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Toxicological Review of Benzo[a]pyrene

[CASRN 50-32-8]

Supplemental Information

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ABBREVIATIONS

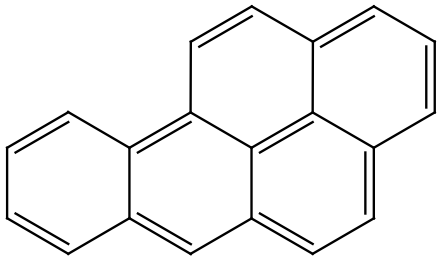
AchE	acetylcholine esterase
ADAF	age-dependent adjustment factor
Ah	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
AhR	aryl hydrocarbon receptor
AIC	Akaike's Information Criterion
AKR	aldo-keto reductase
AMI	acute myocardial infarction
ANOVA	analysis of variance
AST	aspartate transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose, 95% lower bound
BMDS	Benchmark Dose Software
BMR	benchmark response
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide
BPdG	benzo[a]pyrene-7,8-diol-9,10-epoxide-N2-deoxyguanosine
BPQ	benzo[a]pyrene-7.8-quinone
BrdU	bromodeoxyuridine
BSM	benzene-soluble matter
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAAC	Chemical Assessment Advisory Committee
CASRN	Chemical Abstracts Service Registry Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHO	Chinese hamster ovary
CI	confidence interval
CYP	cytochrome
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
dbcAMP	dibutyl cyclic adenosine monophosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC	European Commission
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EROD	7-ethoxyresorufin-O-deethylase
ETS	environmental tobacco smoke
Fe_2O_3	ferrous oxide
FSH	follicle stimulating hormone
GABA	gamma-aminobutyric acid
GD	gestational day
GI	gastrointestinal
GJIC	gap junctional intercellular communication
GSH	reduced glutathione
GST	glutathione-S-transferase
GSTM1	glutathione-S-transferase M1
hCG	human chorionic gonadotropin
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HFC	high-frequency cell
HPLC	high-performance liquid chromatography
hprt	hypoxanthine guanine phosphoribosyl transferase
HR	hazard ratio
Hsp90	heat shock protein 90
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IHD	ischemic heart disease
IRIS	Integrated Risk Information System
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MAP	mitogen-activated protein
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MIAME	Minimum Information About a Microarray Experiment
MLE	maximum likelihood estimate
MMAD	mass median aerodynamic diameter
MN	micronucleus
MPPD	Multi-Path Particle Deposition
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NCE	normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NK	natural killer
NMDA	N-methyl-D-aspartate
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NQO	NADPH:quinone oxidoreductase
NRC	National Research Council

NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
ORD	Office of Research and Development
PAH	polycyclic aromatic hydrocarbon
PBMC	peripheral blood mononuclear cell
PBPK	physiologically based pharmacokinetic
PCA	Principal Components Analysis
PCE	polychromatic erythrocyte
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
PUVA	psoralen plus ultraviolet-A
RBC	red blood cell
$RDDR_{ER}$	regional deposited dose ratio for extrarespiratory effects
RfC	inhalation reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
SAB	Science Advisory Board
s.c.	subcutaneous
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SHE	Syrian hamster embryo
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOAR	Systematic Omics Analysis Review
SOD	superoxide dismutase
SRBC	sheep red blood cells
SSB	single-strand break
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TK	thymidine kinase
ToxR	Toxicological Reliability Assessment
TPA	12-O-tetradecanoylphorbol-13-acetate
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
TWA	time-weighted average
UCL	upper confidence limit
UDP-UGT	uridine diphosphate-glucuronosyltransferase
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UVA	ultraviolet-A
UVB	ultraviolet-B
WBC	white blood cell
WT	wild type
WTC	World Trade Center
XPA	xeroderma pigmentosum group A

APPENDIX A. CHEMICAL PROPERTIES AND EXPOSURE INFORMATION

Benzo[a]pyrene is a polycyclic aromatic hydrocarbon (PAH) with five fused rings and a bay region (Figure A-1). It is a pale yellow crystalline solid with a faint aromatic odor. It is relatively insoluble in water and has low volatility. Benzo[a]pyrene is released to the air from both natural and anthropogenic sources and removed from the atmosphere by photochemical oxidation; reaction with nitrogen oxides, hydroxy and hydroperoxy radicals, ozone, sulfur oxides, and peroxyacetyl nitrate; and wet and dry deposition to land or water. In air, benzo[a]pyrene is predominantly adsorbed to particulates, but may also exist as a vapor at high temperatures (HSDB, 2012). The half-lives for degradation of benzo[a]pyrene in soil, air, water, and sediment are 229–309, 0.02–7, 39–71, and 196–2,293 days, respectively (HSDB, 2012; GLC, 2007).

The structural formula is presented in Figure A-1. The physical and chemical properties of benzo[a]pyrene are shown in Table A-1.



Benzo[a]pyrene

Figure A-1. Structural formula of benzo[a]pyrene.

Table A-1. Chemical and physical properties of benzo[a]pyrene

CASRN 50-32-8		
Synonyms	Benzo[d,e,f]chrysene; 3,4-benzopyrene, 3,4-benzpyrene; benz[a]pyrene; BP; BaP	ChemIDplus (2012)
Melting point	179–179.3°C	O'Neil et al. (2001)
Boiling point	310–312°C at 10 mm Hg	O'Neil et al. (2001)
Vapor pressure, at 20°C	5×10^{-7} mm Hg	Verschueren (2001)
Density	1.351 g/cm^3	IARC (1973)
Flashpoint (open cup)	No data	
Water solubility at 25°C	$1.6–2.3 \times 10^{-3}$ mg/L	Howard and Meylan (1997); ATSDR (1995)
Log K_{ow}	6.04	Verschueren (2001)
Odor threshold	No data	
Molecular weight	252.32	O'Neil et al. (2001)
Conversion factors[a]	1 ppm = 10.32 mg/m^3	Verschueren (2001)
Empirical formula	$C_{20}H_{12}$	ChemIDplus (2012)

[a]Calculated based on the ideal gas law, PV = *n*RT at 25°C: ppm = mg/m^3 × 24.45 ÷ molecular weight.

No reference to any commercial use for purified benzo[a]pyrene, other than for research purposes, was found. The earliest research reference for benzo[a]pyrene was related to the identification of coal tar constituents associated with human skin tumors (Phillips, 1983; Cook et al., 1933). Benzo[a]pyrene is found ubiquitously in the environment, primarily as a result of incomplete combustion emissions (Boström et al., 2002). It is released to the environment via both natural sources (such as forest fires) and anthropogenic sources including stoves/furnaces burning fossil fuels (especially wood and coal), motor vehicle exhaust, cigarette smoke, and various industrial combustion processes (ATSDR, 1995). Benzo[a]pyrene is also found in soot and coal tars. Studies have reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for the majority of PAHs in many watersheds (Rowe and O'Connor, 2011; Van Metre and Mahler, 2010; Mahler et al., 2005). Occupational exposure to PAHs occurs primarily through inhalation and skin contact during the production and use of coal tar and coal-tar-derived products, such as roofing tars, creosote, and asphalt (IARC, 2010). Chimney sweeping can result in exposure to benzo[a]pyrene-contaminated soot (ATSDR, 1995). Workers involved in the production of aluminum, coke, graphite, and silicon carbide may also be exposed to benzo[a]pyrene (see Table A-2).

Benzo[a]pyrene concentrations have been well documented in samples of ground, drinking, and surface water (HSDB, 2012). An assessment of benzo[a]pyrene emissions in the Great Lakes

Region in 2002 indicated that the largest source categories are metal production (33%), petroleum refineries (11%), residential wood burning (28%), open burning (13%), on-road vehicles (6%), and off-highway gasoline engines (3%) (GLC, 2007).

Inhalation Exposure. The Agency for Toxic Substances and Disease Registry (ATSDR, 1995) reported average indoor concentrations of benzo[a]pyrene of 0.37–1.7 ng/m^3 for smokers and 0.27–0.58 ng/m^3 for nonsmokers. Naumova et al. (2002) measured PAHs in 55 nonsmoking residences in three urban areas during June 1999–May 2000. Mean indoor benzo[a]pyrene levels ranged from 0.02 to 0.078 ng/m^3; outdoor levels were 0.025–0.14 ng/m^3. The authors concluded that indoor levels of the 5–7-ring PAHs (such as benzo[a]pyrene) were dominated by outdoor sources and observed an average indoor/outdoor ratio of approximately 0.7 (Naumova et al., 2002). Mitra and Wilson (1992) measured benzo[a]pyrene air levels in Columbus, Ohio, and found elevated indoor levels in homes with smokers. The measured average concentration was 1.38 ng/m^3 for outdoor air; indoor concentrations were 0.07 ng/m^3 for homes with electrical utilities, 0.91 ng/m^3 for homes with gas utilities, 0.80 ng/m^3 for homes with gas utilities and a fireplace, 2.75 ng/m^3 for homes with gas utilities and smokers, and 1.82 ng/m^3 for homes with gas utilities, smokers, and a fireplace (Mitra and Wilson, 1992). Mitra and Ray (1995) evaluated data on benzo[a]pyrene air levels in Columbus, Ohio, and reported average concentrations of 0.77 ng/m^3 inside homes and 0.23 ng/m^3 outdoors. Park et al. (2001) measured an average ambient level of benzo[a]pyrene in Seabrook, Texas during 1995–1996 of 0.05 ng/m^3 (vapor plus particulate). Park et al. (2001) also reported average ambient air levels from earlier studies as 1.0 ng/m^3 for Chicago, 0.19 ng/m^3 for Lake Michigan, 0.01 ng/m^3 for Chesapeake Bay, and 0.02 ng/m^3 for Corpus Christie, Texas. Petry et al. (1996) conducted personal air sampling during 1992 at five workplaces in Switzerland: carbon anode production, graphite production, silicon carbide production, bitumen paving work, and metal recycling. Table A-2 summarizes the benzo[a]pyrene air concentration data from the previous studies.

Table A-2. Benzo[a]pyrene concentrations in air

Setting	Years	n	Concentration (ng/m^3)	Reference
Outdoor, urban				
Los Angeles, California	1999–2000	19	0.065	Naumova et al. (2002)
Houston, Texas	1999–2000	21	0.025	Naumova et al. (2002)
Elizabeth, New Jersey	1999–2000	15	0.14	Naumova et al. (2002)
Seabrook, Texas	1995–1996	NA	0.05	Park et al. (2001)
Columbus, Ohio	1986–1987	8	0.23	Mitra and Ray (1995)
Indoor, residential				
Los Angeles, California	1999–2000	19	0.078	Naumova et al. (2002)
Houston, Texas	1999–2000	21	0.020	Naumova et al. (2002)
Elizabeth, New Jersey	1999–2000	15	0.055	Naumova et al. (2002)
Columbus, Ohio	1986–1987	8	0.77	Mitra and Ray (1995)
Columbus, Ohio		10	0.07–2.75	Mitra and Wilson (1992)
Homes with smokers			0.37–1.7	ATSDR (1995)
Homes without smokers			0.27–0.58	ATSDR (1995)
Occupational				
Aluminum production			30–530	ATSDR (1995)
Coke production			150–6,720; 8,000	Petry et al. (1996); ATSDR (1995)
Carbon anode production, Switzerland	1992	30	1,100	Petry et al. (1996)
Graphite production, Switzerland	1992	16	83	Petry et al. (1996)
Silicon carbide production, Switzerland	1992	14	36	Petry et al. (1996)
Metal recovery, Switzerland	1992	5	14	Petry et al. (1996)
Bitumen paving, Switzerland	1992	9	10	Petry et al. (1996)

NA = not available.

Santodonato et al. (1981) estimated the adult daily intake from inhalation as 9–43 ng/day. The European Commission (EC, 2002) reported benzo[a]pyrene air levels in Europe during the 1990s as 0.1–1 ng/m^3 in rural areas and 0.5–3 ng/m^3 in urban areas. The amount of benzo[a]pyrene is reported to be 5–80 ng per cigarette in mainstream cigarette smoke, but significantly higher, 25–200 ng per cigarette in sidestream smoke. Concentrations of 400–760,000 ng/m^3 have been reported in a cigarette smoke-polluted environment (Cal/EPA, 2010). The mean intake via inhalation for an adult nonsmoker was estimated as 20 ng/day. Naumova et al. (2002) focused on nonsmoking residences and suggested that typical air exposures

are <0.14 ng/m^3, which would result in an intake of <3 ng/day assuming an inhalation rate of 20 m^3/day.

Oral Exposure. The processing and cooking of foods is viewed as the dominant pathway of PAH contamination in foods (Boström et al., 2002). Among the cooking methods that lead to PAH contamination are the grilling, roasting, and frying of meats. Raw meat, milk, poultry, and eggs normally do not contain high levels of PAHs due to rapid metabolism of these compounds in the species of origin. However, some marine organisms, such as mussels and lobsters, are known to adsorb and accumulate PAHs from contaminated water (e.g., oil spills). Vegetables and cereal grains can become contaminated primarily through aerial deposition of PAHs present in the atmosphere (Li et al., 2009).

Kazerouni et al. (2001) measured benzo[a]pyrene in a variety of commonly consumed foods collected from grocery stores and restaurants in Maryland (analyzed as a composite from 4–6 samples of each food type). The foods were tested after various methods of cooking; the results are reported in Table A-3. The concentrations were combined with food consumption data to estimate intake. The intakes of the 228 subjects ranged from approximately 10 to 160 ng/day, with about 30% in the 40–60 ng/day range. The largest contributions to total intake were reported as bread, cereal, and grain (29%) and grilled/barbecued meats (21%).

Table A-3. Benzo[a]pyrene levels in food

Food	Concentration (ng/g)
Meat	
Fried or broiled beef	0.01–0.02
Grilled beef	0.09–4.9
Fried or broiled chicken	0.08–0.48
Grilled chicken	0.39–4.57
Fish	0.01–0.24
Smoked fish	0.1
Bread	0.1
Breakfast cereals	0.02–0.3
Vegetable oil	0.02
Eggs	0.03
Cheese	<0.005
Butter	<0.005
Milk	0.02
Fruit	0.01–0.17

Source: Kazerouni et al. (2001).

Kishikawa et al. (2003) measured benzo[a]pyrene levels in cow milk, infant formula, and human milk from Japan, with means of 0.03 ng/g (n = 14) in cow milk, 0.05 ng/g (n = 3) in infant formula, and 0.002 (n = 51) in human milk.

From the surveys conducted in six European Union countries, the mean or national-averaged dietary intake of benzo[a]pyrene for an adult person was estimated in the range of 0.05–0.29 µg/day (EC, 2002). Children may be subject to higher oral intake of benzo[a]pyrene. In a Spanish study in which benzo[a]pyrene was detected in foods, children ages 4–9 years old were found to have the highest estimated daily intake, as compared to adults and adolescents (Falco et al., 2003). In the United Kingdom, average intakes on a ng kg^{-1} day^{-1} basis were estimated for the following age groups: adults, 1.6; 15–18 years, 1.4; 11–14 years, 1.8; 7–10 years, 2.6; 4–6 years, 3.3; and toddlers, 3.1–3.8. The major contributors were the oils and fats group (50%), cereals (30%), and vegetables (8%) (EC, 2002). The contribution from grilled foods appeared less important in Europe than in the United States because grilled foods are consumed less often (EC, 2002). In the United States, the ingested dose of benzo[a]pyrene may be much higher than the amount inhaled. A study in New Jersey estimated a daily median total ingested dose of 176 ng based on a urinary biomarker study of 14 adult volunteers over 14 consecutive days, which exceeded the winter inhalation dose (11 ng/day) by 16-fold and the summer/fall inhalation dose (2.3 ng/day) by 122-fold (Buckley et al., 1995).

Dermal Exposure. The general population can be exposed dermally to benzo[a]pyrene when contacting soils or materials that contain benzo[a]pyrene, such as soot or tar. Exposure can also occur via the use of dermally applied pharmaceutical products that contain coal tars, including shampoos and formulations used to treat conditions such as eczema and psoriasis (IARC, 2010).

PAHs are commonly found in all types of soils. ATSDR (1995) reported benzo[a]pyrene levels in soil of 2–1,300 µg/kg in rural areas, 4.6–900 µg/kg in agricultural areas, 165–220 µg/kg in urban areas, and 14,000–159,000 µg/kg at contaminated sites (before remediation). The soil levels for all land uses appear highly variable. The levels are affected by proximity to roads/combustion sources, use of sewage-sludge-derived amendments on agricultural lands, particle size, and organic carbon content. Weinberg et al. (1989) reported that PAH levels in soils generally increased during the 1900s and that sediment studies suggest that some declines may have occurred since the 1970s. An illustration of benzo[a]pyrene levels in soil is presented in Table A-4.

Table A-4. Levels of benzo[a]pyrene in soil

Reference	Location	Land type	Concentration mean (µg/kg)
Butler et al. (1984)	United Kingdom	Urban	1,165
Vogt et al. (1987)	Norway	Industrial	321
	Norway	Rural	14
Yang et al. (1991)	Australia	Residential	363
	Poland	Agricultural	22
Trapido (1999)	Estonia	Urban	106
	Estonia	Urban	398
	Estonia	Urban	1,113
	Estonia	Urban	1,224
	Estonia	Rural	6.8
	Estonia	Rural	15
	Estonia	Rural	27
	Estonia	Rural	31
Nam et al. (2008)	United Kingdom	Rural	46
	Norway	Rural	5.3
Mielke et al. (2001)	New Orleans	Urban	276
Nadal et al. (2004)	Spain	Industrial-chemical	100
	Spain	Industrial-petrochemical	18
	Spain	Residential	56
	Spain	Rural	22
Maliszewska-Kordybach et al. (2009)	Poland	Agricultural	30
Wilcke (2000)	Various temperate	Arable	18
	Various temperate	Grassland	19
	Various temperate	Forest	39
	Various temperate	Urban	350
	Bangkok	Urban-tropical	5.5
	Brazil	Forest-tropical	0.3

APPENDIX B. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table B-1. Health assessments and regulatory limits by other national and international agencies

Organization	Toxicity value or determination
Oral value	
WHO (2003); WHO (1996)	The guideline value for benzo[a]pyrene in drinking water of 0.7 µg/L was based on a cancer slope factor of **0.46 (mg/kg-d)$^{-1}$** derived from Neal and Rigdon (1967) and a lifetime excess cancer risk of 10^{-5}.
Health Canada (2010); Health Canada (1998)	The Maximum Acceptable Concentration for benzo[a]pyrene in drinking water of 0.01 µg/L was derived from Neal and Rigdon (1967) using a drinking water consumption rate of 1.5 L/day, a body weight of 70 kg, and a lifetime cancer risk of 5×10^{-7}. (The concentrations of **2, 0.2, and 0.02 µg/L** benzo[a]pyrene correspond to lifetime excess cancer risks of 10^{-4}, 10^{-5}, and 10^{-6}.)
Inhalation value	
WHO (1997); WHO (2000)	Does not recommend specific guideline values for polycyclic aromatic hydrocarbons (PAHs) in air. A unit risk of **87 (mg/m^3)$^{-1}$** for benzo[a]pyrene, as an indicator a PAH mixtures, was derived from U.S. EPA's inhalation unit risk from coke oven emissions.
EU (2005)	Target value of **1 ng/m^3** benzo[a]pyrene (averaged over 1 calendar year) as a marker of PAH carcinogenic risk. Does not include information for how target value was derived.
Cancer characterization	
IARC (2010)	Carcinogenic to humans (Group 1) (based on mechanistic data).
NTP (2011)	Reasonably anticipated to be a human carcinogen. (First classified in 1981.)
Health Canada (1998)	Probably carcinogenic to man.

EPA = U.S. Environmental Protection Agency; EU = European Union; IARC = International Agency for Research on Cancer; NTP = National Toxicology Program; WHO = World Health Organization.

APPENDIX C. LITERATURE SEARCH STRATEGY

Table C-1. Summary of detailed search strategies for benzo[a]pyrene comprehensive literature searches (Pubmed, Toxline, Toxcenter, TSCATS)

Database search date	Query string
PubMed	
08/08/2016	((("Benzo(a)pyrene"[MeSH Terms]) AND (("Benzo(a)pyrene/adverse effects"[MeSH Terms] OR "Benzo(a)pyrene/antagonists and inhibitors"[MeSH Terms] OR "Benzo(a)pyrene/blood"[MeSH Terms] OR "Benzo(a)pyrene/pharmacokinetics"[MeSH Terms] OR "Benzo(a)pyrene/poisoning"[MeSH Terms] OR "Benzo(a)pyrene/toxicity"[MeSH Terms] OR "Benzo(a)pyrene/urine"[MeSH Terms]) OR ("chemically induced"[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR "endocrine disruptors"[MeSH Terms] OR "dose-response relationship, drug"[MeSH Terms] OR ((pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR animals[MeSH Terms])) OR risk[MeSH Terms] OR (cancer[sb] AND "Benzo(a)pyrene"[majr]) OR ("benzo a pyrene/metabolism"[MeSH Terms] AND (humans[MeSH Terms] OR animals[MeSH Terms])))))) AND 2011/12/01 : 3000[mhda]) OR ((("Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR (("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR pah[tw] OR pahs[tw] OR polycyclic aromatic hydrocarbon[tw] OR polycyclic aromatic hydrocarbons[tw]))) NOT medline[sb]) AND (2011/12/01 : 3000[crdat] OR 2011/12/01 : 3000[edat]))
02/14/2012	("Benzo(a)pyrene"[MeSH Terms] AND (("Benzo(a)pyrene/adverse effects"[MeSH Terms] OR "Benzo(a)pyrene/antagonists and inhibitors"[MeSH Terms] OR "Benzo(a)pyrene/blood"[MeSH Terms] OR "Benzo(a)pyrene/pharmacokinetics"[MeSH Terms] OR "Benzo(a)pyrene/poisoning"[MeSH Terms] OR "Benzo(a)pyrene/toxicity"[MeSH Terms] OR "Benzo(a)pyrene/urine"[MeSH Terms]) OR ("chemically induced"[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR "endocrine disruptors"[MeSH Terms] OR "dose-response relationship, drug"[MeSH Terms] OR ((pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR animals[MeSH Terms])) OR risk[MeSH Terms] OR (cancer[sb] AND "Benzo(a)pyrene"[majr]) OR ("benzo a pyrene/metabolism"[MeSH Terms] AND (humans[MeSH Terms] OR animals[MeSH Terms]))))) AND 2008/10/01 : 3000[mhda]) OR ((("Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR (("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR pah[tw] OR pahs[tw] OR polycyclic aromatic hydrocarbon[tw] OR polycyclic aromatic hydrocarbons[tw]))) NOT medline[sb]) AND 2008/10/01 : 3000[edat]) OR ((("Benzo(a)pyrene"[MeSH Terms] AND (("Benzo(a)pyrene/adverse effects"[MeSH Terms] OR "Benzo(a)pyrene/antagonists and inhibitors"[MeSH Terms] OR "Benzo(a)pyrene/blood"[MeSH Terms] OR "Benzo(a)pyrene/pharmacokinetics"[MeSH Terms] OR "Benzo(a)pyrene/poisoning"[MeSH Terms] OR "Benzo(a)pyrene/toxicity"[MeSH Terms] OR

Database search date	Query string
	"Benzo(a)pyrene/urine"[MeSH Terms]) OR ("chemically induced"[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR "endocrine disruptors"[MeSH Terms] OR "dose-response relationship, drug"[MeSH Terms] OR ((pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR animals[MeSH Terms])) OR risk[MeSH Terms] OR (cancer[sb] AND "Benzo(a)pyrene"[majr]) OR ("benzo a pyrene/metabolism"[MeSH Terms] AND (humans[MeSH Terms] OR animals[MeSH Terms])))))) OR (("Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR (("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR pah[tw] OR pahs[tw] OR polycyclic aromatic hydrocarbon[tw] OR polycyclic aromatic hydrocarbons[tw])))AND ("Benzopyrenes/adverse effects"[MeSH Terms] OR "Benzopyrenes/antagonists and inhibitors"[MeSH Terms] OR "Benzopyrenes/blood"[MeSH Terms] OR "Benzopyrenes/pharmacokinetics"[MeSH Terms] OR "Benzopyrenes/poisoning"[MeSH Terms] OR "Benzopyrenes/toxicity"[MeSH Terms] OR "Benzopyrenes/urine"[MeSH Terms] OR ("benzopyrenes"[MeSH Terms] AND ("chemically induced"[Subheading] OR "environmental exposure"[MeSH Terms])) OR "benzopyrenes/metabolism"[Mesh Terms]) AND 1966[PDAT] : 1984[PDAT])) AND (cancer[sb] OR "genes"[MeSH Terms] OR "genetic processes"[MeSH Terms] OR "mutagenicity tests"[MeSH Terms] OR "mutagenesis"[MeSH Terms] OR "mutagens"[MeSH Terms] OR "mutation"[MeSH Terms] OR "neurotoxicity syndromes"[MeSH Terms] OR "nervous system"[MeSH Terms] OR "nervous system diseases"[MeSH Terms] OR "immune system"[MeSH Terms] OR "immune system diseases"[MeSH Terms] OR "immunologic factors"[MeSH Terms] OR "reproductive physiological phenomena"[MeSH Terms] OR ("growth and development"[Subheading] OR "urogenital system"[MeSH Terms] OR "congenital, hereditary, and neonatal diseases and abnormalities"[MeSH Terms] OR "teratogens"[MeSH Terms]))
Toxline	
08/08/2016	"benzo a pyrene" OR "benzo d e f chrysene" OR "benzo def chrysene" OR "3 4 benzopyrene" OR "1 2 benzpyrene" OR "3 4 bp" OR "benz(a)pyrene" OR "3 4 benzpyren" OR "3 4 benzpyrene" OR "4 5 benzpyrene" OR "6 7 benzopyrene" OR benzopirene OR "benzo(alpha)pyrene" OR 50-32-8 [rn] AND 2011:2016 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR PUBDART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] OR PubMed [org]) AND NOT PubMed [org] AND NOT pubdart [org]
02/14/2012	(((50-32-8 [rn] OR "benzo a pyrene" OR "benzo d e f chrysene" OR "benzo def chrysene" OR "3 4 benzopyrene" OR "1 2 benzpyrene" OR "3 4 bp" OR "benz (a) pyrene" OR "3 4 benzpyren" OR "3 4 benzpyrene" OR "4 5 benzpyrene" OR "6 7 benzopyrene" OR benzopirene OR "benzo (alpha) pyrene") AND 2008:2012 [yr] NOT PubMed [org] NOT pubdart [org]) NOT crisp[org]) OR (((50-32-8 [rn] OR "benzo a pyrene" OR "benzo d e f chrysene" OR "benzo def chrysene" OR "3 4 benzopyrene" OR "1 2 benzpyrene" OR "3 4 bp" OR "benz (a) pyrene" OR "3 4 benzpyren" OR "3 4 benzpyrene" OR "4 5 benzpyrene" OR "6 7 benzopyrene" OR benzopirene OR "benzo (alpha) pyrene") NOT PubMed [org] NOT pubdart [org]) AND (brain OR brains OR cephalic OR cerebral OR cerebrum OR cognition OR cognitive OR corpus OR encephalopathies OR encephalopathy OR nerve OR nerves OR nervous OR neural OR neurologic OR neurological OR neurology OR neuronal OR neuropathies OR neuropathy OR neurotoxic OR neurotoxicities OR neurotoxicity OR neurotoxin OR neurotoxins OR spinal cord) OR (antibodies OR antibody OR antigen OR antigenic OR antigens OR autoimmune OR autoimmunities OR autoimmunity OR cytokine OR cytokines OR granulocyte OR granulocytes OR immune OR immunities OR immunity OR immunologic OR immunological OR immunology OR immunoproliferation OR immunosuppression OR immunosuppressive OR

Database search date	Query string
	inflammation OR inflammatory OR interferon OR interferons OR interleukin OR interleukins OR leukocyte OR leukocytes OR lymph OR lymphatic OR lymphocyte OR lymphocytes OR lymphocytosis OR lymphokines OR monocyte OR monocytes) OR (abnormal OR abnormalities OR abnormality OR abort OR aborted OR abortion OR aborts OR cleft OR clefts OR development OR developmental OR embryo OR embryologic OR embryology OR embryonic OR embryos OR fertile OR fertilities OR fertility OR fetal OR fetus OR fetuses OR foetal OR foetus OR foetuses OR gestation OR gestational OR infertile OR infertility OR malform OR malformation OR malformations OR malformed OR malforms OR neonatal OR neonatally OR neonate OR neonates OR newborn OR newborns OR ova OR ovaries OR ovary OR ovum OR perinatal OR perinatally OR placenta OR placental OR placentas OR postnatal OR postnatally OR pregnancies OR pregnancy OR pregnant OR prenatal OR prenatally OR reproduction OR reproductive OR sperm OR spermatid OR spermatids OR spermatocidal OR spermatocyte OR spermatocytes OR spermatogenesis OR spermatogonia OR spermatozoa OR sterile OR sterility OR teratogen OR teratogenesis OR teratogenic OR teratogenicities OR teratogenicity OR teratogens OR weaned OR weaning OR weanling OR weanlings OR zygote OR zygotes) OR (ames OR aneuploid OR aneuploidy OR chromosomal OR chromosome OR chromosomes OR clastogen OR clastogenesis OR clastogenic OR clastogenicities OR clastogenicity OR clastogens OR cytogenesis OR cytogenetic OR cytogenetics OR dna OR dominant lethal OR gene OR genes OR genetic OR genotoxic OR genotoxicities OR genotoxicity OR genotoxin OR genotoxins OR hyperploid OR hyperploidy OR micronuclei OR micronucleus OR mitotic OR mutagen OR mutagenesis OR mutagenicities OR mutagenicity OR mutagens OR mutate OR mutated OR mutating OR mutation OR mutations OR recessive lethal OR sister chromatid) OR (cancer OR cancerous OR cancers OR carcinogen OR carcinogenesis OR carcinogenic OR carcinogenicities OR carcinogenicity OR carcinogens OR carcinoma OR carcinomas OR cocarcinogen OR cocarcinogenesis OR cocarcinogenic OR cocarcinogens OR lymphoma OR lymphomas OR neoplasm OR neoplasms OR neoplastic OR oncogene OR oncogenes OR oncogenic OR precancerous OR tumor OR tumorigenesis OR tumorigenic OR tumorigenicities OR tumorigenicity OR tumors OR tumour OR tumourigenesis OR tumourigenic OR tumourigenicity OR tumours))
Toxcenter	
08/08/2016	L1 S 50-32-8 L2 S L1 NOT (PATENT/DT OR TSCATS/FS) L3 S L2 AND (PY>2011 OR ED>20111201) L15 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG? OR PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT,IT OR ACUTE OR SUBACUTE OR LD50# OR LC50# OR (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT) L16 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR? OR OCCUPATION? OR WORKPLACE? OR WORKER? OR ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER OR (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))) L17 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS? OR FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM OR OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL OR PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L18 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG? OR SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN OR DEVELOPMENT OR DEVELOPMENTAL? OR ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT OR WEAN? OR OFFSPRING OR AGE(W)FACTOR? OR DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)

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	L20 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS? OR TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM? OR GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) L21 QUE (NEPHROTOX? OR HEPATOTOX? OR ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) L22 QUE (RAT OR RATS OR MOUSE OR MICE OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? OR MARMOSET? OR PRIMATE? OR MAMMAL? OR FERRET? OR GERBIL?) L23 QUE (RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? OR OCCUPATION? OR WORKER? OR EPIDEM?) L24 QUE L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 L25 S L3 AND L24 L26 S L25 AND BIOSIS/FS L28 S L25 AND CAPLUS/FS L29 S L28 AND (RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? OR MARMOSET? OR PRIMATE?) L30 S L28 AND (MAMMAL? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? OR OCCUPATION? OR WORKER? OR EPIDEM? OR HUMAN?) L31 S L28 AND (HOMINIDAE OR MAMMAL? OR SUBJECT? OR PATIENT? OR GENOTOX? OR MUTAT? OR MUTAG?) L32 S L29 OR L30 OR L31 L33 S L26 OR L32 L34 DUP REM L33
02/14/2012	L48 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? L49 QUE MARMOSET? OR PRIMATE? OR MAMMAL? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? L50 QUE OCCUPATION? OR WORKER? OR EPIDEM? L51 QUE HUMAN? OR HOMINIDAE OR MAMMAL? L52 QUE SUBJECT? OR PATIENT? L53 QUE GENOTOX? OR MUTAT? OR MUTAG? L54 QUE L48 OR L49 OR L50 OR L51 OR L52 OR L53 L57 S 50-32-8 L58 S L57 NOT PATENT/DT L59 S L58 AND ED>20080930 L60 S L58 AND PY>2007 L61 S L59 OR L60 L62 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L63 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT,IT) L64 QUE (ACUTE OR SUBACUTE OR LD50# OR LC50#) L65 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L66 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L67 QUE (OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS? L68 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)

Database search date	Query string
	L69 QUE MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE) L70 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L71 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L72 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L73 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L74 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L75 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU?) L76 QUE (SPERMI? OR SPERMO?) L77 QUE (NEONAT? OR NEWBORN OR DEVELOPMENT OR DEVELOPMENTAL?) L78 QUE ENDOCRIN? AND DISRUPT? L79 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L80 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L81 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L82 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L83 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L84 QUE (GENETOX? OR GENOTOX? OR MUTAGEN?) L85 QUE GENETIC(W)TOXIC? L86 QUE L62 OR L63 OR L64 OR L65 OR L66 OR L67 OR L68 OR L69 OR L70 OR L71 OR L72 OR L73 OR L74 L87 QUE L75 OR L76 OR L77 OR L78 OR L79 OR L80 OR L81 OR L82 OR L83 OR L84 OR L85 L88 QUE L86 OR L87 L89 QUE NEPHROTOX? OR HEPATOTOX? OR ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON? L90 QUE L88 OR L89 L91 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? L92 QUE MARMOSET? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? L93 QUE OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM? L94 QUE L90 OR L91 OR L92 OR L93 L99 S L61 AND L94 L100 S L99 AND MEDLINE/FS L101 S L99 AND BIOSIS/FS L102 S L99 AND CAPLUS/FS L103 S L99 AND IPA/FS L104 DUP REM L100 L101 L102 L108 S (L104) AND BIOSIS/FS L112 S (L104) AND CAPLUS/FS L113 S L112 AND L54 L114 S L112 NOT L113 L115 S L108 OR L113 OR L114
02/14/2012	L1 S 50-32-8 L2 S L1 NOT PATENT/DT L3 S L2 NOT TSCATS/FS L4 S L3 AND ED>20080930 L5 S L3 AND PY>2007 L6 S L4 OR L5

Database search date	Query string
	L7 S L3 NOT L6 L8 S L7 AND (CANCER? OR CARCINOG? OR CARCINOM? OR COCARCINOG? OR LYMPHOMA? OR NEOPLAS? OR ONCOGEN? OR PRECANCER? OR TUMOR? OR TUMOUR?)/TI,CT,ST,IT L9 S L7 AND (AMES OR ANEUPLOID? OR CHROMOSOM? OR CLASTOGEN? OR CYTOGEN? OR DNA OR DOMINANT LETHAL OR GENETIC OR GENE? OR GENOTOX? OR HYPERPLOID? OR MICRONUCLE? OR MITOTIC OR MUTAGEN? OR MUTAT? OR RECESSIVE LETHAL OR SISTER CHROMATID)/TI,CT,ST,IT L10 S L7 AND (BRAIN OR CEREBRAL OR COGNITION OR COGNITIVE OR ENCEPHAL? OR NERVE? OR NERVOUS OR NEURAL OR NEUROLOG? OR NEURON? OR NEUROP? OR NEUROTOX? OR SPINAL CORD)/TI,CT,ST,IT L11 S L7 AND (ANTIBOD? OR ANTIGEN? OR AUTOIMMUN? OR CYTOKINE? OR GRANULOCYTE? OR IMMUN? OR INFLAMM? OR INTERFERON? OR INTERLEUKIN? OR LEUKOCYTE? OR LYMPH? OR LYMPHOCYT? OR MONOCYT?)/TI,CT,ST,IT L12 S L7 AND (ABNORMAL? OR ABORT? OR CLEFT? OR DEVELOPMENT OR DEVELOPMENTAL OR EMBRYO? OR ENDOCRINE OR FERTIL? OR FETAL? OR FETUS? OR FOETAL? OR FOETUS? OR GESTATION? OR INFERTIL? OR MALFORM? OR NEONAT? OR NEWBORN? OR OVA OR OVARIES OR OVARY OR OVUM)/TI,CT,ST,IT L13 S L7 AND (PERINATAL? OR PLACENTA? OR POSTNATAL? OR PREGNAN? OR PRENATAL? OR REPRODUC? OR SPERM? OR STERIL? OR TERATOGEN? OR WEAN? OR ZYGOTE?)/TI,CT,ST,IT L14 S L8 OR L9 OR L10 OR L11 OR L12 OR L13 L15 S L14 AND MEDLINE/FS L16 S L14 AND BIOSIS/FS L17 S L14 AND CAPLUS/FS L18 S L14 AND IPA/FS L19 DUP REM L15 L16 L18 L17 L29 S L19 NOT MEDLINE/FS L30 S L29 AND (ED>=20000801 OR PY>2000) L31 S L29 AND PY>1999 L32 S 50-32-8 L33 S L32 NOT PATENT/DT L34 S L33 NOT TSCATS/FS L35 S L34 AND ED>20080930 L36 S L34 AND PY>2007 L37 S L35 OR L36 L38 S L34 NOT L37 L39 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L40 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT,IT) L41 QUE (ACUTE OR SUBACUTE OR LD50# OR LC50#) L42 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L43 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L44 QUE (OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS? L45 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L46 QUE MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE) L47 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L48 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L49 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L50 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)

Database search date	Query string
	L51 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L52 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU?) L53 QUE (SPERMI? OR SPERMO?) L54 QUE (NEONAT? OR NEWBORN OR DEVELOPMENT OR DEVELOPMENTAL?) L55 QUE ENDOCRIN? AND DISRUPT? L56 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L57 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L58 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L59 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L60 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L61 QUE (GENETOX? OR GENOTOX? OR MUTAGEN?) L62 QUE GENETIC(W)TOXIC? L63 QUE L39 OR L40 OR L41 OR L42 OR L43 OR L44 OR L45 OR L46 OR L47 OR L48 OR L49 OR L50 OR L51 L64 QUE L52 OR L53 OR L54 OR L55 OR L56 OR L57 OR L58 OR L59 OR L60 OR L61 OR L62 L65 QUE L63 OR L64 L66 QUE NEPHROTOX? OR HEPATOTOX? OR ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON? L67 QUE L65 OR L66 L68 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? L69 QUE MARMOSET? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? L70 QUE OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM? L71 QUE L67 OR L68 OR L69 OR L70 L72 S L38 AND L71 L73 S 50-32-8 L74 S L73 NOT PATENT/DT L75 S L74 NOT TSCATS/FS L76 S L75 AND ED>20080930 L77 S L75 AND PY>2007 L78 S L76 OR L77 L79 S L75 NOT L78 L80 S L79 AND (CANCER? OR CARCINOG? OR CARCINOM? OR COCARCINOG? OR LYMPHOMA? OR NEOPLAS? OR ONCOGEN? OR PRECANCER? OR TUMOR? OR TUMOUR?) L81 S L79 AND (AMES ASSAY OR AMES TEST OR ANEUPLOID? OR CHROMOSOM? OR CLASTOGEN? OR CYTOGEN? OR DNA OR DOMINANT LETHAL OR GENETIC OR GENE? OR GENOTOX? OR HYPERPLOID? OR MICRONUCLE? OR MITOTIC OR MUTAGEN? OR MUTAT? OR RECESSIVE LETHAL OR SISTER CHROMATID) L82 S L79 AND (BRAIN OR CEREBRAL OR COGNITION OR COGNITIVE OR ENCEPHAL? OR NERVE? OR NERVOUS OR NEURAL OR NEUROLOG? OR NEURON? OR NEUROP? OR NEUROTOX? OR SPINAL CORD) L83 S L79 AND (ANTIBOD? OR ANTIGEN? OR AUTOIMMUN? OR CYTOKINE? OR GRANULOCYTE? OR IMMUN? OR INFLAMM? OR INTERFERON? OR INTERLEUKIN? OR LEUKOCYTE? OR LYMPH? OR LYMPHOCYT? OR MONOCYT?) L84 S L79 AND (ABNORMAL? OR ABORT? OR CLEFT? OR DEVELOPMENT OR DEVELOPMENTAL OR EMBRYO? OR ENDOCRINE OR FERTIL? OR FETAL? OR FETUS? OR FOETAL? OR FOETUS? OR

Database search date	Query string
	GESTATION? OR INFERTIL? OR MALFORM? OR NEONAT? OR NEWBORN? OR OVA OR OVARIES OR OVARY OR OVUM) L85 S L79 AND (PERINATAL? OR PLACENTA? OR POSTNATAL? OR PREGNAN? OR PRENATAL? OR REPRODUC? OR SPERM? OR STERIL? OR TERATOGEN? OR WEAN? OR ZYGOTE?) L86 S L80 OR L81 OR L82 OR L83 OR L84 OR L85 L87 S L72 AND L86 L88 S L87 AND PY>1999 L89 S L88 AND MEDLINE/FS L90 S L88 AND BIOSIS/FS L91 S L88 AND CAPLUS/FS L92 S L88 AND IPA/FS L93 DUP REM L89 L90 L92 L91 L98 S (L93) AND BIOSIS/FS L106 S (L93) AND IPA/FS L111 S (L93) AND CAPLUS/FS L112 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE? L113 QUE MARMOSET? OR PRIMATE? OR MAMMAL? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON? L114 QUE OCCUPATION? OR WORKER? OR EPIDEM? L115 QUE HUMAN? OR HOMINIDAE OR MAMMAL? L116 QUE SUBJECT? OR PATIENT? L117 QUE GENOTOX? OR MUTAT? OR MUTAG? L118 QUE L112 OR L113 OR L114 OR L115 OR L116 OR L117 L119 S L111 AND L118 L132 S (L93) AND MEDLINE/FS L133 S L132 OR L98 OR L119 OR L106 OR L31
TSCATS 1	
02/14/2012	50-32-8 Limit: Health Effects
TSCATS 2	
08/08/2016	50-32-8
02/14/2012	50-32-8 Date limited, 2000 to date of search
TSCA 8e/FYI recent submissions	
02/14/2012	Google: 91-20-3 (8e or fyi) tsca
TSCA 8E & FYI via CDAT[a]	
08/08/2016	50-32-8

Database search date	Query string
Secondary Refinement	
02/14/2012	Additional terms applied within Endnote to pre-2008 search results only: forestomach* OR tongue* OR (auditory AND canal*) OR (ear* AND canal*) OR esophagus* OR esophageal* OR larynx* OR laryngeal* OR pharynx* OR pharyngeal* OR ((lung* OR pulmonary OR skin*) AND (neoplasm* OR tumor* OR tumour* OR papilloma* OR carcinoma*)) OR leukemia* OR leukaemia* OR sperm* OR testic* OR fertilit*OR infertilit* OR testosterone OR ((testis OR testes) AND (weight* OR mass*)) OR epididymis* OR epididymal* OR seminiferous OR ((cervical* OR cervix*) AND hyperplasia*) OR ovary OR ovaries OR ovarian OR primordial OR corpora lutea OR corpus luteum OR estrous* OR estrus* OR thymus* OR spleen* OR spleno* OR immunoglobulin* OR immunoglobin* OR ((immune OR immun*) AND (suppress* OR immunosuppress*)) OR (functional AND observational AND battery) OR neurobehavioral*OR neurobehavioural* OR rotarod* OR nerve* AND conduction* OR locomotor* OR neuromuscular* OR weight* OR neurodevelopment* OR ((neuro* OR brain*) AND (development* OR developing)) OR intelligence* OR cognition* OR cognitive* OR learn* OR memory OR righting*

[a]CDAT (Chemical Data Access Tool; http://java.epa.gov/oppt_chemical_search/).

Table C-2. Summary of detailed literature search strategies for benzo(a)pyrene cardiovascular toxicity

Database search date	Query string
PubMed	
3/31/2016	(((((("benzo(a)pyrene"[mh] OR ("benzo(a)pyrene"[tw] OR "Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR (("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR "pah"[tw] OR "pahs"[tw] OR "polycyclic aromatic hydrocarbon"[tw] OR "polycyclic aromatic hydrocarbons"[tw]))))) AND ("macrophages"[mh] OR "Cholesterol"[mh] OR "Ischemia"[mh] OR "Granulocytes"[mh] OR "Myocytes, Smooth Muscle"[mh] OR "Blood supply"[mh] OR "Monocytes"[mh] OR "Lipoprotein"[mh] OR "Triglycerides"[mh] OR "Blood Vessels"[mh] OR "Aorta, Thoracic"[mh] OR "Aorta"[mh] OR "Aortic Diseases"[mh] OR "Atherosclerosis"[mh] OR "Cardiomegaly"[mh] OR "Cardiotoxicity"[mh] OR "Cardiovascular Diseases"[mh] OR "Caveolae"[mh] OR "Endothelial Cells"[mh] OR "Endothelium, Vascular"[mh] OR "Heart Defects, Congenital"[mh] OR "Heart"[mh] OR "Systole"[mh] OR "diastole"[mh] OR "Vascular Endothelial Growth Factor A"[mh] OR "vasoconstriction"[mh] OR "benzo a pyrene/blood"[MeSH Terms] OR "Angiogenesis"[tw] OR "Plaque"[tw] OR "plaques"[tw] OR "Myocardial"[tw] OR "Myocardia"[tw] OR "Myocardiocyte"[tw] OR "Proatherogenic"[tw] OR "Systolic"[tw] OR "diastolic"[tw] OR "Ventricle"[tw] OR "ventricular"[tw])))))) OR ((((("benzo(a)pyrene"[tw] OR "Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR (("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR "pah"[tw] OR "pahs"[tw] OR "polycyclic aromatic hydrocarbon"[tw] OR "polycyclic aromatic hydrocarbons"[tw])))) AND ("artery"[tw] OR "arteries"[tw] OR "arterial"[tw] OR "atherogenesis"[tw] OR "angiogenesis"[tw] OR "plaque"[tw] OR "plaques"[tw] OR "thrombus"[tw]

	OR "thrombosis"[tw] OR "myocardial"[tw] OR "myocardia"[tw] OR "myocardiocyte"[tw] OR "cholesterol"[tw] OR "ischemia"[tw] OR "cardiomyopathy"[tw] OR "lymphocyte"[tw] OR "lymphocytes"[tw] OR "macrophage"[tw] OR "macrophages"[tw] OR "granulocyte"[tw] OR "granulocytes"[tw] OR "smooth muscle cells"[tw] OR "proatherogenic"[tw] OR "hypertension"[tw] OR "neutrophils"[tw] OR "systolic"[tw] OR "systole"[tw] OR "diastolic"[tw] OR "diastole"[tw] OR "ventricle"[tw] OR "ventricles"[tw] OR "ventricular"[tw] OR "vasculature"[tw] OR "monocyte"[tw] OR "monocytes"[tw] OR "lipoprotein"[tw] OR "triglyceride"[tw] OR "triglycerides"[tw])) NOT medline[sb]))

APPENDIX D. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ANALYSIS

D.1. TOXICOKINETICS

D.1.1. Overview

Benzo[a]pyrene is absorbed following exposure by oral, inhalation, and dermal routes. The rate and extent of absorption are dependent upon the exposure medium. The presence of benzo[a]pyrene in body fat, blood, liver, and kidney and the presence of benzo[a]pyrene metabolites in serum and excreta demonstrate wide systemic tissue distribution. Benzo[a]pyrene metabolism occurs in essentially all tissues, with high metabolic capacity in the liver and significant metabolism in tissues at the portal of entry (lung, skin, and gastrointestinal [GI] tract) and in reproductive tissues. Stable metabolic products identified in body tissues and excreta are very diverse and include phenols, quinones, and dihydrodiols. These classes of metabolites are typically isolated as glucuronide or sulfate ester conjugates in the excreta, but can also include glutathione conjugates formed from quinones or intermediary epoxides. The primary route of metabolite elimination is in the feces via biliary excretion, particularly following exposure by the inhalation route. To a lesser degree, benzo[a]pyrene metabolites are eliminated via urine. Overall, benzo[a]pyrene is eliminated quickly with a biological half-life of several hours.

D.1.2. Absorption

The absorption of benzo[a]pyrene has been studied in humans and laboratory animals for inhalation, ingestion, and dermal exposure. In the environment, human exposure to benzo[a]pyrene predominantly occurs via contact with insoluble carbonaceous particles (e.g., soot, diesel particles) to which organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), are adsorbed.

Studies of workers occupationally exposed to benzo[a]pyrene have qualitatively demonstrated absorption via inhalation by correlating concentrations of benzo[a]pyrene in the air and benzo[a]pyrene metabolites in the exposed workers' urine. Occupational exposures to benzo[a]pyrene measured with personal air samplers were correlated to urine concentrations of benzo[a]pyrene-9,10-dihydrodiol, a specific metabolite of benzo[a]pyrene, in 24-hour aggregate urine samples by Grimmer et al. (1994). The amount of benzo[a]pyrene extracted from personal air monitoring devices (a surrogate for ambient PAHs) of coke oven workers was correlated with r-7,t-8,9,c-10 tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (trans-anti-benzo[a]pyrene-tetrol, a

specific metabolite of benzo[a]pyrene) in the workers' urine by Wu et al. (2002). In both of these studies, only a very small fraction (<1%) of the inhaled benzo[a]pyrene was recovered from urine, consistent with studies in animals that found that urine is not a major route of elimination for benzo[a]pyrene (as described in the excretion section below). These occupational studies cannot be used to quantify absorption through inhalation-only exposure in humans because the persistence of benzo[a]pyrene-contaminated particulate matter on surfaces and food may lead to exposures via additional routes (Boström et al., 2002). Nevertheless, the observation of benzo[a]pyrene metabolites in excreta of exposed humans provides qualitative evidence for benzo[a]pyrene absorption, at least some of which is likely to occur via inhalation. This conclusion is supported by studies in experimental animals, which indicate that benzo[a]pyrene is readily absorbed from carbonaceous particles following inhalation exposure (Gerde et al., 2001; Hood et al., 2000).

Results from studies of animals following intratracheal instillation of benzo[a]pyrene provide supporting, quantitative evidence that absorption by the respiratory tract is rapid (Gerde et al., 1993; Bevan and Ulman, 1991; Weyand and Bevan, 1987, 1986). Following intratracheal instillation of 1 µg tritiated benzo[a]pyrene/kg dissolved in triethylene glycol to Sprague-Dawley rats, radioactivity rapidly appeared in the liver (reaching a maximum of about 21% of the administered dose within 10 minutes). Elimination of radioactivity from the lung was biphasic, with elimination half-times of 5 and 116 minutes (Weyand and Bevan, 1986). In bile-cannulated rats, bile collected for 6 hours after instillation accounted for 74% of the administered radioactivity (Weyand and Bevan, 1986). The results are consistent with rapid and extensive absorption by the respiratory tract and rapid entry into hepatobiliary circulation following intratracheal instillation. The respiratory tract absorption may also be affected by the vehicle, since higher amounts of benzo[a]pyrene were excreted in bile when administered with hydrophilic triethylene glycol than with lipophilic solvents ethyl laurate or tricaprylin (Bevan and Ulman, 1991). Particle-bound benzo[a]pyrene deposited in the respiratory tract is absorbed and cleared more slowly than the neat compound (Gerde et al., 2001).

Studies conducted to assess levels of benzo[a]pyrene metabolites or benzo[a]pyrene-deoxyribonucleic acid (DNA) adduct levels in humans exposed to benzo[a]pyrene by the oral route are not adequate to develop quantitative estimates of oral bioavailability. The concentration of benzo[a]pyrene was below detection limits (<0.1 µg/person) in the feces of eight volunteers who had ingested broiled meat containing approximately 8.6 µg of benzo[a]pyrene (Hecht et al., 1979). However, studies in laboratory animals demonstrate that benzo[a]pyrene is absorbed via ingestion. Studies of rats and pigs measured the oral bioavailability of benzo[a]pyrene in the range of 10–40% (Cavret et al., 2003; Ramesh et al., 2001b; Foth et al., 1988; Hecht et al., 1979). The absorption of benzo[a]pyrene may depend on the vehicle. Intestinal absorption of benzo[a]pyrene was enhanced in rats when the compound was solubilized in lipophilic compounds such as triolein, soybean oil, and high-fat diets, as compared with fiber- or protein-rich diets (O'Neill et al., 1991; Kawamura et

al., 1988). Aqueous vehicles, quercetin, chlorogenic acid, or carbon particles reduced biliary excretion of benzo[a]pyrene, while lipid media such as corn oil increased it (Stavric and Klassen, 1994). The addition of wheat bran to the benzo[a]pyrene-containing diets increased fecal excretion of benzo[a]pyrene (Mirvish et al., 1981).

Studies of benzo[a]pyrene metabolites or DNA adducts measured in humans exposed dermally to benzo[a]pyrene-containing PAH mixtures demonstrate that benzo[a]pyrene is absorbed dermally. One study of dermal absorption in volunteers found absorption rate constants ranging from 0.036 to 0.135/hour over a 45-minute exposure, suggesting that 20–56% of the dose would be absorbed within 6 hours (VanRooij et al., 1993). Dermal absorption rates varied 69% between different anatomical sites (forehead, shoulder, volar forearm, palmar side of the hand, groin, and ankle) and only 7% between different individual volunteers (VanRooij et al., 1993). Metabolism is also an important determinant of permeation, with very low rates observed in nonviable skin (Kao et al., 1985). The overall absorbed amount of benzo[a]pyrene in explanted viable skin samples from tissue donors (maintained in short-term organ cultures) exposed for 24 hours ranged from 0.09 to 2.6% of the dose (Wester et al., 1990; Kao et al., 1985). Similar amounts of penetration were measured in skin samples from other species including marmosets, rats, and rabbits (Kao et al., 1985). Skin from mice allowed more of the dose to penetrate (>10%), while that of guinea pig let only a negligible percentage of the dose penetrate (Kao et al., 1985).

The vehicle for benzo[a]pyrene exposure is an important factor in skin penetration. Exposure of female Sprague-Dawley rats and female rhesus monkeys topically to benzo[a]pyrene in crude oil or acetone caused approximately 4-fold more extensive absorption than benzo[a]pyrene in soil (Wester et al., 1990; Yang et al., 1989). The viscosity of oil product used as a vehicle also changed skin penetration with increased uptake of benzo[a]pyrene for oils with decreased viscosity (Potter et al., 1999). Soil properties also greatly impact dermal absorption. Reduced absorption of benzo[a]pyrene occurs with increasing organic carbon content of the soil and increased soil aging (i.e., contact time between soil and chemical) (Turkall et al., 2008; Roy and Singh, 2001; Yang et al., 1989).

D.1.3. Distribution

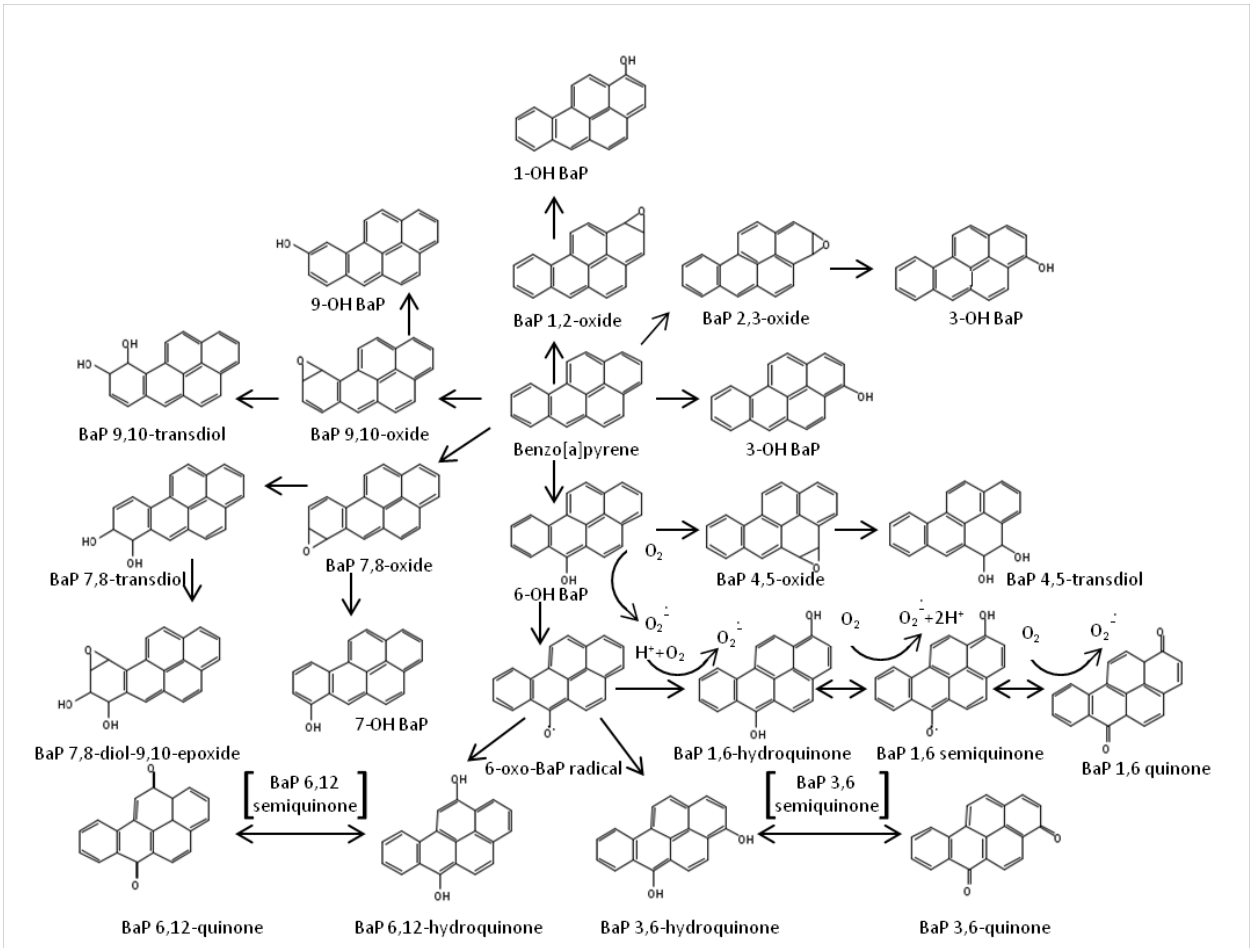
No adequate quantitative studies of benzo[a]pyrene tissue distribution in exposed humans were identified. Obana et al. (1981) observed low levels of benzo[a]pyrene in liver and fat tissues from autopsy samples. However, prior exposure histories were not available for the donors. Nevertheless, the identification of benzo[a]pyrene metabolites or DNA adducts in tissues and excreta of PAH-exposed populations suggest that benzo[a]pyrene is widely distributed.

