the organochlorine pesticides 1,1-dichloro-2,2- bis(p-chlorophenyl)ethylene (DDE) and hexachlorobenzene (HCB) in cultured human lymphocytes. Chemosphere 71: 1335-1339. http://dx.doi.org/10.1016/j.chemosphere.2007.11.040

Freyberger, A; Ellinger-Ziegelbauer, H; Kroetlinger, F. (2007). Evaluation of the rodent Hershberger bioassay: Testing of coded chemicals and supplementary molecular-biological and biochemical investigations. Toxicology 239: 77-88. http://dx.doi.org/10.1016/j.tox.2007.06.093

Freyberger, A; Hartmann, E; Krötlinger, F. (2005). Evaluation of the rodent Hershberger bioassay using three reference (anti)androgens. Arh Hig Rada Toksikol 56: 131-139.

Freyberger, A; Schladt, L. (2009). Evaluation of the rodent Hershberger bioassay on intact juvenile males--testing of coded chemicals and supplementary biochemical investigations. Toxicology 262: 114-120. http://dx.doi.org/10.1016/j.tox.2009.05.013

Gascon, M; Morales, E; Sunyer, J; Vrijheid, M. (2013). Effects of persistent organic pollutants on the developing respiratory and immune systems: a systematic review [Review]. Environ Int 52: 51-65. http://dx.doi.org/10.1016/j.envint.2012.11.005

Gascon, M; Sunyer, J; Casas, M; Martinez, D; Ballester, F; Basterrechea, M; Bonde, JP; Chatzi, L; Chevrier, C; Eggesbo, M; Esplugues, A; Govarts, E; Hannu, K; Ibarluzea, J; Kasper-Sonnenberg, M; Kluemper, C; Koppen, G; Nieuwenhuijsen, MJ; Palkovicova, L; Pele, F; Polder, A; Schoeters, G; Torrent, M; Trnovec, T; Vassilaki, M; Vrijheid, M. (2014). Prenatal exposure to DDE and PCB 153 and respiratory health in early childhood a meta-analysis. Epidemiology 25: 544-553. http://dx.doi.org/10.1097/EDE.0000000000000097

Gerić, M; Ceraj-Cerić, N; Gajski, G; Vasilić, Ž; Capuder, Ž; Garaj-Vrhovac, V. (2012). Cytogenetic status of human lymphocytes after exposure to low concentrations of p,p'-DDT, and its metabolites (p,p'-DDE, and p,p'-DDD) in vitro. Chemosphere 87: 1288-1294. http://dx.doi.org/10.1016/j.chemosphere.2012.01.037

Govarts, E; Nieuwenhuijsen, M; Schoeters, G; Ballester, F; Bloemen, K; de Boer, M; Chevrier, C; Eggesbo, M; Guxens, M; Kraemer, U; Legler, J; Martinez, D; Palkovicova, L; Patelarou, E; Ranft, U; Rautio, A; Petersen, MS; Slama, R; Stigum, H; Toft, G; Trnovec, T; Vandentorren, S; Weihe, P; Kuperus, NW; Wilhelm, M; Wittsiepe, J; Bonde, JP; OBELIX/ENEIECO. (2012). Birth weight and prenatal exposure to polychlorinated biphenyls (PCBS) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European birth cohorts. Environ Health Perspect 120: 162-170. http://dx.doi.org/10.1289/ehp.1103767

Gray, LE, Jr; Wolf, C; Lambright, C; Mann, P; Price, M; Cooper, RL; Ostby, J. (1999). Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol Ind Health 15: 94-118. http://dx.doi.org/10.1177/074823379901500109

Hernik, A; Goralczyk, K; Strucinski, P; Czaja, K; Korcz, W; Minorczyk, M; Lyczewska, M; Ludwicki, JK. (2014). Characterising the individual health risk in infants exposed to organochlorine pesticides via breast milk by applying appropriate margins of safety derived from estimated daily intakes. Chemosphere 94: 158-163. http://dx.doi.org/10.1016/j.chemosphere.2013.09.067

HSDB (Hazardous Substances Data Bank). (2010). DDE. CASRN 72-55-9 [Database]. Bethesda, MD: U.S. National Library of Medicine, Toxicology Data Network (TOXNET). Retrieved from https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm

IARC (International Agency for Research on Cancer). (1991). IARC monographs on the evaluation of carcinogenic risks to humans: Occupational exposures in insecticide application, and some pesticides [IARC Monograph]. In IARC monographs on the evaluation of carcinogenic risks to humans: Occupational exposures in insecticide application, and some pesticides. Lyon, France: World Health Organization.

IARC (International Agency for Research on Cancer). (2017). IARC Monographs on the evaluation of carcinogenic risk to humans. Geneva, Switzerland: International Agency for Research on Cancer, WHO. http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php

Ingber, SZ; Buser, MC; Pohl, HR; Abadin, HG; Murray, HE; Scinicariello, F. (2013). DDT/DDE and breast cancer: a meta-analysis. Regul Toxicol Pharmacol 67: 421-433. http://dx.doi.org/10.1016/j.yrtph.2013.08.021

IPCS (International Programme on Chemical Safety). (2017). INCHEM: Chemical safety information from intergovernmental organizations [Database]: World Health Organization & Canadian Centre for Occupational Health and Safety. Retrieved from http://www.inchem.org/

Jasso-Pineda, Y; Díaz-Barriga, F; Yáñez-Estrada, L; Pérez-Vázquez, FJ; Pérez-Maldonado, IN. (2015). DNA damage in Mexican children living in high-risk contaminated scenarios. Sci Total Environ 518-519C: 38-48. http://dx.doi.org/10.1016/j.scitotenv.2015.02.073

Jeng, HA. (2014). Exposure to endocrine disrupting chemicals and male reproductive health [Review]. Front Public Health 2: 55. http://dx.doi.org/10.3389/fpubh.2014.00055

Jönsson, CJ; Lund, BO; Bergman, A; Brandt, I. (1992). Adrenocortical toxicity of 3-methylsulphonyl-DDE; 3: Studies in fetal and suckling mice. Reprod Toxicol 6: 233-240. http://dx.doi.org/10.1016/0890-6238(92)90178-V

Jönsson, CJ; Rodriguez-Martinez, H; Lund, BO; Bergman, A; Brandt, I. (1991). Adrenocortical toxicity of 3-methylsulfonyl-DDE in mice. II. Mitochondrial changes following ecologically relevant doses. Fundam Appl Toxicol 16: 365-374.

Jossé, R; Rogue, A; Lorge, E; Guillouzo, A. (2012). An adaptation of the human HepaRG cells to the in vitro micronucleus assay. Mutagenesis 27: 295-304. http://dx.doi.org/10.1093/mutage/ger076

Kelce, WR; Lambright, CR; Gray, LEJ, R; Roberts, KP. (1997). Vinclozolin and p,p'-DDE alter androgen-dependent gene expression: In vivo confirmation of an androgen receptor-mediated mechanisms. Toxicol Appl Pharmacol 142: 192-200.

Kelce, WR; Stone, CR; Laws, SC; Gray, LE; Kemppainen, JA; Wilson, EM. (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 375: 581-585. http://dx.doi.org/10.1038/375581a0

Kornbrust, D; Gillis, B; Collins, B; Goehl, T; Gupta, B; Schwetz, B. (1986). Effects of 1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene (DDE) on lactation in rats. J Toxicol Environ Health 17: 23-36. http://dx.doi.org/10.1080/15287398609530799

Legler, J; Fletcher, T; Govarts, E; Porta, M; Blumberg, B; Heindel, JJ; Trasande, L. (2015). Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab 100: 1278-1288. http://dx.doi.org/10.1210/jc.2014-4326

Lewis-Mikhael, AM; Olmedo-Requena, R; Martínez-Ruiz, V; Bueno-Cavanillas, A; Jiménez-Moleón, JJ. (2015). Organochlorine pesticides and prostate cancer, Is there an association? A meta-analysis of epidemiological evidence [Review]. Cancer Causes Control 26: 1375-1392. http://dx.doi.org/10.1007/s10552-015-0643-z

Liu, Q; Shao, W; Zhang, C; Xu, C; Wang, Q; Liu, H; Sun, H; Jiang, Z; Gu, A. (2017a). Organochloride pesticides modulated gut microbiota and influenced bile acid metabolism in mice. Environ Pollut 226: 268-276. http://dx.doi.org/10.1016/j.envpol.2017.03.068

Liu, Q; Wang, Q; Xu, C; Shao, W; Zhang, C; Liu, H; Jiang, Z; Gu, A. (2017b). Organochloride pesticides impaired mitochondrial function in hepatocytes and aggravated disorders of fatty acid metabolism. Sci Rep 7: 46339. http://dx.doi.org/10.1038/srep46339