Distribution of benzo[a]pyrene has been studied in laboratory animals for multiple routes of exposure, including inhalation, ingestion, dermal, and intravenous (i.v.). Exposure to benzo[a]pyrene in various species (Sprague-Dawley rats, Gunn rats, guinea pigs, and hamsters) results in wide distribution throughout the body and rapid uptake into well-perfused tissues (i.e., lung, kidney, and liver) (Weyand and Bevan, 1987, 1986). Benzo[a]pyrene and its metabolites are

distributed systemically after administration via many routes of administration including inhalation (or intratracheal instillation), oral, i.v., and dermal exposures (Saunders et al., 2002; Moir et al., 1998; Neubert and Tapken, 1988; Weyand and Bevan, 1987, 1986; Morse and Carlson, 1985). Intratracheal instillation of radiolabeled benzo[a]pyrene in mice resulted in increased radioactivity in lung-associated lymph nodes, suggesting distribution of benzo[a]pyrene or its metabolites via the lymph (Schnizlein et al., 1987). Rats with biliary cannulas had high excretion of benzo[a]pyrene and benzo[a]pyrene metabolites in bile. The benzo[a]pyrene thioether and glucuronic acid-conjugated metabolites in intestines indicated enterohepatic recirculation of benzo[a]pyrene and benzo[a]pyrene metabolites (Weyand and Bevan, 1986). The vehicle for delivery of inhalated benzo[a]pyrene impacts the distribution, with aerosolized benzo[a]pyrene more readily absorbed directly in the respiratory tract than particle-adsorbed benzo[a]pyrene (which is cleared by the mucociliary and then ingested) (Sun et al., 1982). The reactive metabolites of benzo[a]pyrene are also transported in the blood and may be distributed to tissues incapable of benzo[a]pyrene metabolism. Serum of benzo[a]pyrene-treated mice incubated with splenocytes or salmon sperm DNA resulted in adduct formation, suggesting that reactive benzo[a]pyrene metabolites were systemically distributed and available for interaction with target tissues (Ginsberg and Atherholt, 1989). Exposure of pregnant rats and mice to benzo[a]pyrene via inhalation and ingestion showed a wide tissue distribution of benzo[a]pyrene, consistent with other studies, and demonstrated placental transfer of benzo[a]pyrene and its metabolites (Withey et al., 1993; Neubert and Tapken, 1988; Shendrikova and Aleksandrov, 1974). Data from lactating rats indicate that following injection, distribution of [^{14}C]-labeled benzo[a]pyrene in maternal blood is similar to levels in milk (Lavoie et al., 1987b).

D.1.4. Metabolism

The metabolic pathways of benzo[a]pyrene (Figure D-1) and variation in species, strain, organ system, age, and sex have been studied extensively with in vitro and in vivo experiments. In addition, there have been numerous studies of exposed humans or animals with subsequent detection of benzo[a]pyrene metabolites in tissues or excreta. For example, elevated frequency of a detected urinary metabolite (7,8,9,10-tetrol) was observed in patients treated with coal tar medication (Bowman et al., 1997), demonstrating extensive metabolism of benzo[a]pyrene in humans.



Source: Miller and Ramos (2001).

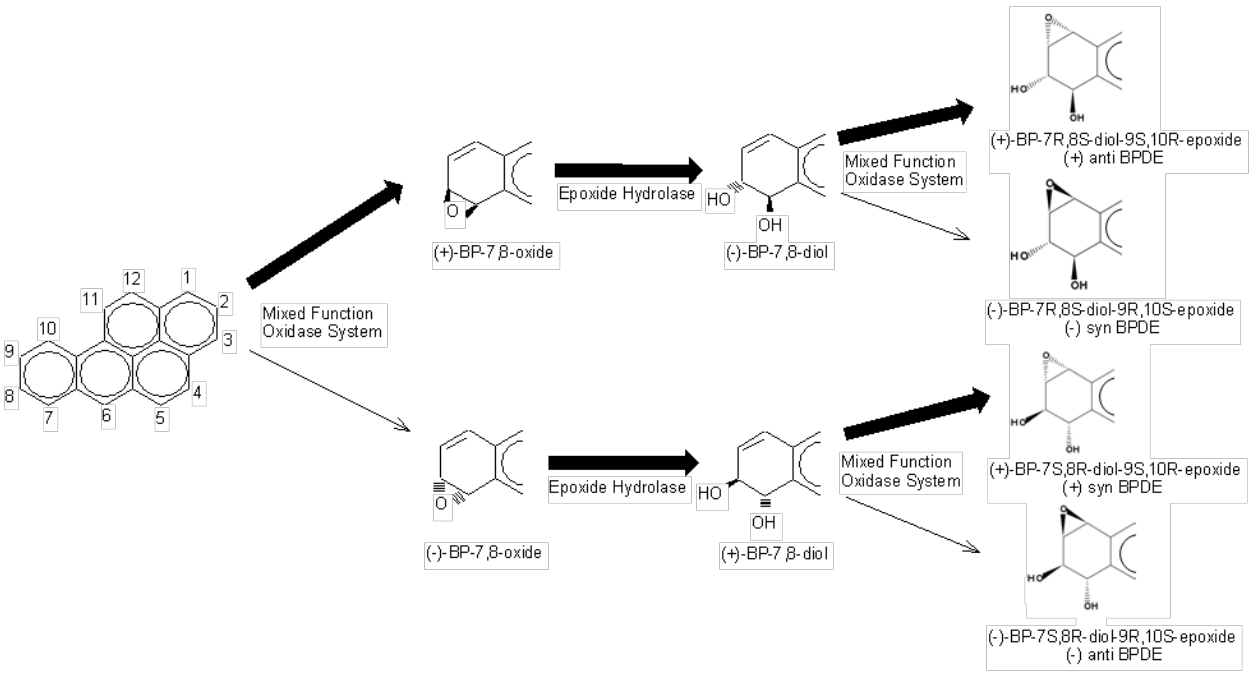
Figure D-1. Metabolic pathways for benzo[a]pyrene.

Phase I metabolism results in a number of reactive metabolites such as epoxides, dihydrodiols, phenols, quinones, and their various combinations that are likely to contribute to the toxic effects of benzo[a]pyrene (e.g., phenols, dihydrodiols, epoxides, and quinones). Phase II metabolism of benzo[a]pyrene metabolites protects the cells and tissues from the toxic effects of benzo[a]pyrene phenols, dihydrodiols and epoxides by converting them to water soluble products that are eliminated. In addition, Phase II metabolism of some benzo[a]pyrene dihydrodiols prevents them from further bioactivation to reactive forms that bind to cellular macromolecules. These metabolic process include glutathione conjugation of diol epoxides, sulfation and glucuronidation of phenols, and reduction of quinones by NADPH:quinone oxidoreductase (NQO). Numerous reviews on the metabolism of benzo[a]pyrene are available (Miller and Ramos, 2001; IPCS, 1998; ATSDR, 1995; Conney et al., 1994; Grover, 1986; Levin et al., 1982; Gelboin, 1980). Key concepts have been adapted largely from these reviews and supplemented with recent findings.

Phase I Metabolism

Phase I reactions of benzo[a]pyrene are catalyzed primarily by cytochrome P450 (CYP450) and produce metabolites including epoxides, dihydrodiols, phenols, and quinones (Figure D-2). The first step of Phase I metabolism is the oxidation of benzo[a]pyrene that forms a series of epoxides, the four major forms of which are the 2,3-, 4,5-, 7,8-, and 9,10-isomers (Gelboin, 1980). Once formed, these epoxides may undergo three different routes of metabolism: (1) spontaneous rearrangement to phenols; (2) hydration to trans-dihydrodiols catalyzed by microsomal epoxide hydrolase (EH); or (3) Phase II detoxification of binding with glutathione (either spontaneously or catalyzed by cytosolic glutathione-S-transferases (GSTs) (IARC, 1983)). The metabolism of benzo[a]pyrene to phenols results in five phenol isomers (1-, 3-, 6-, 7, and 9-OH benzo[a]pyrene) (Pelkonen and Nebert, 1982). Four benzo[a]pyrene epoxides (2,3-, 4,5-, 7,8-, and 9,10-) are hydrated to trans-dihydrodiols. Benzo[a]pyrene-7,8-diol (formed from benzo[a]pyrene-7,8-oxide) has been the focus of much of the study of benzo[a]pyrene metabolism. Benzo[a]pyrene-7,8-diol is the metabolic precursor to the potent DNA-binding metabolite, benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE). BPDE is formed from trans-benzo[a]pyrene 7,8-diol by multiple mechanisms including catalysis by cytochromes (CYPs) (Grover, 1986; Deutsch et al., 1979), myeloperoxidase (Mallet et al., 1991), or prostaglandin h synthase (also known as cyclooxygenase) (Marnett, 1990), and lipid peroxidation (Byczkowski and Kulkarni, 1990). The diolepoxides can react further by spontaneously hydrolyzing to tetrols (Hall and Grover, 1988).

The metabolism of benzo[a]pyrene proceeds with a high degree of stereoselectivity. Liver microsomes from rats stereospecifically oxidize the 7,8-bond of benzo[a]pyrene to yield almost exclusively the (+)-benzo[a]pyrene-(7,8)-oxide (see Figure D-2). Each enantiomer of benzo[a]pyrene-7,8-oxide is stereospecifically converted by EH to a different stereoisomeric trans dihydrodiol. The (+)-benzo[a]pyrene-7,8-oxide is preferentially hydrated to the (−)-trans-benzo[a]pyrene-7,8-dihydrodiol enantiomer by rat CYP enzymes and the (−)-trans-benzo[a]pyrene-7,8-dihydrodiol is preferentially oxidized by CYP enzymes to (+)-benzo[a]pyrene-7R,8S-diol-9S,10R-epoxide [(+)-anti- BPDE], which is the most potent carcinogen among the four stereoisomers (Figure D-2). Formation of these stereoisomers does not occur at equimolar ratios, and the ratios differ between biological systems. For example, a study in rabbit livers demonstrated that purified microsomes oxidized the (-)-benzo[a]pyrene-7,8-dihydrodiol to isomeric diol epoxides in a ratio ranging from 1.8:1 to 11:1 in favor of the (+)-anti-BPDE isomer (Deutsch et al., 1979).



Source: Grover (1986).

Figure D-2. The stereospecific activation of benzo[a]pyrene.

Several studies have attempted to determine which CYP isozyme is predominantly responsible for the metabolism of benzo[a]pyrene. Dermal administration of tritiated benzo[a]pyrene to mice that have an aryl hydrocarbon (Ah) receptor (AhR) knock-out (AhR−/−) had significantly decreased formation of (+)-anti-BPDE-DNA adducts compared to wild type (WT) and 1B1−/− mice (Kleiner et al., 2004). Gavage administration of benzo[a]pyrene in AhR knock-out mice found that the AhR−/− mice (with lower levels of CYP1A1) had higher levels of protein adducts and unmetabolized benzo[a]pyrene than the AhR+/+ or +/− mice (Sagredo et al., 2006). Similarly, CYP1A1 (−/−) knock-out mice administered benzo[a]pyrene in feed for 18 days had higher steady-state blood levels of benzo[a]pyrene and benzo[a]pyrene-DNA adducts (Uno et al., 2006). These findings establish important roles in benzo[a]pyrene metabolism for CYP1A1, but the relationship is not clear between the CYP enzymes and biological activation or detoxification.

Another important factor in evaluating variability in the metabolic activation of benzo[a]pyrene by CYP450s is the effect of functional polymorphisms, which has been the subject of numerous reviews (e.g., Wormhoudt et al., 1999). Recombinant CYP1A1 allelic variants produced BPDE with generally lower catalytic activity and Km values than the WT allele (Schwarz et al., 2001). However, the formation of diol epoxides is stereospecific, with the allelic variants producing about 3 times the amount of (±)-anti-BPDE isomers as compared to the stereoisomer, (±)-syn-BPDE (Schwarz et al., 2001). In a study of occupational exposures to benzo[a]pyrene, no

relationship was observed between benzo[a]pyrene metabolite formation and the CYP1A1 MspI polymorphism (Wu et al., 2002).

Another pathway of benzo[a]pyrene metabolism is the conversion of benzo[a]pyrene to 6-OH benzo[a]pyrene, which can be further oxidized into quinones, primarily the 1,6-, 3,6-, and 6,12- isomers. Trans-benzo[a]pyrene-7,8-dihydrodiol can be converted by aldo-keto reductases (AKR) to 7,8-dihydroxybenzo[a]pyrene (benzo[a]pyrene-7,8-catechol), which auto-oxidizes to benzo[a]pyrene-7,8-quinone (BPQ). BPQ can undergo redox cycling in the presence of cellular reducing equivalents. This reaction pathway produces reactive oxygen species (ROS), including peroxide anion radicals, benzo[a]pyrene semiquinone radicals, hydroxyl radicals, and H_2O_2, which in turn can causes extensive DNA fragmentation (Penning et al., 1999; Flowers et al., 1997; Flowers et al., 1996). 6-Hydroxybenzo[a]pyrene can be oxidized into 6-oxo-benzo[a]pyrene semi-quinone radical and further metabolized into 1,6-, 3,6-, or 6,12-quinones spontaneously, or catalytically by prostaglandin endoperoxide synthetase (Eling et al., 1986). The CYP and AKR enzymes both can metabolize trans-benzo[a]pyrene-7,8-dihydrodiol to different metabolites, BPDE and BPQ. Reconstituted in vitro systems of human lung cells show that CYP enzymes have faster steady-state reaction rate constants than AKR and basal expression of AKR is higher than CYP in lung cells, suggesting that AKR and CYP enzymes compete for metabolism of trans-benzo[a]pyrene-7,8-dihydrodiol (Quinn and Penning, 2008).

Phase II Metabolism

The reactive products of Phase I metabolism are subject to the action of several Phase II conjugation and detoxification enzyme systems that display preferential activity for specific oxidation products of benzo[a]pyrene. These Phase II reactions play a critical role in protecting cellular macromolecules from binding with reactive benzo[a]pyrene diolepoxides, radical cations, or ROS. Therefore, the balance between Phase I activation of benzo[a]pyrene and its metabolites and detoxification by Phase II processes is an important determinant of toxicity.

The diol epoxides formed from benzo[a]pyrene metabolism by Phase I reactions are not usually found as urinary metabolites. Rather, they are detected as adducts of nucleic acids or proteins or further metabolized by glutathione (GSH) conjugation, glucuronidation, and sulfation. These metabolites make up a significant portion of total metabolites in excreta or tissues. For example, the identified metabolites in bile 6 hours after a 2 µg/kg benzo[a]pyrene dose by intratracheal instillation to male Sprague-Dawley rats were 49% glucuronides (quinol diglucuronides or monglucuronides), 30.4% thioether conjugates, 6.2% sulfate conjugates, and 14.4% unconjugated metabolites (Bevan and Sadler, 1992).

Conjugation of benzo[a]pyrene with GSH is catalyzed by GSTs. Numerous studies using human GSTs expressed in mammalian cell lines have demonstrated the ability of GST to metabolize benzo[a]pyrene diol epoxides. Isolated human GSTs have significant catalytic activity toward benzo[a]pyrene-derived diol epoxides and (±)anti-BPDE with variation in activity across GST isoforms (Dreij et al., 2002; Rojas et al., 1998; Robertson et al., 1986). Benzo[a]pyrene quinones

can also be conjugated with GSH (Agarwal et al., 1991; IARC, 1983). This compelling evidence for a role of GSTs in the metabolism of reactive benzo[a]pyrene metabolites has triggered several molecular epidemiology studies. However, recent studies on the impact of polymorphism on adduct levels in PAH-exposed human populations did not show a clear relationship between the Phase I (CYP1A1, EH) or Phase II (GST) enzyme polymorphisms and the formation of DNA adducts (Hemminki et al., 1997) or blood protein adducts (Pastorelli et al., 1998).

Conjugation with uridine diphosphate-glucuronide catalyzed by uridine diphosphate-glucuronosyltransferase (UDP-UGT) enzymes is another important detoxification mechanism for oxidative benzo[a]pyrene metabolites. UGT isoforms, as well as their allelic variants, are expressed, and have glucuronidation activity toward, benzo[a]pyrene-derived phenols and diols in the aerodigestive tract (tongue, tonsil, floor of the mouth, larynx, esophagus), but not in the lung or liver (Fang and Lazarus, 2004; Zheng et al., 2002). UGT activity also shows significant interindividual variability. Incubation of lymphocytes with benzo[a]pyrene resulted in covalent binding to protein with a 143-fold interindividual variability and a statistically significant inverse correlation between glucuronidation and protein binding (Hu and Wells, 2004).

Sulfotransferases can catalyze the formation of sulfates of benzo[a]pyrene metabolites. In rat or mouse liver, cytosolic sulfotransferase (in the presence of 3'-phosphoadenosine 5'-phospho-sulfate) catalyzes formation of sulfates of three benzo[a]pyrene metabolites: benzo[a]pyrene-7,8,9,10-tetrahydro-7-ol, benzo[a]pyrene-7,8-dihydrodiol, and benzo[a]pyrene-7,8,9,10-tetrol. The benzo[a]pyrene-7,8,9,10-tetrahydro-7-ol-sulfate is able to form potentially damaging DNA adducts (Surh and Tannenbaum, 1995). In human lung tissue, 3-hydroxybenzo[a]pyrene conjugation to sulfate produces benzo[a]pyrene-3-yl-hydrogen sulfate, a very lipid-soluble compound that would not be readily excreted in the urine (Cohen et al., 1976).

Although not specific for benzo[a]pyrene, there is now considerable evidence that genetic polymorphisms of the GST, UGT, and EH genes impart an added risk to humans for developing cancer. Of some significance to the assessment of benzo[a]pyrene may be that smoking, in combination with genetic polymorphism at several gene loci, increases the risk for bladder cancer (Moore et al., 2004; Choi et al., 2003; Park et al., 2003) and lung cancer (Alexandrie et al., 2004; Lin et al., 2003). Coke oven workers (who are exposed to PAHs, including benzo[a]pyrene) homozygous at the P187S site of the NQO1 gene (an inhibitor of benzo[a]pyrene-quinone adducts with DNA), or carrying the null variant of the glutathione-S-transferase M1 (GSTM1) gene, had a significantly increased risk of chromosomal damage in peripheral blood lymphocytes. Meanwhile, the risk was much lower than controls in subjects with a variant allele at the H113Y site of the EH gene (Leng et al., 2004).

Tissue-Specific Metabolism

Benzo[a]pyrene metabolism has been demonstrated in vivo in laboratory animals for various tissues via multiple routes including inhalation, ingestion, and dermal absorption. Metabolism of benzo[a]pyrene at the site of administration such as in the respiratory tract, the GI

tract, or the skin impact the amount of benzo[a]pyrene and its form as benzo[a]pyrene or one of the metabolites that reach systemic circulation. Nasal instillation or inhalation of benzo[a]pyrene in monkeys, dogs, rats, and hamsters resulted in the formation of dihydrodiols, phenols, quinones, and tetrols in the nasal mucus and lung (Wolff et al., 1989; Petridou-Fischer et al., 1988; Weyand and Lavoie, 1988; Weyand and Bevan, 1987, 1986; Dahl et al., 1985). In rats, the fractions of metabolites in the lung at 6 hours after instillation were 20% unmetabolized benzo[a]pyrene, 16% conjugates or polyhydroxylated compounds, 10.7% 4,5-, 7,8-, and 9,10-dihydrodiols, 9.3% 1,6-, 3,6-, and 6,12-quinone, and 6.9% 3- and 9-hydroxybenzo[a]pyrene (Weyand and Bevan, 1986). In hamsters, approximately 50% of the benzo[a]pyrene instilled was metabolized in the nose (nasal tissues had the highest metabolic activity per-gram of the respiratory tract tissues), and the metabolites produced were similar to other species (Dahl et al., 1985).

In vitro studies of human and laboratory cells and cell lines provide further quantitative and mechanistic details of the metabolism of benzo[a]pyrene in the cells of the respiratory tract, skin, liver, and other tissues. Tracheobronchial tissues in culture of several species (including humans, mice, rats, hamsters, and bovines) were all found to metabolize benzo[a]pyrene extensively to phenols, diols, tetrols, quinones, and their conjugates (Autrup et al., 1980). The results show a high degree of interindividual variability (a 33-fold difference in human bronchus, a 5-fold variation in human trachea, and a 3-fold difference in bovine bronchus), but minimal variation among individuals of the laboratory animal species (Autrup et al., 1980). Human bronchial epithelial and lung tissue conjugated benzo[a]pyrene metabolites to glutathione and sulfates, but not with glucuronide (Kiefer et al., 1988; Autrup et al., 1978; Cohen et al., 1976). Lung tissue slices exposed to benzo[a]pyrene induced expression of CYP1A1 and CYP1B1 at levels 10−20 times higher than in the liver (Harrigan et al., 2006) and total levels of benzo[a]pyrene-DNA adducts were approximately 2−6 times greater in the lung slices than liver (Harrigan et al., 2004).

Benzo[a]pyrene undergoes extensive metabolism in the GI tract and liver after oral administration. In rats after administration of an oral dose, the majority of benzo[a]pyrene detected in organs is as metabolites (Ramesh et al., 2004; Ramesh et al., 2001b; Yamazaki and Kakiuchi, 1989). In rats administered a 100-nmol dose, >90% was recovered in portal blood as metabolites (Bock et al., 1979). Orally administered benzo[a]pyrene produced strong induction of CYP1A1 in the intestine of mice (Brooks et al., 1999). DNA post-labeling studies of mice administered benzo[a]pyrene by gavage demonstrated higher benzo[a]pyrene-DNA adduct levels in CYP1A1(−/−) than CYP1A1(+/+) mice in small intestines (Uno et al., 2004). To compare the relative roles of the liver and intestine in benzo[a]pyrene metabolism and absorption, a multicompartment perfusion system was developed; it was found that benzo[a]pyrene is extensively metabolized by the intestinal Caco-2 cells and that benzo[a]pyrene and its metabolites are transported to the apical side of the Caco-2 cells away from the liver HepG2 cells (Choi et al., 2004).

Dermal exposure in humans and animals resulted in benzo[a]pyrene metabolism, and the permeation of benzo[a]pyrene in skin is linked to benzo[a]pyrene metabolism. Human skin samples maintained in short-term organ culture (i.e., human epithelial tissue, samples from human hair follicles, and melanocytes isolated from adult human skin) can metabolize benzo[a]pyrene into dihydrodiols, phenols, quinones, and glucuronide and sulfate conjugates (Agarwal et al., 1991; Alexandrov et al., 1990; Hall and Grover, 1988; Merk et al., 1987). Nonviable skin is unable to metabolize benzo[a]pyrene (the permeation into nonviable skin is lower than viable skin) as measured in a range of species including humans, rats, mice, rabbits, and marmosets (Kao et al., 1985). Viable human skin samples treated with 2 μg/cm^2 [^{14}C]-benzo[a]pyrene in acetone and incubated for 24 hours produced the following percentages of benzo[a]pyrene metabolites: 52% water-soluble compounds, 8% polar compounds, 17% diols, 1% phenols, 2.5% quinones, and 18% unmetabolized benzo[a]pyrene (Kao et al., 1985).

Benzo[a]pyrene that reaches systemic circulation is also metabolized by multiple tissues that are targets of benzo[a]pyrene toxicity, including reproductive tissues such as prostate, endometrium, cervical epithelial and stromal, and testes (Ramesh et al., 2003; Bao et al., 2002; Williams et al., 2000; Melikian et al., 1999).

Age-Specific Metabolism

Metabolism of benzo[a]pyrene occurs in the developing fetus and in children, as indicated by DNA or protein adducts or urinary metabolites (Naufal et al., 2010; Ruchirawat et al., 2010; Suter et al., 2010; Mielzyńska et al., 2006; Perera et al., 2005a; Tang et al., 1999; Whyatt et al., 1998). Transport of benzo[a]pyrene and benzo[a]pyrene metabolites to fetal tissues including plasma, liver, hippocampus, and cerebral cortex has been demonstrated in multiple studies (McCabe and Flynn, 1990; Neubert and Tapken, 1988; Shendrikova and Aleksandrov, 1974), and benzo[a]pyrene is metabolized by human fetal esophageal cell culture (Chakradeo et al., 1993). While expression of CYP enzymes is lower in fetuses and infants, the liver to body mass ratio and increased blood flow to liver in fetuses and infants may compensate for the decreased expression of CYP enzymes (Ginsberg et al., 2004). Prenatal exposure to benzo[a]pyrene upregulates CYP1A1 and may increase the formation of benzo[a]pyrene-DNA adducts (Wu et al., 2003a). Activity of Phase II detoxifying enzymes in neonates and children is adequate for sulfation, but decreased for glucuronidation and glutathione conjugation (Ginsberg et al., 2004). The conjugation of benzo[a]pyrene-4,5-oxide with glutathione was approximately one-third less in human fetal than adult liver cytosol (Pacifici et al., 1988). The differential Phase I and II enzyme expression and activity in the developing fetus and in children are consistent with an expectation that these lifestages can be more susceptible to benzo[a]pyrene toxicity.

D.1.5. Elimination

Benzo[a]pyrene metabolites have been detected in the urine of exposed humans, but fecal excretion has not been investigated in any detail. Benzo[a]pyrene and associated metabolites have

also been detected in breast milk, especially in smokers, indicating some portion of dose is excreted via lactation (Yu et al., 2011; Zanieri et al., 2007; Madhavan and Naidu, 1995).

Studies of benzo[a]pyrene elimination in animals following exposure via inhalation, ingestion, and dermal routes have shown that benzo[a]pyrene is excreted preferentially in the feces in multiple species of laboratory animals including rats, mice, hamsters, guinea pigs, monkeys, and dogs (Wang et al., 2003; Likhachev et al., 1992; Wolff et al., 1989; Yang et al., 1989; Petridou-Fischer et al., 1988; Weyand and Bevan, 1987; Sun et al., 1982; Hecht et al., 1979). The metabolites in bile are primarily benzo[a]pyrene conjugates, predominantly thioether conjugates of varying extent in different species (Weyand and Bevan, 1987). Six hours after a single intratracheal instillation of benzo[a]pyrene (2 μg/kg) to male Sprague-Dawley rats, relative metabolite levels were 31.2% diglucuronides, 30.4% thioether conjugates, 17.8% monoglucuronides, 6.2% sulfate conjugates, and 14.4% unconjugated metabolites (Bevan and Sadler, 1992). Rats administered benzo[a]pyrene via i.v. excrete a larger fraction in urine than via inhalation or oral exposure, suggesting an important role for enterohepatic circulation of benzo[a]pyrene metabolite conjugates (Moir et al., 1998; Weyand and Bevan, 1986; Hirom et al., 1983). The vehicle impacts the amount of benzo[a]pyrene excreted and may, in part, be due to the elimination rate or to other factors such as the absorption rate. For tritiated benzo[a]pyrene administered to Sprague-Dawley rats in hydrophilic triethylene glycol, 70.5% of the dose was excreted into bile within 6 hours. When the lipophilic solvents, ethyl laurate and tricaprylin, were used as vehicles, 58.4 and 56.2% of the dose was excreted, respectively (Bevan and Ulman, 1991). In addition to benzo[a]pyrene and its metabolites, adducts of benzo[a]pyrene with nucleotides have also been identified as a small fraction of the administered dose in feces and urine of animals. The level of BPDE adducts with guanine detected in urine of male Wistar rats was dose-dependent. Forty-eight hours after dosing with 100 μg/kg tritiated benzo[a]pyrene, 0.15% of the administered benzo[a]pyrene dose was excreted in the urine as an adduct with guanine (Autrup and Seremet, 1986). Benzo[a]pyrene is also eliminated, to a limited extent, through milk in lactating animals. Levels of benzo[a]pyrene eliminated via lactation after dietary administration constituted <0.003% of the maternal dose in rabbits (West and Horton, 1976) and goats (Lapole et al., 2007), whereas in sheep, lactational elimination represented about 0.014% of the total maternal dose (West and Horton, 1976).

Overall, the data in humans and laboratory animals are sufficient to describe benzo[a]pyrene elimination qualitatively, but are limited in estimating quantitative rates of elimination.

D.2. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS

Several toxicokinetic or pharmacokinetic models of benzo[a]pyrene have been developed for rodents (rat and hamster). However, human models have only been developed via allometric scaling, and metabolic parameters in humans have not been calibrated against in vivo toxicokinetic data or in vitro experiments.

Bevan and Weyand (1988) performed compartmental pharmacokinetic analysis of distribution of radioactivity in male Sprague-Dawley rats, following the intratracheal instillation of benzo[a]pyrene to normal and bile duct-cannulated animals (Weyand and Bevan, 1987, 1986). However, implicit simulation approaches were used, as opposed to physiologically-based approaches. The model calculated linear rate constants among compartments, and assumed that the kinetics of benzo[a]pyrene and its metabolites were the same.

Roth and Vinegar (1990) reviewed the capacity of the lung to impact the disposition of chemicals and used benzo[a]pyrene as a case study. A PBPK model was presented based on data from Wiersma and Roth (1983b); Wiersma and Roth (1983a) and was evaluated against tissue concentration data from Schlede et al. (1970). The model was structured with compartments for arterial blood, venous blood, lung, liver, fat, and slowly and rapidly perfused tissues and an adequate fit was obtained for some compartments; however, tissue-level data for calibration and validation of this model were limited. Metabolism in liver and lung was estimated using kinetic data from control rats and rats pretreated with 3-methylcholanthrene to induce benzo[a]pyrene metabolism. In microsomal preparations from control and 3-methylcholanthrene induced rat livers and lungs, benzo[a]pyrene hydroxylase activity was 1,000-fold greater in liver. In isolated rat lungs, the clearance of benzo[a]pyrene was about one-sixth of the clearance in isolated rat livers and in 3-methylcholanthrene-pretreated rats the clearance in lungs and livers were of similar magnitude. The PBPK simulations model based on these data showed that for a bolus intravascular injection of benzo[a]pyrene in rats, the majority of benzo[a]pyrene metabolism usually occurs in the liver. Except for cases when rats are pretreated with enzyme-inducing agents or where the exposure occurs via inhalation, the metabolic clearance in the lung is minor.

Moir et al. (1998) conducted a pharmacokinetic study on benzo[a]pyrene to obtain data for model development. Rats were injected with varying doses of [^{14}C]-benzo[a]pyrene to 15 mg/kg, and blood, liver, fat, and richly perfused tissue were sampled varying time points after dosing. Moir (1999) then described a model for lung, liver, fat, richly and slowly perfused tissues, and venous blood, with saturable metabolism occurring in the liver. The fat and richly perfused tissues were modeled as diffusion-limited, while the other tissues were flow-limited. The model predicted the blood benzo[a]pyrene concentrations well, although it overestimated the 6 mg/kg results at longer times (>100 minutes). The model also produced a poor fit to the liver data. The model simulations were also compared to data of Schlede et al. (1970), who injected rats with 0.056 mg/kg body weight of benzo[a]pyrene. The model predicted blood and fat benzo[a]pyrene concentrations well, but still poorly predicted liver benzo[a]pyrene concentrations. The model included only one saturable metabolic pathway, and only parent chemical concentrations were used to establish the model. No metabolites were included in the model. This model was re-calibrated by Crowell et al. (2011) by optimizing against additional rodent data and altering partition coefficient derivation. However, it still did not incorporate metabolites, and some tissues continued to exhibit poor model fits.

An attempt to scale the Moir et al. (1998) rodent PBPK model to humans, relevant to risk assessment of oral exposures to benzo[a]pyrene, was presented by Zeilmaker et al. (1999a) and Zeilmaker et al. (1999b). The PBPK model for benzo[a]pyrene was derived from an earlier model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats (Zeilmaker and van Eijkeren, 1997). Most compartments were perfusion-limited, and tissues modeled included blood, adipose (with diffusion limitation), slowly and richly perfused tissues, and liver. However, there was no separate compartment for the lung. The liver compartment featured the AhR-dependent CYP450 induction mechanism and DNA adduct formation as a marker for formation of genotoxic benzo[a]pyrene metabolites. It was assumed that DNA adduct formation and the bulk benzo[a]pyrene metabolism were mediated by two different metabolic pathways. The model was experimentally calibrated in rats with the data for 7-ethoxyresorufin-O-deethylase (EROD) and formation of DNA adducts in the liver after i.v. administration of a single dose and per os administration of a single or repeated doses of benzo[a]pyrene (Zeilmaker et al., 1999a).

Zeilmaker et al. (1999b) assumed identical values for several parameters in rats and humans (i.e., benzo[a]pyrene tissue partition coefficients, AhR concentration in liver, rate constant for the decay of the benzo[a]pyrene-CYP450 complex, half-life of the CYP450 protein, fraction and rate of GI absorption of benzo[a]pyrene, and rates of formation and repair of DNA adducts in liver). The basal CYP450 activity in humans was assumed to be lower than that in rat liver. The mechanism of AhR-dependent induction of CYP450 dominated the simulated benzo[a]pyrene-DNA adduct formation in the liver. The results of PBPK model simulations indicated that the same dose of benzo[a]pyrene administered to rats or humans might produce one order of magnitude higher accumulation of DNA adducts in human liver when compared with the rat (Zeilmaker et al., 1999b).

Even though the model of Zeilmaker et al. (1999b) represents a major improvement in predictive modeling of benzo[a]pyrene toxicokinetics, the interspecies extrapolation introduces significant uncertainties. As emphasized by the authors, the conversion of benzo[a]pyrene to its mutagenic and carcinogenic metabolites could not be explicitly modeled in human liver because no suitable experimental data were available. According to the authors, improvement of the model would require direct measurements of basal activities of CYP1A1 and CYP1A2 and formation of benzo[a]pyrene-DNA adducts in human liver. Metabolic clearance of benzo[a]pyrene in the lungs was also not addressed. Additionally, the toxicokinetic modeling by Zeilmaker et al. (1999b) addressed only one pathway of benzo[a]pyrene metabolic activation, a single target organ (the liver), and one route of administration (oral). In order to model health outcomes of exposures to benzo[a]pyrene, the PBPK model needs to simulate rate of accumulation of benzo[a]pyrene-DNA adducts and/or the distribution and fate of benzo[a]pyrene metabolites (e.g., BPDE) that bind to DNA and other macromolecules. Alternatively, stable toxic metabolites (e.g., trans-anti-tetrol-benzo[a]pyrene) may be used as an internal dose surrogate. While the metabolic pattern of benzo[a]pyrene has been relatively well characterized qualitatively in animals, the quantitative

kinetic relationships between the more complex metabolic reactions in potential target organs are not yet well defined.

D.2.1. Recommendations for the Use of PBPK Models in Toxicity Value Derivation

PBPK models for benzo[a]pyrene were evaluated to determine the capability to extrapolate from rats to humans, or between oral and inhalation exposure routes. Due to significant uncertainties with respect to the inter-species scaling of the metabolic parameters between rats and humans, these models were not used for cross-species extrapolation. Furthermore, no complete mechanistic PBPK model for the inhalation route was identified, nor was there a model for humans that simulates the typical inhalation exposure to benzo[a]pyrene on poorly soluble carbonaceous particles. This precluded the model's use for cross-route extrapolation to the inhalation pathway.

D.3. HUMAN STUDIES

D.3.1. Noncancer Endpoints

Cardiovascular Endpoints

Burstyn et al. (2005) reported the association of death from cardiovascular disease with benzo[a]pyrene exposure in a cohort of 12,367 male European asphalt workers (Table D-1). These workers were first employed in asphalt paving between 1913 and 1999, and worked at least one season. Average duration of follow-up was 17 ± 9 years (mean ± standard deviation [SD]), encompassing 193,889 person-years of observation. Worker exposure to coal tar was estimated using industrial process and hygiene information and modeling (presented in a previous report), and coal tar exposure was found to be the strongest determinant of exposure to benzo[a]pyrene. Benzo[a]pyrene exposure was assessed quantitatively using measurement-driven mixed effects exposure models, using data collected from other asphalt industry workers, and this model was constructed and validated previously. Due to limited data availability, only information regarding the primary cause of death was collected, and this analysis was limited to diseases of the circulatory system (ICD codes 390–459), specifically ischemic heart disease (IHD: ICD codes 410–414). Diesel exhaust exposure was also assessed in this cohort, but varied little among the asphalt pavers, and was not associated with risk of death from cardiovascular disease. Of the initial cohort, 0.25% was lost to follow-up and 0.38% emigrated during the course of observation. Relative risks (RRs) and associated 95% confidence intervals (CIs) were estimated using Poisson regression, and all models included exposure index for agent of interest (coal tar or benzo[a]pyrene), age, calendar period of exit from cohort, total duration of employment, and country, using the category of lowest exposure as the reference. Confounding by tobacco smoke exposure was considered in relation to the strength of its association with cardiovascular disease and the smoking prevalence in the population. The RRs attributed to cigarette smoking in former and current smokers were assumed

to be 1.2 and 2, respectively, based upon literature reports. From analysis of smoking incidence in a subcohort, the following smoking distribution was proposed: in the lowest exposure group, 40% never-smokers, 30% former smokers, and 30% current smokers; and among the highest exposed, the proportion shifted to 20/30/50%, respectively.

Exposed subjects were stratified into quintiles based upon IHD mortality, with 83–86 deaths per exposure category, composing approximately 2/3 of the 660 cardiovascular disease-related deaths. Both cumulative and average exposure indices for benzo[a]pyrene were positively associated with IHD mortality, with a RR of approximately 1.6 in the highest exposure quintile from both metrics, independent of total employment duration. Similar monotonic trends were observed for all cardiovascular diseases (combined), although a dose-response relationship was evident only for IHD and not hypertension or other individual heart disease categories. Similar trends were also observed for coal tar exposure and IHD. Adjusting the RR to account for possible confounding by smoking yields a RR of 1.39 under the assumptions mentioned above, and is still elevated (1.21) if the contribution of smoking to cardiovascular disease etiology was greater than the original assumptions. Furthermore, the RR for the high versus low exposure quintile is 1.24 even if the distribution of nonsmokers/former smokers/current smokers shifts to 0/30/70%, using the original assumptions of cigarette smoke casual potency.

Table D-1. Exposure to benzo[a]pyrene and mortality from cardiovascular diseases in a European cohort of asphalt paving workers

Effect measured	Cumulative exposure (ng/m³-yrs)					*p*-value for trend
	0–189[a]	**189–501**	**502–931**	**932–2,012**	**≥2,013**	
Diseases of the circulatory system						
Deaths RR 95% CI	137 1.00	145 1.08 0.85–1.38	118 1.06 0.80–1.42	132 1.24 0.89–1.71	128 1.42 0.96–2.09	0.09
IHD						
Deaths RR 95% CI	83 1.00	83 0.99 0.72–1.36	84 1.22 0.86–1.74	83 1.24 0.82–1.85	85 1.58 0.98–2.55	0.06
Effect measured	**Average exposure (ng/m³)**					***p*-value for trend**
	0–68[a]	**68–105**	**106–146**	**147–272**	**≥273**	
Diseases of the circulatory system						
Deaths RR 95% CI	128 1.00	142 1.30 1.01–1.67	143 1.55 1.18–2.05	139 1.45 1.09–1.93	108 1.58 1.16–2.15	<0.001

Effect measured	Cumulative exposure (ng/m^3-yrs)					*p*-value for trend
	0–189[a]	189–501	502–931	932–2,012	≥2,013	
IHD						
Deaths RR 95% CI	83 1.00	83 1.13 0.82–1.55	83 1.33 0.94–1.90	86 1.20 0.84–1.71	83 1.64 1.13–2.38	0.02

[a]Reference category.

Source: Burstyn et al. (2005).

Friesen et al. (2010) examined the association between benzo[a]pyrene exposure and deaths from chronic nonmalignant disease in a cohort of 6,423 male and 603 female Canadian aluminum smelter workers (Table D-2). Inclusion criteria required at least 3 years of continuous employment in either the smelter facility or power-generating station from 1954 to 1997, with worker history collected up through 1999. This cohort was probabilistically linked to the Canadian national mortality database for external comparison to the British Columbia population and calculation of standardized mortality ratios (SMRs), which were adjusted for age, sex, and time period. Ninety-five percent CIs were calculated for the SMRs assuming a Poisson distribution. Internal comparisons were also made during the analysis of IHD mortality in male workers, calculating hazard ratios (HRs) for IHD with or without acute myocardial infarction (AMI) after 1969, as AMI could not be differentiated from other IHD on death certificates issued previously. HRs were calculated using Cox regression models, with age as a metamarker of time, also including smoking status, time since first employed and work location status. Smoking information for 77% of this updated cohort was collected by questionnaire, and workers were categorized as 75% ever-smokers and 25% never-smokers. Quantitative exposure to coal tar pitch volatiles were estimated by benzo[a]pyrene measurements, calculated by a job classification and time-based exposure matrix, as described in a previous report; annual arithmetic mean values were calculated for exposures from 1977 to 2000, while pre-1977 levels were backwards-extrapolated from 1977 values, incorporating major technological changes in time periods as appropriate.

Cumulative exposure metrics were highly skewed. Cumulative benzo[a]pyrene with a 5-year lag (past benzo[a]pyrene exposure) and cumulative benzo[a]pyrene in the most recent 5 years (recent benzo[a]pyrene exposure) were only slightly positively correlated ($r = 0.10$, $p < 0.001$). Current benzo[a]pyrene exposure was highly correlated with cumulative exposure for the most recent 5 years of exposure ($r = 0.86$, $p < 0.001$), but not with 5-year lagged cumulative exposure ($r = 0.03$, $p < 0.001$). Lagged cumulative exposure metrics (0–10 years) were all highly correlated with each other ($r = 0.96$, all p-values <0.001); lagged metrics for cumulative exposure were used to distinguish between effects of current versus long-term exposure.

When exposed workers were pooled and compared externally to non-exposed referents, the IHD and AMI SMRs were all ≤1.00 for males, and the only significant association in females was an

SMR of 1.27 for AMI. For internal comparisons, exposed males were stratified into quintiles based upon IHD mortality, with approximately 56 deaths per exposure category. Five-year lagged cumulative benzo[a]pyrene exposure was significantly associated with elevated risk of IHD mortality, HR = 1.62 (95% CI 1.06–2.46) in the highest exposure quintile, while no association was observed between most recent (5 years) exposure and mortality. Restricting IHD events to only AMI (1969 onward) resulted in similar monotonic trends, albeit of lower statistical significance. No association was observed between benzo[a]pyrene exposure and non-AMI IHD. While there was little difference in the exposure-response association among 0-, 2-, and 5-year lagged data, 10-year lagged data resulted in a weaker association. All risk estimates were strengthened by the incorporation of work status and time-since-hire to account for the healthy worker effect, as evidenced by the SMR of 0.87 (95% CI 0.82–0.92) for all chronic nonmalignant diseases combined in male exposed workers versus external referents. Using a continuous variable, the authors calculated the risk of death from IHD as 1.002 (95% CI 1.000–1.005) per μg/m^3 from cumulative benzo[a]pyrene exposure; however, visual inspection of the categorical relationships indicated that the association is nonlinear, suggesting that this value may be an underestimate. Restricting the cohort to only members who died within 30 days of active employment at the worksite, cumulative benzo[a]pyrene exposure was not significantly associated with IHD or AMI, although the HR for the highest exposure group was 2.39 (95% CI 0.95–6.05). Exposure-response relationships were similarly examined in male smelter workers for chronic obstructive pulmonary disease and cerebrovascular disease, but neither was significantly associated with cumulative benzo[a]pyrene exposure in either internal or external comparisons.

Table D-2. Exposure to benzo[a]pyrene and mortality from cardiovascular diseases in a Canadian cohort of male aluminum smelter workers

Effect measured	Categorical cumulative exposure with a 5-yr lag (μg/m^3-yr)					*p*-value for trend[a]	Continuous[b]
	0	0–7.79	7.79–24.3	24.3–66.7	≥66.7		
All IHD (1957 onward)							
Deaths	56	56	57	56	56		281
Person-years of follow-up	33,111	37,581	34,838	31,533	13,688	0.053	150,751
HR	1	1.11	1.48	1.28	1.62		1.002
95% CI	referent	0.76–1.62	1.01–2.17	0.86–1.91	1.06–2.46		1.000–1.005

Effect measured	Categorical cumulative exposure with a 5-yr lag (µg/m³-yr)					*p*-value for trend[a]	Continuous[b]
	0	0–7.79	7.79–24.3	24.3–66.7	≥66.7		
AMI (1969 onward)							
	0	**0–7.51**	**7.51–27.7**	**27.7–67.4**	**≥67.4**		
Deaths	35	37	37	38	37		184
Person-years of follow-up	25,071	30,454	34,621	24,081	13,261	0.19	127,488
HR	1	1.14	1.21	1.36	1.46		1.001
95% CI	referent	0.71–1.82	0.75–1.96	0.84–2.45	0.87–2.45		0.997–1.005

[a]Two-sided test for trend using the person-year-weighted mean value for each category as a linear, continuous variable.
[b]Exposure variable was entered as a continuous, linear variable in the model.

Source: Friesen et al. (2010).

Reproductive and Developmental Endpoints

Wu et al. (2010) conducted a study of benzo[a]pyrene-DNA adduct levels in relation to risk of fetal death in Tianjin, China. This case-control study included women who experienced a delayed miscarriage before 14 weeks gestational age (i.e., a fetal death that remained in utero and therefore required surgical intervention). Cases were matched by age and gravidity to controls (women undergoing induced abortion due to an unplanned or unwanted pregnancy). The study excluded women who smoked, women with chronic disease and pregnancy complications, and women with occupational exposures to PAHs. Residency within Tianjin for at least 1 year was also an eligibility criterion. The participation rate was high: 81/84 eligible cases participated and 81/89 eligible controls participated. Data pertaining to demographic characteristics, reproductive history, and factors relating to potential PAH exposure were collected using a structured interview, and samples from the aborted tissue were obtained. In two of the four hospitals used in the study, blood samples from the women (n = 51 cases and 51 controls) were also collected. The presence of benzo[a]pyrene-BPDE adducts was assessed in the blood and tissue samples using high-performance liquid chromatography (HPLC). There was no correlation between blood and aborted tissue levels of benzo[a]pyrene adducts (r = −0.12 for the 102 blood-tissue pairs, r = −0.02 for the 51 case pairs, and r = −0.21 for the 51 control pairs). (The authors noted that there was little difference between women with and without blood samples in terms of the interview-based measures collected or in terms of the DNA-adduct levels in aborted tissue.) Benzo[a]pyrene-adduct levels were similar but slightly lower in the aborted tissue of cases compared with controls (mean ± SD 4.8 ± 6.0 in cases and 6.0 ± 7.4 in controls, $p = 0.29$). In the blood samples, however, benzo[a]pyrene-adduct levels were higher in cases (6.0 ± 4.7 and 2.7 ± 2.2 in cases and controls, respectively, $p < 0.001$). In logistic regression analyses using a continuous adduct measure, the odds ratio (OR) was 1.35 (95% CI 1.11–1.64) per adduct/10^8 nucleotide. These results were

adjusted for education, household income, and gestational age, but were very similar to the unadjusted results. Categorizing exposure at the median value resulted in an adjusted OR of 4.27 (95% CI 1.41–12.99) in the high compared with low benzo[a]pyrene-adduct group. There was no relation between benzo[a]pyrene-adduct levels in the aborted tissue and miscarriage in the logistic regression analyses using either the continuous (adjusted OR 0.97, 95% CI 0.93–1.02) or dichotomous exposure measure (adjusted OR 0.76, 95% CI 0.37–1.54). Associations between miscarriage and several interview-based measures of potential PAH exposure were also seen: adjusted ORs of 3.07 (95% CI 1.31–7.16) for traffic congestion near residence, 3.52 (95% CI 1.44–8.57) for commuting by walking, 3.78 (95% CI 1.11–12.87) for routinely cooked during pregnancy, and 3.21 (95% CI 0.98–10.48) for industrial site or stack near residence, but there was no association with other types of commuting (e.g., by bike, car, or bus).

Perera et al. (2005a) studied 329 nonsmoking pregnant women (30 ± 5 years old) possibly exposed to PAHs from fires at the World Trade Center (WTC) during the 4 weeks after 09/11/2001. Maternal and umbilical cord blood levels of benzo[a]pyrene (BPDE)-DNA adducts were highest in study participants who lived within 1 mile of the WTC, with an inverse correlation between cord blood levels and distance from the WTC. Neither cord blood adduct level nor environmental tobacco smoke (ETS) alone was positively correlated with adverse birth outcomes. However, the interaction between ETS exposure and cord blood adducts was significantly associated with reduced birth weight and head circumference. Among babies exposed to ETS in utero, a doubling of cord blood benzo[a]pyrene-DNA adducts was associated with an 8% decrease in birth weight (p = 0.03) and a 3% decrease in head circumference (p = 0.04).

Perera et al. (2005b), a reanalysis of Perera et al. (2004), compared various exposures—ETS, nutrition, pesticides, material hardship—with birth outcomes (length, head circumference, cognitive development). ETS exposure and intake of PAH-rich foods by pregnant women were determined by questionnaire. Levels of BPDE-DNA adducts were determined in umbilical cord blood collected at delivery. The study population consisted of Dominican or African-American nonsmoking pregnant women (n = 214; 24 ± 5 years old) free of diabetes, hypertension, HIV, and drug or alcohol abuse. Benzo[a]pyrene adducts, ETS, and dietary PAHs were not significantly correlated with each other. However, the interaction between benzo[a]pyrene-DNA adducts and ETS exposure was significantly associated with reduced birth weights (−6.8%; p = 0.03) and reduced head circumference (−2.9%; p = 0.04).

Tang et al. (2006) measured BPDE-DNA adducts in maternal and umbilical cord blood obtained at delivery from a cohort of 150 nonsmoking women and their newborns in China. Exposure assessment was related to the seasonal operation of a local, coal-fired power plant; however, airborne PAH concentrations were not measured. Dietary PAH intake was not included as a covariate because it did not significantly contribute to the final models, but ETS, sex, and maternal height and weight were considered as covariates. DNA adduct levels were compared to several birth outcomes and physical development parameters, such as gestational age at birth; infant sex,

birth weight, length, head circumference, and malformations; maternal height and pregnancy weight total weight gain; complications of pregnancy and delivery; and medications used during pregnancy.

High cord blood adduct levels were significantly associated with reduced infant/child weight at 18 months (β = −0.048, p = 0.03), 24 months (β = −0.041, p = 0.027), and 30 months of age (β = −0.040, p = 0.049); decreased birth head circumference was marginally associated with DNA adduct levels (β = −0.011, p = 0.057). Maternal adduct levels were correlated neither with cord blood adduct levels nor with fetal and child growth. Among female infants, cord blood adduct levels were significantly associated with smaller birth head circumference (p = 0.022) and with lower weight at 18 months (p = 0.014), 24 months (p = 0.012), and 30 months of age (p = 0.033), and with decreased body length at 18 months of age (p = 0.033). Among male infants, the corresponding associations were also inverse, but were not statistically significant.

Considerable evidence of a deleterious effect of smoking on male and female fertility has accumulated from epidemiological studies of time to pregnancy, ovulatory disorders, semen quality, and spontaneous abortion (reviewed in Waylen et al., 2009; Cooper and Moley, 2008; Soares and Melo, 2008). In addition, the effect of smoking, particularly during the time of the perimenopausal transition, on acceleration of ovarian senescence (menopause) has also been established (Midgette and Baron, 1990). More limited data are available pertaining specifically to measures of benzo[a]pyrene and reproductive outcomes.

Neal et al. (2008) examined levels of benzo[a]pyrene and other PAHs in follicular fluid and serum sample from 36 women undergoing in vitro fertilization at a clinic in Toronto, and compared the successful conception rate in relation to benzo[a]pyrene levels. The women were classified by smoking status, with 19 current cigarette smokers, 7 with passive or sidestream smoke exposure (i.e., nonsmoker with a partner who smoked), and 10 nonsmokers exposed. An early follicular phase blood sample and follicular fluid sample from the follicle at the time of ovum retrieval were collected and analyzed for the presence of benzo[a]pyrene, acenapthelene, phenanthrene, pyrene, and chrysene using gas chromatography/mass spectrometry (MS) (detection limit 5 pg/mL). The frequency of nondetectable levels of serum benzo[a]pyrene was highest in the nonsmoking group (60.0, 14.3, and 21.0% below the detection limit in nonsmoking, sidestream smoke, and active smoking groups, respectively). A similar pattern was seen with follicular fluid benzo[a]pyrene (30.0, 14.3, and 10.5% below the detection limit in nonsmoking, sidestream smoke, and active smoking groups, respectively). In the analyses comparing mean values across groups, an assigned value of 0 was used for nondetectable samples. Follicular fluid benzo[a]pyrene levels were higher in the active smoking group (mean ± standard error [SE], 1.32 ± 0.68 ng/mL) than in the sidestream (0.05 ± 0.01 ng/mL) or nonsmoking (0.03 ± 0.01 ng/mL) groups (p = 0.04). The between-group differences in serum benzo[a]pyrene levels were not statistically significant (0.22 ± 0.15, 0.98 ± 0.56, and 0.40 ± 0.13 ng/mL in nonsmoking, sidestream smoke, and active smoking groups, respectively), and there were no differences in relation to smoking status. Among active smokers,

the number of cigarettes smoked per day was strongly correlated with follicular fluid benzo[a]pyrene levels (r = 0.7, $p < 0.01$). Follicular fluid benzo[a]pyrene levels were significantly higher among the women who did not conceive (1.79 ± 0.86 ng/mL) compared with women who did get pregnant (mean approximately 0.10 ng/mL, as estimated from graph) ($p < 0.001$), but serum levels of benzo[a]pyrene were not associated with successful conception.

A small case-control study conducted between August 2005 and February 2006 in Lucknow city (Uttar Pradesh), India examined PAH concentrations in placental tissues (Singh et al., 2008) in relation to risk of preterm birth. The study included 29 cases (delivery between 28 and <36 weeks of gestation) and 31 term delivery controls. Demographic data on smoking history, reproductive history, and other information were collected by interview, and a 10-g sample of placental tissue was collected from all participants. Concentration of specific PAHs in placental tissue was determined using HPLC. In addition to benzo[a]pyrene, the PAHs assayed were naphthalene, acenaphthylene, phenanthrene, fluorene, anthracene, benzo[a]anthracene, fluoranthene, pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[g,h,i]perylene, and dibenzo[a,h]anthracene. PAH exposure in this population was from environmental sources and from cooking. The age of study participants ranged from 20 to 35 years. There was little difference in birth weight between cases and controls (mean 2.77 and 2.75 kg in the case and control groups, respectively). Placental benzo[a]pyrene levels were lower than the levels of the other PAHs detected (mean 8.83 ppb in controls for benzo[a]pyrene compared with 25–30 ppb for anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, and dibenzo[a,h]anthracene, 59 ppb for acenaphthylene, and 200–380 ppm for naphthalene, phenanthrene, fluoranthene, and pyrene; nondetectable levels of fluorine, benzo[a]anthracene, and benzo[g,h,i]perylene were found). There was little difference in benzo[a]pyrene levels between cases (mean ± SE 13.85 ± 7.06 ppb) and controls (8.83 ± 5.84 ppb), but elevated levels of fluoranthene (325.91 ± 45.14 and 208.6 ± 21.93 ppb in cases and controls, respectively, $p < 0.05$) and benzo[b]fluoranthene (61.91 ± 12.43 and 23.84 ± 7.01 ppb in cases and controls, respectively, $p < 0.05$) were seen.

Neurotoxicity

Niu et al. (2010) studied 176 Chinese coke-oven workers with elevated benzo[a]pyrene exposure and compared them against 48 referents (workers in a supply warehouse), matched by socioeconomic status, lifestyle, and health. Blood levels of monoamine, amino acid, and chlorine neurotransmitters were measured, and the World Health Organization Neurobehavioral Core Test Battery was administered to assess emotional state, learning, memory, and hand-eye coordination. The authors self-designed a study questionnaire to gather information on worker education, vocational history, smoking and drinking habits, and personal habits, and personal and family medical history, as well as any current symptoms and medications used in the previous several weeks. Workers were excluded from the study for any of the following criteria: if they reported feeling depressed at any point during the previous 6 months; if they had taken medicine in the previous 2 weeks that could affect nervous system function; or if they reported undertaking

vigorous exercise less than 48 hours previously. "Smoking" was defined as ≥10 cigarettes/day during the past year. Similarly, "drinking" was defined as wine/beer/spirits consumed ≥3 times/week for the past 6 months. Workplace environmental sampling stations were established at each of the physical work locations, including the referent's warehouse, and dual automatic air sampling pumps collected samples at personal breathing zone height for 6 hours/day, over 3 consecutive days. Benzo[a]pyrene content was determined by HPLC, and relative exposure was compared to post-shift urine levels of a benzo[a]pyrene metabolite, 1-hydroxypyrene. Blood was collected in the morning before breakfast; monoamine (norepinephrine and dopamine) and amino acid (glutamate, aspartate, glycine, and gamma-aminobutyric acid [GABA]) neurotransmitter levels were determined by HPLC, acetylcholine levels were determined by hydroxyamine chromometry, and acetylcholine esterase (AchE) levels were measured in lysed red blood cells (RBCs) using activity kits.

Benzo[a]pyrene mean concentrations were 19.56 ± 13.2, 185.96 ± 38.6, and 1,623.56 ± 435.8 ng/m^3 at the bottom, side, and top of the coke oven, respectively, all of which were higher than the mean at the referents' warehouse (10.26 ± 7.6 ng/m^3). The authors did not report stratified analysis by different levels of benzo[a]pyrene exposure, and reported only comparisons between the referents and all exposed workers combined (Table D-3), or between workers grouped by urinary benzo[a]pyrene metabolite, 1-hydroxypyrene, levels (Table D-4). There were no significant differences in age, education, or smoking or alcohol use between the coke oven and warehouse workers. Urinary 1-hydroxypyrene levels were 32% higher in coke oven workers compared to the referent group, corresponding to the higher levels of benzo[a]pyrene detected in all coke oven workstation compared to the supply warehouse. Performance in two neurobehavioral function tests, digit span and forward digit span, were significantly decreased in the exposed oven workers versus the control group; when stratified by urinary metabolite level, scores significantly decreased with increasing 1-hydroxypyrene levels. Of the neurotransmitters assessed, norepinephrine, dopamine, aspartate, and GABA were significantly decreased in exposed versus control workers; norepinephrine and aspartate were also significantly and inversely related with 1-hydroxypyrene levels. Dopamine levels appeared to decrease with increased urinary metabolite levels, although the relationship was not statistically significant. GABA levels were highly variable, and appeared to increase with increasing 1-hydroxypyrene levels, although this relationship was not statistically significant. Acetylcholine levels were 4-fold higher in coke oven workers compared to referents, and AchE activity was 30% lower; both acetylcholine and AchE were significantly associated with urinary benzo[a]pyrene metabolite levels, although acetylcholine increased and AchE activity decreased with increasing 1-hydroxypyrene. The authors reported the results of correlation analysis, indicating that digit span scores correlated negatively with acetylcholine and positively with AchE (coefficients of −0.230, −0.276 and 0.120, 0.170, respectively), although no indication of statistical significance was given. No other associations were reported.

Table D-3. Exposure-related effects in Chinese coke oven workers or warehouse controls exposed to benzo[a]pyrene in the workplace

Effect measured	Exposure group		*p*-value
	Controls (n = 48)	Exposed workers (n = 176)	
Background information (mean ± SD, incidence or percent)			
Age (yrs)	39.71 ± 7.51	37.86 ± 6.51	0.098
Education (junior/senior)	23/25	110/66	0.068
Smoking	77%	64%	0.093
Drinking	27%	39%	0.140
Urine benzo[a]pyrene metabolite (µmol/mol creatinine; mean ± SD)			
1-Hydroxypyrene	2.77 ± 1.45	3.66 ± 0.67	0.000
Neurobehavioral function tests (mean ± SD)			
Simple reaction time	413.88 ± 95.40	437.39 ± 88.44	0.109
Digit span	17.31 ± 4.54	15.47 ± 4.08	0.006
Forward digit span	10.65 ± 2.42	9.25 ± 2.64	0.001
Neurotransmitter concentrations (mean ± SD)			
Norepinephrine (ng/mL)	62.54 ± 58.07	40.62 ± 29.78	0.000
Dopamine (ng/mL)	1,566.28 ± 317.64	1,425.85 ± 422.66	0.029
Aspartate (µg/mL)	2.13 ± 1.66	1.58 ± 0.99	0.004
Glutamate (µg/mL)	11.21 ± 5.28	9.68 ± 5.72	0.074
GABA (µg/mL)	2.52 ± 5.16	1.01 ± 2.21	0.004
Acetylcholine (µg/mL	172.60 ± 67.19	704.00 ± 393.86	0.000
AchE activity (U/mg protein)	71.31 ± 46.18	50.27 ± 34.02	0.012

Source: Niu et al. (2010).