Liu, Y; Peterson, KE. (2015). Maternal exposure to synthetic chemicals and obesity in the offspring: Recent findings [Review]. Curr Envir Health Rpt 2: 339-347. http://dx.doi.org/10.1007/s40572-015-0068-6

Loeffler, IK; Peterson, RE. (1999). Interactive effects of TCDD and p,p'-DDE on male reproductive tract development in in utero and lactationally exposed rats. Toxicol Appl Pharmacol 154: 28-39. http://dx.doi.org/10.1006/taap.1998.8572

López-Cervantes, M; Torres-Sánchez, L; Tobías, A; López-Carrillo, L. (2004). Dichlorodiphenyldichloroethane burden and breast cancer risk: a meta-analysis of the epidemiologic evidence. Environ Health Perspect 112: 207-214. http://dx.doi.org/10.1289/ehp.6492

Lund, BO; Bergman, A; Brandt, I. (1988). Metabolic activation and toxicity of a DDT-metabolite, 3-methylsulphonyl-DDE, in the adrenal zona fasciculata in mice. Chem Biol Interact 65: 25-40.

Makita, Y. (2008). Effects of perinatal combined exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) and tributyltin (TBT) on rat female reproductive system. Environ Toxicol Pharmacol 25: 380-385. http://dx.doi.org/10.1016/j.etap.2007.12.003

Makita, Y; Omura, M. (2006). Effects of perinatal combined exposure to 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene and tributyltin on male reproductive system. Basic Clin Pharmacol Toxicol 99: 128-132. http://dx.doi.org/10.1111/j.1742-7843.2006.pto_430.x

Makita, Y; Omura, M; Tanaka, A; Kiyohara, C. (2005). Effects of concurrent exposure to tributyltin and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (p,p'-DDE) on immature male Wistar rats. Basic Clin Pharmacol Toxicol 97: 364-368. http://dx.doi.org/10.1111/j.1742-7843.2005.pto_199.x

Mamane, A; Raherison, C; Tessier, JF; Baldi, I; Bouvier, G. (2015). Environmental exposure to pesticides and respiratory health [Review]. Eur Respir Rev 24: 462-473. http://dx.doi.org/10.1183/16000617.00006114

Man, YB; Chan, JK; Wang, HS; Wu, SC; Wong, MH. (2014). DDTs in mothers' milk, placenta and hair, and health risk assessment for infants at two coastal and inland cities in China. Environ Int 65: 73-82. http://dx.doi.org/10.1016/j.envint.2014.01.001

Moon, HJ; Kang, TS; Kim, TS; Kang, IH; Ki, HY; Kim, SH; Han, SY. (2009). OECD validation of phase 3 Hershberger assay in Korea using surgically castrated male rats with coded chemicals. J Appl Toxicol 29: 350-355. http://dx.doi.org/10.1002/jat.1418

Morgan, DP; Roan, CC. (1971). Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man. Arch Environ Health 22: 301-308. http://dx.doi.org/10.1080/00039896.1971.10665848

Morgan, DP; Roan, CC. (1974). The metabolism of DDT in man. In WJ Hayes, Jr. (Ed.), Essays in toxicology (pp. 39-97). New York, NY: Academic Press. http://dx.doi.org/10.1016/B978-0-12-107605-4.50009-3

Mota, PC; Cordeiro, M; Pereira, SP; Oliveira, PJ; Moreno, AJ; Ramalho-Santos, J. (2011). Differential effects of p,p'-DDE on testis and liver mitochondria: implications for reproductive toxicology. Reprod Toxicol 31: 80-85. http://dx.doi.org/10.1016/j.reprotox.2010.09.010

Mühlebach, S; Moor, MJ; Wyss, PA; Bickel, MH. (1991). Kinetics of distribution and elimination of DDE in rats. Xenobiotica 21: 111-120. http://dx.doi.org/10.3109/00498259109039455

NCI (National Cancer Institute). (1978). Bioassays of DDT, TDE, and p,p'-DDE for possible carcinogenicity (pp. 120 pgs). (NCI-CG-TR-131). Washington, DC: U.S. Department of Health, Education, and Welfare, National Institutes of Health. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr131.pdf

NIOSH (National Institute for Occupational Safety and Health). (2016). NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Atlanta, GA: Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare. http://www.cdc.gov/niosh/npg/npgdcas.html

NLM (National Institutes of Health, National Library of Medicine). (2010). Hazardous Substances Data Bank. DDE. CASRN: 72-55-9 [Database]. Retrieved from https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm

NTP (National Toxicology Program). (2014). Report on carcinogens. Thirteenth edition. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.

OSHA (Occupational Safety & Health Administration). (2006). Table Z-1: Limits for air contaminants. Occupational safety and health standards, subpart Z, toxic and hazardous substances. (OSHA standard 1910.1000, 29 CFR). Washington, DC: U.S. Department of Labor. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

OSHA (Occupational Safety & Health Administration). (2011). Air contaminants: Occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. (OSHA Standard 1915.1000). Washington, DC: U.S. Department of Labor. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286

Park, JH; Cha, ES; Ko, Y; Hwang, MS; Hong, JH; Lee, WJ. (2014). Exposure to dichlorodiphenyltrichloroethane and the risk of breast cancer: a systematic review and meta-analysis. Osong Public Health Res Perspect 5: 77-84. http://dx.doi.org/10.1016/j.phrp.2014.02.001

Patrick, SM; Bornman, MS; Joubert, AM; Pitts, N; Naidoo, V; de Jager, C. (2016). Effects of environmental endocrine disruptors, including insecticides used for malaria vector control on reproductive parameters of male rats. Reprod Toxicol 61: 19-27. http://dx.doi.org/10.1016/j.reprotox.2016.02.015

Pestana, D; Teixeira, D; Meireles, M; Marques, C; Norberto, S; Sá, C; Fernandes, VC; Correia-Sá, L; Faria, A; Guardão, L; Guimarães, JT; Cooper, WN; Sandovici, I; Domingues, VF; Delerue-Matos, C; Monteiro, R; Constância, M; Calhau, C. (2017). Adipose tissue dysfunction as a central mechanism leading to dysmetabolic obesity triggered by chronic exposure to p,p'-DDE. Sci Rep 7: 2738. http://dx.doi.org/10.1038/s41598-017-02885-9

Poursafa, P; Ataei, E; Kelishadi, R. (2015). A systematic review on the effects of environmental exposure to some organohalogens and phthalates on early puberty [Review]. J Res Med Sci 20: 613-618. http://dx.doi.org/10.4103/1735-1995.165971

Rogan, WJ; Gladen, BC; McKinney, JD; Carreras, N; Hardy, P; Thullen, J; Tinglestad, J; Tully, M. (1986). Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 109: 335-341. http://dx.doi.org/10.1016/S0022-3476(86)80397-3

Rossi, L; Barbieri, O; Sanguineti, M; Cabral, JR; Bruzzi, P; Santi, L. (1983). Carcinogenicity study with technical-grade dichlorodiphenyltrichloroethane and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene in hamsters. Cancer Res 43: 776-781.

Sagiv, SK; Nugent, JK; Brazelton, TB; Choi, AL; Tolbert, PE; Altshul, LM; Korrick, SA. (2008). Prenatal organochlorine exposure and measures of behavior in infancy using the Neonatal Behavioral Assessment Scale (NBAS). Environ Health Perspect 116: 666-673. http://dx.doi.org/10.1289/ehp.10553

Scheirs, J; De Coen, A; Covaci, A; Beernaert, J; Kayawe, VM; Caturla, M; De Wolf, H; Baert, P; Van Oostveldt, P; Verhagen, R; Blust, R; De Coen, W. (2006). Genotoxicity in wood mice (Apodemus sylvaticus) along a pollution gradient: exposure-, age-, and gender-related effects. Environ Toxicol Chem 25: 2154-2162. http://dx.doi.org/10.1897/05-419R.1

Shen, H; Main, KM; Virtanen, HE; Damggard, IN; Haavisto, AM; Kaleva, M; Boisen, KA; Schmidt, IM; Chellakooty, M; Skakkebaek, NE; Toppari, J; Schramm, KW. (2007). From mother to child: investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere 67: S256-S262. http://dx.doi.org/10.1016/j.chemosphere.2006.05.106

Shi, YQ; Li, HW; Wang, YP; Liu, CJ; Yang, KD. (2013). p,p'-DDE induces apoptosis and mRNA expression of apoptosis-associated genes in testes of pubertal rats. Environ Toxicol 28: 31-41. http://dx.doi.org/10.1002/tox.20694