Table D-4. Exposure-related effects in Chinese coke oven workers or warehouse controls exposed to benzo[a]pyrene in the workplace, stratified by urinary metabolite levels

Effect measured	Exposure group categorized by 1-hydroxypyrene level			*p*-value
	0–3.09 µmol/mol creatinine	3.09–3.90 µmol/mol creatinine	3.90–5.53 µmol/mol creatinine	
Number of subjects	**33**	**72**	**36**	
Neurobehavioral function tests (mean ± SD)				
Digit span	18.24 ± 4.58	16.04 ± 4.24	15.78 ± 3.71	0.003
Forward digit span	10.85 ± 2.12	9.80 ± 2.86	9.58 ± 2.33	0.019
Backward digit span	7.20 ± 3.07	6.38 ± 2.55	6.20 ± 2.15	0.089
Right dotting	152.15 ± 35.43	153.80 ± 31.55	167.22 ± 59.21	0.094

Effect measured	Exposure group categorized by 1-hydroxypyrene level			*p*-value
	0–3.09 µmol/mol creatinine	3.09–3.90 µmol/mol creatinine	3.90–5.53 µmol/mol creatinine	
Number of subjects	**33**	**72**	**36**	
Neurotransmitter concentrations (mean ± SD)				
Norepinephrine (ng/mL)	67.31 ± 67.45	36.97 ± 23.58	46.75 ± 35.88	0.002
Dopamine (ng/mL)	1,614.45 ± 683.57	1,482.30 ± 323.66	1,405.06 ± 332.23	0.134
Aspartate (µg/mL)	2.29 ± 2.13	1.61 ± 0.71	1.47 ± 0.58	0.001
Glutamate (µg/mL)	11.56 ± 8.92	9.93 ± 4.14	9.06 ± 3.30	0.070
GABA (µg/mL)	1.40 ± 3.59	1.42 ± 3.44	1.56 ± 3.24	0.964
Acetylcholine (µg/mL)	334.66 ± 83.75	483.71 ± 57.87	665.85 ± 94.34	0.030
AchE activity (U/mg protein)	68.17 ± 9.28	54.98 ± 4.23	52.64 ± 4.60	0.043

Source: Niu et al. (2010).

Immunotoxicity

Zhang et al. (2012) studied 129 Chinese coke-oven workers with elevated benzo[a]pyrene exposure and compared them against 37 referents (workers in a supply warehouse), matched by socioeconomic status, lifestyle, and health. Area benzo[a]pyrene levels were quantified in the various work areas, and the primary endpoint was the level of early and late apoptosis in peripheral blood mononuclear cells (PBMCs) isolated from each worker subgroup the morning following an overnight fast. The authors self-designed a study questionnaire to gather information on worker education, vocational history, smoking and drinking habits, personal habits, and personal and family medical history, as well as any current symptoms and medications used in the previous several weeks. "Smoking" was defined as ≥10 cigarettes/day during the past year, with "smoking index" defined as cigarettes/day × years smoking. Similarly, "drinking" was defined as wine/beer/spirits consumed ≥3 times/week for the past 6 months, and "drinking index" was defined as grams of alcohol consumed/day × years drinking. Exposed workers were categorized by physical worksite location and expected differences in benzo[a]pyrene exposure: 34 oven bottom workers, 48 oven side workers, and 47 oven top workers. Workplace environmental sampling stations were established at each of the physical work locations, including the referent's warehouse, and dual automatic air sampling pumps collected samples at personal breathing zone height for 6 hours/day, over 3 consecutive days. Benzo[a]pyrene content was determined by HPLC, and relative exposure was compared to post-shift urine levels of a benzo[a]pyrene metabolite, 1-hydroxypyrene. Collected and purified PBMCs were incubated with Annexin-V and PI prior to analysis by flow cytometry; early apoptotic cells were considered to be Annexin V+/PI–, while late apoptotic cells were considered Annexin V+/PI+.

All apoptosis data were displayed graphically, and in all groupings, early:late apoptotic PBMCs occurred at an approximate 2:1 frequency. PBMC apoptosis was similar in each of the three coke oven worker groups, which were all statistically significantly higher than referents

(approximately 2-fold) for both early and late apoptosis. While self-reported smoking incidence varied significantly among the worker groups, stratification by smoking years or smoking index did not reveal any significant association with PBMC apoptosis. Multiple linear stepwise regression analysis suggested that urine 1-hydroxypyrene levels and years of coke oven operation were positively associated with increased early and late PBMC apoptosis (Table D-5), and that years of ethanol consumption was negatively associated with only early apoptosis. These associations were tested by stratifying workers into three groups by urinary 1-hydroxypyrene levels or coke oven operation years, and in both cases, the groups with the highest urinary metabolite levels or longest oven operating experience had statistically significantly higher levels of both early and late apoptotic PBMCs versus the lowest or shortest duration groups, respectively. Likewise, when sorted into groups based upon years of ethanol consumption, the highest ethanol "years of consumption" group had statistically significantly lower early apoptosis rates when compared to the lowest ethanol consuming group.

Table D-5. Background information on Chinese coke oven workers or warehouse controls exposed to benzo[a]pyrene in the workplace

Effect measured	Exposure group (ng/m³; mean ± SD)				
	10.2 ± 7.6	**19.5 ± 13.2**	**185.9 ± 38.6**	**1,623.5 ± 435.8**	
Number of subjects	**37**	**34**	**48**	**47**	***p*-value**
Background information (mean ± SD or %)					
Age (yrs)	37.16 ± 6.00	39.09 ± 5.53	36.98 ± 6.40	37.34 ± 6.78	0.451
Working years	17.35 ± 7.19	18.58 ± 7.23	16.78 ± 6.90	17.26 ± 7.44	0.742
Smoking	62.2	64.7	83.3	53.2	0.017
Drinking	24.3	41.2	39.6	44.7	0.259
Urine benzo[a]pyrene metabolite (µmol/mol creatinine; mean ± SD)					
1-Hydroxypyrene	2.78 ± 1.04	3.22 ± 0.81*	3.51 ± 0.55*	3.66 ± 0.58*	0.000

*$p < 0.05$ significantly different from control mean.

Source: Zhang et al. (2012).

D.3.2. Cancer-related Endpoints

Benzo[a]pyrene-Induced Cytogenetic Damage

Many studies measure cytogenetic damage as biomarkers of early biological effects, which also reflect exposure to genotoxic chemicals. Standard cytogenetic endpoints include chromosomal aberration (CA), sister chromatid exchange (SCE), micronucleus (MN) formation, hypoxanthine guanine phosphoribosyl transferase (hprt) mutation frequency, and glycophorin A mutation frequency (Gyorffy et al., 2008). These biomarkers are often incorporated in multi-endpoint

studies with other biomarkers of exposure. Because they indicate related but different endpoints, there is often a lack of correlation between the different categories of biomarkers.

Merlo et al. (1997) evaluated DNA adduct formation (measured by [^{32}P]-postlabelling) and MN in white blood cells (WBCs) of 94 traffic policemen versus 52 residents from the metropolitan area of Genoa, Italy. All study subjects wore personal air samplers for 5 hours of one work shift, and levels of benzo[a]pyrene and other PAHs were measured. Policemen were exposed to 4.55 ng benzo[a]pyrene/m^3 air, compared with urban residents who were exposed to 0.15 ng/m^3. DNA adduct levels in policemen were 35% higher than in urban residents (p = 0.007), but MN in urban residents were 20% higher than in policemen (p = 0.02). Linear regressions of DNA adducts and MN incidence, respectively, versus benzo[a]pyrene exposure levels did not reveal significant correlations.

Perera and coworkers assessed DNA damage in Finnish iron foundry workers in two separate studies and using three methodologies. Based on results from personal sampling and stationary monitoring in both studies, three levels of benzo[a]pyrene air concentrations were defined: low (<5 ng/m^3 benzo[a]pyrene), medium (5–12 ng/m^3), and high (>12 ng/m^3) (Perera et al., 1994; Perera et al., 1993). In the first study, involving 48 workers, several biomarkers were analyzed for dose-response and interindividual variability (Perera et al., 1993). PAH-DNA adducts were determined in WBCs using an immunoassay and enzyme-linked immunosorbent assay (ELISA) with fluorescence detection. Mutations at the hprt locus were also measured in WBC DNA. The latter assay is based on the fact that each cell contains only one copy of the hprt gene, which is located on the X-chromosome. While male cells have only one X-chromosome, female cells inactivate one of the two X-chromosomes at random. The gene is highly sensitive to mutations such that in the event of a crucial mutation in the gene, enzyme activity disappears completely from the cell. In addition, mutations at the glycophorin A gene locus were measured in RBCs. The glycophorin A mutation frequency was not correlated with either benzo[a]pyrene exposure or PAH-DNA adduct formation. However, both PAH-DNA adduct levels and hprt mutation frequency increased with increasing benzo[a]pyrene exposure. In addition, there was a highly significant correlation between incidence of hprt mutations and PAH-DNA adduct levels (p = 0.004).

In a second study, Perera et al. (1994) surveyed 64 iron foundry workers with assessments conducted in 2 successive years; 24 of the workers provided blood samples in both years. Exposure to benzo[a]pyrene, collected by personal and area sampling in the first year of the study, ranged from <5 to 60 ng/m^3 and was estimated to have decreased by 40% in the second year. The levels of PAH-DNA adducts were roughly 50% lower in the 2nd year, presumably reflecting decreased exposure. The longer-lived hprt mutations were not as strongly influenced by the decreasing exposure to benzo[a]pyrene. Study subjects who did not have detectable levels of DNA adducts were excluded from the study. As in the previous study, a strong correlation between DNA adduct levels and incidence of hprt mutations was observed (Perera et al., 1993).

Kalina et al. (1998) studied several cytogenetic markers in 64 coke oven workers and 34 controls employed at other locations within the same plant. Airborne benzo[a]pyrene and seven other carcinogenic PAHs were collected by personal air samplers, which showed ambient benzo[a]pyrene concentrations ranging widely from 0.002 to 50 μg/m^3 in coke oven workers and from 0.002 to 0.063 μg/m^3 in controls. CAs, SCEs, high-frequency cells (HFCs), and SCE heterogeneity index were all significantly increased with benzo[a]pyrene exposure. Except for increases in HFCs, no effect of smoking was observed. Consistent with studies of PAH-DNA adduct formation, reduced cytogenetic response at high exposure levels produced a nonlinear dose-response relationship. The authors also evaluated the potential influence of polymorphisms in enzymes involved in the metabolism of benzo[a]pyrene. GSTM1 and N-acetyl transferase-2 polymorphisms were studied and no evidence of the two gene polymorphisms having any influence on the incidence of cytogenetic damage was found.

Motykiewicz et al. (1998) conducted a similar study of genotoxicity associated with benzo[a]pyrene exposure in 67 female residents of a highly polluted industrial urban area of Upper Silesia, Poland, and compared the results to those obtained from 72 female residents of another urban but less polluted area in the same province of Poland. Urinary mutagenicity and 1-hydroxypyrene levels, PAH-DNA adducts in oral mucosa cells (detected by immunoperoxidase staining), SCEs, HFCs, CAs, bleomycin sensitivity, and GSTM1 and CYP1A1 polymorphisms in blood lymphocytes were investigated. High volume air samplers and gas chromatography were used to quantify ambient benzo[a]pyrene levels, which were 3.7 ng/m^3 in the polluted area and 0.6 ng/m^3 in the control area during the summer. During winter, levels rose to 43.4 and 7.2 ng/m^3 in the two areas, respectively. The cytogenetic biomarkers (CA and SCE/HFC), urinary mutagenicity, and urinary 1-hydroxypyrene excretion were significantly increased in females from the polluted area, and differences appeared to be more pronounced during winter time. PAH-DNA adduct levels were significantly increased in the study population, when compared to the controls, only in the winter season. No difference in sensitivity to bleomycin-induced lymphocyte chromatid breaks was seen between the two populations. As with the study by Kalina et al. (1998), genetic polymorphisms assumed to affect the metabolic transformation of benzo[a]pyrene were not associated with any difference in the incidence of DNA damage.

In a study of Thai school boys in urban (Bangkok) and rural areas, bulky (including but not limited to BPDE-type) DNA adduct levels were measured in lymphocytes along with DNA single-strand breaks (SSBs), using the comet assay, and DNA repair capacity (Tuntawiroon et al., 2007). Ambient air and personal breathing zone measurements indicated that Bangkok school children experienced significantly higher exposures to benzo[a]pyrene and total PAHs. A significantly higher level of SSBs (tail length 1.93 ± 0.09 versus 1.28 ± 0.12 μm, +51%; $p < 0.001$) was observed in Bangkok school children when compared with rural children, and this parameter was significantly associated with DNA adduct levels. A significantly reduced DNA repair capacity (0.45 ± 0.01 versus 0.26 ± 0.01 γ-radiation-induced deletions per metaphase, −42%; $p < 0.001$) was

also observed in the city school children, again significantly associated with DNA adduct levels. It was not evident why higher environmental PAH exposure would be associated with lowered DNA repair capacity. However, because the personal breathing zone PAH levels and DNA adduct levels were not associated with each other, it is conceivable that the city school children had a priori lower DNA repair capacities that contributed significantly to the high adduct levels. The authors considered genetic differences between the two study populations as a possible reason for this observation.

D.3.3. Epidemiologic Findings in Humans

The association between human cancer and contact with PAH-containing substances, such as soot, coal tar, and pitch, has been widely recognized since the early 1900s (Boström et al., 2002). Although numerous epidemiology studies establish an unequivocal association between PAH exposure and human cancer, defining the causative role for benzo[a]pyrene and other specific PAHs remains a challenge. In essentially all reported studies, either the benzo[a]pyrene exposure and/or internal dose are not known, or the benzo[a]pyrene carcinogenic effect cannot be distinguished from the effects of other PAH and non-PAH carcinogens. Nevertheless, three types of investigations provide support for the involvement of benzo[a]pyrene in some human cancers: molecular epidemiology studies; population- and hospital-based, case-control studies; and occupational cohort studies. In some cohort studies, benzo[a]pyrene exposure concentrations were measured and thus provide a means to link exposure intensity with observed cancer rates. In case-control studies, by their nature, benzo[a]pyrene and total PAH doses can only be estimated.

Molecular Epidemiology and Case-Control Cancer Studies

Defective DNA repair capacity leading to genomic instability and, ultimately, increased cancer risk is well documented (Wu et al., 2007; Wu et al., 2005). Moreover, sensitivity to mutagen-induced DNA damage is highly heritable and thus represents an important factor that determines individual cancer susceptibility. Based on studies comparing monozygotic and dizygotic twins, the genetic contribution to BPDE mutagenic sensitivity was estimated to be 48.0% (Wu et al., 2007). BPDE has been used as an etiologically relevant mutagen in case-control studies to examine the association between elevated lung and bladder cancer risk and individual sensitivity to BPDE-induced DNA damage. Mutagen sensitivity is determined by quantifying chromatid breaks or DNA adducts in phytohemagglutinin-stimulated peripheral blood lymphocytes as an indirect measure of DNA repair capacity.

In a hospital-based, case-control study involving 221 lung cancer cases and 229 healthy controls, DNA adducts were measured in stimulated peripheral blood lymphocytes after incubation with BPDE in vitro (Li et al., 2001). Lung cancer cases showed consistent statistically significant elevations in induced BPDE-DNA adducts in lymphocytes, compared with controls, regardless of subgroup by age, sex, ethnicity, smoking history, weight loss, or family history of cancer. The lymphocyte BPDE-induced DNA adduct levels, when grouped by quartile using the levels in controls

as cutoff points, were significantly dose-related with lung cancer risk (ORs 1.11, 1.62, and 3.23; trend test, $p < 0.001$). In a related hospital-based, case-control study involving 155 lung cancer patients and 153 healthy controls, stimulated peripheral blood lymphocytes were exposed to BPDE in vitro (Wu et al., 2005). DNA damage/repair was evaluated in lymphocytes using the comet assay, and impacts on cell cycle checkpoints were measured using a fluorescence-activated cell-sorting method. The lung cancer cases exhibited significantly higher levels of BPDE-induced DNA damage than the controls ($p < 0.001$), with lung cancer risk positively associated with increasing levels of lymphocyte DNA damage when grouped in quartiles (trend test, $p < 0.001$). In addition, lung cancer patients demonstrated significantly shorter cell cycle delays in response to BPDE exposure to lymphocytes, which correlated with increased DNA damage.

Sensitivity to BPDE-induced DNA damage in bladder cancer patients supports the results observed in lung cancer cases. In a hospital-based, case-control study involving 203 bladder cancer patients and 198 healthy controls, BPDE-induced DNA damage was specifically evaluated at the chromosome 9p21 locus in stimulated peripheral blood lymphocytes (Gu et al., 2008). Deletions of 9p21, which includes critical components of cell cycle control pathways, are associated with a variety of cancers. After adjusting for age, sex, ethnicity, and smoking status, individuals with high BPDE-induced damage at 9p21 were significantly associated with increased bladder cancer risk (OR 5.28; 95% CI 3.26–8.59). Categorization of patients into tertiles for BPDE sensitivity relative to controls demonstrated a dose-related association between BPDE-induced 9p21 damage and bladder cancer risk. Collectively, the results of molecular epidemiology studies with lung and bladder cancer patients indicate that individuals with a defective ability to repair BPDE-DNA adducts are at increased risk for cancer and, moreover, that specific genes linked to tumorigenesis pathways may be molecular targets for benzo[a]pyrene and other carcinogens.

Due to the importance of the diet as a benzo[a]pyrene exposure source, several population- and hospital-based, case-control studies have investigated the implied association between dietary intake of benzo[a]pyrene and risk for several tumor types. In a study involving 193 pancreatic cancer cases and 674 controls (Anderson et al., 2005), another involving 626 pancreatic cancer cases and 530 controls (Li et al., 2007), and a third involving 146 colorectal adenoma cases and 228 controls (Sinha et al., 2005), dietary intake of benzo[a]pyrene was estimated using food frequency questionnaires. In all studies, the primary focus was on estimated intake of benzo[a]pyrene (and other carcinogens) derived from cooked meat. Overall, cases when compared with controls, had higher intakes of benzo[a]pyrene and other food carcinogens, leading to the conclusion that benzo[a]pyrene plays a role in the etiology of these tumors in humans. In a supportive follow-up case-control study of colorectal adenomas, levels of leukocyte PAH-DNA adducts were significantly higher in cases when compared with controls ($p = 0.02$), using a method that recognizes BPDE and several other PAHs bound to DNA (Gunter et al., 2007).

Occupational Cancer Cohort Studies

Epidemiologic studies of workers in PAH-related occupations indicate increased human cancer risks associated with iron and steel production, roofing, carbon black production, and exposure to diesel exhaust (Bosetti et al., 2007). Exposure to benzo[a]pyrene is only one of numerous contributors to the cancer risk from complex PAH-containing mixtures that occur in the workplace. Although some occupational cohort studies report measured or estimated inhalation exposure concentrations for benzo[a]pyrene, none report biomarkers of internal benzo[a]pyrene dose in study subjects (reviewed in Bosetti et al., 2007; Armstrong et al., 2004). Several of these cohort studies (summarized below) demonstrate a positive exposure-response relationship with cumulative PAH exposure using benzo[a]pyrene—or a proxy such as benzene-soluble matter (BSM) that can be converted to benzo[a]pyrene—as an indicator substance. These studies provide insight and support for the causative role of benzo[a]pyrene in human cancer.

Cancer incidence in aluminum and electrode production plants

Exposure to benzo[a]pyrene and BSM in aluminum smelter workers is strongly associated with bladder cancer and weakly associated with lung cancer (Boffetta et al., 1997; Tremblay et al., 1995; Armstrong et al., 1994; Gibbs, 1985; Theriault et al., 1984). In an analysis of pooled data from nine cohorts of aluminum production workers, 688 respiratory tract cancer cases were observed versus 674.1 expected (pooled RR 1.03; CI 0.96–1.11) (Bosetti et al., 2007). A total of 196 bladder cancer cases were observed in eight of the cohorts, compared with 155.7 expected (pooled RR 1.29; CI 1.12–1.49). Based on estimated airborne benzo[a]pyrene exposures from a meta-analysis of eight cohort studies, the predicted lung cancer RR per 100 μg/m^3-years of cumulative benzo[a]pyrene exposure was 1.16 (95% CI 1.05–1.28) (Armstrong et al., 2004).

Spinelli et al. (2006) reported a 14-year update to a previously published historical cohort study (Spinelli et al., 1991) of Canadian aluminum reduction plant workers. The results confirmed and extended the findings from the earlier epidemiology study. The study surveyed a total of 6,423 workers with ≥3 years of employment at an aluminum reduction plant in British Columbia, Canada, between the years 1954 and 1997, and evaluated all types of cancers. The focus was on cumulative exposure to coal tar pitch volatiles, measured as BSM and as benzo[a]pyrene. Benzo[a]pyrene exposure categories were determined from the range of predicted exposures over time from statistical exposure models. There were 662 cancer cases, of which approximately 98% had confirmed diagnoses. The overall cancer mortality rate (SMR 0.97; CI 0.87–1.08) and cancer incidence rate (standardized incidence ratio [SIR] 1.00; CI 0.92–1.08) were not different from that of the British Columbia general population. However, this study identified significantly increased incidence rates for cancers of the bladder (SIR 1.80; CI 1.45–2.21) and stomach (SIR 1.46; CI 1.01–2.04). The lung cancer incidence rate was only slightly higher than expected (SIR 1.10; CI 0.93–1.30). Significant dose-response associations with cumulative benzo[a]pyrene exposure were seen for bladder cancer ($p < 0.001$), stomach cancer ($p < 0.05$), lung cancer ($p < 0.001$), non-

Hodgkin lymphoma ($p < 0.001$), and kidney cancer ($p < 0.01$), although the overall incidence rates for the latter three cancer types were not significantly elevated versus the general population. Similar cancer risk results were obtained using BSM as the exposure measure; the cumulative benzo[a]pyrene and BSM exposures were highly correlated (r = 0.94).

In several occupational cohort studies of workers in Norwegian aluminum production plants, personal and stationary airborne PAH measurements were performed.

In a study covering 11,103 workers and 272,554 person × years of PAH exposure, cancer incidence was evaluated in six Norwegian aluminum smelters (Romundstad et al., 2000a) and (Romundstad et al., 2000b). Reported estimates of PAH exposure concentrations reached a maximum of 3,400 μg/m^3 PAH (680 μg/m^3 benzo[a]pyrene). The overall number of cancers observed in this study did not differ significantly from control values (SIR 1.03; CI 1.0–1.1). The data from this study showed significantly increased incidences for cancer of the bladder (SIR 1.3; CI 1.1–1.5) and elevated, but not significant, SIRs for larynx (SIR 1.3; CI 0.8–1.9), thyroid (SIR 1.4; CI 0.7–2.5), and multiple myeloma (SIR 1.4; CI 0.9–1.9). Incidence rates for bladder, lung, pancreas, and kidney cancer (the latter three with SIRs close to unity) were subjected to a cumulative exposure-response analysis. The incidence rate for bladder cancer showed a trend with increasing cumulative exposure and with increasing lag times (up to 30 years) at the highest exposure level. The incidence of both lung and bladder cancers was greatly increased in smokers. The authors reported that using local county rates rather than national cancer incidence rates as controls increased the SIR for lung cancer (SIR 1.4; CI 1.2–1.6) to a statistically significant level.

Cancer incidence in coke oven, coal gasification, and iron and steel foundry workers

An increased risk of death from lung and bladder cancer is reported in some studies involving coke oven, coal gasification, and iron and steel foundry workers (Boström et al., 2002; Boffetta et al., 1997). An especially consistent risk of lung cancer across occupations is noted when cumulative exposure is taken into consideration (e.g., RR of 1.16 per 100 unity-years for aluminum smelter workers, 1.17 for coke oven workers, and 1.15 for coal gasification workers). In an analysis of pooled data from 10 cohorts of coke production workers, 762 lung cancer cases were observed versus 512.1 expected (pooled RR 1.58; CI 1.47–1.69) (Bosetti et al., 2007). Significant variations in risk estimates among the studies were reported, particularly in the large cohorts (RRs of 1.1, 1.2, 2.0, and 2.6). There was no evidence for increased bladder cancer risk in the coke production workers. Based on estimated airborne benzo[a]pyrene exposures from a meta-analysis of 10 cohort studies, the predicted lung cancer RR per 100 μg/m^3-years of cumulative benzo[a]pyrene exposure was 1.17 (95% CI 1.12–1.22) (Armstrong et al., 2004).

A meta-analysis of data from five cohorts of gasification workers reported 251 deaths from respiratory tract cancer, compared with 104.7 expected (pooled RR 2.58; 95% CI 2.28–2.92) (Bosetti et al., 2007). Pooled data from three of the cohorts indicated 18 deaths from urinary tract cancers, versus 6.0 expected (pooled RR 3.27; 95% CI 2.06–5.19). Based on estimated airborne benzo[a]pyrene exposures from a meta-analysis of four gas worker cohort studies, the predicted

lung cancer RR per 100 μg/m^3-years of cumulative benzo[a]pyrene exposure was 1.15 (95% CI 1.11–1.20) (Armstrong et al., 2004).

Increased risks were reported in iron and steel foundry workers for cancers of the respiratory tract, bladder, and kidney. In an analysis of pooled data from 10 cohorts, 1,004 respiratory tract cancer cases were observed versus 726.0 expected (pooled RR 1.40; CI 1.31–1.49) (Bosetti et al., 2007). A total of 99 bladder cancer cases were observed in seven of the cohorts, compared with 83.0 expected (pooled RR 1.29; CI 1.06–1.57). For kidney cancer, 40 cases were observed compared with 31.0 expected based on four studies (pooled RR 1.30; 95% CI 0.95–1.77).

Xu et al. (1996) conducted a nested case-control study, surveying the cancer incidence among 196,993 active or retired workers from the Anshan Chinese iron and steel production complex. A large number of historical benzo[a]pyrene measurements (1956–1995) were available. The study included 610 cases of lung cancer and 292 cases of stomach cancer, with 959 age- and gender-matched controls from the workforce. After adjusting for nonoccupational risk factors such as smoking and diet, significantly elevated risks for lung cancer and stomach cancer were identified for subjects employed for ≥15 years, with ORs varying among job categories. For either type of cancer, highest risks were seen among coke oven workers: lung cancer, OR = 3.4 (CI 1.4–8.5) and stomach cancer, OR = 5.4 (CI 1.8–16.0).

There were significant trends for long-term, cumulative benzo[a]pyrene exposure versus lung cancer (p = 0.004) or stomach cancer (p = 0.016) incidence. For cumulative total benzo[a]pyrene exposures of <0.84, 0.85–1.96, 1.97–3.2, and ≥3.2 μg/m^3-year, the ORs for lung cancer were 1.1 (CI 0.8–1.7), 1.6 (CI 1.2–2.3), 1.6 (1.1–2.3), and 1.8 (CI 1.2–2.5), respectively. For cumulative total benzo[a]pyrene exposures of <0.84, 0.85–1.96, 1.97–3.2, and ≥3.2 μg/m^3-year, the ORs for stomach cancer were 0.9 (CI 0.5–1.5), 1.7 (CI 1.1–2.6), 1.3 (0.8–2.1), and 1.7 (CI 1.1–2.7), respectively. However, the investigators noted that additional workplace air contaminants were measured, which might have influenced the outcome. Of these, asbestos, silica, quartz, and iron oxide-containing dusts may have been confounders. For lung cancers, cumulative exposures to total dust and silica dust both showed significant dose-response trends (p = 0.001 and 0.007, respectively), while for stomach cancer, only cumulative total dust exposure showed a marginally significant trend (p = 0.061). For cumulative total dust exposures of <69, 69–279, 280–882, and ≥883 mg/m^3, the ORs for lung cancer were 1.4 (CI 1.2–1.9), 1.2 (CI 1.0–2.19), 1.4 (CI 1.0–2.0), and 1.9 (CI 1.3–2.5), respectively. For cumulative silica dust exposures of <3.7, 3.7–10.39, 10.4–27.71, and ≥27.72 mg/m^3, the ORs for lung cancer were 1.7 (CI 1.2–2.4), 1.5 (CI 1.0–2.1), 1.5 (CI 1.0–2.1), and 1.8 (CI 1.2–2.5), respectively. For cumulative total dust exposures of <69, 69–279, 280–882, and ≥883 mg/m^3, ORs for stomach cancer were 1.3 (CI 0.8–2.1), 14 (CI 0.9–2.2), 12 (CI 0.8–1.9), and 1.6 (CI 1.1–2.5), respectively.

Exposure-response data from studies of coke oven workers in the United States have often been used to derive quantitative risk estimates for PAH mixtures, and for benzo[a]pyrene as an

indicator substance (Boström et al., 2002). However, there are numerous studies of coke oven worker cohorts that do not provide estimates of benzo[a]pyrene exposure. An overview of the results of these and other studies can be obtained from the review of Boffetta et al. (1997).

Cancer incidence in asphalt workers and roofers

These groups encompass different types of work (asphalt paving versus roofing) and also different types of historical exposure that have changed from using PAH-rich coal tar pitch to the use of bitumen or asphalt, both of which are rather low in PAHs due to their source (crude oil refinery) and a special purification process. Increased risks for lung cancer were reported in large cohorts of asphalt workers and roofers; evidence for increased bladder cancer risk is weak (Burstyn et al., 2007; Partanen and Boffetta, 1994; Chiazze et al., 1991; Hansen, 1991, 1989; Hammond et al., 1976). In an analysis of pooled data from two cohorts of asphalt workers, 822 lung cancer cases were observed versus 730.7 expected (pooled RR 1.14; 95% CI 1.07–1.22) (Bosetti et al., 2007). In two cohorts of roofers, analysis of pooled data indicated that 138 lung cancer cases were observed, compared with 91.9 expected (pooled RR 1.51; 95% CI 1.28–1.78) (Bosetti et al., 2007).

Epidemiology of patients treated with coal tar containing ointments

In addition to cohorts of workers occupationally exposed to PAH mixtures, another source of potential exposure to benzo[a]pyrene is through topical coal tar formulations used for the treatment of psoriasis, eczema, and dermatitis. Epidemiological studies examining skin cancer risk in relation to various types of topical coal tar exposure are summarized below (see Table D-6); case reports, reviews, and studies that did not include a measure of coal tar use (e.g., Alderson and Clarke, 1983) are not included.

Table D-6. Studies examining skin cancer risk in relation to therapeutic coal tar

Reference and study details	Results
General population studies	
Mitropoulos and Norman (2005) (United States, Arizona) Case-control study (Southeastern Arizona Health Study-2), population-based; n = 404 squamous cell skin cancer cases, 395 controls, 1992–1996, age ≥30 yrs; controls selected using random digit dialing (frequency matched by 5-yr age group and gender); limited to whites; details regarding participation rates not reported	Squamous cell carcinoma (SCC), coal tar/dandruff shampoo use: Cases n (%) | Controls n (%) | OR[a] (95% CI) | OR[b] (95% CI) 101 (25) | 73 (19) | 1.50 (1.05, 2.14) | 1.28 (0.85, 1.9) [a]Adjusted for age and gender. [b]Adjusted for age, gender, actinic keratosis, current number of arm freckles, and reaction of skin to prolonged sun.

Reference and study details	Results
Exposure: Interview, focusing on occupational and other sources of sun exposure, chemical exposures, and coal tar/dandruff shampoo Outcome: Incident squamous cell cancer from regional skin cancer registry	
Studies of patients with skin conditions	
Roelofzen et al. (2010) (Netherlands) Cohort (retrospective); total n = 13,200 (4,315 psoriasis 8,885 eczema patients), identified through hospital records (manual). Diagnosed 1960−1990 (≥3 visits to dermatologist); median age 28 yrs; follow-up through 2003 (median follow-up 21 yrs) Exposure: Coal tar treatment (pix lithantracis and/or liquor carbonis detergens): 8,062 (39%); duration of use obtained from 1,100 users (14%), median = 6 mo Outcome: Skin cancer diagnosis from national cancer registry (operating since 1989) and cause of death registries, with some supplemental questionnaire data from 61% of the cohort	Skin cancer (excluding basal cell carcinoma); includes melanoma and squamous cell [number of cases = 145] HR (95% CI) for use of coal tar; referent category = only used dermatocorticosteroids: Psoriasis 1.08 (0.43, 2.72) Eczema 1.06 (0.62, 1.83) Psoriasis or eczema 1.09 (0.69, 1.72) Proportional hazards models, adjusted for age (continuous), gender, severity (>10% of body area affected), interaction term of coal tar and severity, calendar period, psoralen + ultraviolet-A (PUVA) systemic therapy, and smoking (current and ever versus never). Also examined skin type, history of sun exposure, and alcohol consumption. Smoking data imputed for 58% of the cohort.
Jemec and Østerlind (1994) (Denmark) Cohort (retrospective); n = 88 patients hospitalized for atopic dermatitis/eczema between 1917 and 1937; mean follow-up 38.5 yrs Exposure: Extensive treatment with coal tar was inferred based on the knowledge that this was the recommended treatment at the time of the patients' hospitalization Outcome: Incident cancer diagnosis between January 1943 and December, 1986 as determined by national cancer registry; comparison with general population cancer rates	No skin cancers observed. Authors noted that non-melanoma skin cancers may have been underreported in older records as there was no general record for this endpoint in the registry.

Reference and study details	Results
Jones et al. (1985) (Scotland) Cohort (retrospective); n = 719 psoriasis patients not treated with cytotoxic drugs, ionizing radiation, or UV therapy; age range from <15 to >64 yrs Exposure: Past intermittent treatment with tar for a 10-yr period between 1953 and 1973 as determined by clinic records; median age at start of therapy 27 yrs (male) and 23 yrs (female); exposure not quantified Outcome: Incident skin cancer diagnosis from regional cancer registry	Expected rates calculated from cancer registry data for group of the same size and age as patient population (not further described). Skin cancer with coal tar usage: Observed / Expected Males (n = 305): 3 / 0.9 Females (n = 414): 0 / 0.7
Bhate et al. (1993) (United Kingdom) Prevalence study within cohort of 2,247 psoriasis patients; mean age 41 yrs Exposure: Past treatment with tar and other therapeutics determined from medical records; exposure not quantified and duration not provided Outcome: Skin cancer diagnosis obtained from patient records and confirmed by medical examination	Skin cancer prevalence (percentage) among psoriatic patients treated with coal tar: Male: 9/781 (1%) Female: 21/980 (2%) Referent group not treated with coal tar was not included.
Coal tar use in studies with combined treatment with ultraviolet-B (UVB) therapy (Goeckerman regimen)	
Hannuksela-Svahn et al. (2000) (Finland) Nested case-control study within cohort of 5,687 patients hospitalized with a diagnosis of psoriasis between 1973 and 1984; n = 30 with squamous cell carcinoma (SCC) and n = 137 sex- and age-matched referents without skin cancer; followed until 1995 Exposure: Prior treatment with Goeckerman regimen or its modifications determined from hospital files; magnitude and duration of exposure not reported Outcome: SCC diagnosis determined from national cancer registry and confirmed by review of hospital records	RR (95% CI) of skin cancer with Goeckerman treatment: SCC: 1.5 (0.3–7.3)

Reference and study details	Results
Torinuki and Tagami (1988) (Japan) Cohort (prospective); total n = 151 psoriasis patients including 43 treated with Goeckerman regimen without PUVA treatment, mean age 43 yrs; patients treated between 1976−1986; follow-up: 5/43 Goeckerman patients followed for >6 yrs Exposure: Goeckerman regimen without PUVA treatment; duration of use not reported Outcome: Skin cancer diagnosis from case records	No skin cancers observed.
Maughan et al. (1980) (United States, Mayo Clinic) Cohort (retrospective); n = 426 atopic dermatitis or neurodermatitis patients, treated with Goeckerman regimen between 1950−1954; follow-up: 305 (72%) followed to approximately 1980 (25 yrs) Exposure: Goeckerman regimen (UVB + coal tar treatments) at hospital; follow-up questionnaire inquired about other treatment (including coal tar treatment) after hospitalization; coal tar use ranged from none to every day for 26 yrs Outcome: Skin cancer diagnosis by self-report (follow-up questionnaire) with confirmation through histology specimens; 9 of 11 nonmelanoma skin cancers confirmed	Eleven nonmelanoma skin cancer cases (observed) [eight basal cell, one squamous cell, two unknown] Expected rates from Third National Cancer Survey: Observed/Expected — Expected Minneapolis-St Paul — 6.7 — 1.64 San Francisco-Oakland — 9.4 — 1.17 Iowa — 5.3 — 2.08 Dallas-Fort Worth — 18.8 — 0.59 No difference in duration of coal tar use after hospitalization in skin cancer patients compared to those who did not develop skin cancer.
Pittelkow et al. (1981) (United States, Mayo Clinic) Cohort (retrospective); n = 280 psoriasis patients, hospitalized 1950−1954 at Mayo Clinic; 260 (92%) followed to 1978 (25 yrs) Exposure: Goeckerman regimen (UVB + coal tar treatments) at hospital; other treatment (including coal tar treatment) recorded from clinical records. Median duration use	Among patients reporting coal tar therapy use: n = 19 nonmelanoma squamous cell or basal cell (or unknown) skin cancer cases (observed) Expected rates from Third National Cancer Survey: Observed/Expected — Expected Minneapolis-St Paul — 18.7 — 1.01 San Francisco-Oakland — 23.1 — 0.82 Iowa — 15.5 — 1.22 Dallas-Fort Worth — 49.2 — 0.39

Reference and study details	Results
approximately 15 d in 1951−1955 and 21 d in 1956−1960 Outcome: Skin cancer diagnosis by self-report (follow-up questionnaire) with confirmation through histology specimens; 20 of 22 confirmed	
Coal tar use in studies with combined treatment of PUVA therapy	
Stern et al. (1998); Stern and Laird (1994) (United States, 16 centers) Cohort (prospective); total n = 1,380 psoriasis patients, enrolled between 1975 and 1976 in the PUVA cohort study; mean age 44 yrs; follow-up at 12−15-mo intervals through 1996 (approximately 20 years); 1,049 (91%) patients interviewed at final follow-up Exposure: Non-PUVA treatments (including topical coal tar, ultraviolet B, methotrexate, and ionizing radiation) were collected at start of PUVA treatment and during follow-up; coal tar use was noted to be highly correlated with UVB therapy and thus reported as a single parameter; 'high use' defined as >45 mo topical tar therapy or >300 UVB treatments Outcome: Skin cancer diagnosis reported at follow-up, confirmed by histopathology	From 1996 follow-up (limited to first occurrence 1986−1996): Cancer type — OR (95% CI) [n cases] Squamous — 1.4 (1.0, 2.0) [1,047] Basal cell — 1.5 (1.1, 2.0) [821] OR compares 'high' exposure to UVB/tar to 'low' exposure to UVB/tar, adjusted for age, sex, geographic area, anatomic site (head and neck, other), PUVA treatments through 1985 (five categories from <100 to >336), PUVA treatments after 1985 (≥50, <50), methotrexate (≥208 wks, <208 wks), and Grenz rays or x-rays for therapy (ever/never)
Maier et al. (1996) (Austria) Cohort (retrospective); n = 496 psoriasis patients with more than 5 PUVA treatments and first treatment before 1987; median age 50 yrs; median follow-up was 82 mo Exposure: Non-PUVA treatments (arsenic, x-rays, tar, UVB, and methotrexate) were determined by interview Outcome: Skin cancer diagnosis determined by interview or biopsy at time of follow-up	RR (*p*-value) of skin carcinoma with coal tar usage and more than 5 PUVA treatments (partial analysis): Basal cell and squamous cell — 3.83 (0.04) Squamous cell — 7.85 (0.061) Multivariate partial analysis considered sex, age, skin type, cumulative UVA dose, and exposure to arsenic, x-rays, UVB, and methotrexate.

Reference and study details	Results
Stern et al. (1980) (United States, 16 centers) Nested case-control study based on a study following 1,373 PUVA-treated patients (34 incident cases, 24 prevalent cases; 126 controls); matched by age (within 5 yrs), sex, skin type, geographic area, and ionizing radiation; incident cases also matched for number of PUVA treatments; average follow-up 2.7 yrs Exposure: Exposure to coal tar therapy and/or ultraviolet radiation based on follow-up interview; includes exposures before PUVA trial began; coal tar use quantified as number of months in which crude coal tar preparations was used at least weekly; high coal tar exposure defined as >90 mo of use; high ultraviolet radiation exposure defined as ≥300 sunlamp treatments; assumption made that coal tar and ultraviolet radiation have the same quantitative effect on risk of skin cancer Outcome: Skin cancer, prevalent cases occurred before PUVA trial started; incident cases occurred during follow-up period	RR (95% CI) of skin cancer (skin cancer type not specified) among high exposure (≥90 mo of tar use or ≥300 sunlamp treatments) Matched analysis: All cases (n = 58) 4.7 (2.2, 10.0) Incident cases (n = 34) 5.6 (1.9, 16.2) Prevalent cases (n = 24) 3.8 (1.2, 12.5)
Lindelöf and Sigurgeirsson (1993) (Sweden) Nested case-control study based on a study following 4,799 PUVA-treated patients (24 cases, 96 controls); matched by gender, age, diagnosis, PUVA dose, number of treatments, type of psoralen regimen, site of treatment, and skin type; clinic location matching utilized when possible; mean age 52 yrs Exposure: Non-PUVA treatments (including tar, topical corticosteroids, UVB, and anthralin) collected by questionnaire; exposure not quantified and duration not provided Outcome: Skin cancer diagnosis obtained from Swedish cancer registry	SCC with coal tar usage: Cases n (%) Controls n (%) OR (95% CI) 17 (70) 62 (64) 1.3 (0.5, 3.5) (Similar results were seen for UVB exposure [OR 1.3, 95% CI 0.5, 3.5], reflecting the high correlation between these treatments)

The U.S. Environmental Protection Agency (EPA) noted several limitations with respect to study design and analysis in this literature, precluding the ability to provide a foundation for evaluating the potential association between use of therapeutic coal tar treatment (particularly

long-term treatment) and risk of skin cancer. A primary limitation concerns the quality of the exposure assessment. Only one population-based, case-control study was identified (Mitropoulos and Norman, 2005); this study examined self-reported use of coal tar/dandruff shampoo and incidence of squamous cell cancer in a population in Arizona (adjusted OR 1.28, 95% CI 0.85, 1.9). This exposure measure is likely to be highly susceptible to misclassification bias. EPA considered the likelihood of non-differential misclassification to be high; differential misclassification was also considered to be possible, but of lower likelihood. Non-differential misclassification would arise from lack of awareness of the content of shampoos, inability to recall use of individual shampoos, and the lack of specificity of this particular question. Differential misclassification would arise from differential reporting based on disease status. EPA noted similar concerns regarding exposure quality in the nested case-control study conducted among patients receiving psoralen plus ultraviolet-A (PUVA) treatment (in addition to a variety of other treatments, including coal tar treatments and ultraviolet-B [UVB]) by Lindelöf and Sigurgeirsson (1993). Use of coal tar was collected through a mailed questionnaire, with no information on duration of use and no verification with medical records. A large study of psoriasis and eczema patients (n = 13,200 patients) by Roelofzen et al. (2010) with a 21-year follow-up period obtained data on coal tar treatment through manual chart review; this chart review was conducted in 2003 on medical records going back to 1960. Duration of use (median 6 months) was available for only 14% of the patients who had an indication of use. Thus, considerable non-differential misclassification of exposure (coal tar use) is likely, and the limited exposure data did not allow examination of variation in exposure level. Misclassification of disease was also noted to be a limitation of this study in that Roelofzen et al. (2010) included melanoma, in addition to squamous cell skin cancer, which introduces a lack of specificity of outcome into the analysis as melanoma is not thought to be associated with PAH exposure. Given these issues of exposure and disease misclassification, the RRs from these studies do not provide a sound basis for interpretation as no risk, and would be expected to diminish effect estimates.

Potential misclassifications of both exposure and outcome were also important limitations of the study by Jemec and Østerlind (1994). In this study, coal tar treatment was inferred (not established based on medical records or patient recall) based on the widespread use of this treatment between 1917 and 1937; in addition, the authors noted that nonmelanoma skin cancers were likely underreported in the early years of the cancer registry used to identify cases (Jemec and Østerlind, 1994). While Bhate et al. (1993) used patient medical records to determine exposure and skin cancer diagnosis, this study reported only the prevalence of skin cancer in psoriasis patients treated with coal tar; a referent group of patients not treated with coal tar was not included for comparison. Similarly, Jones et al. (1985) compared the skin cancer incidences in psoriatic patients treated with coal tar with cancer rates estimated from regional cancer registry data for a group of the same size and age as the patient population. Because the referent group did

not consist exclusively of psoriasis patients, the influence of coal tar treatment on skin cancer risk cannot be distinguished from the role of psoriasis in development of skin cancer.

A common regimen for treatment of psoriasis and other skin conditions combines coal tar treatment with UVB radiation (referred to as the Goeckerman regimen). One study of this regimen was very small (n = 43 patients) with only 5 of the patients followed for more than 6 years (Torinuki and Tagami, 1988). Two larger Goeckerman treatment studies (280–426 patients) had a longer follow-up period (25 years), but were limited in terms of the choice of referent groups and differences in disease ascertainment between cases and the reference population (Pittelkow et al., 1981; Maughan et al., 1980). Specifically, dermatology patients were seen at the Mayo Clinic in Rochester, Minnesota, but the reference rates for cancer were obtained from survey data from Minneapolis-St Paul, San Francisco-Oakland, Iowa, and Dallas-Fort Worth. Therefore, it is unclear whether the reference population appropriately represents the case population. In a nested case-control study examining skin cancer and treatment with the Goeckerman regimen, disease ascertainment was accomplished using both a national cancer registry and review of patient files (Hannuksela-Svahn et al., 2000). However, this study is limited by potential misclassification of exposure, because exposure information was obtained only from hospital records, so coal tar treatment in an outpatient setting was not considered. In addition, the combination of UVB and coal tar in the Goeckerman regimen makes it impossible to attribute risk to either individual component. This limitation also affects the interpretation of the results of PUVA trial studies (Stern et al., 1998; Stern and Laird, 1994; Stern et al., 1980) in which the analysis was conducted using a definition of "high" exposure as >4 months of topical tar therapy or >300 UVB treatments. Similarly, the study by Lindelöf and Sigurgeirsson (1993) reported similar prevalence and risk estimates for coal tar use and for UVB, reflecting the high correlation between these treatments. Another study of skin cancer risk in psoriatic patients treated more than 5 times with PUVA did not report similar risk estimates for coal tar and UVB (Maier et al., 1996); however, both exposure to non-PUVA treatments and skin cancer diagnosis were determined by patient recall, a method that is susceptible to both exposure and outcome misclassification.

In summary, the available studies examining therapeutic topical coal tar use and risk of skin cancer were limited by low-quality exposure data with high potential of exposure misclassification (e.g., Roelofzen et al., 2010; Mitropoulos and Norman, 2005; Hannuksela-Svahn et al., 2000; Maier et al., 1996; Jemec and Østerlind, 1994; Lindelöf and Sigurgeirsson, 1993); significant potential for outcome misclassification (e.g., Jemec and Østerlind, 1994); small size (e.g., Jemec and Østerlind, 1994; Torinuki and Tagami, 1988); short duration of follow-up (e.g., Torinuki and Tagami, 1988); choice of referent group (e.g., Bhate et al., 1993; Jones et al., 1985; Pittelkow et al., 1981; Maughan et al., 1980); and/or differences in disease ascertainment between cases and the reference population (e.g., Pittelkow et al., 1981; Maughan et al., 1980). In addition, clinic-based studies focused on the commonly used regimen of coal tar in conjunction with UVB therapy cannot distinguish effects of coal tar from the carcinogenic effects of UVB (e.g., Hannuksela-Svahn et al.,

2000; Torinuki and Tagami, 1988; Pittelkow et al., 1981; Maughan et al., 1980). Likewise, clinic-based studies of coal tar use among patients also treated with PUVA cannot discern the effects of coal tar from those of PUVA (e.g., Stern et al., 1998; Maier et al., 1996; Stern and Laird, 1994; Lindelöf and Sigurgeirsson, 1993; Stern et al., 1980). Therefore, the available studies do not provide an adequate basis for examining the potential association between coal tar treated patients and skin cancer.

D.4. ANIMAL STUDIES

D.4.1. Oral Bioassays

Subchronic Studies

De Jong et al. (1999) treated male Wistar rats (eight/dose group) with benzo[a]pyrene (98.6% purity) dissolved in soybean oil by gavage 5 days/week for 35 days at doses of 0, 3, 10, 30, or 90 mg/kg-day (adjusted doses: 0, 2.14, 7.14, 21.4, and 64.3 mg/kg-day). At the end of the exposure period, rats were necropsied, organ weights were determined, and major organs and tissues were prepared for histological examination (adrenals, brain, bone marrow, colon, caecum, jejunum, heart, kidney, liver, lung, lymph nodes, esophagus, pituitary, spleen, stomach, testis, and thymus). Blood was collected for examination of hematological endpoints, but there was no indication that serum biochemical parameters were analyzed. Immune parameters included determinations of serum immunoglobulin (Ig) levels (IgG, IgM, IgE, and IgA), relative spleen cell distribution, and spontaneous cytotoxicity of spleen cell populations determined in a natural-killer (NK) cell assay.

Body weight gain was decreased beginning at week 2 at the high dose of 90 mg/kg-day; there was no effect at lower doses (De Jong et al., 1999). Hematology revealed a dose-related decrease in RBC count, hemoglobin, and hematocrit at ≥10 mg/kg-day (Table D-7). A minimal but significant increase in mean cell volume and a decrease in mean cell hemoglobin concentration were noted at 90 mg/kg-day, and may indicate dose-related toxicity for the RBCs and/or RBC precursors in the bone marrow. A decrease in WBCs, attributed to a decrease in the number of lymphocytes (approximately 50%) and eosinophils (approximately 90%), was observed at 90 mg/kg-day; however, there was no effect on the number of neutrophils or monocytes. A decrease in the cell number in the bone marrow observed in the 90 mg/kg-day dose group was consistent with the observed decrease in the RBC and WBC counts at this dose level. In the 90 mg/kg-day dose group, brain, heart, kidney, and lymph node weights were decreased and liver weight was increased (Table D-7). Decreases in heart weight at 3 mg/kg-day and in kidney weight at 3 and 30 mg/kg-day were also observed, but these changes did not show dose-dependent responses. Dose-related decreases in thymus weight were statistically significant at ≥10 mg/kg-day (Table D-7).

Table D-7. Exposure-related effects in male Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 5 weeks

Effect	Dose (mg/kg-d)				
	0	3	10	30	90
Hematologic effects (mean ± SD; n = 7–8)					
WBCs (10^9/L)	14.96 ± 1.9	13.84 ± 3.0	13.69 ± 1.8	13.58 ± 2.9	8.53 ± 1.1*
RBCs (10^9/L)	8.7 ± 0.2	8.6 ± 0.2	8.3 ± 0.2*	7.8 ± 0.4*	7.1 ± 0.4*
Hemoglobin (mmol/L)	10.5 ± 0.2	10.4 ± 0.3	9.8 ± 0.2*	9.5 ± 0.4*	8.6 ± 0.6*
Hematocrit (L/L)	0.5 ± 0.01	0.5 ± 0.01	0.47 ± 0.01*	0.46 ± 0.02*	0.43 ± 0.02*
Serum Ig levels (mean ± SD; n = 7–8)					
IgM	100 ± 13	87 ± 16	86 ± 31	67 ± 16*	81 ± 26
IgG	100 ± 40	141 ± 106	104 ± 28	106 ± 19	99 ± 29
IgA	100 ± 28	73 ± 29	78 ± 67	72 ± 22	39 ± 19*
IgE	100 ± 65	50 ± 20	228 ± 351	145 ± 176	75 ± 55
Cellularity (mean ± SD; n = 7–8)					
Spleen (cell number × 10^7)	59 ± 15	71 ± 14	59 ± 13	63 ± 10	41 ± 10*
Bone marrow (G/L)	31 ± 7	36 ± 5	31 ± 8	27 ± 8	19 ± 4*
Spleen cell distribution (%)					
B cells	39± 4	36 ± 2	34 ± 3*	32 ± 4*	23 ± 4*
T cells	40 ± 9	48 ± 12	40 ± 9	36 ± 2	44 ± 6
Th cells	23 ± 7	26 ± 7	24 ± 5	22 ± 4	26 ± 4
Ts cells	24 ± 5	26 ± 6	24 ± 7	19 ± 2	27 ± 5
Body (g) and organ (mg) weights (means; n = 7–8)					
Body weight	305	282*	300	293	250*
Brain	1,858	1,864	1,859	1,784	1,743*
Heart	1,030	934*	1,000	967	863*
Kidney	1,986	1,761*	1,899	1,790*	1,626*
Liver	10,565	9,567	11,250	11,118	12,107*
Thymus	517 ± 47	472 ± 90	438 ± 64*	388 ± 71*	198 ± 65*
Spleen	551	590	538	596	505
Mandibular lymph nodes	152	123	160	141	89*
Mesenteric lymph nodes	165	148	130*	158	107*
Popliteal lymph nodes	19	18	19	17	10*
Thymus cortex surface area *(% of total surface area of thymus; mean ± SD; n = 6–8)*	77.9 ± 3.8	74.4 ± 2.2	79.2 ± 5.9	75.8 ± 4.0	68.9 ± 5.2*

*Significantly ($p < 0.05$) different from control mean. For body weight and organ weight means, SDs were only reported for thymus weights.

Source: De Jong et al. (1999).

Statistically significant reductions were also observed in the relative cortex surface area of the thymus and thymic medullar weight at 90 mg/kg-day, but there was no difference in cell proliferation between treated and control animals using the proliferating cell nuclear antigen (PCNA) technique. Changes in the following immune parameters were noted: dose-related and statistically significant decrease in the relative number of B cells in the spleen at 10 (13%), 30 (18%), and 90 mg/kg-day (41%); significant decreases in absolute number of cells harvested in the spleen (31%), in the number of B cells in the spleen (61%), and NK cell activity in the spleen (E:T ratio was 40.9 ± 28.4% that of the controls) at 90 mg/kg-day; and a decrease in serum IgM (33%) and IgA (61%) in rats treated with 30 and 90 mg/kg-day, respectively. The decrease in the spleen cell count was attributed by the study authors to the decreased B cells and suggested a possible selective toxicity of benzo[a]pyrene to B cell precursors in the bone marrow. The study authors considered the decrease in IgA and IgM to be due to impaired production of antibodies, suggesting a role of thymus toxicity in the decreased (T-cell dependent) antibody production. In addition to the effects on the thymus and spleen, histopathologic examination revealed treatment-related lesions only in the liver and forestomach at the two highest dose levels, but the incidence data for these lesions were not reported by De Jong et al. (1999). Increased incidence for forestomach basal cell hyperplasia ($p < 0.05$ by Fisher's exact test) was reported at 30 and 90 mg/kg-day, and increased incidence for oval cell hyperplasia in the liver was reported at 90 mg/kg-day ($p < 0.01$, Fisher's exact test). The results indicate that 3 mg/kg-day was a no-observed-adverse-effect level (NOAEL) for effects on hematological parameters (decreased RBC count, hemoglobin, and hematocrit) and immune parameters (decreased thymus weight and percent of B cells in the spleen) noted in Wistar rats at 10 mg/kg-day (the lowest-observed-adverse-effect level [LOAEL]) and above. Lesions of the liver (oval cell hyperplasia) and forestomach (basal cell hyperplasia) occurred at doses ≥30 mg/kg-day.

Knuckles et al. (2001) exposed male and female F344 rats (20/sex/dose group) to benzo[a]pyrene (98% purity) at doses of 0, 5, 50, or 100 mg/kg-day in the diet for 90 days. Food consumption and body weight were monitored, and the concentration of benzo[a]pyrene in the food was adjusted every 3–4 days to maintain the target dose. The authors indicated that the actual intake of benzo[a]pyrene by the rats was within 10% of the calculated intake, and the nominal doses were not corrected to actual doses. Hematology and serum chemistry parameters were evaluated. Urinalysis was also performed. Animals were examined for gross pathology, and histopathology was performed on selected organs (stomach, liver, kidney, testes, and ovaries). Statistically significant decreases in RBC counts and hematocrit level (decreases as much as 10 and 12%, respectively) were observed in males at doses ≥50 mg/kg-day and in females at 100 mg/kg-day. A maximum 12% decrease (statistically significant) in hemoglobin level was noted in both sexes at 100 mg/kg-day. Blood chemistry analysis showed a significant increase in blood urea nitrogen (BUN) only in high-dose (100 mg/kg-day) males. Histopathology examination revealed an apparent increase in the incidence of abnormal tubular casts in the kidney in males at

5 mg/kg-day (40%), 50 mg/kg-day (80%), and 100 mg/kg-day (100%), compared to 10% in the controls. Only 10% of the females showed significant kidney tubular changes at the two high-dose levels compared to zero animals in the female control group. The casts were described as molds of distal nephron lumen and were considered by the study authors to be indicative of renal dysfunction. From this study, male F344 rats appeared to be affected more severely by benzo[a]pyrene treatment than the female rats. However, the statistical significance of the kidney lesions is unclear. Several reporting gaps and inconsistencies regarding the reporting of kidney abnormalities in Knuckles et al. (2001) make interpretation of the results difficult. Results of histopathological kidney abnormalities (characterized primarily as kidney casts) were presented graphically and the data were not presented numerically in this report. No indication was given in the graph that any groups were statistically different than controls, although visual examination of the magnitude of response and error bars appears to indicate a 4-fold increase in kidney casts in males compared to the control group (40 compared to 10%). The figure legend reported the data as "percentage incidence of abnormal kidney tissues" and reported values as mean ± SD. However, the text under the materials and methods section stated that Fisher's exact test was used for histopathological data, which would involve the pairwise comparison of incidence and not means. There are additional internal inconsistencies in the data presented. The data appeared to indicate that incidences for males were as follows: control, 10%; 5 mg/kg-day, 40%; 50 mg/kg-day, 80%; and 100 mg/kg-day, 100%; however, these incidences are inconsistent with the size of the study groups, which were reported as 6–8 animals per group. The study authors were contacted, but did not respond to EPA's request for clarification of study design and/or results. Due to issues of data reporting, a LOAEL could not be established for the increased incidence of kidney lesions. Based on the statistically significant hematological effects including decreases in RBC counts, hematocrit, and BUN, the NOAEL in males was 5 mg/kg-day and the LOAEL was 50 mg/kg-day, based on in F344 rats. No exposure-related histological lesions were identified in the stomach, liver, testes, or ovaries in this study.

In a range-finding study, Wistar (specific pathogen-free Riv:TOX) rats (10/sex/dose group) were administered benzo[a]pyrene (97.7% purity) dissolved in soybean oil by gavage at dose levels of 0, 1.5, 5, 15, or 50 mg/kg body weight-day, 5 days/week for 5 weeks (Kroese et al., 2001). Behavior, clinical symptoms, body weight, and food and water consumption were monitored. None of the animals died during the treatment period. Animals were sacrificed 24 hours after the last dose. Urine and blood were collected for standard urinalysis and hematology and clinical chemistry evaluation. Liver enzyme induction was monitored based on EROD activity in plasma. Animals were subjected to macroscopic examination, and organ weights were recorded. The esophagus, stomach, duodenum, liver, kidneys, spleen, thymus, lung, and mammary gland (females only) from the highest-dose and control animals were evaluated for histopathology. Intermediate-dose groups were examined if abnormalities were observed in the higher-dose groups.

A significant, but not dose-dependent, increase in food consumption in males at ≥1.5 mg/kg-day and a decrease in food consumption in females at ≥5 mg/kg-day was observed (Kroese et al., 2001). Water consumption was statistically significantly altered in males only: a decrease at 1.5, 5, and 15 mg/kg-day and an increase at 50 mg/kg-day. Organ weights of lung, spleen, kidneys, adrenals, and ovaries were not affected by treatment. There was a dose-related, statistically significant decrease in thymus weight in males at 15 and 50 mg/kg-day (decreased by 28 and 33%, respectively) and a significant decrease in thymus weight in females at 50 mg/kg-day (decreased by 17%) (Table D-8). In both sexes, liver weight was statistically significantly increased only at 50 mg/kg-day by about 18% (Table D-8).

Table D-8. Exposure-related effects in Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 5 weeks

Organ	Dose (mg/kg-d)				
	0	1.5	5	15	50
Liver weight (g; mean ± SD)					
Males	6.10 ± 0.26	6.19 ± 0.19	6.13 ± 0.10	6.30 ± 0.14	7.20 ± 0.18*
Females	4.28 ± 0.11	4.40 ± 0.73	4.37 ± 0.11	4.67 ± 0.17	5.03 ± 0.15*
Thymus weight (mg; mean ± SD)					
Males	471 ± 19	434 ± 20	418 ± 26	342 ± 20*	317 ± 21*
Females	326 ± 12	367 ± 23	351 ± 25	317 ± 30	271 ± 16*
Basal cell hyperplasia of the forestomach (incidence with slight severity)					
Males	1/10	1/10	4/10	3/10	7/10
Females	0/10	1/10	1/10	3/10*	7/10*

*Significantly ($p < 0.05$) different from control mean; n = 10/sex/group.

Source: Kroese et al. (2001).

Hematological evaluation revealed only statistically nonsignificant, small, dose-related decreases in hemoglobin in both sexes and RBC counts in males. Clinical chemistry analysis showed a small, but statistically significant, increase in creatinine levels in males only at 1.5 mg/kg-day, but this effect was not dose-dependent. A dose-dependent induction of liver microsomal EROD activity was observed, with a 5-fold induction at 1.5 mg/kg-day compared to controls, reaching 36-fold in males at 50 mg/kg-day; the fold induction in females at the top dose was less than in males. At necropsy, significant, dose-dependent macroscopic findings were not observed.

Histopathology examination revealed a statistically significant increase in basal cell hyperplasia in the forestomach of females at doses ≥15 mg/kg-day (Kroese et al., 2001). The induction of liver microsomal EROD was not accompanied by any adverse histopathologic findings

in the liver at the highest dose, 50 mg/kg-day, so the livers from intermediate-dose groups were, therefore, not examined. An increased incidence of brown pigmentation of red pulp (hemosiderin) in the thymus was observed in treated animals of both sexes. However, this tissue was not examined in intermediate-dose groups. This range-finding, 5-week study identified a NOAEL of 5 mg/kg-day and a LOAEL of 15 mg/kg-day, based on decreased thymus weight and forestomach hyperplasia in Wistar rats.

Kroese et al. (2001) exposed Wistar (Riv:TOX) rats (10/sex/dose group) to benzo[a]pyrene (98.6% purity, dissolved in soybean oil) by gavage at 0, 3, 10, or 30 mg/kg body weight-day, 5 days/week for 90 days. The rats were examined daily for behavior and clinical symptoms and by palpation. Food and water consumption, body weights, morbidity, and mortality were monitored. At the end of the exposure period, rats were subjected to macroscopic examination and organ weights were recorded. Blood was collected for hematology and serum chemistry evaluation, and urine was collected for urinalysis. All gross abnormalities, particularly masses and lesions suspected of being tumors, were evaluated. The liver, stomach, esophagus, thymus, lung, spleen, and mesenteric lymph node were examined histopathologically. In addition, cell proliferation in forestomach epithelium was measured as the prevalence of S-phase epithelial cells displaying bromodeoxyuridine (BrdU) incorporation.

There were no obvious effects on behavior of the animals, and no difference was observed in survival or food consumption between exposed animals and controls (Kroese et al., 2001). Higher water consumption and slightly lower body weights than the controls were observed in males, but not females, at the high dose of 30 mg/kg-day. Hematological investigations showed only nonsignificant, small dose-related decreases in RBC count and hemoglobin level in both sexes. Clinical chemistry evaluation did not show any treatment-related group differences or dose-response relationships for alanine aminotransferase, serum aspartate transaminase (AST), lactate dehydrogenase (LDH), or creatinine, but a small dose-related decrease in γ-glutamyl transferase activity was observed in males only. Urinalysis revealed an increase in urine volume in males at 30 mg/kg-day, which was not dose related. At the highest dose, both sexes showed increased levels of urinary creatinine and a dose-related increase in urinary protein. However, no further investigation was conducted to determine the underlying mechanisms for these changes. At necropsy, reddish to brown/gray discoloration of the mandibular lymph nodes was consistently noted in most rats; occasional discoloration was also observed in other regional lymph nodes (axillary). Statistically significant increases in liver weight were observed at 10 and 30 mg/kg-day in males (15 and 29%) and at 30 mg/kg-day in females (17%). A decrease in thymus weight was seen in both sexes at 30 mg/kg-day (17 and 33% decrease in females and males, respectively, compared with controls) (Table D-9). At 10 mg/kg-day, thymus weight in males was decreased by 15%, but the decrease did not reach statistical significance.

Table D-9. Means ± SD[a] for liver and thymus weights in Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 90 days

Organ	Dose (mg/kg-d)			
	0	3	10	30
Liver weight (g)				
Males	7.49 ± 0.97	8.00 ± 0.85	8.62 ± 1.30*	9.67 ± 1.17*
Females	5.54 ± 0.70	5.42 ± 0.76	5.76 ± 0.71	6.48 ± 0.78*
Thymus weight (mg)				
Males	380 ± 60	380 ± 110	330 ± 60	270 ± 40*
Females	320 ± 60	310 ± 50	300 ± 40	230 ± 30*

*Significantly ($p < 0.05$) different from control mean; student t-test (unpaired, two-tailed); n = 10/sex/group.
[a]Reported as SE, but judged to be SD (and confirmed by study authors).

Source: Kroese et al. (2001).

Histopathologic examination revealed what was characterized by Kroese et al. (2001) as basal cell disturbance in the epithelium of the forestomach in males ($p < 0.05$) and females ($p < 0.01$) at 30 mg/kg-day. The basal cell disturbance was characterized by increased number of basal cells, mitotic figures, and remnants of necrotic cells; occasional early nodule development; infiltration by inflammatory cells (mainly histiocytes); and capillary hyperemia, often in combination with the previous changes (Kroese et al., 2001). Incidences for these lesions (also described as "slight basal cell hyperplasia") in the 0, 3, 10, and 30-mg/kg-day groups were 0/10, 2/10, 3/10, and 7/10, respectively, in female rats and 2/10, 0/10, 6/10, and 7/10, respectively, in male rats. Nodular hyperplasia was noted in one animal of each sex at 30 mg/kg-day. A significant ($p < 0.05$) increase in proliferation of forestomach epithelial cells was detected at doses ≥10 mg/kg-day by morphometric of analysis of nuclei with BrdU incorporation. The mean numbers of BrdU-staining nuclei per unit surface area of the underlying lamina muscularis mucosa were increased by about 2- and 3−4-fold at 10 and 30 mg/kg-day, respectively, compared with controls.

A reduction of thymus weight and increase in the incidence of thymus atrophy (the report described the atrophy as slight, but did not specify the full severity scale used in the pathology examination) was observed in males only at 30 mg/kg-day ($p < 0.01$ compared with controls). Respective incidences for thymus atrophy for the control through high-dose groups were 0/10, 0/10, 0/10, and 3/10 for females and 0/10, 2/10, 1/10, and 6/10 for males. No significant differences were observed in the lungs of control and treated animals. In the esophagus, degeneration and regeneration of muscle fibers and focal inflammation of the muscular wall were judged to be a result of the gavage dosing rather than of benzo[a]pyrene treatment.

The target organs of benzo[a]pyrene toxicity in this 90-day dietary study of Wistar rats were the forestomach, thymus, and liver. The LOAEL for forestomach hyperplasia, decreased thymus weight, and thymus atrophy was 30 mg/kg-day and the NOAEL was 10 mg/kg-day.

Chronic Studies and Cancer Bioassays

Kroese et al. (2001) exposed Wistar (Riv:TOX) rats (52/sex/dose group) to benzo[a]pyrene (98.6% purity) in soybean oil by gavage at nominal doses of 0, 3, 10, or 30 mg/kg-day, 5 days/week, for 104 weeks. Mean achieved dose levels were 0, 2.9, 9.6, and 29 mg/kg-day. Additional rats (6/sex/group) were sacrificed after 4 and 5 months of exposure for analysis of DNA adduct formation in blood and major organs and tissues. The rats were 6 weeks old at the start of exposure. The rats were examined daily for behavior and clinical symptoms and by palpation. Food and water consumption, body weights, morbidity, and mortality were monitored during the study. Complete necropsy was performed on all animals that died during the course of the study, that were found moribund, or at terminal sacrifice (organ weight measurement was not mentioned in the report by Kroese et al., 2001). The organs and tissues collected and prepared for microscopic examination included brain, pituitary, heart, thyroid, salivary glands, lungs, stomach, esophagus, duodenum, jejunum, ileum, caecum, colon, rectum, thymus, kidneys, urinary bladder, spleen, lymph nodes, liver pancreas, adrenals, sciatic nerve, nasal cavity, femur, skin including mammary tissue, ovaries/uterus, and testis/accessory sex glands. Some of these tissues were examined only when gross abnormalities were detected. All gross abnormalities, particularly masses and lesions that appeared to be tumors, were also examined.

At 104 weeks, survival in the control group was 65% (males) and 50% (females), whereas mortality in the 30 mg/kg-day dose group was 100% after about week 70. At 80 weeks, survival percentages were about 90, 85, and 75% in female rats in the 0, 3, and 10 mg/kg-day groups, respectively; in males, respective survival percentages were ~95, 90, and 85% at 80 weeks. Survival of 50% of animals occurred at 104, 104, ~90, and 60 weeks for control through high-dose females; for males, the respective times associated with 65% survival were 104, 104, 104, and ~60 weeks. The high mortality rate in high-dose rats was attributed to liver or forestomach tumor development, not to noncancer systemic effects. After 20 weeks, body weight was decreased (compared with controls by >10%) in 30-mg/kg-day males, but not in females. This decrease was accompanied by a decrease in food consumption. Body weights and food consumption were not adversely affected in the other dose groups compared to controls. In males, there was a dose-dependent increase in water consumption starting at week 13, but benzo[a]pyrene treatment had no significant effects on water consumption in females.

Tumors were detected at significantly elevated incidences at several tissue sites in female and male rats at doses ≥10 and ≥3 mg/kg-day, respectively (Table D-10) (Kroese et al., 2001). The tissue sites with the highest incidences of tumors were the liver (hepatocellular adenoma and carcinoma) and forestomach (squamous cell papilloma and carcinoma) in both sexes (Table D-10). The first liver tumors were detected in week 35 in high-dose male rats. Liver tumors were described as complex, with a considerable proportion (59/150 tumors) metastasizing to the lungs. At the highest dose level, 95% of rats with liver tumors had malignant carcinomas (95/100; Table D-10). Forestomach tumors were associated with the basal cell proliferation observed

(without diffuse hyperplasia) in the forestomach of rats in the preliminary range-finding and 90-day exposure studies. At the highest dose level, 59% of rats with forestomach tumors had malignant carcinomas (60/102; Table D-10). Other tissue sites with significantly elevated incidences of tumors in the 30 mg/kg-day dose group included the oral cavity (papilloma and squamous cell carcinoma [SCC]) in both sexes, and the jejunum (adenocarcinoma), kidney (cortical adenoma), and skin (basal cell adenoma and carcinoma) in male rats (Table D-10). In addition, auditory canal tumors (carcinoma or squamous cell papilloma originating from pilo-sebaceous units including the Zymbal's gland) were also detected in both sexes at 30 mg/kg-day, but auditory canal tissue was not histologically examined in the lower dose groups and the controls (Table D-10). Gross examination revealed auditory canal tumors only in the high-dose group.

Table D-10. Incidences of exposure-related neoplasms in Wistar rats treated by gavage with benzo[a]pyrene, 5 days/week, for 104 weeks

	Dose (mg/kg-d)			
	0	**3**	**10**	**30[a]**
Site	**Females[b]**			
Oral cavity				
Papilloma	0/19	0/21	0/9	9/31*
SCC	1/19	0/21	0/9	9/31*
Basal cell adenoma	0/19	0/21	1/9	4/31
Sebaceous cell carcinoma	0/19	0/21	0/9	1/31
Esophagus				
Sarcoma undifferentiated	0/52	0/52	2/52	0/52
Rhabdomyosarcoma	0/52	1/52	4/52	0/52
Fibrosarcoma	0/52	0/52	3/52	0/52
Forestomach				
Squamous cell papilloma	1/52	3/51	20/51*	25/52*
SCC	0/52	3/51	10/51*	25/52*
Liver				
Hepatocellular adenoma	0/52	2/52	7/52*	1/52
Hepatocellular carcinoma	0/52	0/52	32/52*	50/52*
Cholangiocarcinoma	0/52	0/52	1/52	0/52
Anaplastic carcinoma	0/52	0/52	1/52	0/52
Auditory canal				
Benign tumor	0/0	0/0	0/0	1/20
Squamous cell papilloma	0/0	0/1	0/0	1/20
Carcinoma	0/0	0/1	0/0	13/20*

	Dose (mg/kg-d)			
	0	3	10	30[a]
Site	**Males[b]**			
Oral cavity				
Papilloma	0/24	0/24	2/37	10/38*
SCC	1/24	0/24	5/37	11/38*
Basal cell adenoma	0/24	0/24	0/37	2/38
Sebaceous cell carcinoma	0/24	0/24	0/37	2/38
Forestomach				
Squamous cell papilloma	0/52	7/52*	18/52*	17/52*
SCC	0/52	1/52	25/52*	35/52*
Jejunum				
Adenocarcinoma	0/51	0/50	1/51	8/49*
Liver				
Hepatocellular adenoma	0/52	3/52	15/52*	4/52
Hepatocellular carcinoma	0/52	1/52	23/52*	45/52*
Cholangiocarcinoma	0/52	0/52	0/52	1/52
Kidney				
Cortical adenoma	0/52	0/52	7/52*	8/52*
Adenocarcinoma	0/52	0/52	2/52	0/52
Urothelial carcinoma	0/52	0/52	0/52	3/52
Auditory canal				
Benign	0/1	0/0	1/7	0/33
Squamous cell papilloma	0/1	0/0	0/7	4/33
Carcinoma	0/1	0/0	2/7	19/33*
Sebaceous cell adenoma	0/1	0/0	0/7	1/33
Skin and mammary				
Basal cell adenoma	2/52	0/52	1/52	10/51*
Basal cell carcinoma	1/52	1/52	0/52	4/51
SCC	0/52	1/52	1/52	5/51
Keratoacanthoma	1/52	0/52	1/52	4/51
Trichoepithelioma	0/52	1/52	2/52	8/51*
Fibrosarcoma	0/52	3/52	5/52	0/51
Fibrous histiocytoma (malignant)	0/52	0/52	1/52	1/52

*Statistically significant difference ($p \leq 0.01$), Fisher's exact test; analysis of auditory canal tumor incidence was based on assumption of n = 52 and no tumors in the controls.
[a]This group had significantly decreased survival.
[b]Incidences are for number of rats with tumors compared with number of tissues examined histologically. Auditory canal and oral cavity tissues were only examined histologically when abnormalities were observed upon macroscopic examination.

Source: Kroese et al. (2001).

Kroese et al. (2001) did not systematically investigate nonneoplastic lesions detected in rats sacrificed during the 2-year study because the focus was to identify and quantitate tumor

occurrence. However, incidences were reported for nonneoplastic lesions in tissues or organs in which tumors were detected (i.e., oral cavity, esophagus, forestomach, jejunum, liver, kidney, skin, mammary, and auditory canal). The reported nonneoplastic lesions associated with exposure were the forestomach basal cell hyperplasia and clear cell foci of cellular alteration in the liver. Incidences for forestomach basal cell hyperplasia in the control through high-dose groups were 1/52, 8/51, 13/51, and 2/52 for females and 2/50, 8/52, 8/52, and 0/52 for males. Incidences for hepatic clear cell foci of cellular alteration were 22/52, 33/52, 4/52, and 2/52 for females and 8/52, 22/52, 1/52, and 1/52 for males. These results indicate that the lowest dose group, 3 mg/kg-day, was a LOAEL for increased incidence of forestomach hyperplasia and hepatic histological changes in male and female Wistar rats exposed by gavage to benzo[a]pyrene for up to 104 weeks (see Table D-10). The lack of an increase in incidence of these nonneoplastic lesions in the forestomach and liver at the intermediate and high doses (compared with controls) was associated with increased incidences of forestomach and liver tumors at these dose levels. The authors of this study noted that nonneoplastic effects were not quantified in organs with tumors.

As an adjunct study to the 2-year gavage study with Wistar rats, Kroese et al. (2001) sacrificed additional rats (6/sex/group) after 4 and 5 months of exposure (0, 1, 3, 10, or 30 mg/kg-day) for analysis of DNA adduct formation in WBCs and major organs and tissues. Additional rats (6/sex/time period) were exposed to 0.1 mg/kg-day benzo[a]pyrene for 4 and 5 months for analysis of DNA adduct formation. Using the [^{32}P]-postlabeling technique, five benzo[a]pyrene-DNA adducts were identified in all of the examined tissues at 4 months (WBCs, liver, kidney, heart, lung, skin, forestomach, glandular stomach, brain). Only one of these adducts (adduct 2) was identified based on co-chromatography with a standard. This adduct, identified as 10β-(deoxyguanosin-N2-yl)-7β,8α,9α-trihydroxy-7,8,9,10 tetrahydro-benzo[a]pyrene, was the predominant adduct in all organs of female rats exposed to 10 mg/kg-day, except the liver and kidney, in which another adduct (unidentified adduct 4) was predominant. Levels of total adducts (number of benzo[a]pyrene-DNA adducts per 10^{10} nucleotides) in examined tissues (from the single 10 mg/kg-day female rat) showed the following order: liver > heart > kidney > lung > skin > forestomach ≈ WBCs > brain. Mean values for female levels of total benzo[a]pyrene-DNA adducts (number per 10^{10} nucleotides) in four organs showed the same order, regardless of exposure group: liver > lung > forestomach ≈ WBCs; comparable data for males were not reported. Mean total benzo[a]pyrene-DNA adduct levels in livers increased in both sexes from about 100 adducts per 10^{10} nucleotides at 0.1 mg/kg-day to about 70,000 adducts per 10^{10} nucleotides at 30 mg/kg-day. In summary, these results suggest that total benzo[a]pyrene-DNA adduct levels in tissues at 4 months were not independently associated with the carcinogenic responses noted after 2 years of exposure to benzo[a]pyrene. The liver showed the highest total DNA adduct levels and a carcinogenic response, but total DNA adduct levels in heart, kidney, and lung (in which no carcinogenic responses were detected) were higher than levels in forestomach and skin (in which carcinogenic responses were detected).

Groups of Sprague-Dawley rats (32/sex/dose) were fed diets delivering a daily dose of 0.15 mg benzo[a]pyrene/kg body weight every ninth day or 5 times/week (Brune et al., 1981). Other groups (32/sex/dose) were given gavage doses of 0.15 mg benzo[a]pyrene (in aqueous 1.5% caffeine solution)/kg every ninth day, every third day, or 5 times/week. The study included an untreated control group (to compare with the dietary exposed groups) and a gavage vehicle control group (each with 32 rats/sex). Rats were treated until moribundity or death occurred, with average annual doses reported in Table D-11 [mg/kg-year, calculated by Brune et al. (1981)]. The following tissues were prepared for histopathological examination: tongue, larynx, lung, heart, trachea, esophagus, stomach, small intestine, colon, rectum, spleen, liver, urinary bladder, kidney, adrenal gland, and any tissues showing tumors or other gross changes. Survival was similar among the groups, with the exception that the highest gavage-exposure group showed a decreased median time of survival (Table D-11). Significantly increased incidences of portal-of-entry tumors (forestomach, esophagus, and larynx) were observed in all of the gavage-exposed groups and in the highest dietary exposure group (Table D-11). Following dietary administration, all observed tumors were papillomas. Following gavage administration, two malignant forestomach tumors were found (one each in the mid- and high-dose groups) and the remaining tumors were benign. The data in Table D-11 show that the carcinogenic response to benzo[a]pyrene was stronger with the gavage protocol compared with dietary exposure, and that no distinct difference in response was apparent between the sexes. Tumors at distant sites (mammary gland, kidney, pancreas, lung, urinary bladder, testes, hematopoietic, and soft tissue) were not considered treatment-related as they were also observed at similar rates in the control group (data not provided). The study report did not address noncancer systemic effects.

Table D-11. Incidences of alimentary tract tumors in Sprague-Dawley rats chronically exposed to benzo[a]pyrene in the diet or by gavage in caffeine solution

Average annual dose (mg/kg-yr)	Estimated average daily dose[a] (mg/kg-d)	Forestomach tumors[b]	Total alimentary tract tumors[c] (larynx, esophagus, forestomach)	Median survival time (wks)
Benzo[a]pyrene by gavage in 1.5% caffeine solution				
0	0	3/64 (4.7%)	6/64 (9.4%)	102
6	0.016	12/64 (18.8%)*	13/64 (20.3%)	112
18	0.049	26/64 (40.1%)**	26/64 (40.6%)	113
39	0.107	14/64 (21.9%)**	14/64 (21.9%)	87

Average annual dose (mg/kg-yr)	Estimated average daily dose[a] (mg/kg-d)	Forestomach tumors[b]	Total alimentary tract tumors[c] (larynx, esophagus, forestomach)	Median survival time (wks)
Benzo[a]pyrene in diet				
0	0	2/64 (3.1%)	3/64 (4.7%)	129
6	0.016	1/64 (1.6%)	3/64 (4.7%)	128
39	0.107	9/64 (14.1%)*	10/64 (15.6%)	131

*Significantly ($p < 0.1$) different from control using a modified χ^2 test that accounted for group differences in survival time.
**Significantly ($p < 0.05$) different from control using a modified χ^2 test that accounted for group differences in survival time.
[a]Average annual dose divided by 365 days.
[b]No sex-specific forestomach tumor incidence data were reported by Brune et al. (1981).
[c]Sex-specific incidences for total alimentary tract tumors were reported as follows:

Gavage (control, high dose):	Male:	6/32, 7/32, 15/32, 8/32
	Female:	0/32, 6/32, 11/32, 6/32
Diet (control, high dose):	Male:	3/32, 3/32, 8/32
	Female:	0/32, 0/32, 2/32

Source: Brune et al. (1981).

In the other modern cancer bioassay with benzo[a]pyrene, female B6C3F$_1$ mice (48/dose group) were administered benzo[a]pyrene (98.5% purity) at concentrations of 0 (acetone vehicle), 5, 25, or 100 ppm in the diet for 2 years (Beland and Culp, 1998; Culp et al., 1998). This study was designed to compare the carcinogenicity of coal tar mixtures with that of benzo[a]pyrene and it included groups of mice fed diets containing one of several concentrations of two coal tar mixtures. Benzo[a]pyrene was dissolved in acetone before mixing with the feed. Control mice received only acetone-treated feed. Female mice were chosen because they have a lower background incidence of lung tumors than male B6C3F$_1$ mice. Culp et al. (1998) reported that the average daily intakes of benzo[a]pyrene in the 25- and 100-ppm groups were 104 and 430 µg/day, but did not report the intake for the 5-ppm group. Based on the assumption that daily benzo[a]pyrene intake at 5 ppm was one-fifth of the 25-ppm intake (about 21 µg/day), average daily doses for the three benzo[a]pyrene groups are estimated as 0.7, 3.3, and 16.5 mg/kg-day. Estimated doses were calculated using time-weighted average (TWA) body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group (estimated from graphically presented data). Food consumption, body weights, morbidity, and mortality were monitored at intervals, and lung, kidneys, and liver were weighed at sacrifice. Necropsy was performed on all mice that died during the experiment or survived to the end of the study period. Limited histopathologic examinations (liver, lung, small intestine, stomach, tongue, esophagus) were performed on all control and high-dose mice and on all mice that died during the experimental period, regardless of treatment group.

In addition, all gross lesions found in mice of the low- and mid-dose groups were examined histopathologically.

None of the mice administered 100 ppm benzo[a]pyrene survived to the end of the study, and morbidity/mortality was 100% by week 78. Decreased survival was also observed at 25 ppm with only 27% survival at 104 weeks, compared with 56 and 60%, in the 5-ppm and control groups, respectively. In the mid- and high-dose groups, 60% of mice were alive at about 90 and 60 weeks, respectively. Early deaths in exposed mice were attributed to tumor formation rather than other causes of systemic toxicity. Food consumption was not statistically different in benzo[a]pyrene-exposed and control mice. Body weights of mice fed 100 ppm were similar to those of the other treated and control groups up to week 46, and after approximately 52 weeks, body weights were reduced in 100-ppm mice compared with controls. Body weights for the 5- and 25-ppm groups were similar to controls throughout the treatment period. Compared with the control group, no differences in liver, kidney, or lung weights were evident in any of the treated groups (other organ weights were not measured).

Papillomas and/or carcinomas of the forestomach, esophagus, tongue, and larynx at elevated incidences occurred in groups of mice exposed to 25 or 100 ppm, but no exposure-related tumors occurred in the liver or lung (Beland and Culp, 1998; Culp et al., 1998). The forestomach was the most sensitive tissue, demonstrated the highest tumor incidence among the examined tissues, and was the only tissue with an elevated incidence of tumors at 25 ppm (Table D-12). In addition, most of the forestomach tumors in the exposed groups were carcinomas, as 1, 31, and 45 mice had forestomach carcinomas in the 5-, 25-, and 100-ppm groups, respectively. Nonneoplastic lesions were also found in the forestomach at significantly ($p < 0.05$) elevated incidences: hyperplasia and hyperkeratosis at ≥25 ppm (Table D-12). The esophagus was the only other examined tissue showing elevated incidence of a nonneoplastic lesion (basal cell hyperplasia, see Table D-12). Tumors (papillomas and carcinomas) were also significantly elevated in the esophagus and tongue at 100 ppm (Table D-12). Esophageal carcinomas were detected in 1 mouse at 25 ppm and 11 mice at 100 ppm. Tongue carcinomas were detected in seven 100-ppm mice; the remaining tongue tumors were papillomas. Although incidences of tumors of the larynx were not significantly elevated in any of the exposed groups, a significant dose-related trend was apparent (Table D-12).

Table D-12. Incidence of nonneoplastic and neoplastic lesions in female B6C3F$_1$ mice fed benzo[a]pyrene in the diet for up to 2 years

Tissue and lesion	Incidence (%)			
	Benzo[a]pyrene concentration (ppm) in diet			
	0	5	25	100
	Average daily doses (mg/kg-d)			
	0	0.7	3.3	16.5
Liver (hepatocellular adenoma)	2/48 (2)	7/48 (15)	5/47 (11)	0/45 (0)
Lung (alveolar/bronchiolar adenoma and/or carcinoma)	5/48 (10)	0/48 (0)	4/45 (9)	0/48 (0)
Forestomach (papilloma and/or carcinoma)	1/48[a] (2)	3/47 (6)	36/46* (78)	46/47* (98)
Forestomach (hyperplasia)	13/48[a] (27)	23/47 (49)	33/46* (72)	37/47* (79)
Forestomach (hyperkeratosis)	13/48[a] (27)	22/47 (47)	33/46* (72)	38/47* (81)
Esophagus (papilloma and/or carcinoma)	0/48[a] (0)	0/48 (0)	2/45 (0)	27/46* (59)
Esophagus (basal cell hyperplasia)	1/48[a] (2)	0/48 (0)	5/45 (11)	30/46* (65)
Tongue (papilloma and/or carcinoma)	0/49[a] (0)	0/48 (0)	2/46 (4)	23/48* (48)
Larynx (papilloma and/or carcinoma)	0/35[a] (0)	0/35 (0)	3/34 (9)	5/38 (13)

*Significantly different from control incidence ($p < 0.05$); using a modified Bonferonni procedure for multiple comparisons to the same control.
[a]Significant ($p < 0.05$) dose-related trend calculated for incidences of these lesions.

Sources: Beland and Culp (1998); Culp et al. (1998).

Neal and Rigdon (1967) fed benzo[a]pyrene (purity not reported) at concentrations of 0, 1, 10, 20, 30, 40, 45, 50, 100, and 250 ppm to male and female CFW-Swiss mice in the diet. Corresponding doses (in mg/kg-day) were calculated[1] as 0, 0.2, 1.8, 3.6, 5.3, 7.1, 8, 8.9, 17.8, and 44.4 mg/kg-day. The age of the mice ranged from 17 to 180 days old and the treatment time was from 1 to 197 days; the size of the treated groups ranged from 9 to 73. There were 289 mice (number of mice/sex not stated) in the control group. No forestomach tumors were reported at 0, 0.2, or 1.8 mg/kg-day. The incidences of forestomach tumors at 20, 30, 40, 45, 50, 100, and 250 ppm dose groups (3.6, 5.3, 7.1, 8, 8.9, 17.8, and 44.4 mg/kg-day) were 1/23, 0/37, 1/40, 4/40, 23/34, 19/23, and 66/73, respectively.

[1]Calculation: mg/kg-day = (ppm in feed × kg food/day)/kg body weight. Reference food consumption rates of 0.0062 kg/day (males) and 0.0056 kg/day (females) and reference body weights of 0.0356 kg (males) and 0.0305 kg (females) were used by the U.S. EPA (1988) and resulting doses were averaged between males and females.

Other Oral Exposure Cancer Bioassays in Mice

Numerous other oral exposure cancer bioassays in mice have limitations that restrict their usefulness for characterizing dose-response relationships between chronic-duration oral exposure to benzo[a]pyrene and noncancer effects or cancer, but collectively, they provide strong evidence that oral exposure to benzo[a]pyrene can cause portal-of-entry site tumors (see Table D-13 for references).

Table D-13. Other oral exposure cancer bioassays in mice

Species/strain	Exposure	Results	Comments	Reference
Rat/Sprague-Dawley	Groups of rats (32/sex/dose) were fed diets delivering a daily dose of 0.15 mg benzo[a]pyrene/kg body weight every 9th d or 5 times/wk (Brune et al., 1981). Other groups (32/sex/dose) were given gavage doses of 0.15 mg benzo[a]pyrene (in aqueous 1.5% caffeine solution)/kg every 9th d, every 3rd d, or 5 times/wk.	Larynx, esophagus, and forestomach tumors Dose (gavage) 0 6/64 0.016 13/64 0.049 26/64 0.107 14/64 Dose (diet) 0 3/64 0.016 3/64 0.107 10/64	Doses are annual averages. Nonstandard treatment protocol involved animals being treated for ≤5 d/wk; relatively high control incidence compared to other gavage studies.	Brune et al. (1981)
Mouse/HaICR	Groups of 12–20 mice (10 wks old) were fed benzo[a]pyrene in the diet (0.1, 0.3, or 1.0 mg/g diet) for 12–20 wks. Estimated doses were 14.3, 42.0, or 192 mg/kg-d.	Incidence with forestomach tumors: Low, 11/20 (18 wks) Mid, 13/19 (20 wks) High, 12/12 (12 wks)	Less-than-lifetime exposure duration; only stomachs were examined for tumors; tumors found only in forestomach.	Wattenberg (1972)
Mouse/HaICR	Groups of nine mice (9 wks old) were fed benzo[a]pyrene in the diet (0, 0.2, or 0.3 mg/g diet) for 12 wks and sacrificed. Estimated doses were 0, 27.3, or 41 mg/kg-d.	Incidence with forestomach tumors: Control, 0/9 Low, 6/9 High, 9/9	Less-than-lifetime exposure duration; glandular stomach, lung, and livers from control and exposed mice showed no tumors.	Triolo et al. (1977)

Species/strain	Exposure	Results	Comments	Reference
Mouse/HaICR	20 mice (9 wks old) were given benzo[a]pyrene in the diet (0.3 mg benzo[a]pyrene/g diet) for 6 wks and sacrificed after 20 wks in the study.	8/20 exposed mice had forestomach tumors	Less-than-lifetime exposure duration; only stomachs were examined for tumors; tumors found only in forestomach; no nonexposed controls were mentioned.	Wattenberg (1974)
Mouse/CD-1	20 female mice (9 wks old) were given 1 mg benzo[a]pyrene by gavage 2 times/wk for 4 wks and observed for 19 wks. Estimated dose was 33 mg/kg-d, using an average body weight of 0.030 kg from reported data.	Incidence with forestomach tumors: Exposed, 17/20 (85%) Controls, 0/24	Less-than-lifetime exposure duration; only stomach were examined for tumors; tumors found only in forestomach.	El-Bayoumy (1985)
Mouse/BALB	25 mice (8 wks old) were given 0.5 mg benzo[a]pyrene 2 times/wk for 15 wks.	5/25 mice had squamous carcinomas of the forestomach; tumors were detected 28–65 wks after treatment	Less-than-lifetime exposure duration; the following details were not reported: inclusion of controls, methods for detecting tumors, and body weight data.	Biancifiori et al. (1967)
Mouse/C3H	19 mice (about 3 mo old) were given 0.3 mL of 0.5% benzo[a]pyrene in polyethylene glycol-400 by gavage, once/d for 3 d.	By 30 wks, 7/10 mice had papillomas; no carcinomas were evident	Less-than-lifetime exposure duration.	Berenblum and Haran (1955)

Species/strain	Exposure	Results	Comments	Reference
Mouse/albino	Groups of 17–18 mice were given single doses of benzo[a]pyrene and allowed to survive until terminal sacrifice at 569 d.	Incidence of mice (that survived at least to 60 d) with forestomach papillomas: Incidence (Experiment 1) Dose (µg) (Experiment 2) Control 0/17 0/18 12.5 3/17 2/18 50 0/17 1/17 200 8/17 Not evaluated	Less-than-lifetime exposure duration; GI tract examined for tumors with hand lens; body weight data not reported.	Field and Roe (1965)
Mouse/albino	Groups of about 160 female mice (70 d of age; strain unknown) were given 0 or 8 mg benzo[a]pyrene mixed in the diet over a period of 14 mo.	Gastric tumors were observed at the following incidence: Control, 0/158 8 mg benzo[a]pyrene total, 13/160	Close to lifetime exposure duration; daily dose levels and methods of detecting tumors were not clearly reported.	Chouroulinkov et al. (1967)
Mouse/CFW	Groups of mice (mixed sex) were fed benzo[a]pyrene in the diet (dissolved in benzene and mixed with diet) at 0, 1, 10, 20, 30, 40, 45, 50, 100, or 250 ppm in the diet.	ppm / Exposure (d) / Fore-stomach tumor incidence 1 110 0/25 10 110 0/24 20 110 1/23 30 110 0/37 40 110 1/40 45 110 4/40 50 152 24/34 100 110 19/23 250 118 66/73	Less-than-lifetime exposure duration; no vehicle control group; animals ranged from 3 wks to 6 mo old at the start of dosing; only alimentary tract was examined for tumors.	Neal and Rigdon (1967)
Mouse/Swiss albino	Groups of mice (9–14 wks old) were given single doses of 0 or 0.05 mg benzo[a]pyrene in polyethylene glycol-400 by gavage. Surviving mice were killed at 18 mo of age and examined for macroscopic tumors.	Forestomach tumor incidence: Dose (µg) / Carcinoma papilloma 0 0/65 2/65 50 1/61 20/61	Less-than-lifetime duration of exposure; exposure-related tumors only found in forestomach.	Roe et al. (1970)

Species/strain	Exposure	Results	Comments	Reference
Mouse/ICR	Groups of 20 or 24 mice (71 d old) were given 1.5 mg benzo[a]pyrene by gavage 2 times/wk for 4 wks; terminal sacrifice was at 211 d of age. Estimated dose was about 50 mg benzo[a]pyrene/kg, using an average body weight of 0.03 kg during exposure from reported data.	Incidence of mice with forestomach neoplasms Experiment 1, 23/24 Experiment 2, 19/20	Less-than-lifetime duration of exposure; only stomachs were examined for tumors; tumors found only in forestomach; nonexposed controls were not mentioned.	Benjamin et al. (1988)
Mouse/white	Groups of 16–30 mice were given benzo[a]pyrene in triethylene glycol (0.001–10 mg) weekly for 10 wks and observed until 19 mo.	Tumors in stomach antrum Dose (mg) — Carcinoma papilloma 0.001 — 0/16 0/16 0.01 — 0/26 2/26 0.1 — 0/24 5/24 1.0 — 11/30 12/30 10 — 16/27 7/27	Less-than-lifetime exposure duration.	Fedorenko and Yansheva (1967); as cited in U.S. EPA (1991a)
Mouse/A/HeJ	12 female mice (9 wks old) were given standard diet for 25 d, and 3 mg benzo[a]pyrene by gastric intubation on d 7 and 21 of the study. Mice were killed at 31 wks of age and examined for lung tumors.	12/12 exposed mice had lung tumors	Less-than-lifetime exposure duration; only lungs examined for tumors; no nonexposed controls were mentioned.	Wattenberg (1974)
Mouse/A/J	Groups of female mice were fed benzo[a]pyrene in the diet at 0, 16, or 98 ppm for 260 d. Average intakes of benzo[a]pyrene were 0, 40.6, and 256.6 µg/mouse/d. Estimated doses were 0, 1.6, and 9.9 mg/kg-d using a chronic reference body weight value of 0.026 kg (U.S. EPA, 1988).	Incidence of mice surviving to 260 d: Lung tumors Control, 4/21 16 ppm, 9/25 98 ppm, 14/27 Forestomach tumors Control, 0/21 16 ppm, 5/25 98 ppm, 27/27	Close to lifetime exposure duration; A/J strain of mice particularly sensitive to chemically induced cancer; only lungs and stomachs were examined for tumors.	Weyand et al. (1995)

Species/strain	Exposure	Results	Comments	Reference
Mouse/A/J	Groups 40 female mice (8 wks old) were given 0 or 0.25 mg benzo[a]pyrene (in 2% emulphor) by gavage 3 times/wk for 8 wks. Mice were killed at 9 mo of age and examined for lung or forestomach tumors.	Incidence for mice surviving at 9 mo of age: Lung tumors Control, 11/38 Exposed, 22/36 Forestomach tumors Control, 0/38 Exposed, 33/36	Less-than-lifetime duration of exposure; only lungs and GI tract were examined for tumors.	Robinson et al. (1987)

D.4.2. Inhalation Studies

Short-Term and Subchronic Studies

Wolff et al. (1989) exposed groups of 40 male and 40 female F344/Crl rats, via nose only, to 7.5 mg benzo[a]pyrene/m^3 for 2 hours/day, 5 days/week for 4 weeks (corresponding to a TWA of 0.45 mg/m^3). Rats were 10–11 weeks old at the beginning of the experiment. Benzo[a]pyrene (>98% pure) aerosols were formed by heating and then condensing the vaporized benzo[a]pyrene. The particle mass median aerodynamic diameter (MMAD) was 0.21 µm. Subgroups of these animals (six/sex/dose) were exposed for 4 days or 6 months after the end of the 4-week exposure to radiolabeled aluminosilicate particles. Lung injury was assessed by analyzing clearance of radiolabeled aluminosilicate particles and via histopathologic evaluations. Body and lung weights, measured in subgroups from 1 day to 12 months after the exposure did not differ between controls and treated animals. Radiolabeled particle clearance did not differ between the control and treated groups, and there were no significant lung lesions. This study identified a NOAEL for lung effects of 0.45 mg/m^3 for a short-term exposure.

Chronic Studies and Cancer Bioassays

Thyssen et al. (1981) conducted an inhalation study in which male Syrian golden hamsters were exposed to benzo[a]pyrene for their natural lifetime. Groups of 24 animals (8 weeks old) were exposed by nose-only inhalation to sodium chloride aerosols (controls; 240 µg sodium chloride/m^3) or benzo[a]pyrene condensed onto sodium chloride aerosols at three target concentrations of 2, 10, or 50 mg benzo[a]pyrene/m^3 for 3–4.5 hours/day, 5 days/week for 1–41 weeks, followed by 3 hours/day, 7 days/week for the remainder of study (until hamsters died or became moribund). Thyssen et al. (1981) reported average measured benzo[a]pyrene concentrations to be 0, 2.2, 9.5, or 46.5 mg/m^3. More than 99% of the particles were between 0.2 and 0.5 µm in diameter, and over 80% had diameters between 0.2 and 0.3 µm. The particle analysis of the aerosols was not reported to modern standards (MMAD and geometric SD were not reported). Final overall group sizes were larger as animals dying during the first 12 months of the study were replaced.

Review of the individual animal data (including individual animal pathology reports, time-to-death data, and exposure chamber monitoring data) provided by Thyssen et al. to EPA (U.S. EPA,

1990a) revealed several discrepancies in the reported exposure protocol. The actual exposure protocol was as follows: 4.5 hours/day, 5 days/week on weeks 1–12; 3 hours/day, 5 days/week on weeks 13–29; 3.7 hours/day, 5 days/week on week 30; 3 hours/day, 5 days/week on weeks 31–41; and 3 hours/day, 7 days/week for the reminder of the experiment.

Analytical chamber monitoring data were generally recorded about once or twice per week, with some exceptions ranging from no measurements for a 3-week period to as many as five measurements in 1 week. Individual measurements (in mg/m^3) were 0.2–4.52, 1.16–19.2, and 0.96–118.6 in the 2, 10, and 50 mg/m^3 target concentration groups, respectively. Overall, weekly average exposure concentrations varied 2–5-fold from the overall average for each group over the course of the study, with no particular trends over time (data not shown). The 95% confidence limits for the average exposure level over time in each group varied within 4–7% of the averages. Because some animals were started at different times and the exposure protocol changed over time, each individual animal had an exposure history somewhat different than others in the same exposure group. In order to address this variability, U.S. EPA (1990a) used the individual animal data and the chamber monitoring data to calculate a lifetime average continuous exposure for each individual hamster. Group averages of these individual TWA concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3 for the control through high-exposure groups.

Statistical analysis of outcomes was not reported by Thyssen et al. (1981). Survival was similar in the control, low-, and mid-exposure groups, but was decreased about 40% in the high-exposure group. Average survival times in the control, low-, mid-, and high-exposure groups were 96.4 ± 27.6, 95.2 ± 29.1, 96.4 ± 27.8, and 59.5 ± 15.2 weeks, respectively. After the 60^{th} week, body weights decreased and mortality increased steeply in the highest exposure group. Histologic examination of organs[2] revealed an exposure-related increase in the mid- and high-exposure groups of benign and malignant tumors of the upper respiratory tract, including the nasal cavity, larynx, and trachea, and of the upper digestive tract, including the pharynx, esophagus, and forestomach (Table D-14). No lung tumors were observed. Tumors were detected in other sites, but none of these appeared to be related to exposure.

[2]Thyssen et al. (1981) did not report a complete list of organs examined histologically. The individual animal pathology reports documented examination of brain, pituitary, eyes, salivary gland, larynx, pharynx, thyroid, trachea, esophagus, thymus, heart lung, stomach, liver, spleen, pancreas, duodenum, jejunum and ileum, cecum, colon and rectum, kidneys, adrenals, bladder, testicle, epididymides, prostate, submandibular and mesenterial lymph nodes, aorta, sternum, bone, and muscle.

Table D-14. Tumor incidence in the respiratory tract and upper digestive tract for male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime—Thyssen et al. (1981)[a]

Target exposure concentration and (lifetime average continuous exposure)[b], mg/m³	Papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						
	Respiratory tract			Upper digestive tract			Incidence of pharynx or respiratory tract tumors[c]
	Larynx	Trachea	Nasal cavity	Pharynx	Esophagus	Forestomach	
0	0/23[d]	0/24	0/23	0/21	0/24	0/24	0/21[e]
2 (0.25)	0/19	0/20	0/20	0/18	0/20	0/20	0/18
10 (1.01)	11/23 (8)[f]	2/23 (0)	4/23 (1)	9/19 (7)	0/23 (0)	1/23 (1)	17/22 (11)[f]
50 (4.29)	11/23 (8)	3/23 (1)	1/23 (0)	18/22 (17)	2/23 (0)	2/23 (0)	18/22 (17)

[a]Histopathology incidence data from the raw data obtained from the Thyssen study (Clement Associates, 1990), adjusted to show animals only on study long enough to be at risk of tumor development: at least 1 year (0, 2, or 10 mg/m³ groups) or until the first tumor occurrence (week 40 in the 50 mg/m³ group). See Table E-30 for a list of all animals with histopathology results.
[b]See text.
[c]Excludes animals with unexamined tissues, unless a tumor was diagnosed in the tissues that were examined.
[d]Fractions represent the number of animals diagnosed with at least one of the specified tumors, among the animals examined for each tissue.
[e]Statistically significant trends by Cochran-Armitage trend test, conducted by EPA: all tumors: $p < 0.0001$, malignant tumors only: $p < 0.0001$.
[f]Includes one animal with an in situ carcinoma in the larynx.

The tumor types observed in the upper respiratory and upper digestive tract were very similar, characterized as polyps, papillomas, papillary polyps, and squamous carcinomas, with the exceptions of one in situ carcinoma and one adenocarcinoma (both in the mid-exposure group), reflecting similar cell types. Consequently, evaluation of the overall cancer hazard included consideration of the joint incidence of these tumor types. The pharynx and larynx (including the epiglottis), clearly the main cancer targets, can be difficult to distinguish given their close proximity. There were a few instances of nasal cavity or trachea tumors among animals without larynx or pharynx tumors. Tumors of the upper digestive tract may have been a consequence of mucociliary particle clearance (Thyssen et al., 1981), but the tumors in the esophagus and forestomach observed in the mid- and high-exposure groups all occurred in animals that also had pharynx or respiratory tract tumors. Overall, there were increasing trends in tumor incidence with increasing exposure, both for the combined incidence of benign or malignant tumors, or for only malignant tumors (Table D-14), and earlier occurrence of tumors with increasing exposure levels. Several studies have investigated the carcinogenicity of benzo[a]pyrene in hamsters exposed by intratracheal instillation. Single-dose studies verified that benzo[a]pyrene is tumorigenic, but do

not provide data useful for characterizing dose-response relationships because of their design (Kobayashi, 1975; Renzik-Schüller and Mohr, 1974; Henry et al., 1973; Mohr, 1971; Saffiotti et al., 1968; Gross et al., 1965; Herrold and Dunham, 1962). One multiple-dose study, which utilized very low doses (0.005, 0.02, and 0.04 mg once every 2 weeks), failed to find any tumorigenic response (Kunstler, 1983). Tumorigenic responses (mostly in the respiratory tract) were found at higher dosage levels (0.25–2 mg benzo[a]pyrene once per week for 30–52 weeks) in four multiple-dose studies (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Saffiotti et al., 1972). These studies identify the respiratory tract as a cancer target with exposure to benzo[a]pyrene by intratracheal instillation and provide supporting evidence for the carcinogenicity of benzo[a]pyrene at portal-of-entry sites.

D.4.3. Dermal studies

Skin-Tumor Initiation-Promotion Assays

Results from numerous studies indicate that acute dermal exposure to benzo[a]pyrene induces skin tumors in mice when followed by repeated exposure to a potent tumor promoter (Weyand et al., 1992; Cavalieri et al., 1991; Rice et al., 1985; El-Bayoumy et al., 1982; LaVoie et al., 1982; Raveh et al., 1982; Cavalieri et al., 1981; Slaga et al., 1980; Wood et al., 1980; Slaga et al., 1978; Hoffmann et al., 1972). The typical exposure protocol in these studies involved the application of a single dose of benzo[a]pyrene (typically ≥20 nmol per mouse) to dorsal skin of mice followed by repeated exposure to a potent tumor promoter, such as 12-O-tetradecanoylphorbol-13-acetate (TPA).

Carcinogenicity Bioassays

Repeated application of benzo[a]pyrene to skin (in the absence of exogenous promoters) has been variously demonstrated to induce skin tumors in mice, rats, rabbits, and guinea pigs (IARC, 2010; IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973). Mice have been most extensively studied, presumably because of early evidence that they may be more sensitive than other animal species, but comprehensive comparison of species differences in sensitivity to lifetime dermal exposure are not available. Early studies of complete dermal carcinogenicity in other species (rats, hamsters, guinea pigs, and rabbits) have several limitations that make them not useful for dose-response analysis [see IARC (1973) for descriptions of studies]. The limitations in these studies include inadequate reporting of the amount of benzo[a]pyrene applied, use of the carcinogen benzene as a vehicle, and less-than-lifetime exposure duration.

This section discusses complete carcinogenicity bioassays in mice that provide the best available dose-response data for skin tumors caused by repeated dermal exposure to benzo[a]pyrene (Sivak et al., 1997; Higginbotham et al., 1993; Albert et al., 1991; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959). Early studies of benzo[a]pyrene complete carcinogenicity

in mouse skin (Wynder and Hoffmann, 1959; Wynder et al., 1957) are not further described herein, because the investigators applied solutions of benzo[a]pyrene at varying concentrations on the skin, but did not report volumes applied. As such, applied doses in these studies cannot be determined. Other complete carcinogenicity mouse skin tumor bioassays with benzo[a]pyrene are available, but these are not described further in this review, because: (1) they only included one benzo[a]pyrene dose level (e.g., Emmett et al., 1981) or only dose levels inducing 90–100% incidence of mice with tumors (e.g., Wilson and Holland, 1988; Warshawsky and Barkley, 1987) and thus provide no information about the shape of the dose-response relationship; (2) they used a 1-time/week (e.g., Nesnow et al., 1983) or 1-time every 2 weeks (e.g., Levin et al., 1977) exposure protocol, which is less useful for extrapolating to daily human exposure; or (3) they used a vehicle demonstrated to interact with or enhance benzo[a]pyrene carcinogenicity (Bingham and Falk, 1969).

Poel (1959) applied benzo[a]pyrene in toluene to shaved interscapular skin of groups of 13–56 male C57L mice at doses of 0, 0.15, 0.38, 0.75, 3.8, 19, 94, 188, 376, or 752 µg, 3 times/week for up to 103 weeks or until the appearance of a tumor by gross examination (3 times weekly). Some organs (not further specified) and interscapular skin in sacrificed mice were examined histologically. With increasing dose level, the incidence of mice with skin tumors increased and the time of tumor appearance decreased (see Table D-15). Doses >3.8 µg were associated with 100% mortality after increasingly shorter exposure periods, none greater than 44 weeks. Poel (1959) did not mention the appearance of exposure-related tumors in tissues other than interscapular skin.

Table D-15. Skin tumor incidence and time of appearance in male C57L mice dermally exposed to benzo[a]pyrene for up to 103 weeks

Dose (µg)[a]	Incidence of mice with gross skin tumors	Time o first tumor appearance (wks)	Incidence of mice with epidermoid carcinoma[b]	Length of exposure period (wks)
0 (toluene)	0/33 (0%)	–	0/33 (0%)	92
0.15	5/55 (9%)	42–44[c]	0/55 (0%)	98
0.38	11/55 (20%)	24	2/55 (4%)	103
0.75	7/56 (13%)	36	4/56 (7%)	94
3.8	41/49 (84%)	21–25	32/49 (65%)	82
19	38/38 (100%)	11–21	37/38 (97%)	25–44[c]
94	35/35 (100%)	8–19	35/35 (100%)	22–43
188	12/14 (86%)	9–18	10/14 (71%)	20–35
376	14/14 (100%)	4–15	12/14 (86%)	19–35
752	13/13 (100%)	5–13	13/13 (100%)	19–30

[a]Indicated doses were applied to interscapular skin 3 times/week for up to 103 weeks or until time of appearance of a grossly detected skin tumor.
[b]Carcinomas were histologically confirmed.
[c]Ranges reflect differing information in Tables 4 and 6 of Poel (1959).

Source: Poel (1959).

Poel (1963) applied benzo[a]pyrene in a toluene vehicle to shaved interscapular skin of groups of 14–25 male SWR, C3HeB, or A/He mice 3 times/week at doses of 0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 µg benzo[a]pyrene per application, until mice died or a skin tumor was observed. Time ranges for tumor observations were provided, but not times of death for mice without tumors, so it was not possible to evaluate differential mortality among all dose groups or the length of exposure for mice without tumors. With increasing dose level, the incidence of mice with skin tumors increased and the time of tumor appearance decreased (Table D-16). The lowest dose level did not induce an increased incidence of mice with skin tumors in any strain, but strain differences in susceptibility were evident at higher dose levels. SWR and C3HeB mice showed skin tumors at doses ≥0.38 µg benzo[a]pyrene, whereas AH/e mice showed tumors at doses ≥19 µg benzo[a]pyrene (Table D-16). Except for metastases of the skin tumors to lymph nodes and lung, Poel (1963) did not mention the appearance of exposure-related tumors in tissues other than interscapular skin.

Table D-16. Skin tumor incidence and time of appearance in male SWR, C3HeB, and A/He mice dermally exposed to benzo[a]pyrene for life or until a skin tumor was detected

Dose (μg)[a]	SWR Mice		C3HeB Mice		A/He Mice	
	Tumor incidence[b]	Time of tumor appearance (wks)	Tumor incidence[b]	Time of tumor appearance (wks)	Tumor incidence[b]	Time of tumor appearance (wks)
0 (toluene)	0/20 (0%)	–	0/17 (0%)	–	0/17 (0%)	–
0.15	0/25 (0%)	–	0/19 (0%)	–	0/18 (0%)	–
0.38	2/22 (9%)	55	3/17 (18%)	81–93	0/19 (0%)	–
0.75	15/18 (83%)	25–72	4/17 (24%)	51–93	0/17 (0%)	–
3.8	12/17 (70%)	25–51	11/18 (61%)	35–73	0/17 (0%)	–
19.0	16/16 (100%)	12–28	17/17 (100%)	13–32	21/23 (91%)	21–40
94.0	16/17 (94%)	9–17	18/18 (100%)	10–22	11/16 (69%)	14–31
470.0	14/14 (100%)	5–11	17/17 (100%)	4–19	17/17 (100%)	4–21

[a]Indicated doses were applied 3 times/week for life or until a skin tumor was detected. Mice were 10–14 weeks old at initial exposure.
[b]Incidence of mice exposed ≥10 weeks with a skin tumor.

Source: Poel (1963).

Roe et al. (1970) treated groups of 50 female Swiss mice with 0 (acetone vehicle), 0.1, 0.3, 1, 3, or 9 μg benzo[a]pyrene applied to the shaved dorsal skin 3 times/week for up to 93 weeks; all surviving mice were killed and examined for tumors during the following 3 weeks. The dorsal skin of an additional control group was shaved periodically but was not treated with the vehicle. Mice were examined every 2 weeks for the development of skin tumors at the site of application. Histologic examinations included: (1) all skin tumors thought to be possibly malignant; (2) lesions of other tissues thought to be neoplastic; and (3) limited nonneoplastic lesions in other tissues. As shown in Table D-17, markedly elevated incidences of mice with skin tumors were only found in the two highest dose groups (3 and 9 μg), compared with no skin tumors in the control groups. Malignant skin tumors (defined as tumors with invasion or penetration of the panniculus carnosus muscle) were detected in 4/41 and 31/40 mice in the 3- and 9-μg groups, respectively, surviving to at least 300 days. Malignant lymphomas were detected in all groups, but the numbers of cases were not elevated compared with expected numbers after adjustment for survival differences. Lung tumors were likewise detected in control and exposed groups at incidences that were not statistically different.

Table D-17. Tumor incidence in female Swiss mice dermally exposed to benzo[a]pyrene for up to 93 weeks

Dose (µg)[a]	Cumulative number of mice with skin tumor/survivors						Skin tumor incidence[b]	Malignant lymphoma incidence[c]	Lung tumor incidence[c]
	200 d	300 d	400 d	500 d	600 d	700 d			
No treatment	0/48	0/43	0/40	0/31	0/21	0/0	0/43 (0%)	19/44 (43%)	12/41 (29%)
Acetone	0/49	0/47	0/45	0/37	0/23	0/0	0/47 (0%)	12/47 (26%)	10/46 (22%)
0.1	0/45	1/42	1/35	1/31	1/22	1/0	1/42 (2%)	11/43 (26%)	10/40 (25%)
0.3	0/46	0/42	0/37	0/30	0/19	0/0	0/42 (0%)	10/43 (23%)	13/43 (30%)
1	0/48	0/43	0/37	1/30	1/18	1/0	1/43 (2%)	16/44 (36%)	15/43 (35%)
3	0/47	0/41	1/37	7/35	8/24	8/0	8/41 (20%)	23/42 (55%)	12/40 (30%)
9	0/46	4/40	21/32	28/21	33/8	34/0	34/46 (74%)	9/40 (23%)	5/40 (13%)

[a]Doses were applied 3 times/week for up to 93 weeks to shaved dorsal skin.
[b]Numerator: number of mice detected with a skin tumor. Denominator: number of mice surviving to 300 days for all groups except the highest dose group. For the highest dose group (in which skin tumors were first detected between 200 and 300 days), the number of mice surviving to 200 days was used as the denominator.
[c]Numerator: number of mice detected with specified tumor. Denominator: number of mice surviving to 300 days unless a tumor was detected earlier, in which case, the number dying before 300 days without a tumor was subtracted from the number of animals reported to have been examined.

Source: Roe et al. (1970).

Schmidt et al. (1973) dermally administered benzo[a]pyrene in acetone to female NMRI mice (100/group) and female Swiss mice. Benzo[a]pyrene was applied to the shaved dorsal skin twice weekly at doses of 0, 0.05, 0.2, 0.8, or 2 µg until spontaneous death occurred or until an advanced carcinoma was observed. Skin carcinomas were identified by the presence of crater-shaped ulcerations, infiltrative growth, and the beginning of physical wasting (i.e., cachexia). Necropsy was performed for all animals, and histopathological examination of the dermal site of application and any other tissues with gross abnormalities was conducted. Skin tumors were observed at the two highest doses in both strains of female mice (see Table D-18), with induction periods of 53.0 and 75.8 weeks for the 0.8 and 2.0 µg NMRI mice and 57.8 and 60.7 weeks for the Swiss mice, respectively. The authors indicated that the latency period for tumor formation was highly variable, and significant differences among exposure groups could not be identified, but no further timing information was available, including overall survival. Carcinoma was the primary tumor type seen after lifetime application of benzo[a]pyrene to mouse skin.

Table D-18. Skin tumor incidence in female NMRI and Swiss mice dermally exposed to benzo[a]pyrene

Dose (µg)[a]	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma
Female NMRI mice			
0 (acetone)	0/100 (0%)	0/100 (0%)	0/100 (0%)
0.05	0/100 (0%)	0/100 (0%)	0/100 (0%)
0.2	0/100 (0%)	0/100 (0%)	0/100 (0%)
0.8	2/100 (2%)	0/100 (0%)	2/100 (2%)
2	30/100 (30%)	2/100 (2%)	28/100 (28%)
Female Swiss mice			
0 (acetone)	0/80 (0%)	0/80 (0%)	0/80 (0%)
0.05	0/80 (0%)	0/80 (0%)	0/80 (0%)
0.2	0/80 (0%)	0/80 (0%)	0/80 (0%)
0.8	5/80 (6%)	0/80 (0%)	5/80 (6%)
2	45/80 (56%)	3/80 (4%)	42/80 (52%)

[a]Mice were exposed until natural death or until they developed a carcinoma at the site of application; indicated doses were applied 2 times/week to shaved skin of the back.