Shin, JH; Moon, HJ; Kang, IH; Kim, TS; Lee, SJ; Ahn, JY; Bae, H; Jeung, EB; Han, SY. (2007). OECD validation of the rodent Hershberger assay using three reference chemicals; 17alpha-methyltestosterone, procymidone, and p,p'-DDE. Arch Toxicol 81: 309-318. http://dx.doi.org/10.1007/s00204-006-0174-1

Song, S; Ma, X; Tong, L; Tian, Q; Huang, Y; Yin, S; Sun, H. (2013). Residue levels of hexachlorocyclohexane and dichlorodiphenyltrichloroethane in human milk collected from Beijing. Environ Monit Assess 185: 7225-7229. http://dx.doi.org/10.1007/s10661-013-3096-4

Song, Y; Shi, Y; Yu, H; Hu, Y; Wang, Y; Yang, K. (2011). p,p'-Dichlorodiphenoxydichloroethylene induced apoptosis of Sertoli cells through oxidative stress-mediated p38 MAPK and mitochondrial pathway. Toxicol Lett 202: 55-60. http://dx.doi.org/10.1016/j.toxlet.2011.01.020

Song, Y; Wu, N; Wang, S; Gao, M; Song, P; Lou, J; Tan, Y; Liu, K. (2014). Transgenerational impaired male fertility with an Igf2 epigenetic defect in the rat are induced by the endocrine disruptor, p,p'-DDE. Hum Reprod 29: 2512-2521. http://dx.doi.org/10.1093/humrep/deu208

Tang-Péronard, JL; Andersen, HR; Jensen, TK; Heitmann, BL. (2011). Endocrine-disrupting chemicals and obesity development in humans: a review [Review]. Obes Rev 12: 622-636. http://dx.doi.org/10.1111/j.1467-789X.2011.00871.x

Tavares, RS; Mansell, S; Barratt, CL; Wilson, SM; Publicover, SJ; Ramalho-Santos, J. (2013). p,p'-DDE activates CatSper and compromises human sperm function at environmentally relevant concentrations. Hum Reprod 28: 3167-3177. http://dx.doi.org/10.1093/humrep/det372

Taylor, KW; Novak, RF; Anderson, HA; Birnbaum, LS; Blystone, C; Devito, M; Jacobs, D; Köhrle, J; Lee, DH; Rylander, L; Rignell-Hydbom, A; Tornero-Velez, R; Turyk, ME; Boyles, AL; Thayer, KA; Lind, L. (2013). Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review [Review]. Environ Health Perspect 121: 774-783. http://dx.doi.org/10.1289/ehp.1205502

Tinwell, H; Friry-Santini, C; Rouquié, D; Belluco, S; Elies, L; Pallen, C; Bars, R. (2007). Evaluation of the antiandrogenic effects of flutamide, DDE, and linuron in the weanling rat assay using organ weight, histopathological, and proteomic approaches. Toxicol Sci 100: 54-65. http://dx.doi.org/10.1093/toxsci/kfm208

Tomatis, L; Turusov, V; Charles, RT; Boicchi, M. (1974). Effect of long-term exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene, to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane, and to the two chemicals combined on CF-1 mice. J Natl Cancer Inst 52: 883-891.

U.S. APHC (U.S. Army Public Health Command). (2013). Environmental health risk assessment and chemical exposure guidelines for deployed military personnel. Technical guide 230, 2013 revision. Aberdeen Proving Ground, MD. http://phc.amedd.army.mil/PHC%20Resource%20Library/TG230.pdf

U.S. EPA (U.S. Environmental Protection Agency). (1988a). Integrated risk information system (IRIS) Chemical Assessment Summary for dichlorodiphenyldichloroethylene (DDE); CASRN 72-55-9 [EPA Report]. Washington, DC: Office of Research and Development, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0328_summary.pdf

U.S. EPA (U.S. Environmental Protection Agency). (1988b). Integrated risk information system (IRIS) Chemical Assessment Summary for p,p'-dichlorodiphenyl dichloroethane (DDD); CASRN 72-54-8. Washington, DC: Office of Research and Development, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0347_summary.pdf

U.S. EPA (U.S. Environmental Protection Agency). (1988c). Recommendations for and documentation of biological values for use in risk assessment (pp. 1-395). (EPA/600/6-87/008). Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855