Source: Schmidt et al. (1973).

Schmähl et al. (1977) applied benzo[a]pyrene 2 times/week to the shaved dorsal skin of female NMRI mice (100/group) at doses of 0, 1, 1.7, or 3 µg in 20 µL acetone. The authors reported that animals were observed until natural death or until they developed a carcinoma at the site of application. The effective numbers of animals at risk was about 80% of the nominal group sizes, which the authors attributed to autolysis; no information was provided concerning when tumors appeared in the relevant groups, how long treatment lasted in each group, or any times of death. Necropsy was performed on all mice and the skin of the back, as well as any organs that exhibited macroscopic changes, were examined histopathologically. The incidence of all types of skin tumors was increased in a dose-related manner compared to controls (see Table D-19). Carcinoma was the primary tumor type observed following chronic dermal exposure to benzo[a]pyrene, and skin papillomas occurred infrequently. Dermal sarcoma was not observed.

Table D-19. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene

Dose (µg)[a]	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma
0	1/81 (1%)[b]	0/81 (0%)	0/81 (0%)
1	11/77 (14%)	1/77 (1%)	10/77 (13%)
1.7	25/88 (28%)	0/88 (0%)	25/88 (28%)
3	45/81 (56%)	2/81 (3%)	43/81 (53%)

[a]Mice were exposed until natural death or until they developed a carcinoma at the site of application; indicated doses were applied 2 times/week to shaved skin of the back.
[b]Sarcoma.

Source: Schmähl et al. (1977).

Habs et al. (1980) applied benzo[a]pyrene to the shaved interscapular skin of female NMRI mice (40/group) at doses of 0, 1.7, 2.8, or 4.6 µg in 20 µL acetone twice weekly, from 10 weeks of age until natural death or gross observation of infiltrative tumor growth. Latency of tumors, either as time of first appearance or as average time of appearance of tumors, was not reported. Necropsy was performed on all animals, and the dorsal skin, as well as any organs showing gross alterations at autopsy, was prepared for histopathological examination. Age-standardized mortality rates, using the total population of the experiment as the standard population, were used to adjust tumor incidence findings in the study. Benzo[a]pyrene application was associated with a statistically significant increase in the incidence of skin tumors at each dose level (see Table D-20).

Table D-20. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene

Dose (µg)[a]	Skin tumor incidence	Age-standardized tumor incidence[b]
0 (acetone)	0/35 (0%)	0%
1.7	8/34 (24%)	24.8%
2.8	24/35 (68%)	89.3%
4.6	22/36 (61%)	91.7%

[a]Mice were exposed until natural death or until they developed a carcinoma at the site of application; indicated doses were applied 2 times/week to shaved skin of the back.
[b]Mortality data of the total study population were used to derive the age-standardized tumor incidence.

Source: Habs et al. (1980).

Grimmer et al. (1984) and Grimmer et al. (1983) applied benzo[a]pyrene (in 0.1 mL of a 1:3 solution of acetone:dimethyl sulfoxide [DMSO]) to the interscapular skin of female CFLP mice

(65–80/group) 2 times/week for 104 weeks. Doses were 0, 3.9, 7.7, and 15.4 µg in the 1983 experiment, and 0, 3.4, 6.7, and 13.5 µg in the 1984 experiment. Mice were observed until spontaneous death, unless an advanced tumor was observed or if animals were found moribund. Survival information was not provided; incidences reflect the number of animals placed on study. Necropsy was performed on all mice. Histopathological examination of the skin and any other organ showing gross abnormalities was performed. Chronic dermal exposure to benzo[a]pyrene produced a dose-related increase in skin tumor incidence and a decrease in tumor latency (see Table D-21). Carcinoma was the primary tumor type observed and a dose-response relationship was evident for carcinoma formation and incidence of all types of skin tumors.

Table D-21. Skin tumor incidence and time of appearance in female CFLP mice dermally exposed to benzo[a]pyrene for 104 weeks

Dose (µg)[a]	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma	Tumor appearance (wks)
Grimmer et al. (1983)				
0 (1:3 Solution of acetone:DMSO)	0/80 (0%)	0/80 (0%)	0/80 (0%)	–
3.9	22/65 (34%)	7/65 (11%)	15/65 (23%)	74.6 ± 16.78[b]
7.7	39/64 (61%)	5/64 (8%)	34/64 (53%)	60.9 ± 13.90
15.4	56/64 (88%)	2/64 (3%)	54/64 (84%)	44.1 ± 7.66
Grimmer et al. (1984)				
0 (1:3 Solution of acetone:DMSO)	0/65 (0%)	0/65 (0%)	0/65 (0%)	–
3.4	43/64 (67%)	6/64 (9%)	37/64 (58%)	61 (53–65)[c]
6.7	53/65 (82%)	8/65 (12%)	45/65 (69%)	47 (43–50)
13.5	57/65 (88%)	4/65 (6%)	53/65 (82%)	35 (32–36)

[a]Indicated doses were applied twice/week to shaved skin of the back.
[b]Mean ± SD.
[c]Median with 95% CI.

Sources: Grimmer et al. (1984) and Grimmer et al. (1983).

Habs et al. (1984) applied benzo[a]pyrene (in 0.01 mL acetone) to the shaved interscapular skin of female NMRI mice at doses of 0, 2, or 4 µg, 2 times/week for life. Animals were observed twice daily until spontaneous death, unless an invasive tumor was observed. All animals were necropsied and histopathological examination was performed on the dorsal skin and any other organ with gross abnormalities. Chronic dermal exposure to benzo[a]pyrene did not affect body weight gain, but appeared to reduce survival at the highest dose with mean survival times of 691, 648, and 528 days for the 0, 2, and 4 µg/day groups, respectively. The total length of exposure for

each group was not reported, but can be inferred from the survival data. Latency also was not reported. Benzo[a]pyrene application resulted in a dose-related increase the incidence of total skin tumors and skin carcinomas (see Table D-22). Hematopoietic tumors (at 6/20, 3/20, and 3/20) and lung adenomas (at 2/20, 1/20, and 0/20) were observed in the controls and in the benzo[a]pyrene treatment groups, but did not appear to be treatment related according to the study authors.

Table D-22. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene for life

Dose (μg)[a]	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma	Mean survival time, days (95% CI)
0 (Acetone)	0/20 (0%)	0/20 (0%)	0/20 (0%)	691 (600–763)
2	9/20 (45%)	2/20 (10%)	7/20 (35%)	648 (440–729)
4	17/20 (85%)	0/20 (0%)	17/20 (85%)	528 (480–555)

[a]Mice were exposed until natural death or until they developed an invasive tumor at the site of application; indicated doses were applied 2 times/week to shaved interscapular skin.

Source: Habs et al. (1984).

Groups of 23–27 female AhR-responsive Swiss mice were treated on a shaved area of dorsal skin with 0, 1, 4, or 8 nmol (0, 0.25, 1, or 2 μg/treatment) benzo[a]pyrene (>99% pure) in acetone 2 times weekly for 40 weeks (Higginbotham et al., 1993). Surviving animals were sacrificed 8 weeks later. Complete necropsies were performed, and tissues from the treated area, lung, liver, kidney, spleen, urinary bladder, ovary, and uterus were harvested for histopathologic examination. Histopathologic examination was performed on tissues from the treated area, lungs, liver, kidneys, spleen, urinary bladder, uterus, and ovaries, as well as any other grossly abnormal tissue. Lung adenomas occurred in each group (1/27, 2/24, 1/23, 1/23), and other tumors were noted in isolated mice (i.e., malignant lymphoma [spleen] in one low-dose and one mid-dose mouse; malignant lymphoma with middle organ involvement in one high-dose mouse; and hemangioma [liver] in one mid-dose mouse) and were not considered dose related. In addition, benzo[a]pyrene showed no skin tumors under the conditions of this bioassay.

Sivak et al. (1997) designed a study to compare the carcinogenicity of condensed asphalt fumes (including benzo[a]pyrene and other PAHs) with several doses of benzo[a]pyrene alone. For the purposes of this assessment, the exposure groups exposed to PAH mixtures are not discussed. Groups of 30 male C3H/HeJ mice were treated dermally twice/week to 0, 0.0001, 0.001, or 0.01% (0, 0.05, 0.5, or 5 μg) benzo[a]pyrene in a 50 μL volume of cyclohexanone/acetone (1:1) for 104 weeks beginning at 8 weeks of age. Mice dying during the exposure period or sacrificed at the 24-month termination were necropsied; mice with skin tumors that persisted for 4 consecutive

weeks with diameters >3 cm were sacrificed before the study termination and also necropsied. Skin samples and any grossly observed lesions were subjected to histopathological examination. Carcinomas and sarcomas were referred to as carcinomas, whereas papillomas, keratoacanthomas, and fibromas were referred to as papillomas. The incidences of mice with skin tumors and mean survival times for each group are shown in Table D-23. All high-dose mice died before the final sacrifice, and 80% showed scabs and sores at the site of application. The time of first tumor appearance was not reported for the tumor-inducing groups, but from a plot of the tumor incidence in the high-dose group versus treatment days, an estimate of ~320 days (~43 weeks) is obtained for this group. The extent of deaths prior to 1 year in each group was not provided, so the reported incidence may underestimate the tumor rate of animals exposed long enough to develop tumors. However, the crude skin tumor rates show an increasing trend in incidence.

Table D-23. Skin tumor incidence in male C3H/HeJ mice dermally exposed to benzo[a]pyrene for 24 months

Dose (µg)[a]	Skin tumor incidence (all types)[b]	Number of mice that died before final sacrifice	Mean survival time (days)
0 cyclohexanone/acetone (1:1)	0/30 (0%)	19	607
0.05	0/30 (0%)	15	630
0.5	5/30 (20%)	15	666
5.0	27/30 (90%)	30	449

[a]Indicated doses were applied twice/week to shaved dorsal skin.
[b]Number of skin tumor-bearing mice. In the high-dose group, 1 papilloma and 28 carcinomas were detected; in the 0.5 µg group, 2 papillomas and 3 carcinomas were detected.

Source: Sivak et al. (1997).

To examine dose-response relationships and the time course of benzo[a]pyrene-induced skin damage, DNA adduct formation, and tumor formation, groups of 43–85 female Harlan mice were treated dermally with 0, 16, 32, or 64 µg of benzo[a]pyrene in 50 µL of acetone once per week for 29 weeks (Albert et al., 1991). Interscapular skin of each mouse was clipped 3 days before the first application and every 2 weeks thereafter. Additional groups of mice were treated for 9 weeks with 0, 8, 16, 32, or 64 µg radiolabeled benzo[a]pyrene to determine BPDE-DNA adduct formation in the epidermis at several time points (1, 2, 4, and 9 weeks). Tumor formation was monitored only in the skin.

No tumors were present in vehicle-treated or untreated control mice. In exposed groups, incidences of mice with skin tumors were not reported, but time-course data for cumulative number of tumors per mouse, corrected for deaths from nontumor causes, were reported. Tumors began appearing after 12–14 weeks of exposure for the mid- and high-dose groups and at 18 weeks

for the low-dose group. At study termination (35 weeks after start of exposure), the mean number of tumors per mouse was approximately one per mouse in the low- and mid-dose groups and eight per mouse in the high-dose group, indicating that most, if not all, mice in each exposure group developed skin tumors and that the tumorigenic response was greatest in the highest dose group. The majority of tumors were initially benign, with an average time of 8 weeks for progression from benign papillomas to malignant carcinomas. Epidermal damage occurred in a dose-related manner (more severe in the high-dose group than in the low- and mid-dose groups) and included statistically significant increases (compared with controls) in: [^{3}H]-thymidine labeling and mitotic indices; incidence of pyknotic and dark cells (signs of apoptosis); and epidermal thickness. Only a minor expansion of the epidermal cell population was observed. In the high-dose group, indices of epidermal damage increased to a plateau by 2 weeks of exposure. The early time course of epidermal damage indices was not described in the low- or mid-dose groups, since data for these endpoints were only collected at 20, 24, and 30 weeks of exposure. An increased level of BPDE-DNA adducts, compared with controls, was apparent in all exposed groups after 4 weeks of exposure in the following order: 64 > 32 > 16 > 8 μg/week. The time-course data indicate that benzo[a]pyrene-induced increases in epidermal damage indices and BPDE-DNA adducts preceded the appearance of skin tumors.

D.4.4. Reproductive and Developmental Toxicity Studies

Oral

In a study evaluating the combined effects of dibutyl phthalate and benzo[a]pyrene on the male reproductive tract, Chen et al. (2011) administered benzo[a]pyrene alone in corn oil via daily gavage at 5 mg/kg-day to 30 male Sprague-Dawley rats (28–30 days old); a group of 30 rats received only vehicle. Body weight was measured weekly. Groups of 10 rats per group were sacrificed after 4, 8, and 12 weeks of exposure. At sacrifice, blood was collected for analysis of serum testosterone levels by radioimmunoassay. The testes and epididymides were weighed, and the right testis and epididymis were examined microscopically. The left epididymis was used for evaluation of sperm parameters (sperm count and morphology). Oxidative stress, as measured by superoxide dismutase (SOD), glutathione peroxidase, and catalase activity and malondialdehyde levels, was evaluated in the left testis of each rat. Exposure to benzo[a]pyrene did not affect body weight, and no signs of toxicity were seen. Testes and epididymides weights of exposed rats were similar to controls at all time points. Sperm counts and percent abnormal sperm were also similar to controls at 4 and 8 weeks of exposure, but were significantly ($p < 0.05$) different from controls after 12 weeks of exposure to benzo[a]pyrene (29% decrease in sperm count and 54% increase in percent abnormal sperm). Serum testosterone levels were significantly increased relative to controls after 4 weeks (>2-fold higher) and 8 weeks (~1.5-fold higher) of benzo[a]pyrene exposure, but were comparable to controls after 12 weeks. Histopathology evaluation of the testes revealed irregular and disordered arrangement of germ cells in the seminiferous tubules of treated rats; the

authors did not report incidence or severity of these changes. Among measures of testicular oxidative stress, only catalase activity was significantly affected by benzo[a]pyrene exposure, showing an increase of ~50% after 12 weeks of exposure. These data suggest a LOAEL of 5 mg/kg-day (the only dose tested) for decreased sperm count, increased percentage of abnormal sperm, altered testosterone levels, and histopathology changes in the testes following 13 weeks of exposure.

Chung et al. (2011) evaluated the effects of low-dose benzo[a]pyrene exposure on spermatogenesis and the role of altered steroidogenesis on the sperm effects. Groups of 20–25 male Sprague-Dawley rats (8 weeks old) were given daily gavage doses of 0, 0.001, 0.01, or 0.1 mg/kg-day benzo[a]pyrene in DMSO for 90 consecutive days. At the end of exposure, the animals were sacrificed for removal of the pituitary, testes, and epididymides, and collection of serum and testicular interstitial fluid. Subgroups of each exposure group were used for various analyses. Serum levels of testosterone and luteinizing hormone (LH) were measured, as was testosterone concentration in the interstitial fluid (ELISA). Body and testes weights were recorded. Sections of the testis were analyzed for apoptotic germ cells using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Evaluation of the epididymis included histopathology as well as measurement of caput and caudal epididymal tubule diameters. In addition, sperm were isolated from the cauda epididymis for analysis of sperm number and motility, acrosomal integrity, and immunocytochemistry for ADAM3 (a disintegrin and metallopeptidase domain 3; a sperm surface protein associated with fertilization).

Leydig cells were isolated from the right testis of animals from each dose group and cultured with or without human chorionic gonadotropin (hCG) or dibutyl cyclic adenosine monophosphate (dbcAMP) to evaluate testosterone production (Chung et al., 2011). Cultured Leydig cells were also subjected to western blot and immunocytochemistry analyses to evaluate changes in the expression of genes involved in steroidogenesis (steroidogenic acute regulatory protein, p450 side-chain cleavage, and 3β-hydroxysteroid dehydrogenase isomerase). Finally, pituitary gland extracts were evaluated for LH protein content using immunohistochemistry. Data were reported graphically and analyzed by analysis of variance (ANOVA) followed by Duncan's post hoc test, using a *p*-value cutoff of 0.05 for significant difference.

At termination of exposure, body weights of treated animals were similar to controls, as were absolute testes weights (Chung et al., 2011). Testosterone concentrations in both serum and testicular interstitial fluid were significantly reduced at the high dose of benzo[a]pyrene (0.1 mg/kg-day); based on visual inspection of the data, the mean serum concentration in this group was ~20% of the control and the mean interstitial fluid concentration was ~60% of the control (n = 9 animals/dose for these evaluations). In addition, baseline production of testosterone by cultured Leydig cells was significantly decreased (~50% based on data shown graphically) at 0.1 mg/kg-day. Both hCG- and dbcAMP-stimulated testosterone production measurements were lower (~60% lower than controls) in Leydig cells from rats exposed to either 0.01 or

0.1 mg/kg-day. Serum LH was significantly increased at both 0.01 and 0.1 mg/kg-day (~65–75% higher than controls based on visual inspection of graphs); concordant increases in the intensity of LH immunoreactivity were evident in pituitary extracts from exposed rats.

Dose-related increases in the number of apoptotic germ cells, primarily spermatogonia, were demonstrated both via TUNEL assay and caspase-3 staining; the number per tubule was significantly increased over control at all doses (Chung et al., 2011). Numbers of sperm were lower in the treatment groups, but did not differ significantly from the control group. However, sperm motility was significantly reduced in exposed groups compared with controls. The authors did not report sperm motility for all dose groups, but showed only the significant decrease in the 0.01 mg/kg-day mid-dose group (~30% lower than controls based on visual inspection of graph). Acrosomal integrity (measured by LysoTracker staining) was diminished in sperm heads from exposed rats; likewise, the expression of ADAM3 protein was downregulated by exposure to benzo[a]pyrene; the authors reported a significant decrease in the 0.01 mg/kg-day group, but did not provide details of the analysis of other exposure groups. Histopathology examination of the caput and cauda epididymides revealed dose-related decreases in both cauda and caput tubule diameters that were statistically significantly lower than controls at all doses (~10–30% smaller mean diameter than control based on measurements of 175 tubules collected from five samples in each group; data reported graphically).

Statistically significant effects observed at the lowest dose (0.001 mg/kg-day) of benzo[a]pyrene in this study included decreased caput and cauda epididymal tubule diameters (~10–15% lower than controls) and increased numbers of apoptotic germ cells (~2-fold higher than controls) by TUNEL assay (Chung et al., 2011). The authors reported that "sperm motility was significantly reduced in the benzo[a]pyrene-exposed groups in comparison to that of the control" but provided quantitative data only for the middle dose group, which exhibited a ~30% decrease in percent motile sperm. No statistically significant decrease in sperm count was reported at any dose. The middle dose (0.01 mg/kg-day) is considered to be a LOAEL based on reduced sperm motility.

Gao et al. (2011) examined effects of benzo[a]pyrene exposure via on cervical cell morphology within the uterus. Female ICR mice (18–22 g) were exposed to doses of 0, 2.5, 5, or 10 mg/kg twice per week for 14 weeks, either by gavage or by intraperitoneal (i.p.) injection (for this review, only oral results are reported). After adjustment for equivalent continuous dosing (2/7 days/week), the equivalent daily doses are estimated to be 0.7, 1.4, and 2.9 mg/kg-day. Both vehicle (sesame oil) and untreated control groups were maintained. Body weights were determined weekly. Groups of 26 mice per dose per exposure route were sacrificed at the end of exposure for evaluation of cervical weight and histopathology. Additional groups of 10 mice were exposed for 14 weeks and used for determination of lipid peroxidation (malondialdehyde and glutathione-S-transferase levels) and CYP1A1 activity (EROD) in both liver and cervix, as well as creatine kinase activity, AST activity, and IL-6 levels in cervix and serum.

Mortality was observed in all exposure groups with the exception of the low-dose oral exposure group; the authors did not indicate the timing or causes of death (Gao et al., 2011). There were no control deaths. Mortality incidences in the oral exposure groups (low to high dose) were 0/26 (untreated control), 0/26 (vehicle control), 0/26, 1/36, and 2/26. Benzo[a]pyrene treatment resulted in dose-dependent decreases in body weight gain. In the high-dose group of both treatments, body weight began to decline after ~7 weeks of exposure. Based on visual examination of data presented graphically, mean terminal body weights in the low-, mid-, and high-dose oral exposure groups were ~10, 15, and 30% lower (respectively) than the vehicle control mean. The untreated control mean body weight for the oral exposure group was similar to the vehicle control mean body weight. Uterine weight as a function of body weight was not affected by oral benzo[a]pyrene exposure. Microscopic examination of the cervix revealed increased incidences of epithelial hyperplasia and inflammatory cells in the cervix of all groups of exposed mice, and atypical hyperplasia of the cervix in mice exposed to 1.4 or 2.9 mg/kg -day benzo[a]pyrene. Statistical analysis of the findings was conducted, but was poorly reported in the publication. Table D-24 shows the incidences in the oral exposure groups, along with the results of Fisher's exact tests performed for this review.

Table D-24. Mortality and cervical histopathology incidences in female ICR mice exposed to benzo[a]pyrene via gavage for 14 weeks

Endpoint	Dose (mg/kg-d)				
	Untreated control	Vehicle control	0.7	1.4	2.9
Mortality	0/26	0/26	0/26	1/26	2/26
Cervical epithelial hyperplasia	0/26	0/26	4/26	6/25*	7/24*
Atypical hyperplasia of cervix	0/26	0/26	0/26	2/25	4/24*
Inflammatory cells in cervix	2/26	3/26	10/26*	12/25*	18/24*

*Significantly different from vehicle control by Fisher's exact test performed for this review (one-sided $p < 0.05$).

Source: Gao et al. (2011).

Levels of malondialdehyde in both the cervix and liver were significantly higher than controls in all dose groups of animals treated by either oral (1.5–2-fold higher in the cervix and ~3–7-fold higher in the liver after oral exposure, $p < 0.05$) or i.p. exposure. Concomitant decreases in GST activity (~15–50% lower than controls in the cervix and ~30–60% lower in the liver after oral exposure, $p < 0.05$) were also observed at all doses and in both organs and both treatments. EROD activity was increased in the cervix (~4–~12-fold) and liver (~12–~35-fold) of all exposure groups. Measurement of creatine kinase and AST activity in the cervix and serum also showed significant increases at all doses and after both exposures (~1.5–2-fold in the cervix, and ~20–50%

higher than controls in the liver after oral exposure). Finally, levels of the inflammatory cytokine IL-6 were significantly ($p < 0.05$) increased in the cervix of all treated mice, and were markedly increased (from more than 2-fold higher than untreated or vehicle controls at the low dose, to ~6-fold higher at the high dose) in the serum of treated mice.

Based on the observations of decreased body weight and increased cervical epithelial inflammation and hyperplasia, a LOAEL of 0.7 mg/kg-day (the lowest dose tested) is identified for this study.

Mohamed et al. (2010) investigated multi-generational effects in male mice following exposure of 6-week-old C57BL/6 mice (10/group) to 0 (corn oil), 1, or 10 mg/kg-day benzo[a]pyrene for 6 weeks by gavage. Following final treatment, male mice were allowed to stabilize for 1 week prior to being mated with two untreated female mice to produce an F1 generation. Male mice were sacrificed 1 week after mating. F1 males were also mated with untreated female mice, as were F2 males. The mice of the F1, F2, and F3 generations were not exposed to benzo[a]pyrene. The F0, F1, F2, and F3 mice were all sacrificed at the same age (14 weeks) and endpoints including testis histology, sperm count, sperm motility, and in vitro sperm penetration (of hamster oocytes) were evaluated. These endpoints were analyzed statistically using ANOVA and Tukey's honest significance test and results were reported graphically as means ± SD.

Testicular atrophy was observed in the benzo[a]pyrene treatment groups, but was not statistically different than controls. Statistically significant reductions were observed in epididymal sperm counts of F0 and F1 generations treated with the high or low dose of benzo[a]pyrene. For F0 and F1 generations, epididymal sperm counts were reduced approximately 50 and 70%, respectively, in the low- and high-dose groups. Additionally, sperm motility was statistically significantly decreased at the high dose in the F0 and F1 generations. Sperm parameters of the F3 generation were not statistically different from controls. An in vitro sperm penetration assay revealed statistically significantly reduced fertilization in F0 and F1 generations of the low- and high-dose groups. However, the value of this in vitro test is limited as it bypasses essential components of the intact animal system (U.S. EPA, 1996). Based on decreased epididymal sperm counts of F0 and F1 generations, a LOAEL of 1 mg/kg-day was established from this study (no NOAEL was identified).

Arafa et al. (2009) exposed groups of 12 male Swiss albino rats to benzo[a]pyrene in olive oil (0 or 50 mg/kg-day via gavage) for 10 consecutive days, either alone or after similar treatment with 200 mg/kg-day of the flavonoid hesperidin, which has been shown to exert anti-inflammatory, antioxidant, and anticarcinogenic activity. One day after the final dose, the animals were sacrificed for removal of the cauda epididymides and testes. Epididymal sperm count and motility were assessed, as was daily sperm production in the testes. The study authors also investigated the testicular activity of LDH, SOD, and GST, as well as GSH, malondialdehyde, and protein content. The testes were examined under light microscope.

Relative testes weights (normalized to body weight) of benzo[a]pyrene exposed-animals were significantly decreased compared with controls (35% lower, $p < 0.05$) (Arafa et al., 2009). In addition, exposure to benzo[a]pyrene alone resulted in significantly decreased sperm count, numbers of motile sperm, and daily sperm production (~40% decrease from control in each parameter, $p < 0.05$). Effects on sperm count and production were abolished by hesperidin pretreatment, but the number of motile sperm remained significantly depressed (compared with the control group) in the group exposed to both benzo[a]pyrene and hesperidin. Measures of antioxidant enzymes and lipid peroxidation showed statistically significant induction of oxidative stress in the testes of benzo[a]pyrene-exposed rats. With the exception of the decrease in testicular GSH content (which was partially mitigated), pretreatment with hesperidin eliminated the effects of benzo[a]pyrene on lipid peroxidation and antioxidant enzymes.

Xu et al. (2010) treated female Sprague-Dawley rats (6/group) to 0 (corn oil only), 5, or 10 mg/kg-day benzo[a]pyrene by gavage every other day for a duration of 60 days. This resulted in TWA doses of 0, 2.5, and 5 mg/kg-day over the study period of 60 days. Endpoints examined included ovary weight, estrous cycle, 17β-estradiol blood level, and ovarian follicle populations (including primordial, primary, secondary, atretic, and corpora lutea). Animals were observed daily for any clinical signs of toxicity and following sacrifice, gross pathological examinations were made and any findings were recorded. All animals survived to necropsy. A difference in clinical signs was not observed for the treated groups and body weights were not statistically different in treated animals (although they appear to be depressed 6% at the high dose). Absolute ovary weight was statistically significantly reduced in both the low- and high-dose groups (11 and 15%, respectively) (see Table D-25). Animals treated with the high dose were noted to have a statistically significantly prolonged duration of the estrous cycle and nonestrus phase compared to controls. Animals in the high-dose group also had statistically significantly depressed levels of estradiol (by approximately 25%) and decreased numbers of primordial follicles (by approximately 20%). This study also indicated a strong apoptotic response of ovarian granulosa cells as visualized through TUNEL labeling; however, the strongest response was seen at the low dose; decreased apoptosis was also observed at the high dose. Based on decreased ovary weight following a 60-day oral exposure to benzo[a]pyrene, a LOAEL of 2.5 mg/kg-day was established from this study (no NOAEL was identified).

Table D-25. Means ± SD for ovary weight in female Sprague-Dawley rats

	Dose (mg/kg-d)[a]		
	0	**2.5**	**5**
Ovary weight (g)	0.160 ± 0.0146	0.143 ± 0.0098*	0.136 ± 0.0098*
Body weight (g)	261.67 ± 12.0	249.17 ± 11.2	247.25 ± 11.2

*Statistically different from controls ($p < 0.05$) using one-way ANOVA.
[a]TWA doses over the 60-day study period.

Source: Xu et al. (2010).

Zheng et al. (2010) treated male Sprague-Dawley rats to 0 (corn oil only), 1, or 5 mg/kg-day benzo[a]pyrene by daily gavage for a duration of 30 (8/group) or 90 days (8/group). At necropsy, the left testis of each animal was collected and weighed. Testes testosterone concentrations were determined by radioimmunoassay and results were expressed as ng/g testis and reported graphically. Testicular testosterone was statistically significantly decreased in the high-dose group approximately 15% following 90 days of exposure. The low-dose group also appeared to have a similar average depression of testosterone levels; however, the change did not reach statistical significance. Testosterone levels measured in animals sacrificed following 30 days of benzo[a]pyrene exposure were not statistically different than controls. Based on decreased testicular testosterone levels following a 90-day oral exposure to benzo[a]pyrene, a LOAEL of 5 mg/kg-day and a NOAEL of 1 mg/kg-day were identified.

McCallister et al. (2008) administered 0 or 300 µg/kg-day benzo[a]pyrene by gavage in peanut oil to pregnant Long-Evans rats (n = 5 or 6) on gestation days (GDs) 14–17. At this exposure level, no significant changes were see in number of pups per litter, pup growth, or liver to body weight ratios in control compared to benzo[a]pyrene exposed offspring. Treatment-related differences in brain to body weight ratios were observed only on postnatal days (PNDs) 15 and 30. Decreases in cerebrocortical messenger ribonucleic acid (mRNA) expression of the glutamatergic N-methyl-D-aspartate (NMDA) receptor subunit was significantly reduced (50%) in treated offspring compared to controls. In addition, in utero exposed offspring exhibited decreased evoked cortical neuronal activity in the barrel field cortex when tested at PNDs 90–120.

Rigdon and Neal (1965) administered diets containing 1,000 ppm benzo[a]pyrene to pregnant mice (nine/group) on GDs 10–21 or 5–21. The pups were reported as appearing generally normal at birth, but cannibalism was elevated in the exposed groups. These results are in contrast with an earlier study (Rigdon and Rennels, 1964) in which rats (strain not specified) were fed diets containing benzo[a]pyrene at 1,000 ppm for approximately 28 days prior to mating and during gestation. In the earlier study, five of eight treated females mated with untreated males became pregnant, but only one delivered live young. The treated dam that delivered had two live and two stillborn pups; one dead pup was grossly malformed. In the remaining treated females,

vaginal bleeding was observed on GDs 23 or 24. In the inverse experimental design, three of six controls mated to benzo[a]pyrene-treated males became pregnant and delivered live young. Visceral and skeletal examinations of the pups were not conducted. These studies were limited by the small numbers of animals, minimal evaluation of the pups, lack of details on days of treatment (food consumption, weight gain), and occurrence of cannibalism.

Reproductive Effects of In Utero Exposure Via Oral Route

Mackenzie and Angevine (1981) conducted a two-generation reproductive and developmental toxicity study for benzo[a]pyrene in CD-1 mice. Benzo[a]pyrene was administered by gavage in 0.2 mL of corn oil to groups of 30 or 60 pregnant (the F0 generation) mice at doses of 0, 10, 40, or 160 mg/kg-day on GDs 7–16 only. Therefore, unlike the standard two-generation study, F1 animals were exposed only in utero. F1 offspring were evaluated for postnatal development and reproductive function as follows. F1 pups (four/sex when possible) were allowed to remain with their mothers until weaning on PND 20. Crossover mating studies were then conducted. Beginning at 7 weeks of age, each F1 male mouse (n = 20–45/group) was allowed to mate with two untreated virgin females for 5-day periods for 25 days (for a total exposure of 10 untreated females/F1 male), after which time the males were separated from the females. Fourteen days after separation from the males (i.e., on days 14–19 of gestation), the females were sacrificed and the numbers of implants, fetuses, and resorptions were recorded. The F2 fetuses were then examined for gross abnormalities. Similarly, each F1 female mouse (n = 20–55/group), beginning at 6 weeks of age, was paired with an untreated male for a period of 6 months. Males were replaced if the females failed to produce a litter during the first 30-day period. All F2 young were examined for gross abnormalities on day 1 of life and their weights were recorded on day 4. This F2 group was sacrificed on day 20 postpartum, while the F1 female was left with a male until the conclusion of the study. At 6 weeks of age, gonads of groups of 10 male and 10 female F1 mice exposed to 0, 10, or 40 mg/kg-day benzo[a]pyrene in utero were subjected to gross pathology and histologic examinations.

No maternal toxicity was observed. The number of F0 females with viable litters at parturition at the highest dose was statistically significantly reduced by about 35% (Table D-26), but progeny were normal by gross observation. Parturition rates of the low- and mid-dose groups were unaffected by treatment, and litter sizes of all treated groups were similar to the control group throughout lactation. However, body weights of the F1 pups in the mid- and high-dose groups were statistically significantly decreased on PND 20, by 7 and 13%, respectively, and in all treated pups on PND 42, 6, 6, and 10% for the low, mid, and high dose, respectively (Table D-26). The number of F1 pups surviving to PNDs 20 and 42 was significantly reduced at the high dose ($p < 0.01$), by 8 and 16%, respectively. When F1 males were bred to untreated females and F1 females were mated with untreated males, a marked dose-related decrease in fertility of >30% was observed in both sexes, starting at the lowest exposure. There were no treatment-associated gross abnormalities or differences in body weights in the F2 offspring.

Table D-26. Reproductive effects in male and female CD-1 F1 mice exposed in utero to benzo[a]pyrene

Effect	Dose (mg/kg-d)[a]			
	0	10	40	160
F0 mice with viable litters at parturition	46/60 (77%)	21/30 (70%)	44/60 (73%)	13/30 (43%)*
Mean ± SEM pup weight (g) at PND 20	11.2 ± 0.1	11.6 ± 0.1	10.4 ± 0.1*	9.7 ± 0.2*
Mean ± SEM pup weight (g) at PND 42	29.9 ± 0.2	28.2 ± 0.3*	28.0 ± 0.2*	26.8 ± 0.4*
F1 male fertility index[b]	80.4	52.0*	4.7*	0.0*
F1 female fertility index[c]	100.0	65.7*	0.0*	0.0*

*Significantly ($p < 0.05$) different from control by unspecified tests.
[a]Pregnant F0 mice were administered daily doses of benzo[a]pyrene in corn oil on GDs 7–16.
[b]Beginning at 7 weeks of age, each F1 male mouse (20–45/group) was exposed to 10 untreated females over a period of 25 days. Index = (females pregnant/females exposed to males) × 100.
[c]Beginning at 6 weeks of age, each F1 female mouse (20–55/group) was cohabitated with an untreated male for a period of 6 months.

SEM = standard error of the mean.

Source: Mackenzie and Angevine (1981).

Exposure to benzo[a]pyrene caused a marked dose-related decrease in the size of the gonads. In F1 males, testes weights were statistically significantly reduced. Testes from animals exposed in utero to 10 and 40 mg/kg-day weighed approximately 42 and 82%, respectively, of the weight of testes from the control animals (no F2 offspring were produced in the high-dose group). This was confirmed by histopathologic observation of atrophic seminiferous tubules in the 40 mg/kg-day group that were smaller than those of controls and were empty except for a basal layer of cells. The number of interstitial cells in the testes was also increased in this group. Males from the 10 mg/kg-day group showed limited testicular damage; although all exhibited evidence of tubular injury, each animal had some seminiferous tubules that displayed active spermatogenesis. Ovarian tissue was absent or reduced in F1 females such that organ weights were not possible to obtain. Examination of available tissue in these females revealed hypoplastic ovaries with few follicles and corpora lutea (10 mg/kg-day) or with no evidence of folliculogenesis (40 mg/kg-day). Ovarian tissue was not examined in highest-dose females.

The LOAEL in this study was 10 mg/kg-day based on decreases in mean pup weight (<5%) at PND 42 of F1 offspring of dams treated with 10, 40, or 160 mg/kg-day benzo[a]pyrene, marked decreases in the reproductive capacity (as measured by fertility index) of both male and female F1 offspring exposed at all three treatment levels of benzo[a]pyrene (by approximately 30% in males and females), decreased litter size (by about 20%) in offspring of F1 dams, and the dramatic

decrease in size and alteration in anatomy of the gonads of both male and female F1 mice exposed to 10 and 40 mg/kg-day benzo[a]pyrene in utero. A NOAEL was not identified.

In another reproductive and developmental toxicity study, benzo[a]pyrene was administered by gavage in corn oil to nine female NMRI mice at a dose of 10 mg/kg-day on GDs 7–16; a group of nine controls received corn oil (Kristensen et al., 1995). Body weights were monitored. F0 females were kept with their offspring until after weaning (21 days after delivery). At 6 weeks of age, one F1 female from each litter (n = 9) was caged with an untreated male. The F2 offspring were inspected for gross deformities at birth, weight and sex were recorded 2 days after birth, and the pups were sacrificed. The F1 females were sacrificed after 6 months of continuous breeding. The effects of benzo[a]pyrene treatment on fertility, ovary weights, follicles, and corpora lutea were evaluated. F0 females showed no signs of general toxicity, and there was no effect on fertility. F1 females had statistically significantly lower median numbers of offspring, number of litters, and litter sizes and a statistically significantly greater median number of days between litters as compared with the controls (Table D-27). At necropsy, the F1 females from treated F0 females had statistically significantly reduced ovary weights; histologic examination of the ovaries revealed decreased numbers of small, medium, or large follicles and corpora lutea (Table D-27). Only one dose group was used in this study, with decreased F1 female fertility observed following in utero exposure at the LOAEL of 10 mg/kg-day; no NOAEL was identified.

Table D-27. Effect of prenatal exposure to benzo[a]pyrene on indices of reproductive performance in F1 female NMRI mice

Endpoint (median with range in parentheses)	Control[a]	Benzo[a]pyrene exposed[a] (10 mg/kg-d)
Number of F2 offspring	92 (26–121)	22* (0–86)
Number of F2 litters	8 (3–8)	3* (0–8)
F2 litter size (number of pups per litter)	11.5 (6–15)	8* (3–11)
Number of d between F2 litters	20.5 (20–21)	21* (20–23)
F1 ovary weight (mg)	13 (13–20)	9* (7–13)
Number of small follicles	44 (1–137)	0* (0–68)
Number of medium follicles	9 (5–25)	0* (0–57)
Number of large follicles	14 (6–23)	0* (0–19)
Number of corpora lutea	16 (6–35)	0* (0–14)

*Significantly ($p < 0.05$) different from control group by Wilcoxon rank sum test or Kruskall-Wallis two-tailed test.
[a]Groups of nine female NMRI F0 mice were administered 0 or 10 mg benzo[a]pyrene/kg-day by gavage in corn oil on GDs 7–16. One F1 female from each litter was continuously bred with an untreated male for 6 months.

Source: Kristensen et al. (1995).

Chen et al. (2012) treated male and female neonatal Sprague-Dawley rats (10/sex/group) with benzo[a]pyrene (unspecified purity) dissolved in peanut oil by gavage daily on PNDs 5–11, at doses of 0.02, 0.2, or 2 mg/kg in 3 mL vehicle/kg body weight, determined individually based upon daily measurements. This time period was described as representing the brain growth spurt in rodents, analogous to brain developmental occurring from the third trimester to 2 years of age in human infants. Breeding was performed by pairs of 9-week-old rats, with delivery designated as PND 0. Litters were culled to eight pups/dam (four males and four females, when possible) and randomly redistributed at PND 1 among the nursing dams; dams themselves were rotated every 2–3 days to control for caretaking differences, and cage-side observations of maternal behavior were made daily. One male and female from each litter were assigned per treatment group, and the following physical maturation landmarks were assessed daily in all treatment groups until weaning at PND 21: incisor eruption, eye opening, development of fur, testis decent, and vaginal opening.

Neonatal sensory and motor developmental tests were administered to pups during the preweaning period at PNDs 12, 14, 16, and 18, and were behavioral tests administered to rats as adolescents (PNDs 35 and 36) or as adults (PNDs 70 and 71): each rat was only tested during one developmental period. All dosing was performed from 1300 to 1600 hours, and behavioral testing was during the "dark" period from 1900 to 2300 hours, although tests were performed in a lighted environment. Pups were observed individually and weighed daily, the order of testing litters was randomized each day, and all observations were recorded by investigators blinded to group treatment.

Sensory and motor developmental tests, including the surface righting reflex test, negative geotaxis test, and cliff aversion test, were performed only once, while the forelimb grip strength test was assessed during three 60-second trials on PND 12. Rat movements during the open-field test were recorded by camera, and two blinded investigators scored movement and rearing separately during a 5-minute evaluation period. Blinded investigators directly observed video monitoring of rat movements during the elevated plus maze, and after a 5-minute free exploration period, recorded number of entries into the closed and open arms, time spent in the open arms, and latency to the first arm entry. Assessment of the Morris water maze was slightly different, in that the rats were habituated to the testing pool by a 60-second swim without a platform on the day prior to testing. The rats were then tested during a 60-second swim with a hidden platform present at a constant position each day for 4 days; on the 5th day, the rats were evaluated during a 60-second probe swim without a platform. The number of times each animal crossed the original platform location and the duration of time spent in the platform quadrant were recorded during this final evaluation. One pup/sex/litter were assigned for behavioral testing to each of four tracks: Track 1, surface righting reflex test, cliff aversion test, and open-field test (PNDs 12–18); Track 2, negative geotaxis test, forelimb grip strength test, and open-field test (PNDs 12–20); Track 3, elevated plus maze, Morris water maze, and open-field test (PNDs 34–36); and Track 4, elevated plus maze,

Morris water maze, and open-field test (PNDs 69–71). All results were presented in graphic form only.

No significant effects on pup body weight were observed during the 7-day treatment period (PNDs 5–11). Three-way ANOVA (time × benzo[a]pyrene treatment × sex) indicated that effects of benzo[a]pyrene were not sex-dependent throughout the 71-day experiment, so both sexes were pooled together. From this pooled analysis, pups in the 2 mg/kg-day treatment group gained significantly less weight at both PND 36 and 71. There were no differences among treatment groups in incisor eruption, eye opening, development of fur, testis decent, or vaginal opening.

For all measurements of neonatal sensory and motor development, results from both sexes were analyzed together since benzo[a]pyrene was reported to have no significant interaction with sex by 3-way ANOVA. No significant differences were observed in either the cliff aversion or forelimb grip strength tests. In the surface righting reflex test, latency was increased in the 0.2 mg/kg-day group at PND 12, in the 0.02 and 2 mg/kg-day groups at PND 14, and in only the high-dose group at PND 16; latency was not significantly different in any group at PND 18. At PND 12, there was a dose-related increase in negative geotaxis latency associated with 0.02, 2, and 2 mg/kg-day benzo[a]pyrene, which was also present in the 2 mg/kg-day group at PND 14, but returned to control levels at PND 16 and 18. In the open field test, there were no significant differences in either locomotion or rearing activity at PND 18 or 20. At PND 34, the 2 mg/kg-day group exhibited significantly increased movement, but increases in rearing were not significant. At PND 69, increased locomotion was observed in both the 0.2 and 2 mg/kg-day groups, while rearing was significantly increased in only the 2 mg/kg-day treatment group.

The elevated plus maze performance was only evaluated in adolescent and adult rats. Unlike the previous tests, 3-way ANOVA revealed a statistically significant interaction between neonatal benzo[a]pyrene treatment and sex, so male and female performance was analyzed independently. No significant differences in PND 35 males were observed, and the only significant observation in PND 35 females was increased time spent in the open maze arms by the 2 mg/kg-day treatment group. Significantly decreased latency time to first open arm entry was observed in PND 70 males and females in both 0.2 and 2 mg/kg-day treatment groups; these groups also spent significantly more time in open maze arms, along with the 0.02 mg/kg-day female group. At PND 70, the 2 mg/kg-day males, along with the 0.2 and 2 mg/kg-day females, entered more frequently into open arms and less frequently into closed arms than the vehicle controls. In the Morris water maze, escape latency (time to reach the platform during each of the four testing days) was consistently increased in the 2 mg/kg-day treatment group of both sexes, in both adolescent and adult animals. These increases were statistically significant in both males and females treated with 2 mg/kg-day benzo[a]pyrene at both PNDs 39 and 74, and were also significantly elevated in 0.2 mg/kg-day animals of both sexes at PND 74. Likewise, performance during the 5th test day, in the absence of the escape platform, was significantly adversely affected by both metrics (decreased time spent in the target quadrant and decreased number of attempts to cross the platform location)

in 2 mg/kg-day rats of both sexes at both PNDs 40 and 75. PND 75 females treated with 0.2 mg/kg-day benzo[a]pyrene also showed significant decreases in both performance metrics, while PND 75 0.2 mg/kg-day males only demonstrated significant differences in "time spent in target quadrant." Swim speed was also assessed, but there were no differences among any treatment group at either age evaluated.

Jules et al. (2012) treated pregnant Long-Evans Hooded rats with benzo[a]pyrene (unspecified purity) dissolved in 0.875 mL peanut oil by gavage daily on GDs 14–17, at doses of 150, 300, 600, and 1,200 μg benzo[a]pyrene/kg body weight, with animals weighed daily. Cage-side observations were performed until pup weaning, and litter size was evaluated for each treatment group. Pups from four to five individual litters were analyzed for each endpoint, which was independently repeated for a total of three replicates. Delivery was designated PND 0, and pups were harvested on PNDs 0–15 for benzo[a]pyrene metabolite identification, or for other endpoints as young adults at PND 53. Systolic/diastolic blood pressure and heart rate was recorded by a volume pressure recording sensor and occlusion tail-cuff applied to conscious, non-anesthetized animals. Animals were preconditioned to the restraint device and tail-cuff by daily acclimatization sessions during PNDs 46–50, to minimize stress effects during data collection. Cardiac function values were averaged from 15 readings each collected over a 1-minute interval every other minute for 30 minutes on PND 53.

No significant differences in litter size or pup weight gain from PND 0 to 15 were reported in any treatment group, and no convulsions, tremors, or abnormal movements were reproducibly observed. Most analytical data were reported graphically, as mean ± standard error of the mean (SEM) of three replicates of 3–5 offspring measured/group. Plasma and heart tissue total benzo[a]pyrene metabolite levels were maximal at PND 0 (the first time point sampled) and progressively decreased from PNDs 0 to 13. Compared to the low-dose group (150 μg/kg), plasma metabolite levels were significantly elevated in the 600 and 1,200 μg/kg-day benzo[a]pyrene groups through PND 13, while heart metabolite levels were significantly increased through PND 11. Metabolites in mid-dose group, 300 μg/kg-day, trended between the 150 and 600 μg/kg-day group levels from PND 0 to 7, while not achieving statistically significant differences in pair-wise comparisons. Three principal groups of benzo[a]pyrene metabolites were identified. More than 70% of the total heart metabolite burden was composed of diol metabolites through PND 13, while the more reactive hydroxyl metabolites increased in relative composition from PND 9 to 13, and the dione population remained constant at ≤5%.

Cardiovascular function was evaluated in pups exposed in utero to 600 or 1,200 μg/kg-day benzo[a]pyrene versus controls (see Table D-28). A dose-related and statistically significant increase in both systolic (20, 50%) and diastolic pressure (30, 80%) was observed in mid- and high-dose pups, respectively. Heart rate was also significantly altered; a 10% increased heart rate was reported in the 600 μg/kg-day benzo[a]pyrene group, while the average heart rate of the 1,200 μg/kg-day benzo[a]pyrene groups decreased 8%.

Table D-28. Exposure-related effects in Long-Evans Hooded rats exposed to benzo[a]pyrene by gavage daily in utero from GD 14 to 17

Effect measured	Dose (mg/kg-d)		
	0	0.600	1.20
Heart rate (bpm; mean ± SEM)	504.6 ± 15.7	554.6 ± 26.2*	466.3 ± 16.9*
Blood pressure measured by tail cuff (mmHg; mean ± SEM)			
Systolic pressure	131.6 ± 1.2	151.6 ± 45*	200.4 ± 2.4*
Diastolic pressure	85.0 ± 4.2	113.0 ± 3.3*	155.6 ± 3.2*

*Significantly ($p < 0.05$) different from control mean; n = 4–5/replicate, 3 replicates performed.

Source: Jules et al. (2012).

Bouayed et al. (2009a) treated nursing female Swiss Albino OF1 mice (5/dose group) with benzo[a]pyrene (unspecified purity) dissolved in avocado oil by gavage daily while nursing pups from PND 1 to 14 at 0, 2, or 20 mg/kg-day in 10 mL/kg body weight, individually determined each day. Prior to benzo[a]pyrene treatment, litters were culled to 10 pups (5/sex when possible), and nurturing females were assigned to litters that were stratified randomly to achieve equivalent mean pup litter body weights across the designated treatment groups. As the effects of benzo[a]pyrene on maternal nurturing behavior was unknown, dam behavior was visually monitored daily until weaning. Furthermore, maternal nurturing performance from PND 0 to 21 was assessed by two methods: a nest-building test administered twice a day where nest quality/ complexity was scored 15 minutes after cotton material was supplied; and pup retrieval, in which latency to return the displaced pup to the nest was measured twice and averaged, was evaluated once daily At the indicated times, two mice/sex/litter were randomly selected and weighed, and their brains were resected for later mRNA expression analysis (n = 20/group).

Pup neuromotor maturation and behavior was assessed during pre-weaning by four standard methods (administered between 10 am and 1 pm on testing days, and in temporal order as indicated): (1) *righting reflex test*, maximum duration of 120 seconds, administered on PNDs 3, 5, 7, and 9; (2) *negative geotaxis test*, maximum duration of 120 seconds, administered on PNDs 5, 7, 9, and 11; (3) *forelimb grip test*, duration until failure, administered on PNDs 9 and 11; and (4) *open field test*, 6-minute evaluation of locomotor activity and rearing following a 1-minute habituation period, administered on PND 15. Adolescent function was evaluated by three methods: (1) *water escape pole climbing test*, administered at PND 20, in which the time to find the pole, time to climb the pole, and time to reach the safety platform were reported; (2) *elevated plus maze*, administered at PND 32 for 5 minutes, in which the latency time to first open arm entry, number of entries into open arms, total number of entries, percent of time spent in open arms, and percent of entries into open arms was determined; and (3) *Y-maze spontaneous alternation test*, administered at PND 40

for 5 minutes, in which the percentage of spontaneous alternation was calculated by: [(the number of successful overlapping triplets)/(total number of arm entries − 2) × 100%].

Benzo[a]pyrene treatment did not significantly affect the body weight of nursing mothers during the 2-week treatment period. Since 3-way ANOVA indicated that changes in pup weight as a result of benzo[a]pyrene treatment were not sex-dependent, data from male and female pups were combined. Benzo[a]pyrene treatment of nursing mothers was associated with a 8−9% weight gain in pups nursing from the 2 mg/kg-day group and a 10−12% weight gain in pups from the 20 mg/kg-day group at PNDs 12−20 (see Table D-29). While not significantly different from PND 26 to 40, pup weight in the 20 mg/kg-day group was continuously higher than either the 2 mg/kg-day group or vehicle-treated controls. There were no significant differences in pup brain weight or eye opening observed. Likewise, benzo[a]pyrene treatment of nursing mothers did not affect nest-building interest or quality, and while not significantly impacting pup retrieval time, the retrieval latency period was observed to increase with increasing treatment duration in both benzo[a]pyrene groups versus controls.

Table D-29. Exposure-related pup body weight effects in Swiss Albino OF1 mice exposed as pups to benzo[a]pyrene in breast milk from dams treated by gavage daily from PND 1 to 14

Pup body weight (g; mean ± SEM, n = 20)	Dose (mg/kg-d)		
	0	2	20
PND 0	1.70 ± 0.02	1.73 ± 0.02	1.74 ± 0.02
PND 4	3.01 ± 0.08	3.08 ± 0.06	3.16 ± 0.04
PND 8	5.08 ± 0.1	5.26 ± 0.09	5.30 ± 0.08
PND 12	6.57 ± 0.12	7.16 ± 0.06*	7.39 ± 0.05*
PND 20	12.51 ± 0.24	13.55 ± 0.25**	13.79 ± 0.14*
PND 26	17.71 ± 0.49	18.60 ± 0.36	18.35 ± 0.34
PND 32	24.47 ± 0.55	25.59 ± 0.57	25.38 ± 0.54
PND 40	30.55 ± 0.94	30.90 ± 0.93	31.78 ± 0.97

*$p < 0.001$ significantly different from control mean.
**$p < 0.01$.

Source: Bouayed et al. (2009a).

Behavioral test data was reported graphically, as mean ± SEM of n = 20/group. For the pre-weaning neuromotor developmental tests, benzo[a]pyrene treatment was found to not depend on sex; therefore, data from male and female pups were combined. Pups nursing from mothers administered 2 or 20 mg/kg-day benzo[a]pyrene had significantly elevated righting reflex times at PNDs 3−5, which decreased to control times at PNDs 7−9. Only pups from the 20 mg/kg-day

treatment group demonstrated significantly increased negative geotaxis latency, which was 2-fold greater than controls at PNDs 5, 7, and 9, but returned to control levels at PND 11. Interestingly, mice in the 20 mg/kg-day group had increased forelimb grip strength, which was significantly greater than control mice at PNDs 9 and 11, corresponding to increased body weight in the benzo[a]pyrene-treated mice versus controls. Mice in the 2 mg/kg-day group also performed better than controls at PND 9, but were equivalent at PND 11. No treatment or sex-related effects were reported on locomotion or rearing activity during the open field test. Sex-dependency on test performance became evident during the analysis of the water escape pole climbing test data: female pups were not significantly affected using any metric, while males in the 20 mg/kg-day group demonstrated a statistically significantly longer pole-grasping latency (3-fold), and took 13 times longer to escape the pole and board the safety platform versus vehicle controls. While performance of male pups from the 2 mg/kg-day group was not statistically significantly worse than vehicle controls by pair-wise comparison, latency for both pole-grasping and escape in this treatment group contributed to a significant trend for treatment dose and these effects. In the evaluation of the elevated plus maze, treatment effects did not appear to depend upon sex, so both male and female performance was analyzed together. Mice in both benzo[a]pyrene treatment groups demonstrated decreased latency time to first entering an open arm (30–50%), as well as entered open arms 2 times more frequently and spent twice as much time there versus vehicle controls. While mice in the 2 mg/kg-day treatment group entered into closed arms 20% less frequently than controls, mice in the 20 mg/kg-day group were not significantly different. Likewise, mice nursing from mothers treated with 2 mg/kg-day benzo[a]pyrene performed 15% more spontaneous alternations in the Y-maze spontaneous alternation test compared to controls, while mice in the high-dose group were not significantly different. The brains of pups nursing from the 20 mg/kg-day group expressed approximately 50% lower levels of 5-hydroxytryptamine (serotonin) 1A (5HT1A), and mu 1-opioid (MOR1) mRNA, and a trend was observed in the low-dose group as well. No significant changes in alpha-1D adrenergic or GABA-A mRNA levels were detected.

Reproductive Effects in Adults and Repeated Oral Exposure

Rigdon and Neal (1965) conducted a series of experiments to assess the reproductive effects of orally administered benzo[a]pyrene to Ah-responsive white Swiss mice. Female animals (number not stated) were administered benzo[a]pyrene at 250, 500, or 1,000 ppm in the feed before or during a 5-day mating period. Based on the initial body weight, the doses can be estimated as 32, 56, and 122 mg/kg-day, respectively. No effect on fertility was observed at any treatment dose, even when animals were fed 1,000 ppm benzo[a]pyrene for 20 days prior to mating, but interpretation of this finding was marred by large variability in numbers of pregnant females and litter sizes for both treated and control mice. In separate experiments, the fertility of five male mice/group was not affected by exposure to 1,000 ppm in food for up to 30 days prior to mating with untreated females. Histologic examinations showed that male mice fed 500 ppm

benzo[a]pyrene for 30 days had spermatozoa present in their testes; further details were not provided. The only treatment-related effect was a lack of weight gain related to feed unpalatability. While this study suggests that premating exposure of male or female mice to doses up to 122 mg/kg-day for 20 days may not affect fertility, the sample sizes were too small and the study designs were too inconsistent to provide reliable NOAELs and LOAELs for reproductive/ developmental toxicity.

In an earlier study (Rigdon and Rennels, 1964), rats (strain not specified) were fed diets containing benzo[a]pyrene at 1,000 ppm for approximately 28 days prior to mating and during gestation. In this study, five of eight treated females mated with untreated males became pregnant, but only one delivered live young. The treated dam that delivered had two live and two stillborn pups; one dead pup was grossly malformed. In the remaining treated females, vaginal bleeding was observed on GDs 23 or 24. In the inverse experimental design, three of six controls mated to benzo[a]pyrene-treated males became pregnant and delivered live young. Visceral and skeletal examinations of the pups were not conducted. These studies are insufficiently reported and of insufficient design (e.g., inadequate numbers of animals for statistical analysis) to provide reliable NOAELs or LOAELs for reproductive effects from repeated oral exposure to benzo[a]pyrene.

D.4.5. Inhalation

Reproductive Toxicity and In Utero Exposure via Inhalation

Archibong et al. (2002) evaluated the effect of exposure to inhaled benzo[a]pyrene on fetal survival and luteal maintenance in timed-pregnant F344 rats. Prior to exposure on GD 8, laparotomy was performed to determine the number of implantation sites, and confirmed pregnant rats were divided into three groups, consisting of rats that had four to six, seven to nine, or more than nine conceptuses in utero. Rats in these groups were then assigned randomly to the treatment groups or control groups to ensure a similar distribution of litter sizes. Animals (10/group) were exposed to benzo[a]pyrene:carbon black aerosols at concentrations of 25, 75, or 100 μg/m^3 via nose-only inhalation, 4 hours/day on GDs 11–20. Control animals were either sham-exposed to carbon black or remained entirely unexposed. Results of particle size analysis of generated aerosols were reported by several other reports from this laboratory (Inyang et al., 2003; Ramesh et al., 2001a; Hood et al., 2000). Aerosols showed a trimodal distribution (average of cumulative mass, diameter) <95%, 15.85 μm; 89%, <10 μm; 55%, <2.5 μm; and 38%, <1 μm (Inyang et al., 2003). Ramesh et al. (2001a) reported that the MMAD (± geometric SD) for the 55% mass fraction with diameters <2.5 μm was 1.7 ± 0.085. Progesterone, estradiol-17β, and prolactin concentrations were determined in plasma collected on GDs 15 and 17. Fetal survival was calculated as the total number of pups divided by the number of all implantation sites determined on GD 8. Individual pup weights and crown-rump length per litter per treatment were determined on PND 4 (PND 0 = day of parturition).

Archibong et al. (2002) reported that exposure of rats to benzo[a]pyrene caused biologically and statistically significant ($p \leq 0.05$) reductions in fetal survival compared with the two control groups; fetal survival rates were 78.3, 38.0, and 33.8% per litter at 25, 75, and 100 μg/m^3, respectively, and 96.7% with carbon black or 98.8% per litter in untreated controls (see Table D-30). Consequently, the number of pups per litter was also decreased in a concentration-dependent manner. The decrease was ~50% at 75 μg/m^3 and ~65% at 100 μg/m^3, compared with sham-exposed and unexposed control groups. No effects on hormone levels were observed on GDs 15 or 17 at the low dose. Biologically significant decreases in mean pup weights (expressed as g per litter) of >5% relative to the untreated control group were observed at doses ≥75 μg/m^3 (14 and 16% decreases at 75 and 100 μg/m^3, respectively, $p < 0.05$). There were no statistically significant differences from the control groups in crown-rump length (see Table D-30).

Table D-30. Pregnancy outcomes in female F344 rats treated with benzo[a]pyrene on GDs 11–21 by inhalation

	Administered concentration of benzo[a]pyrene (μg/m^3)				
Parameter[a]	0 (unexposed control)	0 (carbon black)	25	75	100
Implantation sites	8.6 ± 0.2	8.8 ± 0.1	8.8 ± 0.5	9.0 ± 0.2	8.8 ± 0.1
Pups per litter	8.5 ± 0.2	8.7 ± 0.2	7.4 ± 0.5*	4.2 ± 0.1*	3.0 ± 0.2*
Survival (litter %)	98.9 ± 1.1	96.7 ± 1.7	78.3 ± 4.1*	38.0 ± 2.1*	33.8 ± 1.3*
Pup weight (g/litter)	10.6 ± 0.1	8.8 ± 0.1	10.5 ± 0.2	9.1 ± 0.2*	8.9 ± 0.1*
Crown-rump length (mm/litter)	29.4 ± 0.6	29.3 ± 0.5	28.0 ± 0.6	27.3 ± 0.7	27.9 ± 0.7

*Significantly different from controls at $p < 0.05$ by one-tailed post-hoc t-testing following ANOVA.
[a]Values presented as means ± SEM.

Source: Archibong et al. (2002).

Benzo[a]pyrene exposure at 75 μg/m^3 caused a statistically significant decrease in plasma progesterone, estradiol, and prolactin on GD 17; these levels were not determined in the rats exposed to 100 μg/m^3 (Archibong et al., 2002). Plasma prolactin is an indirect measure of the activity of decidual luteotropin, a prolactin-like hormone whose activity is necessary for luteal maintenance during pregnancy in rats. Control levels of prolactin increased from GD 15 to 17, but this increase did not occur in the rats exposed to 75 μg/m^3. Although the progesterone concentration at 75 μg/m^3 was significantly lower than in controls on GD 17, the authors thought that the circulating levels should have been sufficient to maintain pregnancy; thus, the increased loss of fetuses was thought to be caused by the lower prolactin levels rather than progesterone deficiency. The reduced circulating levels of progesterone and estradiol-17β among

benzo[a]pyrene-treated rats were thought to be a result of limited decidual luteotropic support for the corpora lutea. The authors proposed the following mechanism for the effects of benzo[a]pyrene on fertility: benzo[a]pyrene or its metabolites decreased prolactin and decidual luteotropin levels, compromising the luteotropic support for the corpora lutea and thereby decreasing the plasma levels of progesterone and estradiol-17β. The low estradiol-17β may decrease uterine levels of progesterone receptors, thereby resulting in fetal mortality. Based on biologically and statistically significant decreases in pups/litter and percent fetal survival/per litter, the LOAEL was 25 μg/m^3; no NOAEL was identified.

Neurotoxicity and In Utero Exposure via Inhalation

To evaluate the effects of benzo[a]pyrene on the developing central nervous system, Wormley et al. (2004) studied rat offspring from those produced by the Archibong et al. (2002) investigation (personal communication, D. Hood to K. Hogan, 5/11/2016), in which exposed timed-pregnant F344 rats (10/group) to benzo[a]pyrene:carbon black aerosols by nose-only inhalation on GDs 11–21 for 4 hours/day at a concentration of 100 μg/m^3. Results of particle size analysis of generated aerosols were reported by other reports from this laboratory (Ramesh et al., 2001a; Hood et al., 2000). Particle size analysis of a 100-μg/m^3 aerosol showed a trimodal distribution (average of cumulative mass, diameter): <95%, 15.85 μm; 90%, <10 μm; 67.5%, <2.5 μm; and 66.2%, <1 μm; the MMAD ± geometric SD for the latter fraction was 0.4 ± 0.02 μm (Hood et al., 2000). As noted by Archibong et al. (2002), benzo[a]pyrene reduced the number of live pups at this exposure level to one-third of control values. During PNDs 60–70, electrical stimulation and evoked field potential responses were recorded via electrodes implanted into the brains of the animals. Direct stimulation of perforant paths in the entorhinal region revealed a diminution in long-term potentiation of population spikes across the perforant path-granular cell synapses in the dentate gyrus of the hippocampus of F1 generation benzo[a]pyrene-exposed animals; responses in exposed offspring were about 25% weaker than in control offspring. Additionally, NMDA receptor subunit 1 protein (important for synaptic functioning) was down-regulated in the hippocampus of benzo[a]pyrene-exposed F1 pups. The authors interpreted their results as suggesting that gestational exposure to benzo[a]pyrene inhalation attenuates the capacity for long-term potentiation (a cellular correlate of learning and memory) in the F1 generation.

In another study by this same group of investigators, Wu et al. (2003a) evaluated the generation of benzo[a]pyrene metabolites in F1 generation pups, as well as the developmental profile for AhR and mRNA. From each litter generated in the Archibong et al. (2002) investigation (personal communication, D. Hood to K. Hogan, 12/30/2016), two randomly selected pups were sacrificed on each of PNDs 0, 3, 5, 10, 15, 20, and 30. Body, brain, and liver weights were recorded. Benzo[a]pyrene metabolites were analyzed in the cerebral cortex, hippocampus, liver, and plasma. A dose-related increase in plasma and cortex benzo[a]pyrene metabolite concentrations in pups was observed. Dihydrodiols (4,5-; 7,8-; 9,10-) dominated the metabolite distribution profile up to

PND 15 and the hydroxy (3-OH-benzo[a]pyrene; 9-OH-benzo[a]pyrene) metabolites after PND 15 at 100 μg/m^3 (the only exposure concentration reported).

Adult Male Reproductive Effects and Repeated Inhalation Exposure

Inyang et al. (2003) evaluated the effect of subacute exposure to inhaled benzo[a]pyrene on testicular steroidogenesis and epididymal function in rats. Male F344 rats (10/group), 13 weeks of age, were exposed to benzo[a]pyrene:carbon black aerosols at 25, 75, or 100 μg/m^3 via nose-only inhalation, 4 hours/day for 10 days. Control animals either were exposed to carbon black (sham) to control for exposure to the inert carrier or remained untreated. Each benzo[a]pyrene concentration had its own set of controls (carbon black and untreated). Aerosols showed a trimodal distribution (average of cumulative mass, diameter): 95%, <15.85 μm; 89%, <10 μm; 55%, <2.5 μm; and 38%, <1 μm (Inyang et al., 2003); an earlier report from this laboratory indicated that the 55% mass fraction had a MMAD ± geometric SD of 1.7 ± 0.085 (Ramesh et al., 2001a). Blood samples were collected at 0, 24, 48, and 72 hours after cessation of exposure to assess the effect of benzo[a]pyrene on systemic concentrations of testosterone and LH, hormones that regulate testosterone synthesis. Reproductive endpoints such as testis weight and motility and density of stored (epididymal) sperm were evaluated.

Regardless of the exposure concentration, inhaled benzo[a]pyrene did not affect testis weight or the density of stored sperm compared with controls. However, inhaled benzo[a]pyrene caused a concentration-dependent reduction in the progressive motility of stored sperm. Progressive motility was similar at 75 and 100 μg/m^3, but these values were significantly lower ($p < 0.05$) than in any other group. The reduction in sperm motility postcessation of exposure was thought to be the result of benzo[a]pyrene limiting epididymal function. Benzo[a]pyrene exposure to 75 μg/m^3 caused a decrease in circulating concentrations of testosterone compared with controls from the time of cessation of exposure (time 0) to 48 hours posttermination of exposure ($p < 0.05$). However, the decrease was followed by a compensatory increase in testosterone concentration at 72 hours postcessation of exposure. Exposure to 75 μg/m^3 caused a nonsignificant increase in plasma LH concentrations at the end of exposure compared with controls, which increased further and turned significant ($p < 0.05$) for the remaining time of the study period. The decreased plasma concentration of testosterone, accompanied by an increased plasma LH level, was thought to indicate that benzo[a]pyrene did not have a direct effect on LH. The authors also noted that the decreased circulating testosterone may have been secondary to induction of liver CYP450 enzymes by benzo[a]pyrene. The authors concluded that subacute exposure to benzo[a]pyrene contributed to impaired testicular endocrine function that ultimately impaired epididymal function. For this study, the NOAEL was 25 μg/m^3 and the LOAEL was 75 μg/m^3, based on a statistically significant reduction in the progressive motility of stored sperm and impairment of testicular function with 10 days of exposure at 75 μg/m^3.

In a follow-up study with longer exposure duration, adult male F344 rats (10 per group) were exposed to benzo[a]pyrene:carbon black aerosols at 75 μg/m^3 via nose-only inhalation,

4 hours/day for 60 days (Archibong et al., 2008; Ramesh et al., 2008). Rats in the control group were subjected to the nose-only restraint, but were not exposed to the carbon black carrier. Blood samples were collected at 0, 24, 48, and 72 hours after exposure terminated, and the animals were sacrificed for tissue analyses following the last blood sampling. Data were analyzed statistically for benzo[a]pyrene effects on weekly body weights, total plasma testosterone and LH concentrations, testis weights, density of stored spermatozoa, sperm morphological forms and motility, benzo[a]pyrene metabolite concentrations and aryl hydrocarbon hydroxylase (AHH) activity, and morphometric assessments of testicular histologies. Relative to controls, the results indicated 34% reduced testis weight ($p < 0.025$), reduced daily sperm production ($p < 0.025$), and reduced intratesticular testosterone concentrations ($p < 0.025$). Plasma testosterone concentrations were reduced to about one-third of the level in controls on the last day of exposure (day 60) and at 24, 48, and 72 hours later ($p < 0.05$). However, plasma LH concentrations in benzo[a]pyrene-exposed rats were elevated throughout the blood sampling time periods compared with controls ($p < 0.05$). In testis, lung, and liver, AHH activity and benzo[a]pyrene-7,8-dihydrodiol (precursor to the DNA-reactive BPDE) and benzo[a]pyrene-3,6-dione metabolites were significantly ($p < 0.05$) elevated relative to controls. Progressive motility and mean density of stored spermatozoa were significantly reduced ($p < 0.05$). Weekly body weight gains were not affected by benzo[a]pyrene exposure. These results indicate that a 60-day exposure of adult male rats to benzo[a]pyrene: carbon black aerosols at 75 μg/m^3 produced decreased testis weight; decreased intratesticular and plasma testosterone concentrations; and decreased sperm production, motility, and density.

D.5. OTHER PERTINENT TOXICITY INFORMATION

D.5.1. Genotoxicity Information

Information summarizing methods commonly used to detect DNA adducts following PAH or benzo[a]pyrene exposure is presented in Table D-31. Information regarding the genotoxicity of benzo[a]pyrene in in vitro and in vivo systems is presented in Tables D-32, D-33, D-34, and D-35.

Table D-31. Select PAH-DNA adduct detection methods[a]

Adduct detection method	Adduct detection limit (nucleotides)	Quantitation	Adduct identification
Radiolabeled compounds			
Accelerator mass spectroscopy (typically ^{14}C or ^{3}H); with or without separation	10^{12}	Highest sensitivity	High specificity due to radiolabeled chemical exposure (no structural information)
Dosing with radiolabeled compound (typically ^{14}C or ^{3}H) + quantification of radioactive DNA using liquid scintillation counting	10^{9}	High to moderate sensitivity (potential isotope artefacts may lower sensitivity)	Moderate specificity (additional characterization may be required)

Adduct detection method	Adduct detection limit (nucleotides)	Quantitation	Adduct identification
Unlabeled adduct detection			
^{32}P-postlabeling + separation by thin-layer chromatography or HPLC	10^9	High sensitivity	Low specificity (chemical nature of adducts unknown—additional characterization required)
Separation by chromatography (gas chromatography or liquid chromatography) + MS	10^9	High sensitivity	Highest specificity; structural identification possible
Separation (HPLC or electrophoresis) + fluorescence spectroscopy, electrochemical, or UV detection	10^8	Moderate to high sensitivity for PAH adducts	High specificity and structural identification (depending on quality of standard)
Immunoassays			
Immunoassay using antisera raised against BP-modified DNA or adducts	10^8	High sensitivity	Broad specificity for family of carcinogenic PAH-DNA adducts
Immunohistochemistry (in situ detection in intact tissues)	10^7	Low sensitivity	Broad specificity for family of carcinogenic PAH-DNA adducts

[a]Summarized from Himmelstein et al. (2009); Arlt et al. (2006); Phillips et al. (2000); Poirier et al. (2000).

Table D-32. In vitro genotoxicity studies of benzo[a]pyrene in non-mammalian cells

	Result		Reference
	+S9	−S9	
Endpoint/test system: *prokaryotic cells*			
Forward mutation			
Salmonella typhimurium TM677	+	–	Rastetter et al. (1982)
S. typhimurium TM677	+	ND	Babson et al. (1986)
Reverse mutation			
S. typhimurium TA98, TA1538	+	ND	Ames et al. (1975)
S. typhimurium TA98, TA100, TA1538	+	ND	Mccann et al. (1975)
S. typhimurium TA1538, TA98	+	–	Wood et al. (1976)
S. typhimurium TA98, TA100, TA1537	+	–	Epler et al. (1977)
S. typhimurium TA98, TA100	+	–	Obermeier and Frohberg (1977)
S. typhimurium TA98	+	–	Pitts et al. (1978)
S. typhimurium TA98, TA100	+	ND	Lavoie et al. (1979)
S. typhimurium TA98, TA100	+	–	Simmon (1979a)

	Result		Reference
	+S9	−S9	
S. typhimurium TA98	+	ND	Hermann (1981)
S. typhimurium TA98, TA100	+	ND	Alfheim and Ramdahl (1984)
S. typhimurium TA98, TA100, TA1538	ND	–	Glatt et al. (1985)
S. typhimurium TA97, TA98, TA100	+	–	Sakai et al. (1985)
S. typhimurium TA97, TA98, TA100, TA1537	+	–	Glatt et al. (1987)
S. typhimurium TA97, TA98, TA100	+	ND	Marino (1987)
S. typhimurium TA98	+	–	Alzieu et al. (1987)
S. typhimurium TA98, TA100	+	–	Prasanna et al. (1987)
S. typhimurium TA98	+	ND	Ampy et al. (1988)
S. typhimurium TA98, TA100	+	ND	Bos et al. (1988)
S. typhimurium TA98	+	ND	Lee and Lin (1988)
S. typhimurium TA98	+	ND	Antignac et al. (1990)
S. typhimurium TA98	–	ND	Gao et al. (1991)
S. typhimurium TA98	+	ND	Balansky et al. (1994)
S. typhimurium TA100	+	ND	Norpoth et al. (1984)
S. typhimurium TA100	+	–	Carver et al. (1986)
S. typhimurium TA100	+	ND	Pahlman and Pelkonen (1987)
S. typhimurium TA100	+	ND	Tang and Friedman (1977)
S. typhimurium TA100	+	ND	Bruce and Heddle (1979)
S. typhimurium TA100	+	ND	Phillipson and Ioannides (1989)
S. typhimurium TA100	–	ND	Balansky et al. (1994)
S. typhimurium TA1537, TA1538	+	–	Ames et al. (1973)
S. typhimurium TA1537, TA1538	+	–	Glatt et al. (1975)
S. typhimurium TA1537	+	ND	Oesch et al. (1976)
S. typhimurium TA1538	+	ND	Egert and Greim (1976)
S. typhimurium TA1538	+	–	Rosenkranz and Poirier (1979)
S. typhimurium TA1535	–	–	Ames et al. (1973)
S. typhimurium TA 1535	–	–	Glatt et al. (1975)
S. typhimurium TA 1535	–	ND	Mccann et al. (1975)
S. typhimurium TA1535	–	–	Epler et al. (1977)
DNA damage			
Escherichia coli/pol A	+	–	Rosenkranz and Poirier (1979)

	Result		Reference
	+S9	−S9	
E. coli/differential killing test	+	−	Tweats (1981)
E. coli WP2-WP100/rec-assay	+	ND	Mamber et al. (1983)
E. coli/SOS chromotest Pq37	+	−	Mersch-Sundermann et al. (1992)
Endpoint/test system: *nonmammalian eukaryotes*			
Mitotic recombination			
Saccharomyces cerevisiae D4-RDII	ND	−	Siebert et al. (1981)
S. cerevisiae D3	−	−	Simmon (1979b)

+ = positive; − = negative; ND = not determined.

Table D-33. In vitro genotoxicity studies of benzo[a]pyrene in mammalian cells

Assay/test system	Result		Reference
	+S9	−S9	
Forward mutation			
Human AHH-1 lymphoblastoid cells	ND	+	Danheiser et al. (1989)
Human lymphoblast (AHH-1) cells (*hprt*)	ND	+	Crespi et al. (1985)
Human lymphoblastoid (AHH-1) cell line	ND	+	Chen et al. (1996)
Human fibroblast (MRC5CV1) cell line (*hprt*)	−	ND	Hanelt et al. (1997)
Human lymphoblast (TK) cells	ND	+	Barfknecht et al. (1982)
Human lymphoblast (TK6) cells	+	ND	Crespi et al. (1985)
Human embryonic epithelial (EUE) cells	ND	+	Rocchi et al. (1980)
Human HSC172 lung fibroblasts	+	−	Gupta and Goldstein (1981)
Human Q3-wp normal lung keratinocytes	+	ND	Allen-Hoffmann and Rheinwald (1984)
Human SCC-13Y lung keratinocytes	ND	+	Allen-Hoffmann and Rheinwald (1984)
Mouse *lacZ* transgenic Muta™Mouse primary hepatocytes	ND	+	Chen et al. (2010)
Mouse L5178Y/HGPRT	+	−	Clive et al. (1979)
Mouse lymphoma (L5178Y/TK+/-) cells	+	−	Clive et al. (1979)
Mouse lymphoma (L5178Y/TK+/-) cells	+	ND	Amacher et al. (1980); Amacher and Turner (1980)
Mouse lymphoma (L5178Y/TK+/-) cells	+	−	Amacher and Paillet (1983)
Mouse lymphoma (L5178Y/TK+/-) cells	+	ND	Arce et al. (1987)
Chinese hamster ovary (CHO) cells (*aprt*)	+	ND	Yang et al. (1999)
CHO cells (5 marker loci)	+	+	Gupta and Singh (1982)

Assay/test system	Result		Reference
	+S9	−S9	
Chinese hamster V79 cells (co-cultured with irradiated HepG2 cells)	+	ND	Diamond et al. (1980)
Chinese hamster V79 lung epithelial cells	+	ND	Huberman et al. (1976)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Chinese hamster V79 lung epithelial cells	+	ND	O'Donovan (1990)
Rat/Fischer, embryo cells/OuaR	ND	+	Mishra et al. (1978)
DNA damage			
DNA adducts			
Human peripheral blood lymphocytes	ND	+	Wiencke et al. (1990)
Human peripheral blood lymphocytes	ND	+	Li et al. (2001)
Human peripheral blood lymphocytes	ND	+	Wu et al. (2005)
Human peripheral blood lymphocytes	ND	+	Gu et al. (2008)
Human fibroblast (MRC5CV1) cell line	+	ND	Hanelt et al. (1997)
Human hepatoma (HepG2) cell line	ND	+	Tarantini et al. (2009)
Hamster tracheal cells	ND	+	Roggeband et al. (1994)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Virus transformed SHE and mouse C3H10T1/2 cells	ND	+	Arce et al. (1987)
Mouse lymphoma (L5178Y/TK+/-) cells	+	ND	Arce et al. (1987)
Rat tracheal cells	ND	+	Roggeband et al. (1994)
Unscheduled DNA synthesis			
HeLa cells	+	ND	Martin et al. (1978)
Human fibroblasts	+	ND	Agrelo and Amos (1981)
Human fibroblasts	+	–	Robinson and Mitchell (1981)
Human HepG2	ND	+	Valentin-Severin et al. (2004)
Hamster primary embryo cells	ND	+	Casto et al. (1976)
Hamster tracheal cells	ND	+	Roggeband et al. (1994)
Rat hepatocytes	ND	+	Michalopoulos et al. (1978)
Rat tracheal cells	ND	–	Roggeband et al. (1994)
DNA repair			
Human mammary epithelial cells	ND	+	Leadon et al. (1988)
Human skin fibroblasts	ND	+	Milo et al. (1978)
Baby hamster kidney (BHK21/c13) cells	ND	+	Feldman et al. (1978)
secondary mouse embryo fibroblasts (C57BL/6) and human lymphocytes	ND	+	Shinohara and Cerutti (1977)
Rat/F344 hepatocytes	ND	+	Williams et al. (1982)

Assay/test system	Result		Reference
	+S9	−S9	
Cytogenetic damage			
CAs			
Human blood cells	ND	+	Salama et al. (2001)
Human WI38 fibroblasts	+	−	Weinstein et al. (1977)
Chinese hamster lung cells	+	−	Matsuoka et al. (1979)
Chinese hamster V79-4 lung epithelial cells	−	−	Popescu et al. (1977)
Mouse lymphoma (L5178Y/TK+/-) cells	+	ND	Arce et al. (1987)
Rat Liver RL1 cells	+	ND	Dean (1981)
MN			
Human AHH-1 lymphoblastoid cells	ND	+	Crofton-Sleigh et al. (1993)
Human HepG2 liver cells	ND	+	Wu et al. (2003a)
Human lymphoblastoid (TK) cells	ND	+	Fowler et al. (2010)
Human MCL-5 lymphoblastoid cells	ND	+	Crofton-Sleigh et al. (1993)
Human peripheral blood lymphocytes	+	ND	Lo Jacono et al. (1992)
Chinese hamster V79 cells	ND	+	Whitwell et al. (2010)
Chinese hamster V79-MZ cells	ND	+	Matsuoka et al. (1999)
DNA strand breaks			
Human sperm	+	+	Sipinen et al. (2010)
Human peripheral blood lymphocytes	+	+	Rodriguez-Romero et al. (2012)
Human fibroblast (MRC5CV1) cell line	+	ND	Hanelt et al. (1997)
Human hepatoma (HepG2) cell line	ND	+	Tarantini et al. (2009)
Human prostrate carcinoma (DU145) cell line	ND	+	Nwagbara et al. (2007)
Mouse embryo fibroblast (C3H/10T1/2 CL 8) cells	ND	+	Lubet et al. (1983)
Rat C18 trachea epithelial cells	ND	+	Cosma and Marchok (1988); Cosma et al. (1988)
Rat lymphocytes	ND	+	Gao et al. (1991)
SCEs			
Human C-HC-4 and C-HC-20 hepatoma cells	ND	+	Abe et al. (1983b); Abe et al. (1983a)
Human diploid fibroblast (TIG-II) cell line	+	+	Huh et al. (1982)
Human fibroblasts	ND	+	Juhl et al. (1978)
Human blood cells	ND	+	Salama et al. (2001)
Human peripheral blood lymphocytes	ND	+	Rudiger et al. (1976)
Human peripheral blood lymphocytes	ND	+	Craig-Holmes and Shaw (1977)
Human peripheral blood lymphocytes	ND	+	Schonwald et al. (1977)
Human peripheral blood lymphocytes	ND	+	Wiencke et al. (1990)
Human peripheral blood lymphocytes	+	−	Tohda et al. (1980)

Assay/test system	Result		Reference
	+S9	−S9	
Human peripheral blood lymphocytes	+	ND	Lo Jacono et al. (1992)
Chinese hamster Don-6 cells	ND	+	Abe et al. (1983b); Abe et al. (1983a)
Chinese hamster V79 lung epithelial cells	+	–	Popescu et al. (1977)
Chinese hamster V79 lung epithelial cells	+	ND	Mane et al. (1990)
Chinese hamster V79 lung epithelial cells	+	ND	Wojciechowski et al. (1981)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Chinese hamster V79 lung epithelial cells	ND	+	Kulka et al. (1993a)
CHO cells	+	–	de Raat (1979)
CHO cells	+	–	Husgafvel-Pursiainen et al. (1986)
CHO cells	ND	+	Wolff and Takehisa (1977)
CHO cells	ND	+	Pal et al. (1978)
Chinese hamster lung cells	ND	+	Shimizu et al. (1984)
Rabbit peripheral blood lymphocytes	ND	+	Takehisa and Wolff (1978)
Rat ascites hepatoma AH66-B	ND	+	Abe et al. (1983b); Abe et al. (1983a)
Rat esophageal tumor R1	ND	+	Abe et al. (1983b); Abe et al. (1983a)
Rat hepatocyte (immortalized) cell lines (NRL cl-B, NRL cl-C, and ARL)	+	ND	Kulka et al. (1993b)
Rat hepatoma (Reuber H4-II-E) cells	ND	+	Dean et al. (1983)
Rat liver cell line ARL18	ND	+	Tong et al. (1981)
Rat pleural mesothelial cells	ND	+	Achard et al. (1987)
Aneuploidy			
Chinese hamster V79-MZ cells	ND	+	Matsuoka et al. (1998)
Cell transformation			
Human BEAS-2B lung cells	ND	+	van Agen et al. (1997)
Human breast epithelial (MCF-10F, MCF-7, T24) cell lines	ND	+	Calaf and Russo (1993)
Baby hamster kidney (BHK21/c13) cells	+	ND	Greb et al. (1980)
Golden hamster embryo cells	+	ND	Mager et al. (1977)
Syrian hamster embryo (SHE) cells	ND	+	Dipaolo et al. (1971); Dipaolo et al. (1969)
SHE cells	ND	+	Dunkel et al. (1981)
SHE cells	ND	+	Leboeuf et al. (1990)
SHE cells/focus assay	ND	+	Casto et al. (1977)
Fetal Syrian hamster lung cells	ND	+	Emura et al. (1987); Emura et al. (1980)
Virus infected rat embryo RLV/RE and RAT cells; mouse embryo AKR/Me cells; Syrian hamster embryo cells	ND	+	Heidelberger et al. (1983)

Assay/test system	Result		Reference
	+S9	−S9	
Virus transformed SHE and mouse C3H10T1/2 cells	ND	+	Arce et al. (1987)
Mouse C3H/10T1/2 embryo fibroblasts	ND	+	Nesnow et al. (2002); Nesnow et al. (1997)
Mouse embryo fibroblast (C3H/10T1/2 CL 8) cells	ND	+	Peterson et al. (1981)
Mouse embryo fibroblast (C3H/10T1/2 CL 8) cells	ND	+	Lubet et al. (1983)
Mouse SHE cells; BALB/c-3T3 cells; C3H/10T1/2 cells; prostate cells	ND	+	Heidelberger et al. (1983)
Mouse BALB/c-3T3 cells	ND	+	Dunkel et al. (1981)
Mouse BALB/c-3T3 cells	ND	+	Matthews (1993)
Mouse BALB/c-3T3 clone A31-1-1	ND	+	Little and Vetrovs (1988)
Rat/Fischer, embryo cells (leukemia virus transformed)	ND	+	Dunkel et al. (1981)
Rat/Fischer, embryo cells/Oua^{R}	ND	+	Mishra et al. (1978)

+ = positive; − = negative; CHO = Chinese hamster ovary; ND = not determined; SHE = Syrian hamster embryo; TK = thymidine kinase.

Table D-34. Studies of benzo[a]pyrene-induced genotoxicity in humans exposed to PAHs

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Human, hprt locus mutation assay in T lymphocytes	T-cells of lung cancer patients (smokers and nonsmokers from lung cancer patients and population controls with known smoking status) analyzed for hprt locus mutations.	+		Splicing mutations, base-pair substitutions, frameshift, and deletion mutations observed. Smokers and nonsmokers had GC→TA transversions (13 and 6%, respectively) and GC→AT transitions (24 and 35%, respectively) in hprt gene consistent with in vitro mutagenicity of benzo[a]pyrene.	Hackman et al. (2000)
Mutation	Human, *K-ras* and *p53* mutations in tumor tissues	Lung tumors from 24 nonsmoking women who used smoky coal in their homes in Xuan Wei County, Yunnan Province, China. Mutations determined by multiplex PCR amplification and cycle-sequencing.	+		86% of KRAS mutations and 76% of TP53 mutations were G→T transversions.	Demarini et al. (2001)
Mutation	Human, *K-ras* mutations in tumor tissues	Comparison of lung tumors or sputum samples from 102 lung cancer patients (41 nonsmoking women and 61 smoking men) who used smoky coal in their homes in Xuan Wei County, Yunnan Province, China, and 50 lung cancer patients (14 nonsmoking women, 33 smoking men, 3 nonsmoking men) from Beijing and Henan using natural gas in the home.	+		The frequency of nonsmoking women in Xuan Wei county with mutations (21.9%) and G→T transversions (66.7%) were similar to that of smoking men in Beijing and Henan (16.7% and 66.7%, respectively).	Keohavong et al. (2003)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	96 people occupationally or medically exposed to PAH mixtures (psoriatic patients, coke oven workers, chimney sweeps, and aluminum anode plant workers); anti-BPDE-DNA adducts in lymphocyte plus monocyte fraction measured by HPLC/fluorescence analysis.	+		Percentages of subjects with BPDE-DNA adduct levels greater than the 95th percentile control value were 47% (7/15) in coke oven workers and 21% (4/19) in chimney sweeps, compared to 3% (1/34) in controls.	Pavanello et al. (1999)
BPDE-DNA adducts	Human, WBCs	95 male coke-oven workers from two plants were tested for GSTM1 polymorphisms and anti-BPDE-DNA adducts in lymphocyte plus monocyte fraction measured by HPLC/fluorescence analysis.	+		Compared to GSTM1-active, GSTM1*0/*0 workers had significantly higher BPDE-DNA adducts ($p = 0.011$); these were significantly related to exposures to PAHs ($p < 0.01$) and to lack of GSTM1 ($p < 0.001$) and not to other sources of exposure.	Pavanello et al. (2004)
BPDE-DNA adducts	Human, WBCs	67 highly exposed coke oven workers were tested for genetic factors that can modulate individual responses to carcinogenic PAHs; adducts measured by HPLC/fluorescence analysis.	+		Levels of BPDE-DNA adducts were significantly associated with workplace PAH exposure (as correlated with urinary excretion of 1-pyrenol), lack of GSTM1 activity, and low nucleotide excision repair capacity.	Pavanello et al. (2005)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	585 Caucasian municipal workers (52% males, 20−62 years old) from northeast Italy environmentally exposed to PAH mixtures were screened for anti-BPDE-DNA adducts in lymphocyte plus monocyte fraction measured by HPLC/fluorescence analysis.	+		42% of the participants had elevated anti-BPDE-DNA adduct levels, defined as >0.5 adducts/10^8 nucleotides (mean, 1.28 ± 2.80 adducts/ 108 nucleotides). Comparison of adduct levels with questionnaire responses indicated that smoking, frequent consumption of PAH-rich meals (>52 versus <52 times/yr), and long time periods spent outdoors (>4 versus <4 hrs/d) were risk factors as all increased BPDE-DNA adduct levels significantly.	Pavanello et al. (2006)
BPDE-DNA adducts	Human, WBCs	39 male coke oven workers and 39 matched controls, smokers and nonsmokers, exposed to PAHs for 6−8 hrs/d for at least 4−6 mo before blood collection; leukocyte DNA isolated and digested, and benzo[a]pyrene tetrols analyzed by HPLC with fluorescent detection. Low, medium, and high exposure groups correspond to <0.15, 0.15−4, and >4 mg/m3 of benzo[a]pyrene, respectively.	+	<0.15, 0.15−4, or >4 µg/m^3 benzo[a]pyrene	Anti-BPDE-DNA adducts detected in 51% of coke oven workers (mean 15.7 ± 37.8/ 108 nucleotides) versus 18% non-exposed (mean 2.0 ± 8.7/108 nucleotides). Interindividual variation of adduct levels was 100-fold in workers and 50-fold in control; smokers had 3.5-fold more adducts than nonsmokers.	Rojas et al. (1995)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	20 male coke oven workers, all smokers, were selected from workers studied in Rojas et al. (1995); workers were exposed to PAHs for 6–8 hrs/d for at least 4–6 mo before blood collection; leukocyte DNA isolated and digested, and benzo[a]pyrene tetrols analyzed by HPLC with fluorescent detection. Low, medium, and high exposure groups correspond to <0.15, 0.15–4, and >4 mg/m^3 of benzo[a]pyrene, respectively.	+	<0.15, 0.15–4, or >4 µg/m^3 benzo[a]pyrene	Levels of anti-BPDE-DNA adducts significantly correlated with genotype: GSTM1*0/*0 + CYP1A1*2A/*2A or *2A/*2B >> GSTM1*0/*0 + CYP1A1*1/*1 or *1/*2A or *1/*2B >> GSTM1-active (no detectable adducts). Results correlated with adduct levels in non-tumorous lung tissues from 20 lung cancer patients.	Rojas et al. (1998)
BPDE-DNA adducts	Human, WBCs	89 male coke oven workers and 44 power plant workers were exposed to PAHs for 6–8 hrs/d for at least 4–6 mo before blood collection; leukocyte DNA isolated and digested, and benzo[a]pyrene tetrols analyzed by HPLC with fluorescent detection. Low, medium, and high exposure groups correspond to <0.15, 0.15–4, and >4 mg/m^3 of benzo[a]pyrene, respectively.	+	<0.15, 0.15–4, or >4 µg/m^3 benzo[a]pyrene	PAH exposure, CYP1A1 status and smoking significantly affected DNA adduct levels, i.e., CYP1A1(*1/*2 or *2A/*2a) > CYP1A1*1/*1; occupational > environmental exposure; smokers > nonsmokers; adducts increased with dose and duration of smoking.	Rojas et al. (2000)
BPDE-DNA adducts	Human, WBCs	Coke oven workers were exposed to PAHs and benzo[a]pyrene-WBC DNA analyzed by HPLC-fluorescence detection for BPDE-DNA adducts.	±	0.14 µg/m^3	Median detectable BPDE-DNA adducts in workers versus controls not significant (p = 0.51) due to low number of subjects (9 workers, 26 controls); 4/9 workers had adducts substantially higher than all controls. No significant difference between smokers and nonsmokers; no correlation with air benzo[a]pyrene levels and adduct levels.	Mensing et al. (2005)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	35 iron foundry workers (12 nonsmokers and 23 smokers) and 10 matched controls (6 nonsmokers and 4 smokers) between August 1985 and May 1986; workers stratified according to job title and assigned exposure category. BPDE-DNA adducts measured by ELISA (immunoassay).	+	<0.05, 0.05–0.2, or >0.2 µg/m^3 benzo[a]pyrene	Benzo[a]pyrene exposures significantly associated with adduct formation (p = 0.0001). Low, medium, and high exposure groups all significantly elevated compared to controls; low group significantly higher than medium or high categories.	Perera et al. (1988)
BPDE-DNA adducts	Human, WBCs from maternal and umbilical cord blood	Cohort study of 329 nonsmoking pregnant women exposed to emissions from fires during the 4 wks following the collapse of the WTC building in New York City on 09/11/2001; BPDE-DNA adducts measured by HPLC/fluorescence analysis.	+		BPDE-DNA adduct levels in cord and maternal blood were highest in study participants who lived within 1 mile of the WTC, with inverse correlation between cord blood levels and distance from WTC.	Perera et al. (2005b); Perera et al. (2004)
BPDE-DNA adducts	Human, WBCs from umbilical cord blood	164 pregnant women in NYC wearing personal air monitors during the third trimester; umbilical cord blood was screened for BPDE-DNA adducts and global DNA methylation levels using HPLC/fluorescence analysis.	±	50% above and 50% below median of 5.314 ng/m^3 (all PAHs including pyrene)	BPDE-DNA adducts were not significantly associated with individual PAH exposures, but did correlate with increased global DNA methylation.	Herbstman et al. (2012)
BPDE-DNA adducts	Human, placenta	28 smoking (15) and nonsmoking (13) pregnant women with uncomplicated pregnancies; placental nuclei analyzed by immunoaffinity chromatography, HPLC/SFS and GC/MS to identify BPDE-DNA adducts.	+		BPDE-DNA adducts detected; no correlation with smoking history.	Manchester et al. (1988)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, skin punch biopsies	10 eczema patients (3 males and 7 females) treated with coal tar ointment 2 times/day for 7–33 days were biopsied and DNA from skin analyzed by HPLC-fluorescence detection for BPDE-DNA adducts.	+	3–10% coal tar ointment	Presence of BPDE-DNA adducts significantly correlated with normal myeloperoxidase levels (wild-type MPO-463GG) compared to reduced levels in patients with the MPO-463AA/AG polymorphism	Rojas et al. (2001)
BPDE-DNA adducts	Human, lung parenchyma	13 lung cancer patients (11 smokers, 2 exsmokers); nontumorous lung parenchyma analyzed by HPLC-fluorescence detection for anti- and syn-BPDE-DNA adducts.	+		Anti- and syn-BPDE-DNA adducts detected in 9 of 11 smokers and 2 of 2 exsmokers.	Alexandrov et al., 1992
BPDE-DNA adducts	Human, lung tissues	39 lung cancer patients (26 smokers, 11 exsmokers, 2 nonsmokers); tumor and nontumor tissues (not specified) analyzed by ^{32}P-postlabelling and synchronous fluorescence spectrophotometry after immunoaffinity chromatography and HPLC to detect BPDE-DNA adducts.	+		Detectable adducts in 33/39 by postlabelling, 11/39 by SFS+IAC, and 6 of these 11 when adding HPLC. Significantly higher levels of adducts in heavy smokers; weak association between adducts and TP53 mutations.	Andreassen et al. (1996)
BPDE-DNA adducts	Human, lung tissues	24 lung cancer patients (13 smokers, 11 nonsmokers); nontumorous lung tissues adjacent to tumor tissue analyzed for PAH-DNA adducts by 32P-postlabeling and chromatographic co-migration with BPDE standard.	+		Putative BPDE-DNA adducts were significantly higher in smokers ($1.5 \pm 1.0/10^8$ nucleotides) than nonsmokers ($0.2 \pm 0.2/10^8$ nucleotides) ($p < 0.001$); may be overestimation due to co-migration of other PAH adducts.	Godschalk et al. (2002)

Table D-35. Non-human in vivo genotoxicity studies of benzo[a]pyrene

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation, germline	Mouse, T-stock, (SEC × C57BL)F1, (C3H × 101)F1, or (C3H × C57BL)F1 for females; (101 × C3H)F1 or (C3H × 101)F1 for males; dominant-lethal mutation assay	12-wk-old males dosed with benzo[a]pyrene i.p. and mated 3.5−6.5 d posttreatment with 12-wk-old females from different stocks; sacrificed on d 12−15 after vaginal plug was observed; females kept in a 5-hr dark phase to synchronize ovulation 5 wks before the start of the experiment; fertilized eggs collected 9−11 hrs after mating and first-cleavage metaphase chromosomes prepared 20 hrs after mating.	+	500 mg/kg	The percent of dominant lethal mutations were in the order of T-stock = (C3H × 101)F1 > (SEC × C57BL)F1 > (C3H × C57BL)F1.	Generoso et al. (1979)
Mutation, germline	Mouse, male stocks: (101 × C3H)F1; female stocks (A): (101 × C3H)F1, (B): (C3H × 101)F1, (C): (C3H × C57BL)F1, (D):(SEC × C57BL)F1, (E):T-stock females; dominant lethal mutations	In dominant lethal assay, 12-wk-old males dosed i.p. with benzo[a]pyrene and mated with 10−12-wk-old (#1) stock A females; or (#2) stock B females on the day of dosing; or with (#3a) with stocks B, C, and D females 3.5−7.5 d postdosing, or with (#3b) with stocks B, C, D, and E females 3.5−6.5 d postdosing. Control group mated at time corresponding to 1.5−4.5 d posttreatment in the test groups.	+	500 mg/kg	Dominant lethal effects were observed in early to middle (4.5−5.5 and 6.5−7.5 d posttreatment, respectively) spermatozoa and in preleptotene spermatocytes (32.5−33.5 and 34.5−35.5 d posttreatment, respectively).	Generoso et al. (1982)
Mutation, germline	Mouse, male stocks: (101 × C3H)F1; female stocks (A): (101 × C3H)F1, (B): (C3H × 101)F1, (C): (C3H × C57BL)F1, (D): (SEC × C57BL)F1, (E): T-stock females; heritable translocations	For heritable translocation assay, males were mated with stocks B and D females 3.5−7.7 d post-benzo[a]pyrene treatment and male progeny screened for translocation heterozygosity.	−	500 mg/kg	No significant differences were observed between treated and control progeny.	Generoso et al. (1982)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutations and BPDE-DNA adducts, germline	Mouse, C57BL/6, *cII* transgenic (Big Blue®)	Benzo[a]pyrene administered i.p. in corn oil on d 0, 1, and 2; sacrificed at d 4, 16, 30, 44, or 119. Caput and cauda epididymal spermatozoa analyzed for *cII* mutation frequency, and DNA adducts analyzed in testis by liquid chromatography-MS/MS selected reaction monitoring with ^{15}N-deoxyguanosine labeling.	+	50 mg/kg	Exposed spermatocytes acquired persistent BPDE-DNA adducts; exposed spermatogonia gave rise to spermatocytes with mutations consistent with a benzo[a]pyrene spectrum (GC>TA transversions).	Olsen et al. (2010)
Mutations and BPDE-DNA adducts, germline	Mouse, C57BL/6 males, WT and Xpc$^{-/-}$ with pUR288 *lacZ* reporter gene	Benzo[a]pyrene given via gavage in sunflower oil 3 times/wk for 1, 4, or 6 wks (Xpc$^{-/-}$) or 6 wks (WT). Spleen, testis, and sperm cells analyzed for *lacZ* mutation frequency, and DNA adducts analyzed in testis by [^{32}P]-postlabeling.	+	13 mg/kg	Statistically significant increases in lacZ mutation frequencies in Xpc$^{-/-}$ spleen at 4 and 6 wks (dose dependent) and in WT spleen and sperm at 6 wks; DNA adducts were statistically significant in testis in all exposed groups.	Verhofstad et al. (2011)
Mutations and BPDE-DNA adducts	Mouse, C57BL/6 *lacZ* transgenic	Mice dosed with single i.p. injection of benzo[a]pyrene in DMSO; sacrificed 1, 3, 5, 7, 14, 21, and 28 d posttreatment; spleen, lung, liver, kidney, and brain collected, DNA isolated and analyzed for mutations in *lacZ* reporter gene in *E. coli* and adducts by [^{32}P]-postlabeling assay.	+	50 mg/kg	BPDE-dG adduct levels peaked between 5 and 7 d posttreatment, followed by gradual decline; rate of removal highest in lung, liver, and spleen and lowest in kidney and brain; mutant frequencies peaked between 7 and 14 d in lung, spleen, liver, and kidney; brain was not significant at any time point.	Boerrigter (1999)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Mouse, C57BL female × T-strain male; somatic mutation assay	Mice mated for a 5-d period; 10.25 d post-appearance of vaginal plug, females injected i.p. with benzo[a]pyrene or vehicle; offspring (pups) scored for survival, morphology, and presence of white near-midline ventral spots and recessive spots.	+	100 or 500 mg/kg	Induced coat color mosaics represent genetic changes (e.g., point mutations) in somatic cells. White near-midline ventral spots and recessive spots represent melanocyte cell killing and mutagenicity, respectively. Benzo[a]pyrene caused high incidence of recessive spots but did not correlate with white near-midline ventral spots.	Russell (1977)
Mutation	Mouse, *lacZ* transgenic (Muta™Mouse)	Benzo[a]pyrene given via gavage in olive oil daily for 28 consecutive d; sacrificed 3 d after last dosing; four organs analyzed for *lacZ* mutation frequency.	+	25, 50, or 75 mg/kg-d	Highest *lacZ* mutation frequency observed in small intestine, followed by bone marrow, glandular stomach, and liver.	Lemieux et al. (2011)
Mutation	Mouse, *lacZ* transgenic (Muta™Mouse)	Benzo[a]pyrene given orally in corn oil for 5 consecutive d; sacrificed 14 d after last dosing; 11 organs analyzed for *lacZ* mutation frequency.	+	125 mg/kg-d	Highest mutation frequency observed in colon followed by ileum > forestomach > bone marrow = spleen > glandular stomach > liver = lung > kidney = heart.	Hakura et al. (1998)
Mutation	Mouse, C57BL/6J *Dlb-1* congenic; *Dlb-1* locus assay	Animals dosed: (1) i.p. with vehicle or benzo[a]pyrene two, four, or six doses at 96-hr intervals; or (2) single dose of benzo[a]pyrene given i.p. or orally alone or 96 hrs following a single i.p. dosing with 10 µg/kg TCDD.	+	40 mg/kg	Benzo[a]pyrene caused a dose-dependent increase in mutant frequency; i.p. route showed higher mutant frequency than oral route; induction of mutations were associated with Ah-responsiveness.	Brooks et al. (1999)
Mutation	Mouse, C57BL/6 (*lacZ* negative and *XPA*$^{+/+}$ and *XPA*$^{-/-}$); hprt mutations in T lymphocytes	Gavage in corn oil 3 times/wk for 0, 1, 5, 9, or 13 wks; sacrificed 7 wks after last treatment.	+	13 mg/kg	Mutation sensitivity: *XPA*$^{-/-}$ > *XPA*$^{+/+}$.	Bol et al. (1998)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Mouse, Cockayne syndrome-deficient *(Csb$^{-/-}$)*; heterozygous *(Csb$^{+/-}$)* and WT controls *(Csb$^{+/+}$)*; hprt mutation frequency assay	*Csb$^{-/-}$/lacZ$^{+/-}$* and *Csb$^{+/-}$/lacZ$^{+/-}$* mice were dosed i.p. with benzo[a]pyrene 3 times/wk for 5, 9, or 13 wks; for hprt mutation frequency analysis mice were sacrificed 3 wks after last treatment; splenocytes collected; for *lacZ* mutation frequency analysis, mice were sacrificed 3 d after last treatment and liver, lung, and spleen were collected.	+	13 mg/kg	*lacZ* mutation frequency detected in all tissues but no differences between WT and *Csb$^{-/-}$* mice; hprt mutations significantly higher in *Csb$^{-/-}$* mice than control mice. BPDE-dGuo adducts in hprt gene are preferentially removed in WT mice than *Csb$^{-/-}$* mice.	Wijnhoven et al. (2000)
Mutation	Mouse, B6C3F$_1$, forestomach H-*ras*, K-*ras*, and *p53* mutations	Benzo[a]pyrene given in feed in a 2-yr chronic feeding study.	+	5, 25, or 100 ppm	68% K-*ras* (codons 12, 13), 10% H-*ras* (codon 13), 10% *p53* mutations; all G→T transversions.	Culp et al. (2000)
Mutation	Mouse, *lacZ/galE* (Muta™ Mouse); skin painting study	Mice topically treated with a single dose or in five divided doses daily; sacrificed 7 or 21 d after the single or final treatment; DNA from skin, liver, and lung analyzed for mutations.	+ (skin) − (liver, lung)	1.25 or 2.5 mg/kg (25 or 50 µg/mouse)	Skin showed significant dose- and time-dependent increase in mutation frequency; liver and lung showed no mutations; mutation frequency for single- or multiple-dose regimens was similar.	Dean et al. (1998)
Mutation	Mouse, T-strain	Benzo[a]pyrene given to pregnant mice by gavage in 0.5 mL corn oil on GDs 5−10.	+	10 mg/mouse (5 × 2 mg)		Davidson and Dawson (1976)
Mutation	Mouse, 129/Ola (WT); hprt mutations in splenic T lymphocytes	Single i.p. injection followed by sacrifice 7 wks posttreatment.	+	0, 50, 100, 200, or 400 mg/kg	Dose-dependent increase in hprt mutation frequency.	Bol et al. (1998)
Mutation	Mouse, A/J, male	Single i.p. injection followed by sacrifice 28 days posttreatment.	+	0, 0.05, 0.5, 5, or 50 mg/kg	Dose-dependent increase in lung tissue K-*ras* codon 12 G→T mutation frequency.	Meng et al. (2010)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Mouse, CD-1; skin papillomas (Ha-*ras* mutations)	Female mice were initiated topically with a single dose of benzo[a]pyrene and 1 wk after initiation promoted twice weekly with 5 nmol TPA for 14 wks. One month after stopping TPA application, papillomas were collected and DNA from 10 individual papillomas was analyzed for Ha-*ras* mutations by polymerase chain reaction and direct sequencing.	+	600 nmol/mouse	About 90% of papillomas contained Ha-*ras* mutations, all of them being transversions at codons 12 (20% GGA→GTA), 13 (50% GGC→GTC), and 61 (20% CAA→CTA).	Colapietro et al. (1993)
Mutation	Rat, Wistar	Single dose by gavage; urine and feces collected 0–24, 24–48, and 48–72 hrs posttreatment; urine and extracts of feces tested in *S. typhimurium* TA100 strain with or without S9 mix and β-glucuronidase.	+	0, 1, 5, 10, or 100 mg/kg	Fecal extracts and urine showed mutagenicity ≥1 and 10 mg/kg body weight benzo[a]pyrene, respectively. Highest mutagenic activity observed for 0–24 hrs posttreatment for feces and 24–48 hrs posttreatment for urine with β-glucuronidase ± S9 mix.	Willems et al. (1991)
BPDE-DNA adducts	Mouse, *lacZ* transgenic (Muta^{TM}Mouse)	Benzo[a]pyrene given via gavage in olive oil daily for 28 consecutive d; sacrificed 3 d after last dosing; four organs analyzed for DNA adducts using [^{32}P]-postlabeling with nuclease P1 digestion enrichment.	+	25, 50, or 75 mg/kg-day	Highest adduct levels observed in liver, followed by glandular stomach, small intestine, and bone marrow.	Lemieux et al. (2011)
BPDE-DNA adducts	Mouse, (*Ahr*+/+, *Ahr*+/-, *Ahr*-/-)	Gavage; sacrificed 24 hrs posttreatment.	+	100 mg/kg	No induction of CYP in *Ahr-/-*, but all alleles positive for adduct formation.	Sagredo et al. (2006)
BPDE-DNA adducts	Mouse, C57BL/6J *Cyp1a1*(+/-) and *Cyp1a1* (-/-)	Single i.p. injection; sacrificed 24 hrs posttreatment; liver DNA analyzed by [^{32}P]-postlabeling assay.	+	500 mg/kg	BPDE-DNA adduct levels 4-fold higher in *Cyp1a1*(-/-) mice than *Cyp1a1*(+/-) mice.	Uno et al. (2001)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Mouse, B6C3F$_1$	Benzo[a]pyrene fed in diet for 4 wks (100 ppm) or for 1, 2, 8, 16, and 32 wks (5 ppm); sacrificed and liver, lungs, forestomach, and small intestine collected; DNA analyzed by [^{32}P]-postlabeling assay.	+	5 ppm (32 wks) or 100 ppm (4 wks)	Linear dose-response in 4-wk study; the 5 ppm groups showed a plateau after 4 wks of feeding.	Culp et al. (2000)
BPDE-DNA adducts	Mouse, BALB/c	Single i.p. injection; sacrificed 12 hrs postinjection; liver and forestomach collected; DNA binding of [^{3}H]-benzo[a]-pyrene analyzed by scintillation counting.	+	140 µCi/100 g body weight	Liver DNA had 3-fold higher binding of benzo[a]pyrene than that of forestomach.	Gangar et al. (2006)
BPDE-DNA adducts	Mouse, BALB/cAnN (BALB), CBA/JN (CBA); [^{32}P]-postlabeling assay	Animals dosed i.p. with or without 24-hr pretreatment with TCDD.	+	50 or 200 mg/kg	Adduct levels similar in both strains dosed with benzo[a]pyrene alone. TCDD pretreatment had a greater suppressive effect on adduct formation in BALB relative to CBA mice at low dose but resulted in no significant difference in adduct levels at high dose.	Wu et al. (2008)
BPDE-DNA adducts	Mouse, BALB/c, skin	Four doses of benzo[a]pyrene topically applied to the shaved backs of animals at 0, 6, 30, and 54 hrs; sacrificed 1 d after last treatment; DNA analyzed by [^{32}P]-postlabeling assay.	+	4 × 1.2 µmol/ animal	Five adducts spots detected.	Reddy et al. (1984)
BPDE-DNA adducts	Mouse, Swiss, epidermal and dermal skin	Single topical application on shaved backs; sacrificed 1, 3, and 7 d posttreatment; epidermal and dermal cells separated; DNA isolated, digested with DNAseI, and estimated DNA binding; adducts separated by HPLC.	+	250 nmol in 150 µL acetone	Both cells positive for benzo[a]pyrene adducts; epidermis > dermis; adducts persisted up to 7 d with a gradual decline in levels.	Oueslati et al. (1992)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Rat, CD, peripheral blood lymphocytes, lungs, and liver	Single i.p. injection; sacrificed 3 d posttreatment; DNA analyzed by Nuclease P1-enhanced [^{32}P]-postlabeling assay.	+	2.5 mg/animal	BPDE-dG as major adducts and several minor adducts detected in all tissues.	Ross et al. (1991)
BPDE-DNA adducts	Rat, Sprague-Dawley, liver	Single i.p. injection followed by sacrifice at 4 hrs posttreatment; liver DNA isolated and analyzed by [^{32}P]-postlabeling assay.	+	100 mg/kg	Two adduct spots detected.	Reddy et al. (1984)
BPDE-DNA adducts	Rat, Lewis, lung and liver	Animals received a single oral dose of benzo[a]pyrene in tricaprylin; sacrificed 1, 2, 4, 11, and 21 d postdosing; analyzed liver and lung DNA for BP-DNA adducts by [^{32}P]-postlabeling assay and urine for 8-oxo-7,8-dihydro-2'-deoxyguanosine adducts by HPLC-electrochemical detection.	+	10 mg/kg	BPDE-dG levels peaked 2 d after exposure in both tissues, higher in lungs than liver at all time points, decline faster in liver than lung; Increased 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in urine and decreased levels in liver and lung.	Briedé et al. (2004)
BPDE-DNA adducts	Rat, F344; [^{32}P]-postlabeling assay	Benzo[a]pyrene given in the diet for 30, 60, or 90 d; animals sacrificed and liver and lung isolated and DNA extracted and analyzed for adducts.	+	0, 5, 50, or 100 mg/kg	Adduct levels linear at low and intermediate doses, nonlinear at high dose.	Ramesh and Knuckles (2006)
BPDE-DNA adducts	Rat, Wistar; liver and peripheral blood lymphocyte adducts	Single dose by gavage; sacrificed 24 hrs postdosing; peripheral blood lymphocytes and liver DNA analyzed by [^{32}P]-postlabeling for BPDE-DNA adducts.	+	0, 10, or 100 mg/kg	At 100 mg/kg dose, total adduct levels in peripheral blood lymphocytes were 2-fold higher than the levels in liver; adduct profiles differed between peripheral blood lymphocytes and liver.	Willems et al. (1991)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
CAs	Mouse, C57 (high AHH inducible) and DBA (low AHH inducible) strains; 11-d-old embryos; adult bone marrows	Study used four matings (female × male): C57 × C57; DBA × DBA; C57 × DBA; and DBA × C57; pregnant mice treated orally on GD 11 with benzo[a]pyrene; sacrificed 15 hrs posttreatment; material liver, bone marrow and placenta and embryos collected; male mice dosed similarly and bone marrows collected; individual embryo cell suspensions and bone marrow preparations scored for CAs. Tissue AHH activity measured.	+	150 mg/kg	Levels of CAs: hybrid embryos > homozygous DBA embryos > homozygous C57 embryos; tissue AHH activity: C57 mothers and their embryos > DBA females and their homozygous embryos. No quantitative correlation between benzo[a]pyrene-induced CAs and AHH inducibility. No differences in bone marrow mitotic index of males of different strains between control and treatment groups.	Adler et al. (1989)
CAs	Mouse, 1C3F1 hybrid (101/E1 × C31 × E1)F1; CAs in bone marrow	Single dose by gavage; sacrificed 30 hrs postdosing; bone marrow from femur isolated and analyzed for CAs.	+	63 mg/kg	Significant increase in CAs in benzo[a]pyrene-treated animals compared to controls.	Adler and Ingwersen (1989)
CAs	Rat, Wistar; peripheral blood lymphocytes	Single dose by gavage; sacrificed 6, 24, and 48 hrs posttreatment; blood from abdominal aorta collected, whole blood cultures set up, CAs scored in 100 first-division peripheral blood lymphocytes per animal.	–	0, 10, 100, or 200 mg/kg	No difference between control and treatment groups at any dose or at any sampling time observed.	Willems et al. (1991)
CAs	Hamster; bone marrow	Single, i.p. injection of benzo[a]pyrene dissolved in tricapryline; animals sacrificed 24 hrs post-exposure.	+	25, 50, or 100 mg/kg	Benzo[a]pyrene induced CAs at 50 mg/kg body weight only, with negative responses at the low and high dose.	Bayer (1978)
MN	Mouse, *lacZ* transgenic (Muta^{TM}Mouse)	Benzo[a]pyrene given via gavage in olive oil daily for 28 consecutive d; blood samples were collected 48 h after last dose; percent of PCEs and NCEs reported.	+	25, 50, and 75 mg/kg-d	Statistically significant, dose-dependent increases in percent of PCEs and NCEs at all doses.	Lemieux et al. (2011)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
MN	Mouse, CD-1 and BDF1; bone marrow	Dosed orally once, twice, or thrice at 24-hr intervals; sacrificed 24 hrs after last treatment.	+	250, 500, 1,000, or 2,000 mg/kg	Significant increase at all doses; no dose-response; double dosing at 500 mg/kg dose gave best response.	Shimada et al. (1990)
MN	Mouse, CD-1 and BDF1, peripheral blood reticulocytes	Given single i.p injection; tail blood collected at 24-hr intervals from 0 to 72 hrs.	+	62.5, 125, 250, or 500 mg/kg	Maximum response seen at 48 hrs posttreatment.	Shimada et al. (1992)
MN	Mouse, ICR [Hsd: (ICR)Br]	Benzo[a]pyrene was heated in olive oil and given orally as a single dose; males, females, and pregnant mothers used; pregnant mice dosed on GDs 16−17 and sacrificed on GDs 17−18; micronuclei evaluated in adult bone marrow and fetal liver.	+	150 mg/kg	All groups significantly higher than controls for MN; fetal liver more sensitive than any other group.	Harper et al. (1989)
MN	Mouse, Swiss albino; bone marrow	Given orally in corn oil; sacrificed 24 hrs post-exposure.	+	75 mg/kg		Koratkar et al. (1993)
MN	Mouse, Swiss; bone marrow polychromatic erythrocytes	Given by gavage and sacrificed 36 hrs posttreatment.	+	75 mg/kg		Rao and Nandan (1990)
MN	Mouse, CD-1 and MS/Ae strains	i.p. and oral administration.	+	62.5, 125, 250, or 500 mg/kg	Good dose-response by both routes, strains; i.p. better than oral; MS/Ae strain more sensitive than CD-1 strain.	Awogi and Sato (1989)
MN	Mouse, BDF1, bone marrow	Male and female mice aged 12−15 wks given single i.p. injection of benzo[a]pyrene or corn oil; sacrificed 24, 48, and 72 hrs posttreatment; bone marrow smears prepared, stained with May-Grunwald-Giemsa technique and scored for MN PCEs.	+	0, 25, 50, or 60 mg/kg	Positive at all doses, time points, and sexes tested. Dose-dependent increase in MN observed in both sexes; males responded better than females; highest positive response observed at 72 hrs postinjection.	Balansky et al. (1994)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
MN	Mouse, HRA/Skh hairless, keratinocytes	Single topical application.	+	0.5, 5, 50, 100, or 500 mg/mouse		He and Baker (1991)
MN	Mouse, HOS:HR-1, hairless; skin micronuclei	Topical application once daily for 3 d; sacrificed 24 hrs after last treatment.	+	0.4, 1, 2, or 4 mg		Nishikawa et al. (2005)
MN	Mouse, HR-1 hairless, skin (benzo[a]pyrene with slight radiation)		+		Exposure to sunlight simulator to evaluate photogenotoxicity and chemical exposure.	Hara et al. (2007)
MN	Rat, Sprague-Dawley, peripheral blood reticulocytes	Given single i.p injection; tail blood collected at 24-hr intervals from 0 to 96 hrs.	+	62.5, 125, 250, 500, or 1,000 mg/kg	Maximum response seen at 72 hrs posttreatment.	Shimada et al. (1992)
MN	Rat, Sprague-Dawley, pulmonary alveolar macrophages	Intratracheal instillation, once/day for 3 d.	+	25 mg/kg		De Flora et al. (1991)
MN	Rat, Sprague-Dawley, bone marrow cells	Intratracheal instillation, once/day for 3 d.	–	25 mg/kg		De Flora et al. (1991)
MN	Hamster; bone marrow	Single, i.p. injection of benzo[a]pyrene dissolved in tricaprylin; animals sacrificed 30 hrs post-exposure.	–	100, 300, or 500 mg/kg		Bayer (1978)
MN	Fish (carp, rainbow trout, clams); blood and hemolymph		+	0.05, 0.25, 0.5, or 1 ppm		Kim and Hyun (2006)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
DNA strand breaks	Rat, Sprague-Dawley; comet assay	Instilled intratracheally with: (1) single dose of benzo[a]pyrene in aqueous suspension; sacrificed at 3, 24, and 48 hrs posttreatment; alveolar macrophages, lung cells, lymphocytes, and hepatocytes collected or (2) dose-response study and sacrificed at 24 hrs posttreatment; lungs collected; controls received normal saline instillation; all cells analyzed by comet assay.	+	Experiment #1: 3 mg of benzo[a]pyrene; Experiment #2: dose-response study with 0.75, 1.5, or 3 mg benzo[a]pyrene	All time points showed significant increase in SSBs (Experiment #1); a dose-response in SSBs was observed (Experiment #2).	Garry et al. (2003a); Garry et al. (2003b)
DNA strand breaks	Aquatic organisms: carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and clams (*Spisula sachalinensis*); Comet assay	All organisms acclimatized in tanks for 2 d, water changed every 24 hrs; exposed to benzo[a]pyrene in DMSO in a tank; one-third volume of tank contents changed every 12 hrs; organisms sacrificed at 24, 48, 72, and 96 hrs posttreatment; cell suspensions prepared from liver (carp and trout) or digestive gland (clam) for comet assay.	+	0.05, 0.25, 0.5, or 1 ppm	Significant dose-response for strand breaks observed; carp and trout liver showed highest response at 48 hrs and clam digestive gland showed time-dependent increase at highest concentration.	Kim and Hyun (2006)
DNA strand breaks	Rat, Brown Norway	UDS determined after 5 and 18 hrs of a single intragastric dosing.	−	62.5 mg/kg	Negative at both time points.	Mullaart et al. (1989)
UDS	Rat, F344	Single i.p. injection of benzo[a]pyrene or DMSO; sacrificed at 2 or 12 hrs post-exposure; liver isolated, hepatocyte cultures were set up and incubated with 10 mCi/mL [^{3}H]-thymidine for 4 hrs; washed and autoradiography performed.	−	100 mg/kg	Benzo[a]pyrene was negative at both time points.	Mirsalis et al. (1982)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
UDS	Mouse, HOS:HR-1 hairless; skin	Single topical application on two spots on the backs after stripping stratum corneum with adhesive tape to enhance penetration; sacrificed 24 hrs posttreatment, skin isolated [^{3}H]-thymidine; cultured; epidermal UDS measured.	+	0, 0.25, 0.5, or 1% (w/v) in acetone	UDS index showed a dose-dependent increase up to 0.5% benzo[a]pyrene dose and then plateaued.	Mori et al. (1999)
UDS	Rat, Brown Norway; liver	Single intragastric injection; sacrificed at 5 and 18 hrs post-injection.	–	62.5 mg/kg	Benzo[a]pyrene was negative at both time points.	Mullaart et al. (1989)
UDS	Mouse, (C3Hf × 101)F1 hybrid, germ cells	i.p. injection of benzo[a]pyrene; [^{3}H]-thymidine injection later.	–	0.3 mL	Concentration not specified.	Sega (1979)
UDS	Mouse, early spermatid	i.p. injection.	–	250–500 mg/kg	Reviewed by Sotomayor and Sega (2000).	Sega (1982)
SCEs	Hamster; SCEs in bone marrow	8–12-wk-old animals dosed with two i.p. injections of benzo[a]pyrene given 24 hrs apart; animals sacrificed 24 hrs after last treatment; bone marrow from femur isolated and metaphases analyzed.	+	450 mg/kg	Significant increase in metaphase SCEs in benzo[a]pyrene-treated animals compared to vehicle-treated controls.	Roszinsky-Koecher et al. (1979)
SCEs	Hamster	Animals implanted subcutaneously (s.c.) with BrdU tablet; 2 hrs later given phorone (125 or 250 mg/kg) i.p.; another 2 hrs later dosed i.p. with benzo[a]pyrene; 24 hrs post-BrdU dosing, animals injected with colchicine 10 mg/kg body weight, sacrificed 2 hrs later; bone marrow from femur prepared for SCE assay.	+	50 or 100 mg/kg	SCEs significantly increased with low dose of phorone.	Bayer et al. (1981)
SCEs	Hamster; fetal liver	i.p. injection to pregnant animals on GDs 11, 13, or 15; fetal liver SCEs were analyzed.	+	50 or 125 mg/kg	Produced doubling of SCE frequency.	Pereira et al. (1982)
SCEs	Hamster; bone marrow	Not available	+	2.5, 25, 40, 50, 75, or 100 mg/kg	Frequency of SCEs increased ≥40 mg/kg body weight.	Bayer (1978)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
SCEs	Mouse, DBA/2 and C57BL/6, bone marrow cells	Two intragastric injections given; mice implanted with BrdU tablets, sacrificed on d 5, SCEs estimated.	+	10 or 100 mg/kg	SCEs and benzo[a]pyrene-DNA adducts in the order of C57Bl/6 (AHH-inducible) < DBA/2 (AHH-noninducible).	Wielgosz et al. (1991)
SCEs	Mouse, DBA/2 and C57BL/6, splenic lymphocytes	Two intragastric injections given; mice killed on 5th day and cells cultured for 48 hrs with BrdU.	+	10 or 100 mg/kg	SCEs and benzo[a]pyrene-DNA adducts in the order of C57Bl/6 (AHH-inducible) < DBA/2 (AHH-noninducible).	Wielgosz et al. (1991)
SCEs	Rat, Wistar; peripheral blood lymphocytes	Single dose by gavage; sacrificed 6, 24, and 48 hrs posttreatment; blood from abdominal aorta collected, whole blood cultures set up, SCEs scored in 50 second-division metaphases in peripheral blood lymphocytes per animal.	+	0, 10, 100, or 200 mg/kg	Linear dose-response at any sampling time; however, significant at the highest dose only; no interaction between dose and sampling time.	Willems et al. (1991)
Mutation	*Drosophila melanogaster,* sex-linked recessive lethal test	*Basc* males exposed to benzo[a]pyrene were mated with virgin females of Berlin K or *mei-9*^{L1} strains.	±	10 mM	Data inconclusive due to low fertility rates of *mei-9*^{L1} females.	Vogel et al. (1983)
Mutation	*D. melanogaster*, sex-linked recessive lethal test	Adult Berlin males treated orally with benzo[a]pyrene.	+	5 or 7.5 mM	Low mutagenic activity.	Vogel et al. (1983)
Mutation	*D. melanogaster*, Berlin-K and Oregon-K strains; sex-linked recessive lethal test	Benzo[a]pyrene dissolved in special fat and injected into the abdomen of flies.	−	2 or 5 mM	Negative at both doses.	Zijlstra and Vogel (1984)
Mutation	*D. melanogaster*, sex-linked recessive lethal test	Male Berlin K larvae treated with benzo[a]pyrene for 9−11 d.	+	0.1−4 mM	3-Fold enhancement in lethals in treated versus controls.	Vogel et al. (1983)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	*D. melanogaster*, Canton-S (WT) males, FM6 (homozygous for an X-chromosome) females; sex-linked recessive lethal test	Adult male flies were fed on filters soaked in benzo[a]pyrene for 48 or 72 hrs; treated and control males mated with FM6[a] females, males transferred to new groups of females at intervals of 3, 2, 2, and 3 d; four broods obtained; a group of 100 daughters of each male were mated again; scored for percent lethal.	–	250 or 500 ppm	Authors report incomplete dissolution of benzo[a]pyrene in DMSO as a possible cause of negative result.	Valencia and Houtchens (1981)
Mutation	*D. melanogaster;* somatic mutation, eye color mosaicism	Fifty females and 20 females were mated in a culture bottle for 48 hrs allowing females to oviposit; adults were then discarded and the eggs were allowed to hatch; larvae fed on benzo[a]pyrene deposited on food surface and the emerging adult males were scored for mosaic eye sectors.	+	1, 2, or 3 mM	Benzo[a]pyrene was effective as a mutagen; no dose-response observed.	Fahmy and Fahmy (1980)
Cell trans-formation	Hamster, LVG:LAK strain (virus free); transplacental host-mediated assay	Pregnant animals dosed i.p. with benzo[a]pyrene on GD 10; sacrificed on GD 13, fetal cell cultures prepared, 10×10^6 cells/plate; 5 d post-culture trypsinized; subcultured every 4–6 d thereafter and scored for plating efficiency and transformation.	+	3 mg/100 g body weight		Quarles et al. (1979)