U.S. EPA (U.S. Environmental Protection Agency). (2011a). Health effects assessment summary tables (HEAST). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. http://epa-heast.ornl.gov/heast.php

U.S. EPA (U.S. Environmental Protection Agency). (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose (pp. 1-50). (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum, Office of the Science Advisor. https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose

U.S. EPA (U.S. Environmental Protection Agency). (2012a). 2012 Edition of the drinking water standards and health advisories [EPA Report]. (EPA/822/S-12/001). Washington, DC: U.S. Environmental Protection Agency, Office of Water. http://www.epa.gov/sites/production/files/2015-09/documents/dwstandards2012.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Benchmark dose technical guidance. In Risk Assessment Forum. (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

U.S. EPA (U.S. Environmental Protection Agency). (2012c). Estimation Programs Interface Suite for Microsoft Windows, v 4.11: p,p-DDE (CASRN 72-55-9) [Fact Sheet]. Washington, DC. https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

U.S. EPA (U.S. Environmental Protection Agency). (2015a). About the TSCA chemical substance inventory. Download the non-confidential TSCA inventory [Database]. Retrieved from http://www2.epa.gov/tsca-inventory/how-access-tsca-inventory

U.S. EPA (U.S. Environmental Protection Agency). (2015b). List of lists: Consolidated list of chemicals subject to the Emergency Planning and Community Right- To-Know Act (EPCRA), Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and Section 112(r) of the Clean Air Act [EPA Report]. (EPA 550-B-15-001). Wasington, DC: Office of Solid Waste and Remedial Response. https://www.epa.gov/sites/production/files/2015-03/documents/list_of_lists.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2017). Integrated risk information system. IRIS assessments [Database]. Washington, DC: Integrated Risk Information 'System. Retrieved from http://www.epa.gov/iris/

Veeramachaneni, DN. (2006). Germ cell atypia in undescended testes hinges on the aetiology of cryptorchidism but not the abdominal location per se. Int J Androl 29: 235-240. http://dx.doi.org/10.1111/j.1365-2605.2005.00565.x

Wang, N; Shi, L; Kong, D; Cai, D; Cao, Y; Liu, Y; Pang, G; Yu, R. (2011). Accumulation levels and characteristics of some pesticides in human adipose tissue samples from Southeast China. Chemosphere 84: 964-971. http://dx.doi.org/10.1016/j.chemosphere.2011.05.062

WHO (World Health Organization). (2011). DDT in indoor residual spraying: Human health aspects [WHO EHC]. (Environmental health criteria: 241). Geneva, Switzerland. http://www.who.int/ipcs/publications/ehc/ehc241.pdf

WHO (World Health Organization). (2017). Online catalog for the Environmental Health Criteria (EHC) monographs. Geneva, Switzerland: World Health Organization (WHO). http://www.who.int/ipcs/publications/ehc/en/

Xu, C; Tang, M; Zhang, H; Zhang, C; Liu, W. (2015). Levels and patterns of DDTs in maternal colostrum from an island population and exposure of neonates. Environ Pollut 209: 132-139. http://dx.doi.org/10.1016/j.envpol.2015.11.028

Yamasaki, K; Okuda, H; Takeuchi, T; Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicol Lett 189: 14-20. http://dx.doi.org/10.1016/j.toxlet.2009.04.023

Yan, M; Shi, Y; Wang, Y; Wang, C; Zhou, J; Quan, C; Liu, C; Yang, K. (2013). Effects of p,p'-DDE on the mRNA and protein expressions of vimentin, N-cadherin and FSHR in rats testes: an in vivo and in vitro study. Environ Toxicol Pharmacol 35: 486-494. http://dx.doi.org/10.1016/j.etap.2013.02.008

You, L; Brenneman, KA; Heck, H. (1999a). In utero exposure to antiandrogens alters the responsiveness of the prostate to p,p'-DDE in adult rats and may induce prostatic inflammation. Toxicol Appl Pharmacol 161: 258-266. http://dx.doi.org/10.1006/taap.1999.8804

You, L; Casanova, M; Archibeque-Engle, S; Sar, M; Fan, LQ; Heck, HA. (1998). Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE. Toxicol Sci 45: 162-173.

You, L; Gazi, E; Archibeque-Engle, S; Casanova, M; Conolly, RB; Heck, HA. (1999b). Transplacental and lactational transfer of p,p'-DDE in Sprague-Dawley rats. Toxicol Appl Pharmacol 157: 134-144.