[a]FM6 = First Multiple No. 6 is an X-chromosome with a complex of inversions (to suppress cross-over) and visible markers such as yellow body and white and narrow eyes.

NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte; UDS = unscheduled DNA synthesis; XPA = xeroderma pigmentosum group A.

D.5.2. Tumor Promotion and Progression

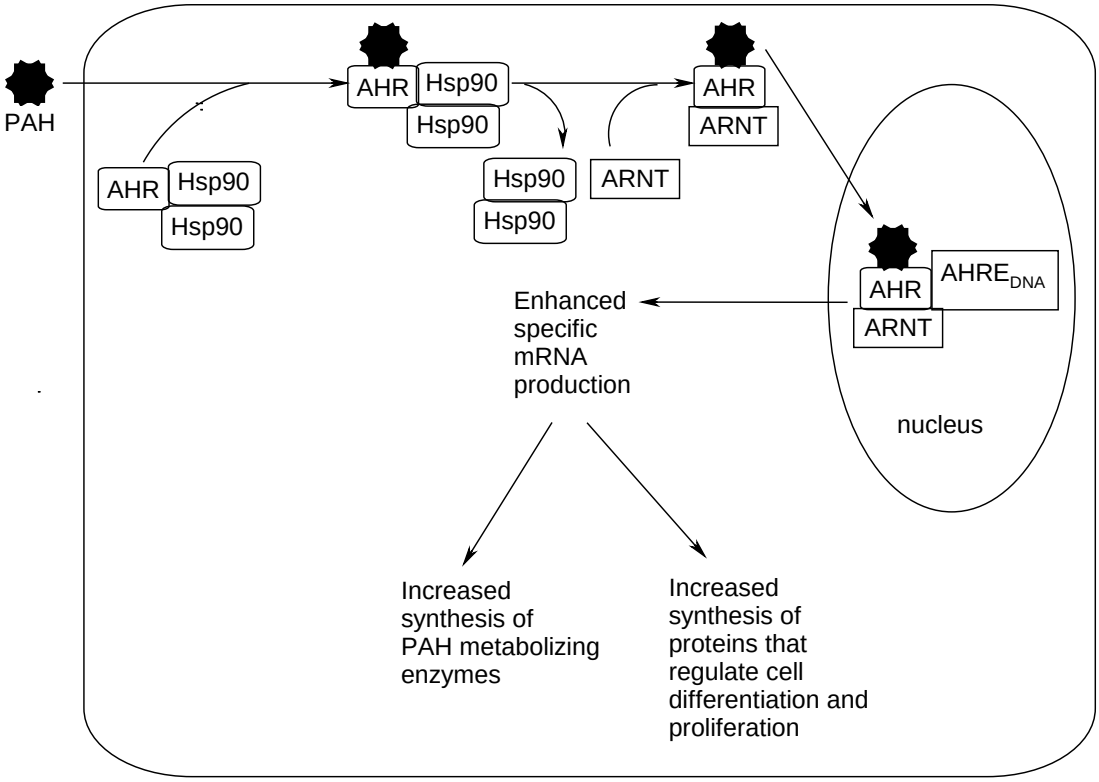
Cytotoxicity and Inflammatory Response

The cytotoxicity of benzo[a]pyrene metabolites may contribute to tumor promotion via inflammatory responses leading to cell proliferation (Burdick et al., 2003). Benzo[a]pyrene is metabolized to *o*-quinones, which are cytotoxic and can generate ROS (Bolton et al., 2000; Penning et al., 1999). Benzo[a]pyrene *o*-quinones reduce the viability and survival of rat and human hepatoma cells (Flowers-Geary et al., 1996; Flowers-Geary et al., 1993). Cytotoxicity was also induced by benzo[a]pyrene and BPDE in a human prostate carcinoma cell line (Nwagbara et al., 2007). Inflammatory responses to cytotoxicity may contribute to the tumor promotion process. For example, benzo[a]pyrene quinones (1,6-, 3,6-, and 6,12-benzo[a]pyrene-quinone) generated ROS and increased cell proliferation by enhancing the epidermal growth factor receptor pathway in cultured breast epithelial cells (Burdick et al., 2003).

Several studies have demonstrated that exposure to benzo[a]pyrene increases the production of inflammatory cytokines, which may contribute to cancer progression. Garçon et al. (2001a) and Garçon et al. (2001b) exposed Sprague-Dawley rats by inhalation to benzo[a]pyrene with or without ferrous oxide (Fe_2O_3) particles. They found that benzo[a]pyrene alone or in combination with Fe_2O_3 particles elicited mRNA and protein synthesis of the inflammatory cytokine, IL-1. Tamaki et al. (2004) also demonstrated a benzo[a]pyrene-induced increase in IL-1 expression in a human fibroblast-like synoviocyte cell line (MH7A). Benzo[a]pyrene increases the expression of the mRNA for CCL1, an inflammatory chemokine, in human macrophages (N'Diaye et al., 2006). The benzo[a]pyrene-induced increase in CCL1 mRNA was inhibited by the potent AhR antagonist, 3′-methoxy-4′-nitroflavone.

AhR-Mediated Effects

The promotional effects of benzo[a]pyrene may also be related to AhR affinity and the upregulation of genes related to biotransformation (i.e., induction of CYP1A1), growth, and differentiation (Boström et al., 2002). Figure D-3 illustrates the function of the AhR and depicts the genes regulated by this receptor as belonging to two major functional groups (i.e., induction of metabolism or regulation cell differentiation and proliferation). PAHs bind to the cytosolic AhR in complex with heat shock protein 90 (Hsp90). The ligand-bound receptor is then transported to nucleus in complex with the AhR nuclear translocator. The AhR complex interacts with the Ah-responsive elements of the DNA to increase the transcription of proteins associated with induction of metabolism and regulation of cell differentiation and proliferation.



AHRE$_{DNA}$ = Ah-responsive elements of DNA; ARNT = AhR nuclear translocator.

Source: Okey et al. (1994).

Figure D-3. Interaction of PAHs with the AhR.

Binding to the AhR induces enzymes that increase the formation of reactive metabolites, resulting in DNA binding and, eventually, tumor initiation. In addition, with persistent exposure, the ligand-activated AhR triggers epithelial hyperplasia, which provides the second step leading from tumor initiation to promotion and progression (Nebert et al., 1993). Ma and Lu (2007) reviewed several studies of benzo[a]pyrene toxicity and tumorigenicity in mouse strains with high and low affinity AhRs. Disparities were observed in the tumor pattern and toxicity of Ah-responsive (+/+ and +/−) and Ah-nonresponsive (−/−) mice. Ah-responsive mice were more susceptible to toxicity and tumorigenicity in proximal target tissues such as the liver, lung, and skin. For example, Shimizu et al. (2000) reported that AhR knock-out mice (−/−), treated with benzo[a]pyrene by subcutaneous (s.c.) injection or dermal painting, did not develop skin cancers at the treatment site, while AhR-responsive (+/+) or heterozygous (+/−) mice developed tumors within 18–25 weeks after treatment. Benzo[a]pyrene treatment increased CYP1A1 expression in the skin and liver of AhR-positive mice (+/− or +/+), but CYP1A1 expression was not altered by benzo[a]pyrene treatment in AhR knock-out mice (−/−). Talaska et al. (2006) also showed that benzo[a]pyrene adduct levels in skin were reduced by 50% in CYP1A2 knock-out mice and by 90%

in AhR knock-out mice compared with WT C57Bl6/J mice following a single dermal application of 33 mg/kg benzo[a]pyrene for 24 hours. Ma and Lu (2007) further noted that Ah-nonresponsive mice were at greater risk of toxicity and tumorigenicity in remote organs, distant from the site of exposure (i.e., bone marrow). As an example, Uno et al. (2006) showed that benzo[a]pyrene (125 mg/kg-day, orally for 18 days) caused marked wasting, immunosuppression, and bone marrow hypocellularity in CYP1A1 knock-out mice, but not in WT mice.

Some studies have demonstrated the formation of DNA adducts in the liver of AhR knock-out mice following i.p. or oral exposure to benzo[a]pyrene (Sagredo et al., 2006; Uno et al., 2006; Kondraganti et al., 2003). These findings suggest that there may be alternative (i.e., non-AhR-mediated) mechanisms of benzo[a]pyrene activation in the mouse liver. Sagredo et al. (2006) studied the relationship between the AhR genotype and CYP metabolism in different organs of the mouse. AhR+/+, +/−, and −/− mice were treated once with 100 mg/kg benzo[a]pyrene by gavage. CYP1A1, CYP1B1, and AhR expression was evaluated in the lung, liver, spleen, kidney, heart, and blood, via real-time or reverse transcriptase polymerase chain reaction, 24 hours after treatment. CYP1A1 RNA was increased in the lung and liver and CYP1B1 RNA was increased in the lung following benzo[a]pyrene treatment in AhR+/+ and +/− mice (generally higher in heterozygotes). Benzo[a]pyrene treatment did not induce CYP1A1 or CYP1B1 enzymes in AhR−/− mice. The expression of CYP1A1 RNA, as standardized to β-actin expression, was generally about 40 times that of CYP1B1. The concentration of benzo[a]pyrene metabolites and the levels of DNA and protein adducts were increased in mice lacking the AhR, suggesting that there may be an AhR-independent pathway for benzo[a]pyrene metabolism and activation. The high levels of benzo[a]pyrene DNA adducts in organs other than the liver of AhR−/− mice may be the result of slow detoxification of benzo[a]pyrene in the liver, allowing high concentrations of the parent compound to reach distant tissues.

Uno et al. (2006) also demonstrated a paradoxical increase in liver DNA adducts in AhR knock-out mice following oral exposure to benzo[a]pyrene. WT C57BL/6 mice and several knock-out mouse strains (CYP1A2−/− and CYP1B1−/− single knock-out, CYP1A1/1B1−/− and CYP1A2/1B1−/− double knock-out) were studied. Benzo[a]pyrene was administered in the feed at 1.25, 12.5, or 125 mg/kg for 18 days (this dose is well-tolerated by WT C57BL/6 mice for 1 year, but lethal within 30 days to the CYP1A1−/− mice). Steady-state blood levels of benzo[a]pyrene, reached within 5 days of treatment, were ~25 times higher in CYP1A1−/− and ~75 times higher in CYP1A1/1B1−/− than in WT mice, while clearance was similar to WT mice in the other knock-out mouse strains. DNA adduct levels, measured by [32P]-postlabeling in liver, spleen, and bone marrow, were highest in the CYP1A1−/− mice at the two higher doses, and in the CYP1A1/1B1−/− mice at the mid dose only. Adduct patterns, as revealed by 2-dimensional chromatography, differed substantially between organs in the various knock-out types.

AhR signaling may play a role in cytogenetic damage caused by benzo[a]pyrene (Dertinger et al., 2001; Dertinger et al., 2000). The in vivo formation of MN in peripheral blood reticulocytes of

C57Bl/6J mice induced by a single i.p. injection of benzo[a]pyrene (150 mg/kg) was eliminated by prior treatment with the potent AhR antagonist, 3'-methoxy-4'-nitroflavone. This antagonist also protected AhR-null allele mice from benzo[a]pyrene-induced increases in MN formation, suggesting that 3'-methoxy-4'-nitroflavone may also act through a mechanism independent of the AhR (Dertinger et al., 2000).

Several in vitro studies have suggested that the AhR plays a role in the disruption of cell cycle control, possibly leading to cell proliferation and tumor promotion following exposure to benzo[a]pyrene (Andrysík et al., 2007; Chung et al., 2007; Chen et al., 2003). Chung et al. (2007) showed that benzo[a]pyrene-induced cytotoxicity and apoptosis in mouse hepatoma (Hepa1c1c7) cells occurred through a *p53* and caspase-dependent process requiring the AhR. An accumulation of cells in the S-phase of the cell cycle (i.e., DNA synthesis and replication) was also observed, suggesting that this process may be related to cell proliferation. Chen et al. (2003) also demonstrated the importance of the AhR in benzo[a]pyrene-7,8-dihydrodiol- and BPDE-induced apoptosis in human HepG2 cells. Both the dihydrodiol and BPDE affected Bcl2 (a member of a family of apoptosis suppressors) and activated caspase and p38 mitogen-activated protein (MAP) kinases, both enzymes that promote apoptosis. When the experiments were conducted in a cell line that does not contain AhR nuclear translocator (see Figure D-3), the dihydrodiol was not able to initiate apoptotic event sequences, indicating that activation to BPDE by CYP1A1 was required. BPDE did not induce apoptosis-related events in a p38-defective cell line, illustrating the importance of MAP kinases in this process. In rat liver epithelial cells (WB-F344 cells), in vitro exposure to benzo[a]pyrene resulted in apoptosis, a decrease in cell number, an increase in the percentage of cells in S-phase (comparable to a proliferating population of WB-F334 cells), and increased expression of cell cycle proteins (e.g., cyclin A) (Andrysík et al., 2007). Benzo[a]pyrene-induced apoptosis was attenuated in cells transfected with a dominant-negative mutation of the AhR.

Inhibition of gap junctional intercellular communication (GJIC)

Gap junctions are channels between cells that allow substances of a molecular weight up to roughly 1 kDa to pass from one cell to the other. This process of metabolic cooperation is crucial for differentiation, proliferation, apoptosis, and cell death and consequently for the two epigenetic steps of tumor formation, promotion, and progression. Chronic exposure to many toxicants results in down-regulation of gap junctions. For tumor promoters, such as TPA or TCDD, inhibition of intercellular communication is correlated with their promoting potency (Sharovskaya et al., 2006; Yamasaki, 1990).

Bláha et al. (2002) surveyed the potency of 35 PAHs, including benzo[a]pyrene, to inhibit GJIC. The scrape loading/dye transfer assay was employed using a rat liver epithelial cell line that was incubated in vitro for 15, 30, or 60 minutes with 50 μM benzo[a]pyrene. After incubation, cells were washed, and then a line was scraped through the cells with a surgical blade. Cells were exposed to the fluorescent dye lucifer yellow for 4 minutes and then fixed with formalin. Spread of

the dye from the scrape line into cells remote from the scrape was estimated under a fluorescence microscope. Benzo[a]pyrene reduced spread of the dye after 30 minutes of exposure (approximately 50% of control). Recovery of GJIC was observed 60 minutes after exposure.

Sharovskaya et al. (2006) studied the effects of carcinogenic and noncarcinogenic PAHs on GJIC in HepG2 cells. Individual carcinogenic PAHs inhibited GJIC in a temporary fashion (70–100% within 24 hours), but removal of the PAH from culture reversed the effect. Noncarcinogenic PAHs had very little effect on GJIC. Benzo[a]pyrene at 20 μM inhibited GJIC completely within 24 hours, while its noncarcinogenic homolog, benzo[e]pyrene, produced <20% inhibition. The effect was not AhR-dependent, because benzo[a]pyrene inhibited GJIC in HepG2 cells to the same extent as in hepatoma G27 cells, which express neither CYP1A1 nor AhR. The authors concluded that the effects of benzo[a]pyrene and benzo[e]pyrene on GJIC were direct (i.e., not caused by metabolites).

D.5.3. Benzo[a]pyrene Transcriptomic Microarray Analysis

The objective of this analysis was to use transcriptomic microarray analysis to help inform the cancer mode of action for benzo[a]pyrene. A systematic review and meta-analysis approach was used to: (1) identify studies; (2) analyze the raw data; (3) assess data quality; and (4) combine evidence from multiple studies to identify genes that were reproducibly active across all of the studies.

The Gene Expression Omnibus and Array Express microarray repositories were searched for studies that used benzo[a]pyrene as a test chemical and raw data were available. The search terms used and the number of studies retrieved are listed in Table D-36. Many of the search terms included terms for specific PAH mixtures, as benzo[a]pyrene is commonly used as a reference chemical in PAH mixture studies, to ensure the available and usable benzo[a]pyrene microarray data were identified.

Table D-36. Search terms and the number of studies retrieved from the gene expression omnibus and array express microarray repositories

Search term	Number of microarray studies retrieved
Coal tar	2
Polycyclic aromatic hydrocarbons	13
B[a]P	52
Diesel	11
Smoke NOT cigarette	16
Benzo[a]pyrene	53
Fuel oil	1
Cigarette smoke	63
Tobacco smoke	16

Forty responsive gene expression datasets were identified, representing 26 peer-reviewed publications. These datasets were further culled for analysis by focusing on publicly available results and species and organs represented by more than one available dataset on the same microarray platform. Crossing microarray platforms and species boundaries adds significant uncertainty to the interpretation with respect to comparisons of the probes being measured and how those different probes align to the genome and are mapped to specific genes, and creates an open question regarding the discovery and mapping of orthologous genes across species. Thus, the analysis included two studies that focused on mouse in vivo transcriptomic studies of the liver (Gene Expression Omnibus accessions: GSE24907 and GSE18789).

The first study (Malik et al., 2012), GSE24907, exposed five male Muta mice (a LacZ transgenic mouse line) per group to 25, 50, or 75 mg/kg-day benzo[a]pyrene or olive oil vehicle for 28 days by gavage. The second study (Yauk et al., 2011), GSE18789, exposed 27–30-day-old male $B6C3F_1$ mice to 150 mg/kg-day benzo[a]pyrene by gavage for 3 days and sacrificed 4 or 24 hours after the final dose. Both studies were subjected to study quality evaluation by the Systematic Omics Analysis Review (SOAR) tool.

SOAR was developed to assist in the quick and transparent identification of studies that are suitable for hazard assessment development. SOAR consists of a series of objective questions that examine the overall study quality of a transcriptomic microarray study. SOAR combines questions from the Toxicological Reliability Assessment (ToxR) Tool, the Minimum Information About a Microarray Experiment (MIAME) standard, and the Checklist for Exchange and Interpretation of Data from a Toxicology Study. Both studies were determined to be relevant and suitable for hazard assessment development using SOAR.

Data Analysis Overview

Raw data for both studies were obtained from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) using the GEOquery package (Davis and Meltzer, 2007) in Bioconductor (a bioinformatics software repository for packages that may be used in the R statistical environment). Each study was pre-processed, normalized, subjected to quality control analysis (see below), and analyzed independently to determine the number of active genes using a fold-change cut-off, and then a subsequent *p*-value cut-off.

Pre-processing involves the acquisition of data, background subtraction (not performed here), and synthesis of gene expression data across multiple probesets (only for Affymetrix data, and only if analysis is performed on a probeset basis). Normalization is the mathematical adjustment of data to correct. Data were normalized using fastlo within-groups to control for technical variance (Eckel et al., 2005).

The raw microarray data from both studies were analyzed for quality using Principal Components Analysis (PCA) and boxplot analysis. PCA is commonly used for cluster analysis based on the variance within the dataset. The PCA algorithm (in this case, singular value decomposition

was used) can be thought of projecting the data into a multidimensional space, and drawing an axis through the data cloud to explain the largest amount of variance. The next axis is drawn through the cloud to explain the next largest amount of variance while also being orthogonal to the first axis (e.g., the Y-axis is orthogonal to the X-axis in a Cartesian plane). The idea is that samples will naturally cluster in a way that is easily visualized in a simple 2-dimensional plot, where the axis representing the largest variance is the X-axis. For quality control purposes, observation of samples from the same biological grouping (e.g., all of the controls, or all of the samples treated the same way for the same duration) clustered in the X–Y plane is preferable. The samples in GSE24907 separated mostly by group when the normalized data were visualized by PCA. The boxplots exhibited a somewhat compressed interquartile range. Overall, the data were deemed to be of high enough quality to continue analysis, although the compressed interquartile range could manifest data compression issues, which may decrease the overall statistical power.

The normalized samples in GSE18709 also separated mostly by group; however, one benzo[a]pyrene treated 24-hour sample and one 4-hour control sample clustered distantly from the rest of their groups. This raises concerns that there remains a significant amount of variance in the data that the normalization could not overcome. This variance may decrease the overall statistical power of the meta-analysis. The boxplots of normalized data for this study were more compressed than that for GSE24907.

Data were analyzed using limma and an empirical Bayes moderated t-test (Smyth, 2004). Following analysis, active genes were identified. A gene was considered active if it exhibited a 1.5-fold-change and a *p*-value <0.1 in at least one condition or group (e.g., time-point or dose).

A data mining/pathway analysis approach was undertaken using the GeneGo Metacore software and using the active gene lists. This approach compares the pathways identified from bioinformatics analyses of the active gene lists from both studies. The active gene lists from both studies were analyzed using the GeneGo Metacore software. The data were mined to identify GeneGo Metacore pathways that represent a large number of genes from both datasets. Gene expression data were overlaid only for those conditions where the gene was at least 1.5-fold up- or down-regulated. The GeneGo pathways were analyzed for relevance to the hypothesized mode of action for benzo[a]pyrene, and for pathways that may illustrate new modes of action. This analysis is strictly an exploratory pathway analysis to help inform the interpretation of the transcriptomics data.

The pathway analysis is a powerful method for comparing study results and identifying consistency than a direct comparison of the active gene list. For instance, differentially expressed gene lists reported in the peer-reviewed literature are not reproducible across similar studies (Shi et al., 2008; Chuang et al., 2007; Ein-Dor et al., 2005; Lossos et al., 2004; Fortunel et al., 2003). In one example, three different studies aimed at identifying genes that confer "stemness" (i.e., genes which are responsible for conferring stem-cell like capabilities) each yielded 230, 283, and 385 active genes, yet the overlap between them was only one gene (Fortunel et al., 2003). This

demonstrates that the use of simple Venn diagrams to show the overlap of genes across studies are not as informative as pathway analysis, and are less likely to provide support to potential mode-of-action hypotheses.

Three candidate pathways were identified. These are:

- AhR signaling
- DNA damage regulation of the G1/S phase transition
- Nrf2 regulation of oxidative stress

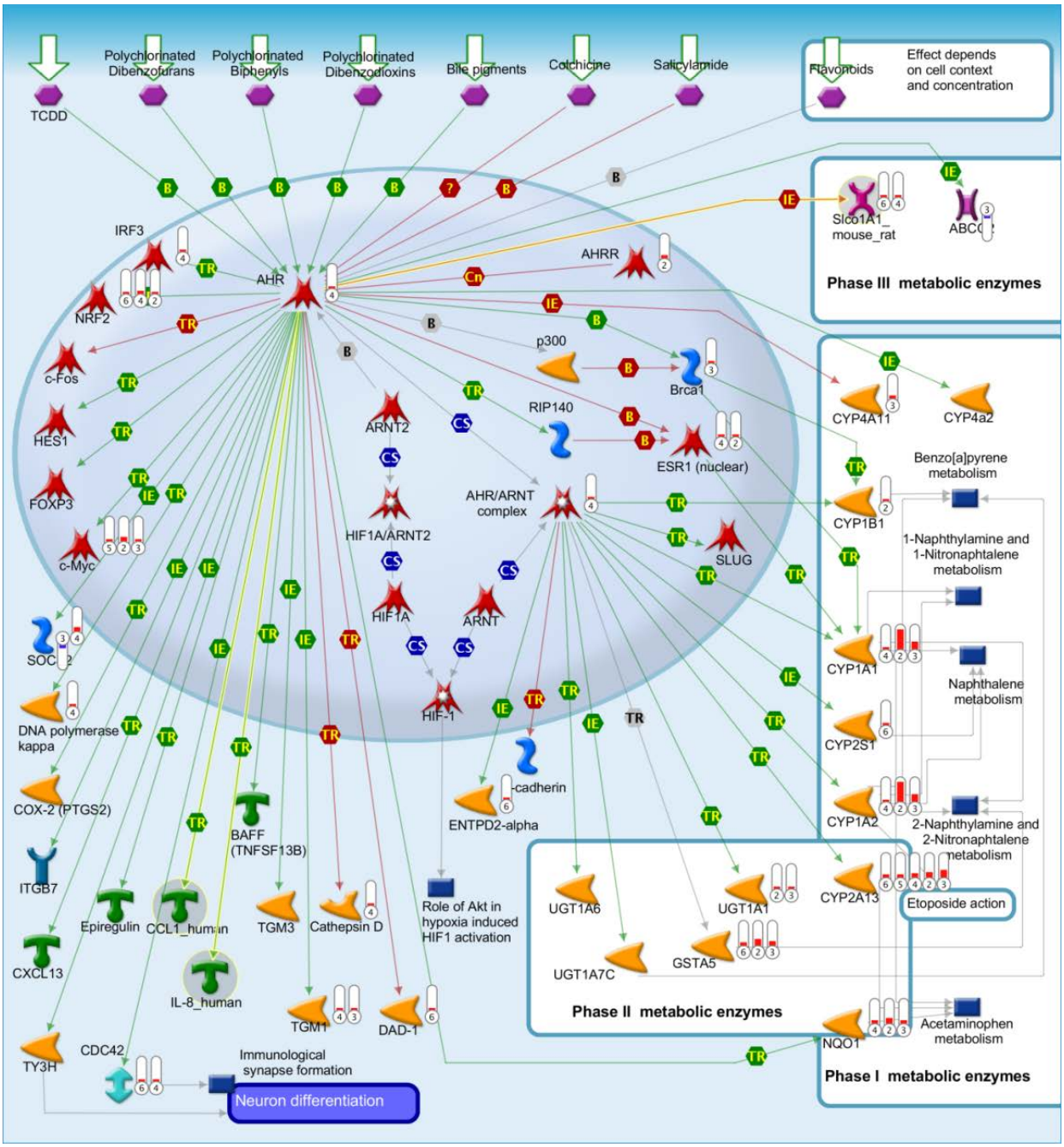
Gene differential expression is represented on the pathway map as a "thermometer" next to the protein symbol. Upregulation is symbolized by an upward pointing thermometer, where the length of the red bar represents a relative log2 fold-change. Downregulation is symbolized by a downward pointing thermometer, where the length of the blue bar represents a relative log2 fold-change. A red line connecting proteins represents inhibition. A green line connecting proteins represents activation. A symbol legend accompanies this report.

Table D-37. Mapping of group numbers to time/dose groups

Number under Thermometer in Figures D-4–D-6	Dose	Time point	Reference
2	150 mg/kg	3-d exposure (sacrificed 4 hrs after final dose)	Yauk et al. (2011)
3	150 mg/kg	3-d exposure (sacrificed 24 hrs after final dose)	Yauk et al. (2011)
4	75 mg/kg	28-d exposure	Malik et al. (2012)
5	50 mg/kg	28-d exposure	Malik et al. (2012)
6	25 mg/kg	28-d exposure	Malik et al. (2012)

AhR Signaling

The AhR regulates the transcription of several genes, including xenobiotic metabolism genes (Figure D-4). It appears that benzo[a]pyrene is activating the AhR in these studies based on the expression of many of its transcriptional targets. Relevant to further analysis and investigating the mode of action, the c-Myc gene is upregulated at 4 and 24 hours in the time-course and at the 50 mg/kg dose in the dose-response, while Nrf2 is upregulated at the 4-hour time-point and at the 25 and 75 mg/kg-day doses. c-Myc has been shown to be upregulated following exposure to TCDD, and a putative dioxin response element has been detected in the c-Myc promoter (Dere et al., 2011; Kim et al., 2000). The AhR has been demonstrated to bind and regulate the Nrf2 promoter (Dere et al., 2011; Lo et al., 2011; Nair et al., 2008).



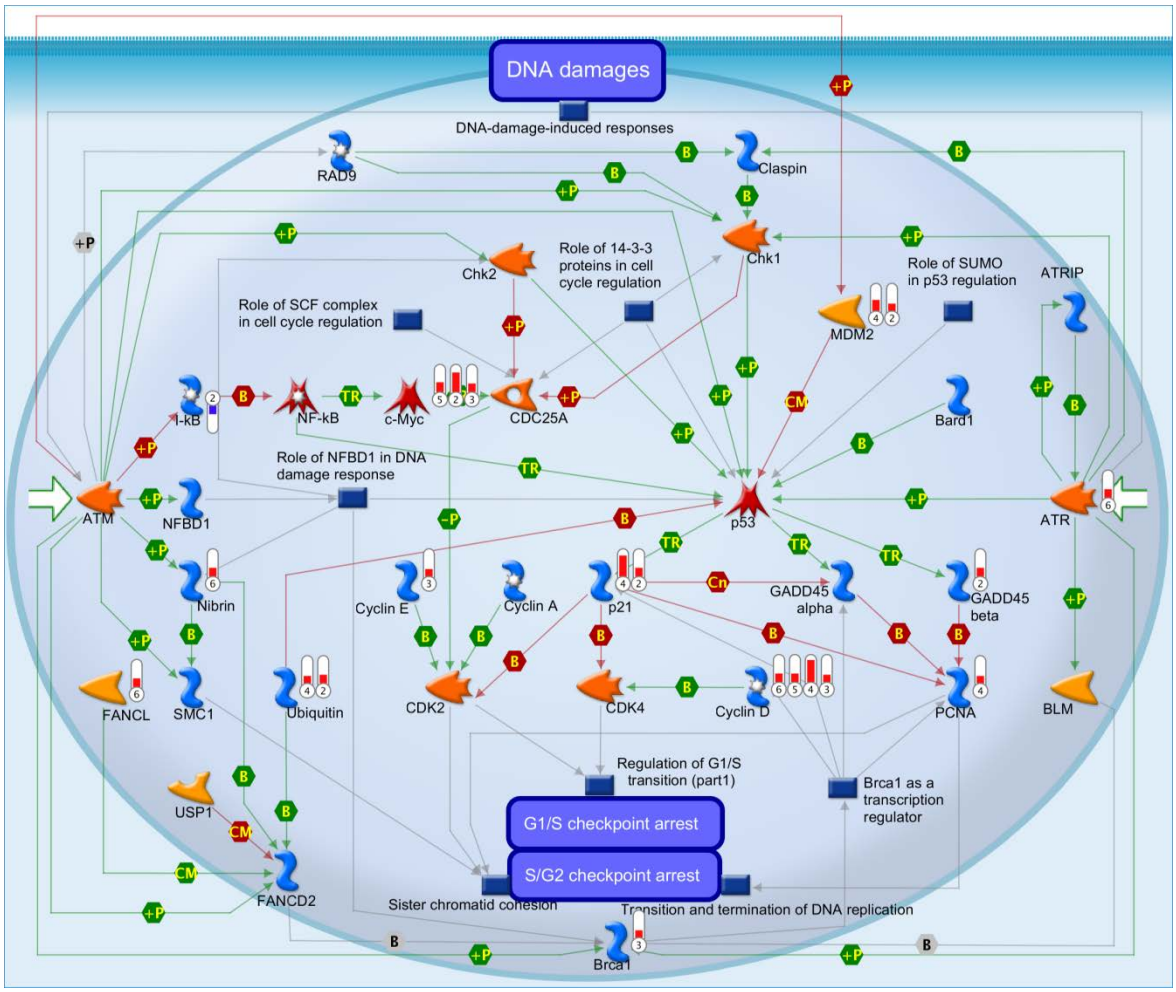
For Figures D-4–D-6, the "thermometers" display the fold change gene expression. The numbers under the thermometer represent the group within the two studies (see Table D-37). For instance, NRF2 is upregulated in the 25 mg/kg-day group.

Figure D-4. AhR pathway.

DNA Damage Signaling

The strong upregulation of p21 and MDM2 at 4 hours and 75 mg/kg-day suggests that *p53* is activated following exposure to benzo[a]pyrene, suggesting that benzo[a]pyrene induces DNA damage as early as the 4-hour time-point, and at 75 mg/kg-day in mice (Figure D-5). MDM2 is a

target gene of *p53*, and also negatively inhibits *p53* signaling through ubiquitination. Ubiquitin is also upregulated at 4 hours and 75 mg/kg-day, further suggesting that that *p53* may initially be upregulated at times prior to 4 hours and prior to sacrifice in the 75 mg/kg-day groups, and that at the time of sacrifice, the *p53* signal may be degraded due to MDM2-mediated ubiquitination. Coupled with the upregulation of Cyclin D and PCNA at 75 mg/kg-day (among other conditions), this suggests that a pro-mitotic shift may be occurring, which could lead to cellular proliferation in the liver in the mice exposed to 75 mg/kg-day.



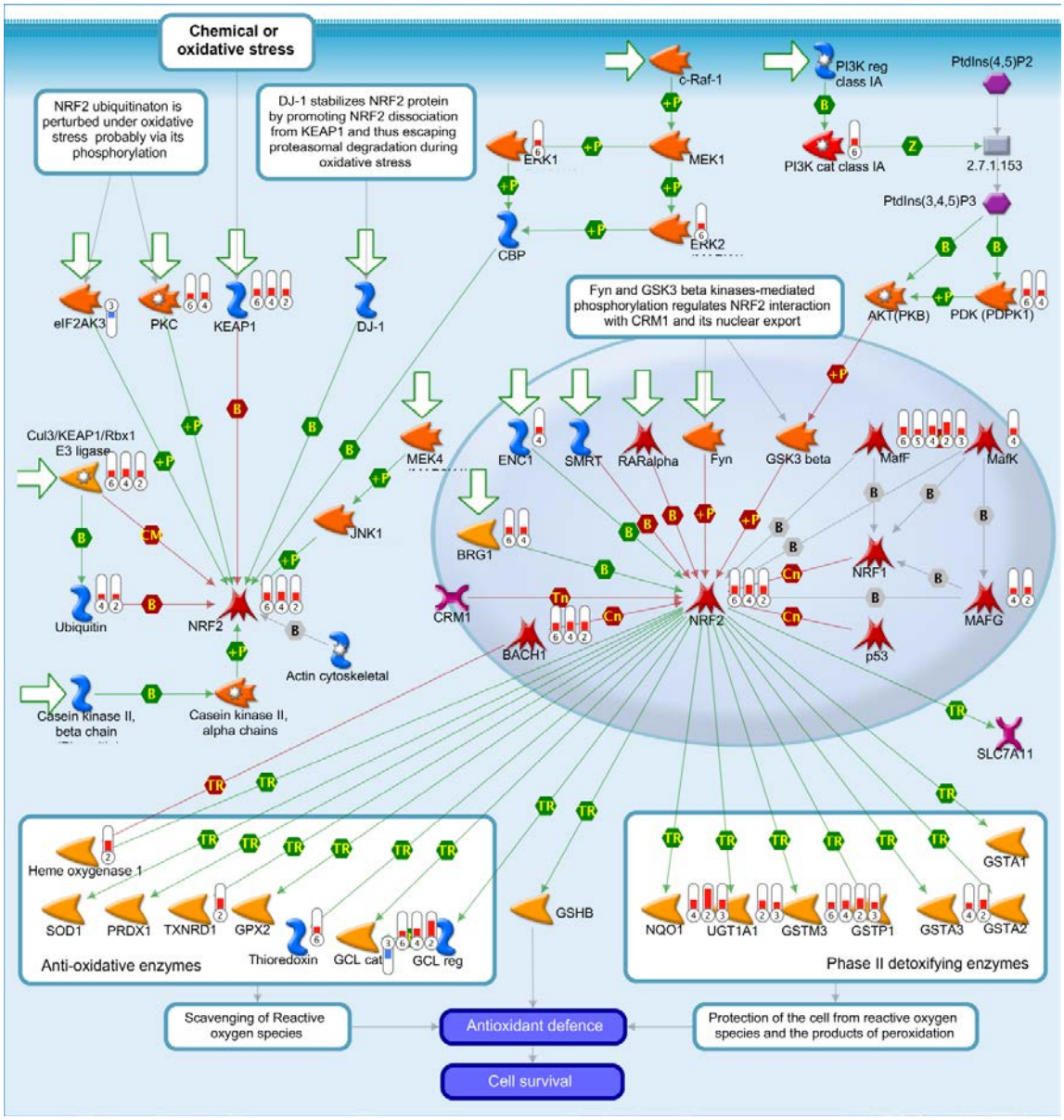
Activation of transcriptional targets of *p53*, including p21 and GADD45, and upregulation of the downstream transcriptional target, PCNA, suggests that *p53* is activated.

Figure D-5. DNA damage pathway.

Nrf2 Signaling

Nrf2 transcription may be upregulated by benzo[a]pyrene through activation of the AhR (Figure D-4). The Nrf2 protein heterodimerizes with the MafF protein (Surh et al., 2008; Marini et al., 2002; Kim et al., 2000) to regulate the transcription of Phase II metabolism and anti-oxidative

enzymes (Figure D-6). Activated *p53* competes with Nrf2 anti-oxidant signaling, perhaps to ensure a large oxidative stress response is present in the cell to promote the induction of apoptosis (Faraonio et al., 2006). Upregulation of Cul3 at 4 hours and the 75 mg/kg-day dose in concert with the upregulation of ubiquitin at the same time and dose suggests that repression of Nrf2 activity may occur. This would support the *p53*-mediated pro-oxidant hypothesis, which is further substantiated by the lack of upregulation of anti-oxidant genes at 75 mg/kg-day, with the exception of GCL cat.



Nrf2 is upregulated by benzo[a]pyrene exposure, which results in the upregulation of Phase II detoxifying enzymes. This appears to be a compensatory response due to increased oxidative status within cells.

Figure D-6. Nrf2 pathway.

Pathway Analysis Summary

Activation of the AhR appears to be present based on the transcriptional data. This may lead to formation of oxidative metabolites and radicals, which may lead to oxidative damage and DNA damage. Although the alterations to the Nrf2 pathway suggest cells are gearing up for a pro-apoptotic environment, there is no transcriptional evidence that the apoptotic pathways are being activated. Thus, there is significant uncertainty as to whether or not apoptosis may occur.

The transcriptomics data support a potential mutagenic and cellular proliferation mode of action. The transcriptomics data support the hypothesis that DNA damage is occurring at 4 hours following three daily doses of 150 mg/kg-day of benzo[a]pyrene and 75 mg/kg-day for 28 days. This is supported by the transcriptional activation of *p53* target genes, including p21 and MDM2. The transcriptional data further suggest that *p53* signaling may be waning under these conditions, as ubiquitin and MDM2 are both upregulated, and work together to degrade *p53*. Furthermore, the transcriptional upregulation of Cyclin D in the 75 mg/kg-day exposure may result in enough Cyclin D protein to overcome the p21 inhibitory competition for CDK4, allowing for G1/S phase transition to occur. In addition, the upregulation of PCNA in the 75 mg/kg-day exposure group, together with upregulation of ubiquitin, further supports the argument that cells are moving towards a more G1/S phase transition friendly environment. Translesion synthesis (i.e., a DNA repair/bypass mechanism, whereby DNA adducts are allowed to remain in newly synthesized DNA, so as to allow the cell to continue with DNA synthesis and complete the cell cycle) by ubiquitinated PCNA may favor mutagenesis if the G1/S phase transition occurs by allowing DNA adducts to persist in daughter cells.

There are a number of areas of uncertainty within the transcriptomics data that require additional research. For instance, transcriptomics data only measure changes in gene expression; these studies did not monitor changes in protein or metabolite expression, which would be more indicative of an actual cellular state change. Inferences of protein activation and changes in protein activity and cellular signaling are made based on the transcriptomics data. Further research is required at the molecular level to demonstrate that the cellular signaling events being inferred are actually taking place, and that these events result in phenotypic changes, consistent with the overall mode of action. The studies also have inherent uncertainty with respect to extrapolation from short-term, high-dose studies to low-dose exposures across a lifetime. In addition, this work uses a hypothesized mode of action in the liver to support an overall mode of action.

APPENDIX E. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints, organized by risk value (reference value or cancer risk value). Except where other software is noted, all endpoints were modeled using the U.S. Environmental Protection Agency's (EPA's) Benchmark Dose Software (BMDS) (U.S. EPA, 2012a); version 2.0 or later. The preambles for the cancer and noncancer parts below describe the practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b).

E.1. NONCANCER ENDPOINTS

E.1.1. Data Sets

The noncancer endpoints that were considered for dose-response modeling are presented in Tables E-1 (for the reference dose [RfD], from oral exposure) and E-2 (for the reference concentration [RfC], from inhalation exposure). For each endpoint, the exposures and response data used for the modeling are presented. See Sections 2.1 and 2.2 for discussion of selecting these particular data sets. Further details for some data sets—e.g., regarding data transformations or digitization from figures, highlighting particular subsets or combining similar subsets of data from an investigation—are provided below.

All data reported by Chen et al. (2012) were presented graphically; dose group means and standard deviations (SDs) were digitized from the publication. For the Morris water maze data, individual animal data for postnatal day (PND) 74 were provided upon request by the study authors. For the elevated plus maze data (Chen et al., 2012), the results from female rats at PND 70 were chosen for dose-response analyses, as effects in females and older animals were greater relative to control than in males or at PND 35. For the other outcomes from this study considered for dose-response analysis, data for male and female rats were combined because there was no substantive difference between males and females for each dose group (supported by the authors' statistical testing using two-way analysis of variance [ANOVA], and allowing for interactions), and because there was no rationale or information available suggesting that there would be sex-mediated differences for these tests. However, although there were then 20 rats in each dose

group, there were 10 litters, with 1 male and 1 female from each litter that were not technically independent due to intralitter correlation. These analyses were carried out using N = 20/group, then repeated using N = 10/group, under a bounding assumption of 100% intralitter correlation.

Table E-1. Noncancer endpoints selected for dose-response modeling for benzo[a]pyrene: RfD

Study, species (strain), endpoint	Doses (mg/kg-d) and effect data				
Kroese et al. (2001); Rat (Wistar)	Dose	0	3	10	30
	N	10	10	10	10
Thymus weight (mg), male Thymus weight (mg), female	Mean ± SD[a] Mean ± SD[a]	380 ± 60 320 ± 60	380 ± 110 310 ± 50	330 ± 60 300 ± 40	270 ± 40 230 ± 30
Xu et al. (2010); Rat (Sprague-Dawley)/ female	Dose[b]	0	2.5	5	
	N	6	6	6	
Ovary weight (mg) Primordial follicles (count)	Mean ± SD Mean ± SD	0.160 ± 0.0146 147 ± 13.8	0.143 ± 0.0098 138 ± 23.0	0.136 ± 0.0098 115 ± 12.3	
Chen et al. (2012); Rat (Sprague-Dawley)	Dose	0	0.02	0.2	2.0
Open field, number of crossed squares, male and female, PND 69	Mean ± SD N	68.1 ± 16.2 (20)	68.4 ± 13.2 (20)	82.5 ± 19.3 (20)	94.5 ± 17.1 (20)
Elevated plus maze—Number of open arm entries, female—PND 70	Mean ± SD N	10.1 ± 2.3 (10)	10.2 ± 3.2 (10)	12.8 ± 3.1 (10)	16.3 ± 3.2 (10)
Morris water maze, male and female:	N	(20)	(20)	(20)	(20)
Escape latency (sec), PND 71 PND 72 PND 73 PND 74	Mean ± SD Mean ± SD Mean ± SD Mean ± SD	33.1 ± 11.4 24.4 ± 9.9 18.0 ± 9.9 9.9 ± 5.8	35.8 ± 11.6 26.5 ± 7.9 19.7 ± 10.1 12.5 ± 5.1	38.6 ± 9.9 31.0 ± 8.4 25.5 ± 7.2 19.1 ± 5.9	50.8 ± 9.3 47.8 ± 8.4 39.7 ± 11.3 33.5 ± 9.9
Gao et al. (2011); Mouse (ICR)/female	Dose[c]	0	0.71	1.4	2.9
	N	26	26	25	24
Cervical epithelial hyperplasia	Incidence	0/26	4/26	6/25	7/24

[a]Reported as standard error (SE), but confirmed to be SD by study authors.
[b]Time-weighted average (TWA) doses corresponding to dosing every other day.
[c]TWA doses corresponding to dosing twice per week (2/7 days/week).

Table E-2. Noncancer endpoints selected for dose-response modeling for benzo[a]pyrene: RfC

Study, species (strain), endpoint	Doses (mg/kg-d) and effect data				
Archibong et al. (2002); Rat (F344)/ female	Exposure level, µg/m^3	0 (Carbon black)	25	75	100
	N	10	10	10	10
Fetal survival (litter %)	Mean ± SE[a]	96.7 ± 1.7	78.3 ± 4.1	38.0 ± 2.1	33.8 ± 1.3
Archibong et al. (2012); Rat (F344)/ female	Exposure level, µg/m^3	0	50	75	100
	N	5	5	5	5
Ovary weight (g) Ovulation rate (eggs/dam)	Mean ± SE[a] Mean ± SE[a]	0.68 ± 0.004 15.3 ± 2.0	0.61 ± 0.003 13.9 ± 3.0	0.59 ± 0.002 12.8 ± 2.5	0.60 ± 0.003 8.3 ± 1.0

[a]SE reported in source, converted to SD for modeling using SD = SE × $N^{1/2}$.

While the preferred measure for elevated plus maze results is percent of open arm entries or percent of time in the open arms, as a function of total arm entries or time, in order to rule out potential differences in motor activity or general exploration (Hogg, 1996), the data reported by Chen et al. (2012) were not normalized by either quantity. However, since sufficient information was reported to rule out an impact of treatment on total arm entries, the number of open arm entries was considered a suitable measure for dose-response analysis.

E.1.2. Dose-Response Modeling for Noncancer Endpoints

E.1.2.1. *Models and Evaluation of Model Fit*

For each dichotomous endpoint, BMDS dichotomous models were fitted to the data using the maximum likelihood method. For the log-logistic and dichotomous Hill models, slope parameters were restricted to be ≥1; for the gamma and Weibull models, power parameters were restricted to be ≥1; and for the multistage models, betas were restricted to be non-negative ($b_i \geq 0$). Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value <0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

For each continuous endpoint, BMDS continuous models were fitted to the data using the maximum likelihood method. For the polynomial models, betas were restricted to be non-negative (in the case of increasing response) or non-positive (in the case of decreasing response data); and for the Hill, power, and exponential models, power parameters were restricted to be ≥1. Model fit was assessed by a series of tests as follows. First the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value ≥0.10), then the model

was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value <0.10), then the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value <0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

E.1.2.2. *Model selection*

For each endpoint selected for modeling, the BMDL estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and Akaike's Information Criterion (AIC) value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, then the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, then the lowest BMDL was selected as the POD.

E.1.2.3. *Modeling results*

The following tables and figures summarize the modeling results for the noncancer endpoints modeled (RfD: Tables E-3 through E-14, Figures E-1 through E-12; RfC: Tables E-15 through E-18, Figures E-13 and E-14). Note that for inhalation outcomes, while the abbreviations BMC and BMCL (for benchmark concentration and benchmark concentration lower bound, respectively) describe the exposures being modeled, the abbreviations BMD and BMDL are used generically in this appendix to denote the results of benchmark dose modeling.

For the dose-response analyses of the Chen et al. (2012) outcomes involving combined male and female responses, the alternate analyses allowing for 100% intralitter correlation yielded BMDLs up to 30% lower than assuming complete independence of the pups (analyses not shown).

Table E-3. Summary of BMD modeling results for decreased thymus weight in male Wistar rats exposed to benzo[a]pyrene by gavage for 90 days (Kroese et al., 2001); BMR = 1 SD change from the control mean

Model	Variance *p*-value	Goodness of fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
		p-value	AIC		
Constant variance					
Linear	0.01	0.74	384.84	12.97	8.97
Nonconstant variance					
Hill	Insufficient degrees of freedom				
Linear, polynomial (2-degree), power	**0.30**	**0.23**	**380.71**	**16.40**	**11.30**

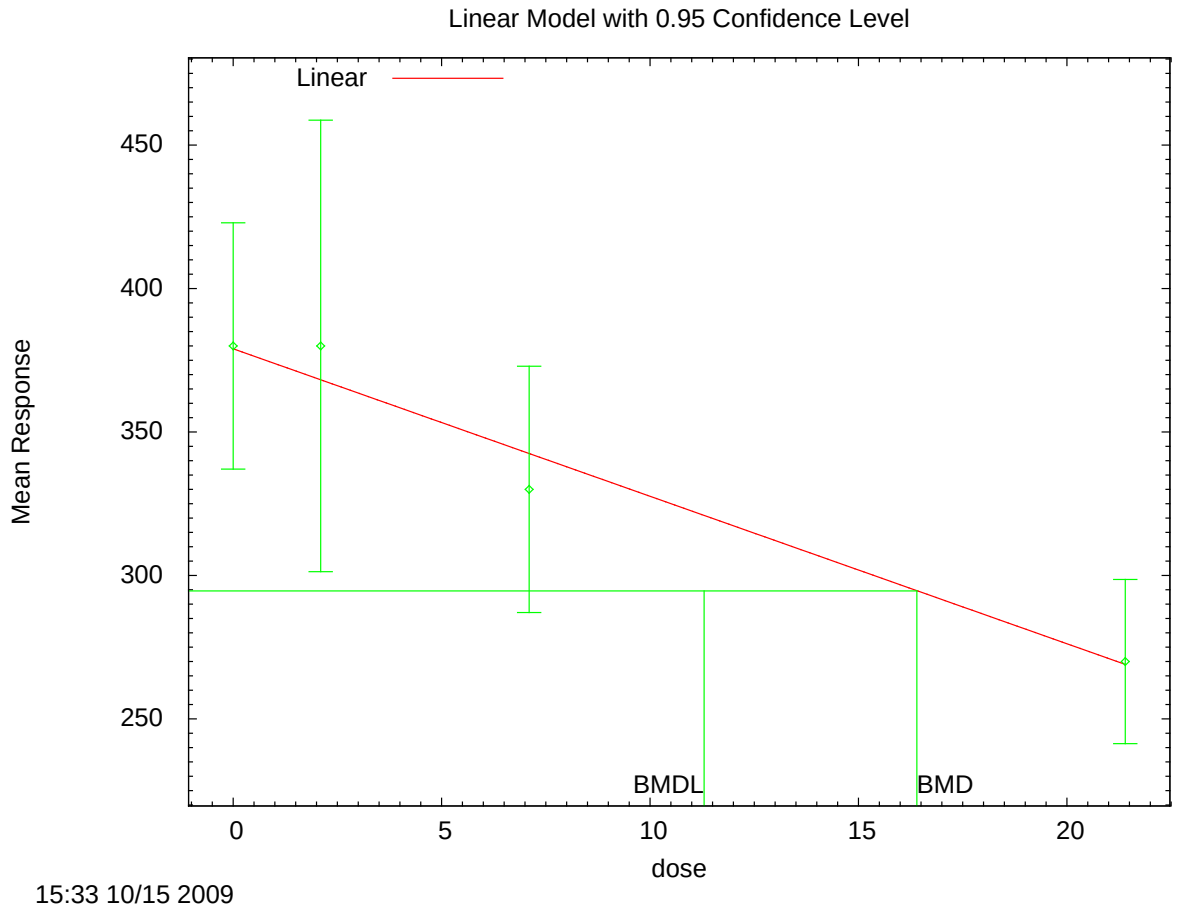


Figure E-1. Fit of linear model (nonconstant variance) to data on decreased thymus weight in male Wistar rats—90 days (Kroese et al., 2001); BMR = 1 SD change from control; dose in mg/kg-day.

```
================================================================
Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2Linkrolin.(
d)
Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2Linkrolin.p
lt
================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = mean
   Independent variable = dose
   The polynomial coefficients are restricted to be negative
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        lalpha =      8.56121
                           rho =            0
                        beta_0 =      380.763
                        beta_1 =      -5.3285


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho       beta_0       beta_1

    lalpha          1           -1        0.048       -0.061

       rho         -1            1       -0.048        0.061

    beta_0      0.048       -0.048            1        -0.84

    beta_1     -0.061        0.061        -0.84            1

                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         lalpha         -18.8293          9.75429           -37.9473            0.288754
            rho          4.66515          1.67581            1.38062             7.94967
         beta_0          378.954          16.5291            346.558             411.351
         beta_1         -5.14219          1.00497           -7.11189            -3.17249


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10        380          379           60         84.3         0.0392
  2.1    10        380          368          110         78.8          0.475
  7.1    10        330          342           60         66.6         -0.591
 21.4    10        270          269           40         37.9         0.0908


 Model Descriptions for likelihoods 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 + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var$$e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -189.116991           5     388.233982
             A2         -183.673279           8     383.346558
             A3         -184.883626           6     381.767253
         fitted         -186.353541           4     380.707081
              R         -196.353362           2     396.706723


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              25.3602          6       0.0002928
   Test 2              10.8874          3         0.01235
   Test 3              2.42069          2          0.2981
   Test 4              2.93983          2          0.2299

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 less than .1.  A non-homogeneous variance
model appears to be appropriate

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 greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        16.4008

             BMDL =        11.2965
```

Table E-4. Summary of BMD modeling results for decreased thymus weight in female Wistar rats exposed to benzo[a]pyrene by gavage for 90 days (Kroese et al., 2001); BMR = 1 SD change from the control mean

Model (constant variance)	Goodness of fit			BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
	Variance *p*-value	Mean *p*-value	AIC		
Hill	NA				
Linear	**0.17**	**0.81**	**349.12**	**10.52**	**7.64**
Polynomial (2-degree)[a]	0.17	0.77	350.80	13.29	7.77
Power	NA				

[a]Lowest degree polynomial with an adequate fit is reported.
NA = No available degrees of freedom to calculate a goodness-of-fit value.

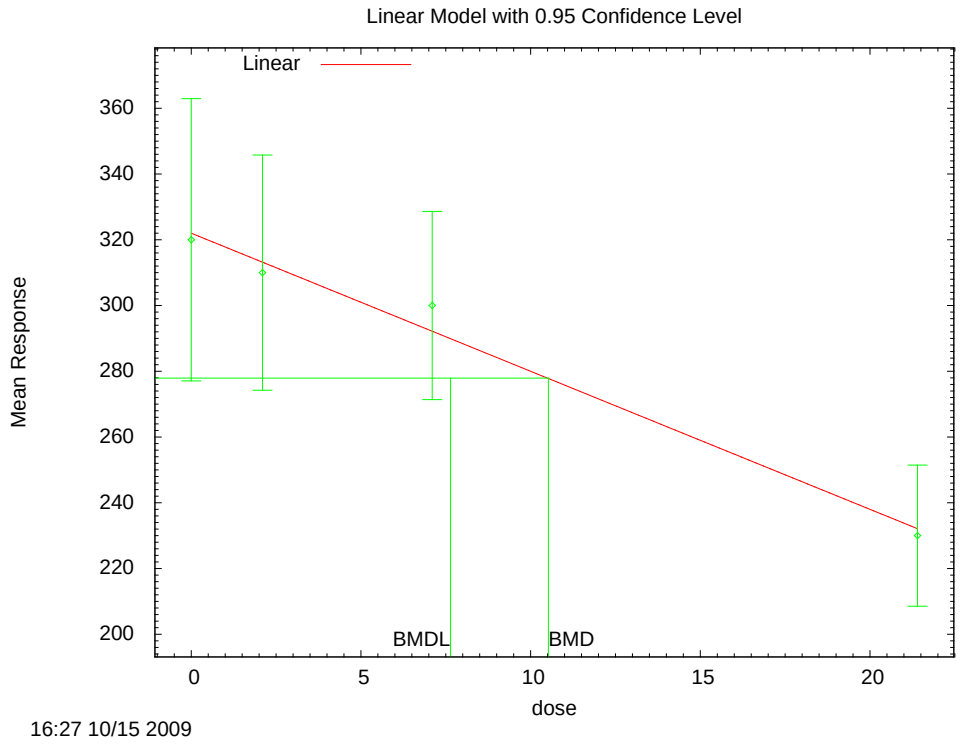


Figure E-2. Fit of linear model (constant variance) to decreased thymus weight in female Wistar rats exposed to benzo[a]pyrene by gavage for 90 days (Kroese et al., 2001); BMR = 1 SD change from control; dose in mg/kg-day.

```
================================================================
Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\female\durationadjusted\2Linkrolin
.(d)
Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\female\durationadjusted\2Linkrolin
.plt                                        Thu Oct 15 16:27:44 2009
 ================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = mean
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        alpha =            1
                          rho =            0   Specified
                       beta_0 =      322.144
                       beta_1 =      -4.2018


          Asymptotic Correlation Matrix of Parameter Estimates

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

                 alpha       beta_0       beta_1

    alpha            1     2.4e-008    -2.3e-008

   beta_0     2.4e-008            1        -0.68

   beta_1    -2.3e-008        -0.68            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha          1954.92          437.134            1098.16             2811.69
       beta_0           322.144          9.48287            303.558              340.73
       beta_1          -4.2018         0.837537           -5.84334            -2.56026



     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10        320          322           60         44.2         -0.153
  2.1    10        310          313           50         44.2         -0.237
  7.1    10        300          292           40         44.2           0.55
 21.4    10        230          232           30         44.2         -0.159
```

```
 Model Descriptions for likelihoods 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)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var$$e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -171.357252            5     352.714504
              A2         -168.857234            8     353.714467
              A3         -171.357252            5     352.714504
          fitted         -171.562118            3     349.124237
               R         -181.324151            2     366.648303


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              24.9338          6       0.0003512
   Test 2              5.00004          3          0.1718
   Test 3              5.00004          3          0.1718
   Test 4             0.409733          2          0.8148

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 greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        10.5228

            BMDL =        7.64037
```

Table E-5. Summary of BMD modeling results for decreased ovary weight in female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 60 days (Xu et al., 2010); BMR = 1 SD change from the control mean

Model	Goodness of fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
	p-value	AIC		
Power	NA			
Linear (polynomial 1°)[a]	**0.39**	**-138.67**	**2.27**	**1.49**

[a]Lowest degree polynomial with an adequate fit is reported.
NA = No available degrees of freedom to calculate a goodness-of-fit value.

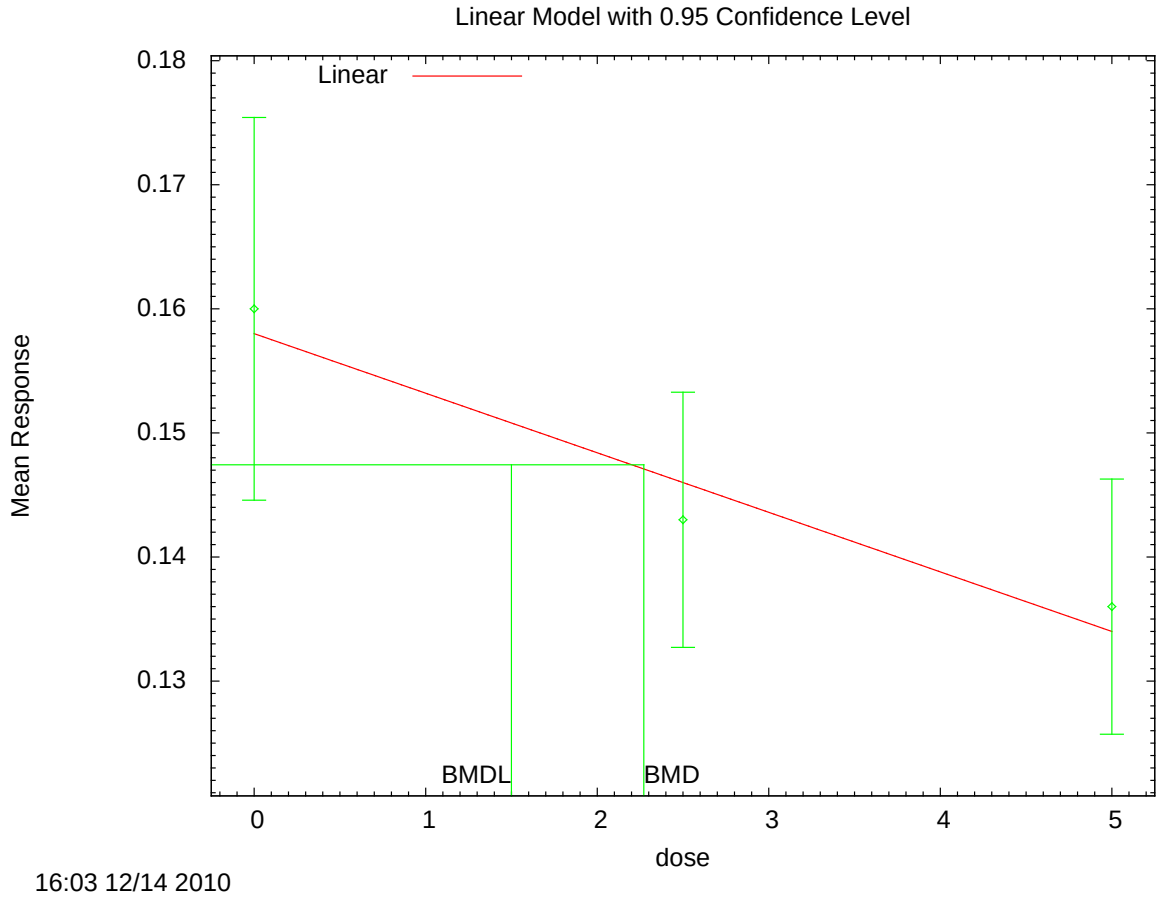


Figure E-3. Fit of linear/polynomial (1°) model to data on decreased ovary weight in female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 60 days (Xu et al., 2010); BMR = 1 SD change from control; dose in mg/kg-day.

```
================================================================
Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
Input Data File:
C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1SD.(d)
Gnuplot Plotting File:
C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1SD.plt
                                                Tue Dec 14 13:51:32 2010
 ================================================================

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        alpha =     0.000136
                          rho =            0   Specified
                       beta_0 =     0.158333
                       beta_1 =      -0.0048


          Asymptotic Correlation Matrix of Parameter Estimates

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

                 alpha       beta_0       beta_1

    alpha            1       4e-010    -4.5e-010

   beta_0       4e-010            1        -0.77

   beta_1    -4.5e-010        -0.77            1



                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha      0.000118889     3.96296e-005        4.12162e-005         0.000196562
        beta_0         0.158333       0.00406354            0.150369            0.166298
        beta_1          -0.0048       0.00125904         -0.00726768         -0.00233232



     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     6       0.16        0.158       0.0147       0.0109          0.374
  2.5     6      0.143        0.146       0.0098       0.0109         -0.749
    5     6      0.136        0.134       0.0098       0.0109          0.374


 Model Descriptions for likelihoods 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)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var$$e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          72.766595            4    -137.533190
              A2          73.468565            6    -134.937129
              A3          72.766595            4    -137.533190
          fitted          72.335891            3    -138.671782
               R          67.008505            2    -130.017010


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              12.9201          4         0.01167
   Test 2              1.40394          2          0.4956
   Test 3              1.40394          2          0.4956
   Test 4             0.861408          1          0.3533

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 greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        2.27159

            BMDL =        1.49968
```

Table E-6. Summary of BMD modeling results for decreased primordial follicles in female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 60 days (Xu et al., 2010); BMR = 1 SD change and 10% relative deviation from the control mean

Model	Goodness of fit		BMD_{1SD} mg/kg-d	$BMDL_{1SD}$ mg/kg-d	Basis for model selection	
	p-value	AIC				
Constant variance						
Exponential (M2)	0.31	123.82	2.40	1.47	Among adequately fitting models, with narrow range of BMDLs, Linear model had lowest AIC.	
Exponential (M3)	NA	124.80	3.35	1.60		
Exponential (M4)	0.31	123.82	2.40	1.24		
Power	NA	124.80	3.37	1.70	**BMD_{10RD} mg/kg-d**	**$BMDL_{10RD}$ mg/kg-d**
Polynomial 2°	NA	124.80	3.39	1.70		
Linear (polynomial 1°)	**0.37**	**123.59**	**2.48**	**1.60**	**2.33**	**1.64**

NA = No available degrees of freedom to calculate a goodness-of-fit value.

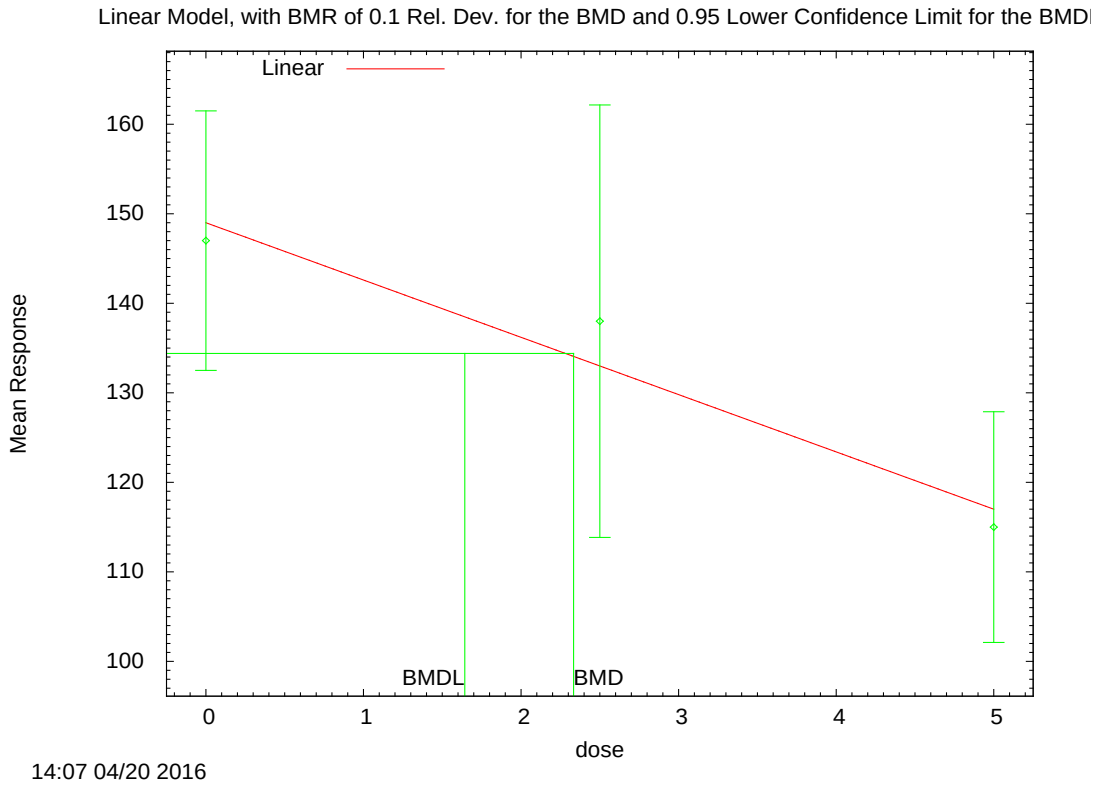


Figure E-4. Fit of linear/polynomial (1°) model to primordial follicle count data for female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 60 days (Xu et al., 2010); BMR = 10% relative deviation from control; dose in mg/kg-day.

```
================================================================
Polynomial Model. (Version: 2.18;  Date: 05/19/2014)
Input Data File: C:/BMDS250_2014/Data/BenzoaPyrene_iris2016/lin_IRIS_BaP_ovafollicles_adj_Lin-
ConstantVariance-BMR1Std-down.(d)
Gnuplot Plotting File:
C:/BMDS250_2014/Data/BenzoaPyrene_iris2016/lin_IRIS_BaP_ovafollicles_adj_Lin-ConstantVariance-
BMR1Std-down.plt
                                                Wed Apr 20 13:50:20 2016
 ================================================================

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 3
   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



                 Default Initial Parameter Values
                        alpha =      290.414
                          rho =            0   Specified
                       beta_0 =      149.333
                       beta_1 =         -6.4


          Asymptotic Correlation Matrix of Parameter Estimates

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

                 alpha       beta_0       beta_1

    alpha            1     1.1e-007     1.6e-009
   beta_0     1.1e-007            1        -0.77
   beta_1     1.6e-009        -0.77            1


                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha            252.9          84.2997            87.6755             418.124
        beta_0          149.333          5.92663             137.717             160.949
        beta_1             -6.4           1.8363            -9.99908            -2.80092


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     6        147          149         13.8         15.9         -0.359
  2.5     6        138          133           23         15.9          0.719
    5     6        115          117         12.3         15.9         -0.359


 Model Descriptions for likelihoods 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)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

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


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          -58.400878            4     124.801756
              A2          -56.975162            6     125.950323
              A3          -58.400878            4     124.801756
          fitted          -58.796972            3     123.593943
               R          -63.438413            2     130.876825


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              12.9265          4         0.01164
   Test 2              2.85143          2          0.2403
   Test 3              2.85143          2          0.2403
   Test 4             0.792187          1          0.3734

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 greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        2.48482
            BMDL =        1.60424
```

Table E-7. Summary of BMD modeling results for mean number of squares crossed on PND 69 by male and female Sprague Dawley rats exposed to benzo[a]pyrene by gavage, PNDs 5–11 (Chen et al., 2012); BMR = 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0244	538.60	1.52	1.18	One model provided an adequate fit and a valid BMDL estimate—the Exponential M4 constant variance model was selected.
Exponential (M4)	**0.727**	**533.30**	**0.225**	**0.105**	
Exponential (M5)	NA	535.18	0.221	0.107	
Hill	NA	535.18	0.229	0.0839	
Linear, Power[c], Polynomial 2°, 3°[c]	0.0285	538.29	1.44	1.08	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.404), selected model in bold; scaled residuals for selected model for doses 0, 0.02, 0.2, and 2 mg/kg were 0.22, −0.27, 0.05, and −0.01, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary); this model reduced to the Exponential (M2) model.

[c]For the Power model, the power parameter estimate was 1; for the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space). These models reduced to the Linear model.

NA = No available degrees of freedom to calculate a goodness-of-fit value.

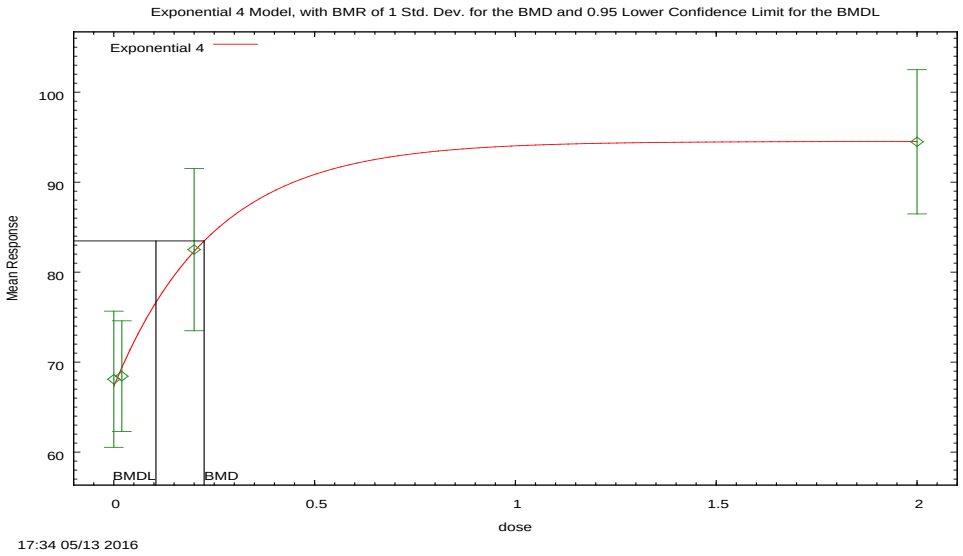


Figure E-5. Plot of mean squares crossed on PND 69 by male and female Sprague Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5–11, by dose, with fitted curve for Exponential (M4) model with constant variance (Chen et al., 2012); BMR = 1 SD change from control mean; dose in mg/kg-day.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation.
BMR = 1.0000 Estimated standard deviations from control
BMD = 0.224896
BMDL at the 95% confidence level = 0.104872

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	5.56624	5.56471
rho	N/A	0
a	67.303	64.695
b	4.00574	1.02094
c	1.4046	1.53357
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	20	68.1	67.3	16.17	16.17	0.2204
0.02	20	68.44	69.4	13.15	16.17	−0.2654
0.2	20	82.51	82.31	19.27	16.17	0.05465
2	20	94.49	94.53	17.13	16.17	−0.009693

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−262.5886	5	535.1772
A2	−261.1275	8	538.2549
A3	−262.5886	5	535.1772
R	−277.7454	2	559.4908
4	−262.6497	4	533.2994

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	33.24	6	<0.0001
Test 2	2.922	3	0.4038
Test 3	2.922	3	0.4038
Test 6a	0.1222	1	0.7267

Table E-8. Summary of BMD modeling results for elevated plus maze: open arm entries at PND 70 for female Sprague Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD

Model[a]	Goodness of fit		BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.154	132.71	1.17	0.898	Among adequately fitting models, the BMDLs covered a 10-fold range. The Exponential 4 model had the lowest BMDL (and the lowest AIC).
Exponential (M4)	**0.848**	**131.00**	**0.208**	**0.0917**	
Exponential (M5)	N/A[c]	132.96	0.212	0.0921	
Hill	N/A[c]	132.96	0.214	0.0692	
Linear; Power Polynomial 2°, 3°[c]	0.180	132.39	1.04	0.759	

[a]Constant variance case presented (BMDS Tests 2 and 3 *p*-value = 0.719).

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). This model reduced to the Exponential (M2) model.

[c]For the Power model, the power parameter estimate was 1; for the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space). All models in this row reduced to the Linear model.

NA = No available degrees of freedom to calculate a goodness-of-fit value.

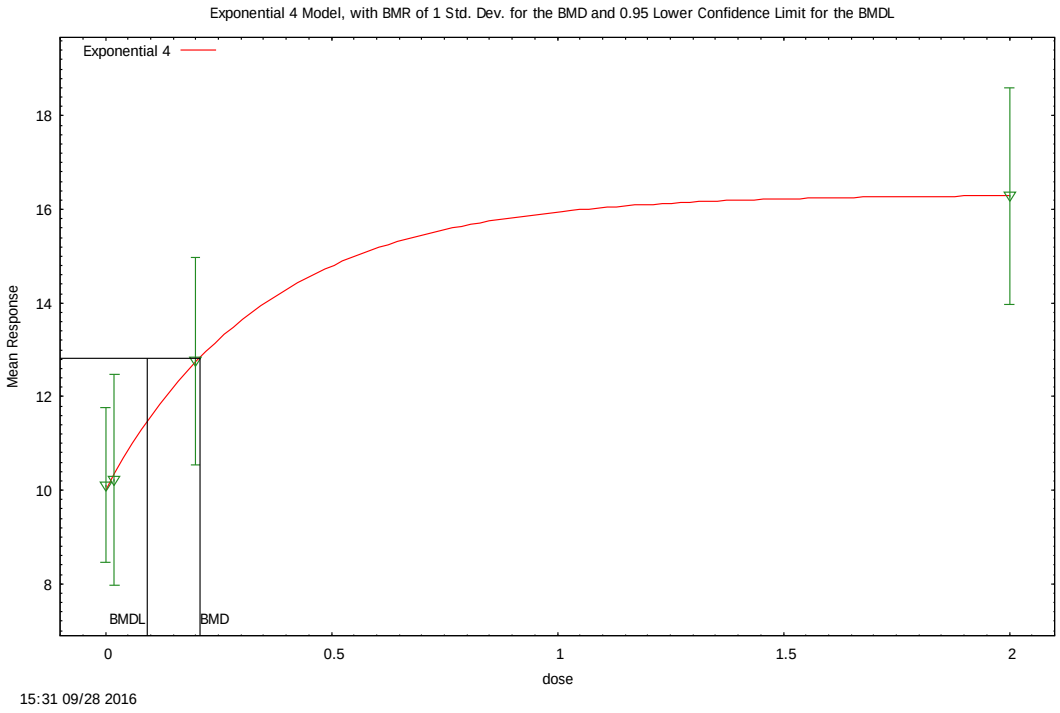


Figure E-6. Fit of Exponential 4 model for elevated plus maze: open arm maze entries on PND 70 for female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD, dose in mg/kg .

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated standard deviations from control
BMD = 0.208365
BMDL at the 95% confidence level = 0.0916703

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	2.07497	2.07406
rho	N/A	0
a	10.0002	9.6045
b	2.84307	1.12639
c	1.63133	1.78088
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	10.11	10	2.31	2.82	0.123
0.02	10	10.22	10.35	3.16	2.82	−0.1448
0.2	10	12.76	12.74	3.1	2.82	0.0243
2	10	16.29	16.29	3.23	2.82	−0.002547

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−61.48113	5	132.9623
A2	−60.80983	8	137.6197
A3	−61.48113	5	132.9623
R	−73.16117	2	150.3223
4	−61.49948	4	130.999

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	24.7	6	0.0003876
Test 2	1.343	3	0.719
Test 3	1.343	3	0.719
Test 6a	0.0367	1	0.8481

Table E-9. Summary of BMD Modeling Results for escape latency of male and female Sprague-Dawley rats at PND 71 exposed to benzo[a]pyrene by gavage on PNDs 5–11, (Chen et al., 2012); BMR = 1 SD[a] change from the control mean

Model[b]	Goodness of fit		BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[c]	0.433	461.23	1.24	1.01	Among adequately fitting models, BMDLs ranged up to ~6-fold above that of the Hill model; Hill model selected for POD derivation.
Exponential (M4) Exponential (M5)[c]	0.503	462.01	0.466	0.178	
Hill	**0.51**	**461.99**	**0.494**	**0.163**	
Linear, Power, Polynomial 2°, 3°[d]	0.474	461.05	1.14	0.883	

[a]A common estimate of SD across all trial days for escape latency, PNDs 71–74, yielded a SD of 9 seconds. In order to implement this value as a BMR across all trial days, the value was treated equivalently as an absolute deviation of 9 seconds. Also see Section 2.1.2.

[b]Constant variance case presented (BMDS Test 2 *p*-value = 0.711, BMDS Test 3 *p*-value = 0.711).

[c]For the Exponential (M3) and (M5) models, the estimate of d was 1 (boundary); these models reduced to the (M2) and (M4) models, respectively.

[d]For the Power model, the power parameter estimate was 1. For the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space). The models in this row reduced to the Linear model.

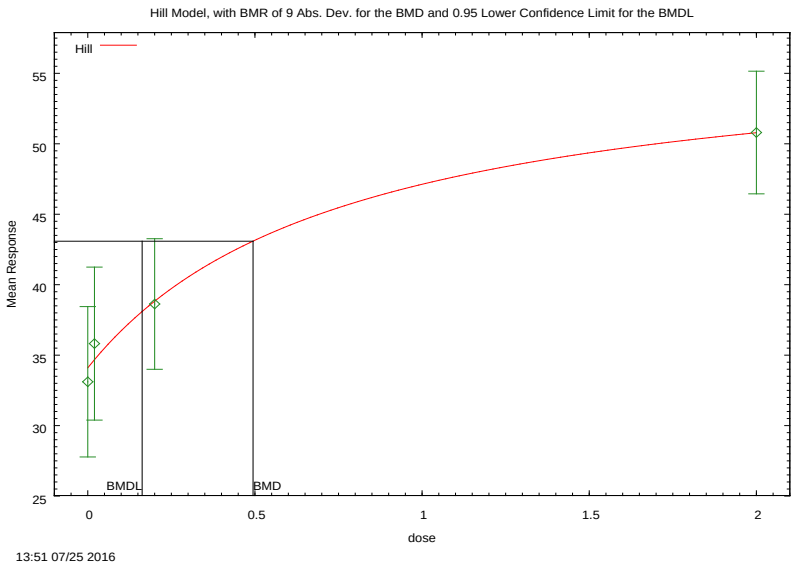


Figure E-7. Plot of escape latency at PND 71 by dose, with fitted curve for Hill model using constant variance, for male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD change from control mean; dose in mg/kg-day.

Hill Model (Version: 2.17; Date: 01/28/2013)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A constant variance model is fit

Benchmark Dose Computation
BMR = 9 seconds absolute deviation
BMD = 0.494368
BMDL at the 95% confidence level = 0.162618

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	107.223	112.255
rho	N/A	0
intercept	34.087	33.11
v	23.2217	17.69
n	1	0.308231
k	0.781197	3.30822

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	20	33.1	34.1	11.4	10.4	−0.422
0.02	20	35.8	34.7	11.6	10.4	0.498
0.2	20	38.6	38.8	9.9	10.4	−0.0822
2	20	50.8	50.8	9.3	10.4	0.00601

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−226.779191	5	463.558382
A2	−226.09162	8	468.183241
A3	−226.779191	5	463.558382
fitted	−226.996255	4	461.99251
R	−241.044463	2	486.088927

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	29.9057	6	<0.0001
Test 2	1.37514	3	0.7114
Test 3	1.37514	3	0.7114
Test 4	0.434128	1	0.51

Table E-10. Summary of BMD Modeling Results for escape latency of male and female Sprague-Dawley rats at PND 72 exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD[a] change from control mean

Model[b]	Goodness of fit		BMD_{9AD} (mg/kg)	$BMDL_{9AD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[c]	0.170	430.81	0.991	0.883	Among adequately fitting models, BMDLs ranged up to ~5-fold that of the Hill model; Hill selected for POD derivation.
Exponential (M4) Exponential (M5)[c]	0.587	429.56	0.322	0.170	
Hill	**0.598**	**429.54**	**0.329**	**0.162**	
Linear, Power, Polynomial 2°, 3°[d]	0.244	430.08	0.833	0.708	

[a]A common estimate of SD across all trial days for escape latency, PNDs 71–74, yielded a SD of 9 seconds. In order to implement this value as a BMR across all trial days, the value was treated equivalently as an absolute deviation of 9 seconds. Also see Section 2.1.2.

[b]Constant variance case presented (BMDS Test 2 *p*-value = 0.751, BMDS Test 3 *p*-value = 0.751).

[c]For the Exponential (M3) and (M5) models, the estimate of d was 1 (boundary); these models reduced to the (M2) and (M4) models, respectively.

[d]For the Power model, the power parameter estimate was 1. For the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space). The models in this row reduced to the Linear model.

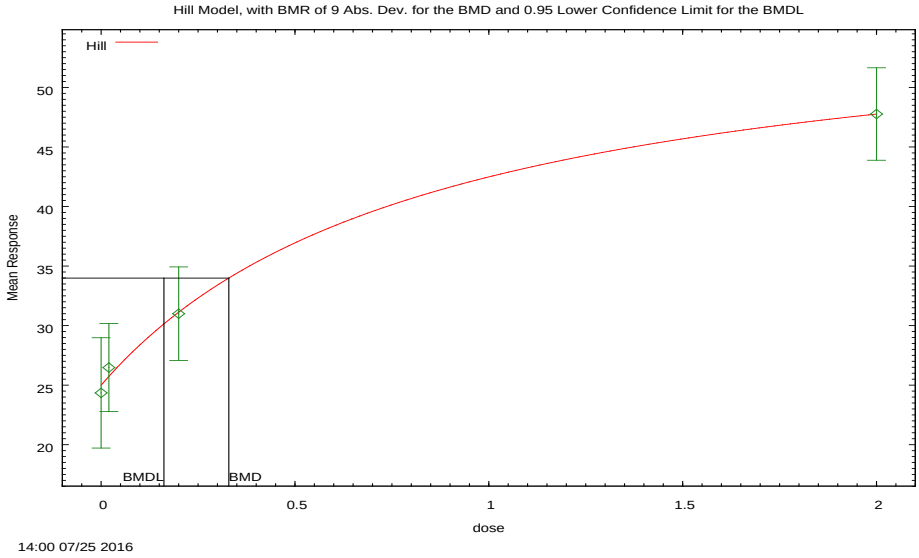


Figure E-8. Plot of mean escape latency at PND 72 by dose, with fitted curve for Hill model with constant variance for male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD from control mean; dose in mg/kg-day.

Hill Model (Version: 2.17; Date: 01/28/2013)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A constant variance model is fit

Benchmark Dose Computation
BMR = 9 seconds absolute deviation
BMD = 0.329352
BMDL at the 95% confidence level = 0.162043

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	71.4666	74.9675
rho	N/A	0
intercept	24.9901	24.35
v	32.6091	23.42
n	1	0.391771
k	0.863966	3.25689

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	20	24.4	25	9.9	8.45	−0.339
0.02	20	26.5	25.7	7.9	8.45	0.398
0.2	20	31	31.1	8.4	8.45	−0.0634
2	20	47.8	47.8	8.3	8.45	0.00418

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−210.630456	5	431.260911
A2	−210.025963	8	436.051926
A3	−210.630456	5	431.260911
fitted	−210.769197	4	429.538393
R	−241.925097	2	487.850194

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	63.7983	6	<0.0001
Test 2	1.20899	3	0.7509
Test 3	1.20899	3	0.7509
Test 4	0.277482	1	0.5984

Table E-11. Summary of BMD Modeling Results for escape latency of male and female Sprague-Dawley rats at PND 73 exposed to benzo[a]pyrene by gavage on PNDs 5–11 (Chen et al., 2012); BMR = 1 SD[a] change from control mean

Model[a]	Goodness of fit		BMD_{9AD} (mg/kg)	$BMDL_{9AD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2), Exponential (M3)[c]	0.113	450.51	1.09	0.956	Among adequately fitting models, BMDLs ranged ~8-fold from that of the Hill model; Hill selected for POD derivation.
Exponential (M4) Exponential (M5)[c]	0.762	448.24	0.266	0.137	
Hill	**0.786**	**448.22**	**0.272**	**0.122**	
Linear, Power, Polynomial 2°, 3°[d]	0.166	449.74	0.909	0.747	

[a]A common estimate of SD across all trial days for escape latency, PNDs 71–74, yielded a SD of 9 seconds. In order to implement this value as a BMR across all trial days, the value was treated equivalently as an absolute deviation of 9 seconds. Also see Section 2.1.2.

[b]Constant variance case presented (BMDS Test 2 *p*-value = 0.262, BMDS Test 3 *p*-value = 0.262), no model was selected as a best-fitting model.

[c]For the Exponential (M3) and (M5) models, the estimate of d was 1 (boundary); these models reduced to the (M2) and (M4) models, respectively.

[d]For the Power model, the power parameter estimate was 1. For the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space). The models in this row reduced to the Linear model.

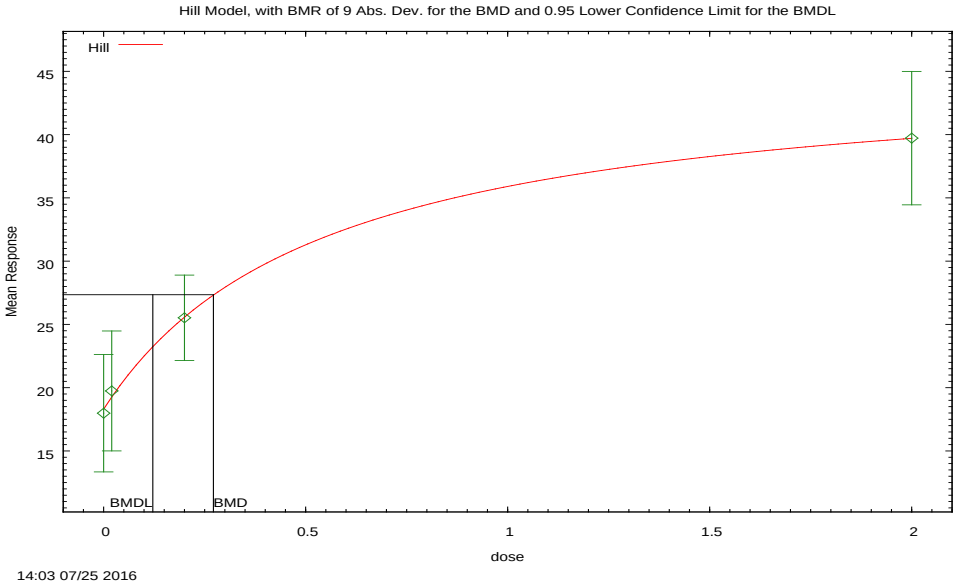


Figure E-9. Plot of mean escape latency at PND 73 by dose, with fitted curve for Hill model with constant variance, for male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage PNDs 5–11 (Chen et al., 2012); BMR = 1 SD change from control mean; dose in mg/kg-day.

Hill Model. (Version: 2.17; Date: 01/28/2013)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A constant variance model is fit

Benchmark Dose Computation
BMR = 9 seconds absolute deviation
BMD = 0.271642
BMDL at the 95% confidence level = 0.121722

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	90.2658	94.9287
rho	N/A	0
intercept	18.3451	17.98
v	27.2509	21.74
n	1	0.348791
k	0.550858	3.37789

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	20	18	18.3	9.91	9.5	−0.172
0.02	20	19.7	19.3	10.1	9.5	0.207
0.2	20	25.5	25.6	7.21	9.5	−0.0394
2	20	39.7	39.7	11.3	9.5	0.00413

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−220.073327	5	450.146655
A2	−218.073516	8	452.147032
A3	−220.073327	5	450.146655
fitted	−220.11036	4	448.220721
R	−243.776723	2	491.553446

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	51.4064	6	<0.0001
Test 2	3.99962	3	0.2615
Test 3	3.99962	3	0.2615
Test 4	0.0740662	1	0.7855

Table E-12. Summary of BMD modeling results for escape latency at PND 74 for male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage PNDs 5−11 (Chen et al., 2012); BMR = 1 SD[a] change from control mean

Model[b]	Goodness of fit		BMD_{9AD} (mg/kg)	$BMDL_{9AD}$ (mg/kg)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[c]	2.80×10^{-4}	400.22	1.10	0.988	Among adequately fitting models, BMDLs ranged up to ~7-fold above that of the Hill model; Hill model selected for POD derivation.
Exponential (M4) Exponential (M5)[c]	0.466	386.39	0.227	0.147	
Hill	**0.515**	**386.28**	**0.226**	**0.134**	
Linear, Power, Polynomial 2°, 3°[d]	0.00166	396.66	0.825	0.689	

[a]A common estimate of SD across all trial days for escape latency, PNDs 71−74, yielded a SD of 9 seconds. In order to implement this value as a BMR across all trial days, the value was treated equivalently as an absolute deviation of 9 seconds. Also see Section 2.1.2.

[b]Modeled variance case presented (BMDS Test 2 *p*-value = 0.00736, BMDS Test 3 *p*-value = 0.314).

[c]For the Exponential (M3) and (M5) models, the estimate of d was 1 (boundary); these models reduced to the (M2) and (M4) models, respectively.

[d]For the Power model, the power parameter estimate was 1; for the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameter space); all models reduced to the Linear model.

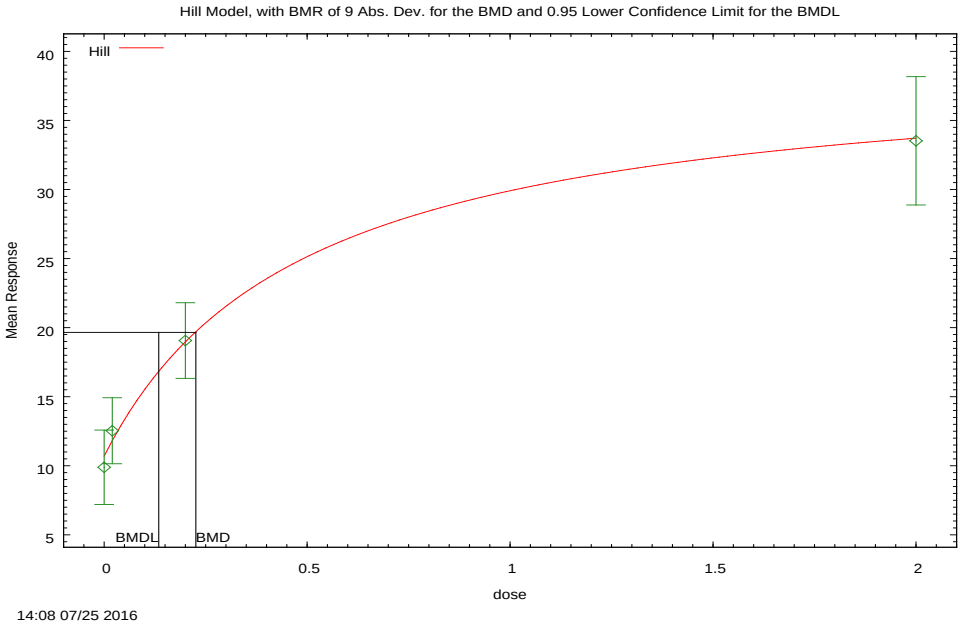


Figure E-10. Plot of mean response by dose for Hill model, with modeled variance, fit to escape latency at PND 74 of male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage on PNDs 5−11 (Chen et al., 2012); BMR = 1 SD from control mean; dose in mg/kg-day.

Hill Model (Version: 2.17; Date: 01/28/2013)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A modeled variance is fit

Benchmark Dose Computation
BMR = 9 seconds absolute deviation
BMD = 0.225851
BMDL at the 95% confidence level = 0.134475

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	0.885005	3.87067
rho	0.998715	0
intercept	10.6552	9.89
v	28.6997	23.635
n	1	0.28055
k	0.494355	3.47106

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	20	9.89	10.7	5.75	5.07	−0.675
0.02	20	12.5	11.8	5.1	5.33	0.641
0.2	20	19.1	18.9	5.85	6.76	0.0948
2	20	33.5	33.7	9.93	9.01	−0.0704

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−192.775022	5	395.550043
A2	−186.77088	8	389.54176
A3	−187.928576	6	387.857153
fitted	−188.14043	5	386.280859
R	−234.533996	2	473.067991

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	95.5262	6	<0.0001
Test 2	12.0083	3	0.007355
Test 3	2.31539	2	0.3142
Test 4	0.423706	1	0.5151

Table E-13. Summary of BMD modeling results for incidence of cervical epithelial hyperplasia in female ICR mice exposed to benzo[a]pyrene by oral exposure for 98 days (Gao et al., 2011); BMR = 10% extra risk

Model	Goodness of fit		BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
	p-value	AIC		
Gamma	0.6874	82.2821	0.659	0.452
Logistic	0.1422	88.4607	1.422	1.052
Log-logistic	**0.8360**	**81.7004**	**0.578**	**0.369**
Probit	0.1544	88.1151	1.326	0.979
Log-probit	0.0775	88.2004	1.012	0.686
Multistage	0.6874	82.2821	0.659	0.452

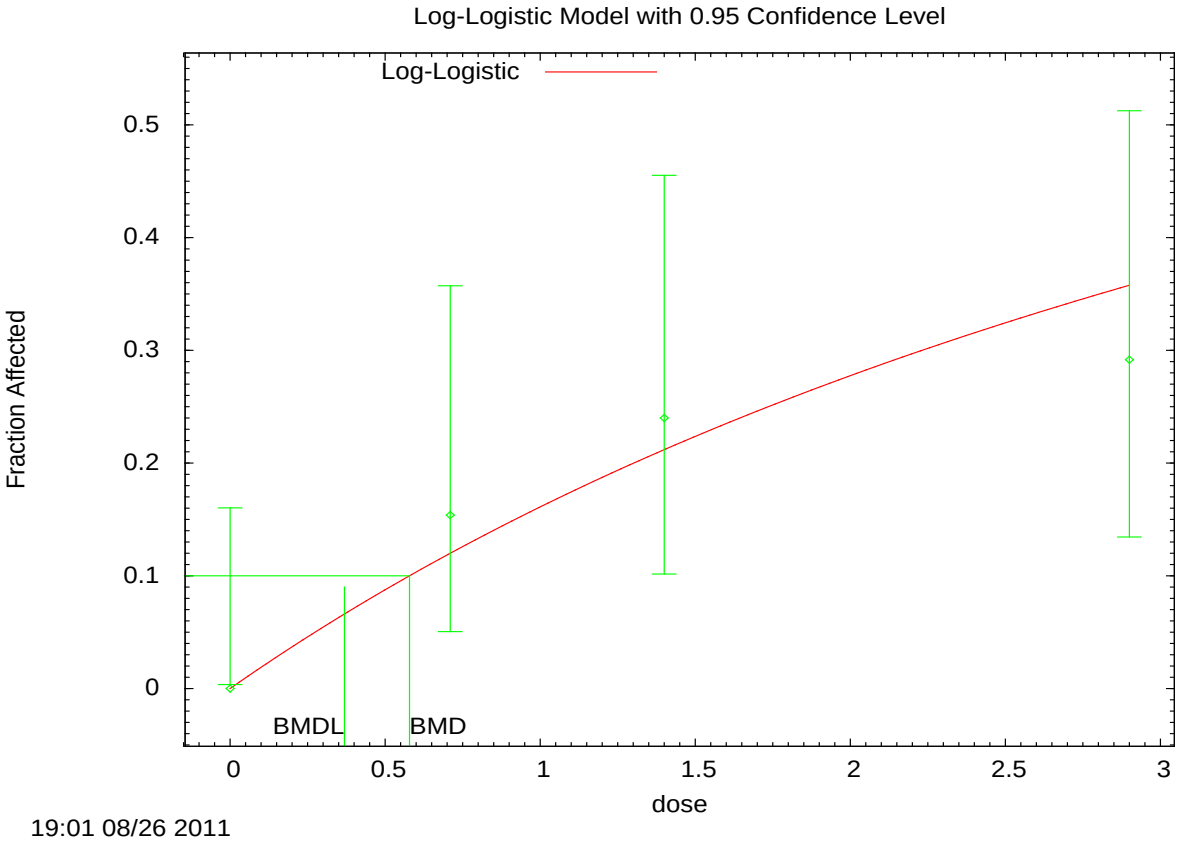


Figure E-11. Fit of log-logistic model to data on cervical epithelial hyperplasia (Gao et al., 2011); BMR = 10% extra risk; dose in mg/kg-day.

```
================================================================================
Logistic Model. (Version: 2.13; Date: 10/28/2009)
Input Data File: C:\Users\hclynch\Documents\_Active Projects\_FA498 IRIS\xBaP\IASC Aug 2011\bmd
modeling\lnl_gao 2011 inflamm cells_Opt.(d)
         Gnuplot Plotting File: C:\Users\hclynch\Documents\_Active Projects\_FA498 IRIS\xBaP\IASC
Aug 2011\bmd modeling\lnl_gao 2011 inflamm cells_Opt.plt
================================================================================

   The form of the probability function is:

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

   Dependent variable = Col3
   Independent variable = Col1
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   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 =     -1.60901
                        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
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background                0            *                *                   *
     intercept          -1.6502            *                *                   *
         slope                1            *                *                   *

* - Indicates that this value is not calculated.


                      Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -39.4267         4
   Fitted model        -39.8502         1      0.847034      3          0.8382
  Reduced model        -45.7739         1       12.6945      3        0.005346

          AIC:         81.7004

                                  Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          26         0.000
    0.7100     0.1200         3.119     4.000          26         0.532
    1.4000     0.2119         5.297     6.000          25         0.344
    2.9000     0.3577         8.584     7.000          24        -0.675

 Chi^2 = 0.86      d.f. = 3        P-value = 0.8360
```

```
   Benchmark Dose Computation

Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95

             BMD =       0.578668
            BMDL =       0.368701
```

Table E-14. Summary of BMD modeling results of embryo/fetal survival for female F344 rats exposed to benzo[a]pyrene via inhalation on GDs 11–20 (Archibong et al., 2002); BMR = 10 percentage points absolute deviation from control mean

Model[a]	Goodness of fit		BMD_{10AD} ($\mu g/m^3$)	$BMDL_{10AD}$ ($\mu g/m^3$)	Basis for model selection
	p-value	AIC			
Constant variances assumed[a]					No adequate fit: variances for the variability among litter mean percentages could not be fit as a function of exposure; no model selected.
Exponential (M2)	0.0382	214.98	9.49	8.40	
Exponential (M3)	0.0239	215.55	12.6	8.73	
Exponential (M4)	0.0382	214.98	9.49	7.76	
Exponential (M5)	NA	212.45	17.1	12.3	
Hill	NA	212.45	18.7	13.5	
Linear, Power, Polynomial 2°, 3°[c]	0.00240	220.52	15.1	13.8	
Variances modeled as a function of exposure[a]					
Exponential (M2)	0.0148	213.79	9.42	8.32	
Exponential (M3)	0.00515	215.18	11.7	8.44	
Exponential (M4)	0.00376	215.75	9.12	6.68	
Exponential (M5)	NA	209.36	17.9	12.4	
Hill	NA	209.36	19.3	14.0	
Linear, Power, Polynomial 2°, 3°[b]	2.61×10^{-4}	221.86	15.4	14.0	

[a]Under constant variance assumption (BMDS Test 2 *p*-value = 0.000134) and modeled variances (BMDS Test 3 *p*-value = 0.00512), no model was selected as a best-fitting model.
[b]For the Power model, the power parameter estimate was 1. For the Polynomial 2° and 3° models, the coefficients of higher order than b1 were estimated to be 0 (boundary of parameters space). These models reduced to the Linear model.
NA = No available degrees of freedom to calculate a goodness-of-fit value.

As detailed in Table E-14, continuous dose-response models were not successful in fitting the percentage survival data from Archibong et al. (2002) due to non-monotonic variances. Continuous models rely on assuming that a normal distribution can adequately characterize the observed data. However, for dichotomous responses that cover a broad range of responses, as here, the variances may not be straightforward to address. Consequently, characterizing the data in terms of the underlying binomial responses, in order to apply binomial models, was considered. However, the individual animal data needed for applying a nested model were not available, and an approximation of the proportions affected, adjusted for litter effect (the tendency of littermates to respond more like each other than those in other similarly treated litters), was used.

To approximate the underlying incidence data from data reported as the mean of litter percentages, the following steps were taken:

- Total number of embryos/fetuses in each group—this was estimated from the number of litters in each group and the mean number of implantations (see Table E-15).
- Total number of affected embryos/fetuses in each group—The mean of litter-specific survival percentages was taken to be the overall estimate of surviving embryos/fetuses for each exposure group. As BMDS dichotomous models only address increasing responses, these percentages were converted to the equivalent percentage not surviving (see Table E-15).
- Allowance for litter effect, or intralitter correlation—Although the approach of applying continuous models to means and SDs appropriately gave equal weight to each litter as the experimental unit, the proportion of affected embryos/fetuses among all in a dose group does not. Consequently, this transformation of the reported data to total affected embryos/fetuses among total exposed embryos/fetuses needed to address litter effect. A data adjustment has been developed that reduces the total numbers of fetuses to account for litter effect (Fox et al., 2016). The adjustment reduces the sample size, here total number of implantations, as a means of addressing litter effect.

Dose-response modeling of the adjusted data by BMDS dichotomous models was carried out using the percentage affected in the "% Positive" option, which calculates incidence from inputs of percentage and sample size for each group. The data inputs are bolded in Table E-16.

Table E-15. Derivation of incidence data adjusted for design effect, for embryo/fetal resorption data in Archibong et al. (2002)

Endpoint	Exposures and effect data				
Embryo/fetus survival (%)	Exposure (µg/m^3), continuous equivalent	0 (carbon black)	4.6	13.8	18.4
	Mean ± SE, Number of litters (N_L) Mean number of implantations	96.7 ± 1.7 (10) 8.8	78.3 ± 4.1 (10) 8.8	38.0 ± 2.1 (10) 9.0	33.8 ± 1.3 (10) 8.8
Embryo/fetus resoptions[a] (%)	Estimated percentage, P_F Number of embryos/fetuses (N_F)	**3.3** (88)	**21.7** (88)	**62.0** (90)	**66.2** (88)
	Design effect D[b]	1.87	3.61	5.22	5.34
	Adjusted N (N_F/D)	**47.2**	**24.4**	**17.3**	**16.5**

[a]Embryo/fetus resorptions were calculated by subtracting the reported percentage survival from 100%.

[b]The design effect was estimated using the average of two estimates of the relationship between fetal proportions and design effect developed from historical data from National Toxicology Program (NTP) developmental studies of rats (Fox et al., 2016). Using the model $D_i = \exp(a + b*\log(P_F) + 0.5* \sigma_{res}^2)$: D_{LS} used the parameter values a = 1.6852, b = 0.3310, and $\sigma_{res}^2 = 0.1248$; D_{OR} used the parameter values a = 1.8327, b = 0.3690, and $\sigma_{res}^2 = 0.1090$.

Table E-16. Summary of BMD modeling results for estimated incidence of embryo/fetal resorptions (Archibong et al., 2002), adjusted for design effect; BMR = 1, 5, or 20% extra risk[a]

Model[b]	Goodness of fit		BMD_1 ($\mu g/m^3$)	$BMDL_1$ ($\mu g/m^3$)	BMD_5 ($\mu g/m^3$)	$BMDL_5$ ($\mu g/m^3$)	BMD_{20} ($\mu g/m^3$)	$BMDL_{20}$ ($\mu g/m^3$)
	p-value	AIC						
Gamma	0.634	89.529	2.17	0.681	7.76	3.47	25.2	15.1
Dichotomous-Hill	N/A	91.303	7.35	0.430	13.8	2.26	25.7	18.7
Logistic	0.254	90.102	3.82	2.53	15.7	11.3	41.9	33.7
LogLogistic	0.716	89.436	3.23	0.462	9.34	2.41	25.4	11.4
Probit	0.320	89.593	3.41	2.31	14.2	10.4	39.6	32.2
LogProbit	0.721	89.431	5.34	0.427	10.9	1.85	25.4	10.0
Weibull	0.621	89.548	1.81	0.680	7.19	3.47	24.9	15.1
Multistage 2, 3°[c]	0.583	89.605	1.18	0.677	5.93	3.46	24.2	15.0
Quantal-Linear	0.805	87.748	0.934	0.672	4.77	3.43	20.7	14.9

[a]Multiple BMRs provided in order to inform low-dose extrapolation; the BMR of 20% provides the basis for judging fit to the observed data. Note that the BMDLs do not reflect allowance for simultaneous predictions; only one BMDL from the selected model is used to derive a reference value, depending on the degree of low-dose extrapolation that is justified.

[b]**Basis for Model Selection**: Among the adequately fitting models, $BMDL_{20}$ values ranged close to 3-fold; the Quantal-Linear model had the lowest AIC. However, in the response range where a POD is needed, model uncertainty increases, as shown by increasing range of BMDLs and greater BMD/BMDL range for several models.

[c]For Multstage 3°, the b3 parameter was estimated at the boundary of the parameter space (0), and the model reduced to Multstage 2°.

NA = No available degrees of freedom to calculate a goodness-of-fit value.

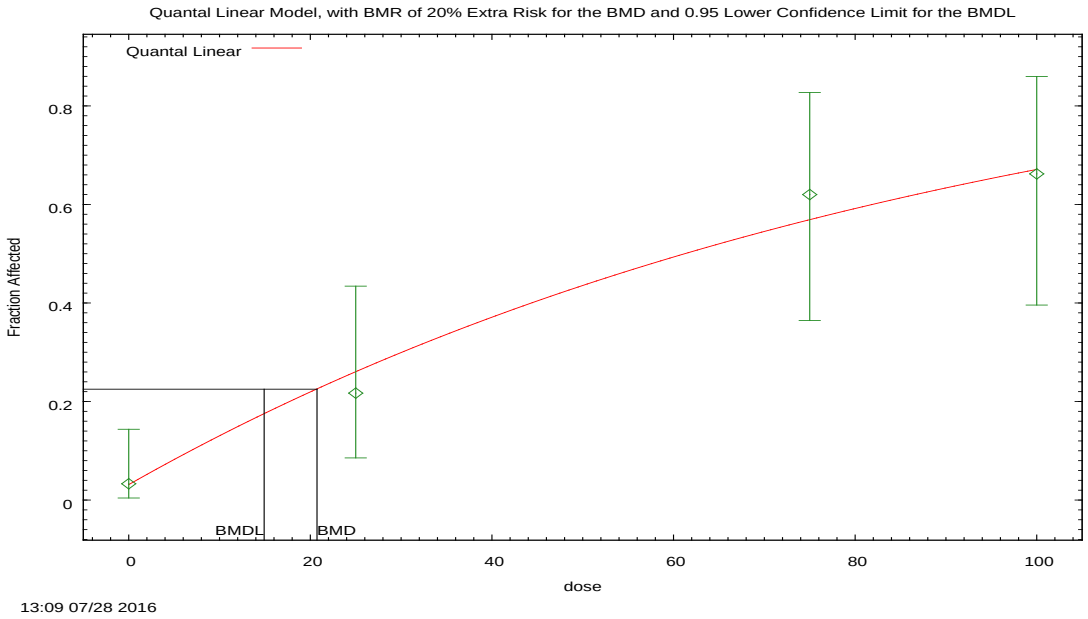


Figure E-12. Plot of incidence of embryo/fetal resorptions by dose, with fitted curve for Quantal-Linear model, for F344 female rats exposed to benzo[a]pyrene by inhalation on GDs 11–20 (Archibong et al., 2012; Archibong et al., 2002); BMR = 20% extra risk; dose in µg/m³.

Quantal Linear Model using Weibull Model (Version: 2.16; Date: 2/28/2013)
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-slope*dose)]

Benchmark Dose Computation
BMR = 20% Extra risk
BMD = 20.7381
BMDL at the 95% confidence level = 14.9171

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0312684	0.0519837
Slope	0.0107601	0.00980808
Power	N/A	1

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test df	*p*-value
Full model	−41.65	4			
Fitted model	−41.87	2	0.444722	2	0.8
Reduced model	−61.52	1	39.734	3	<0.0001

AIC: = 87.7479

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0313	1.476	1.558	47.2	0.07
25	0.2598	6.338	5.295	24.4	−0.48
75	0.5678	9.822	10.726	17.3	0.44
100	0.6697	11.05	10.923	16.5	−0.07

Chi^2 = 0.43 df = 2 *p*-value = 0.8052

Table E-17. Summary of BMD Modeling Results for ovarian weight in F344 rats exposed to benzo[a]pyrene via inhalation for 14 days prior to mating (Archibong et al., 2012); BMR = 10% relative deviation from control mean

Model[a]	Goodness of fit		BMD_{10RD} (µg/m³)	$BMDL_{10RD}$ (µg/m³)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	<0.0001	-140.91	74.7	63.3	No adequate fit; Exponential (M4) fit shown as illustration only. Dropping the high-dose group reduced the number of applicable models; no adequate fits. (Equivalent coefficients of variation were <1%.)
Exponential (M4)	0.00117	-165.02	41.0	28.6	
Exponential (M5)	NA	-167.87	49.4	42.9	
Hill	0.0170	-169.86	49.6	44.9	
Power, Polynomial 2°, 3°, Linear[d]	<0.0001	-139.36	77.0	65.6	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.520, BMDS Test 3 *p*-value = 0.520), no model was selected as a best-fitting model.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and reduced to the Exponential (M2) model.
[c]For the Power model, the power parameter estimate was 1; for the Polynomial 2° and 3° models, the coefficient estimates of higher order than b1 were 0 (boundary of parameters space). All models reduced to the Linear model.
NA = No available degrees of freedom to calculate a goodness-of-fit value.

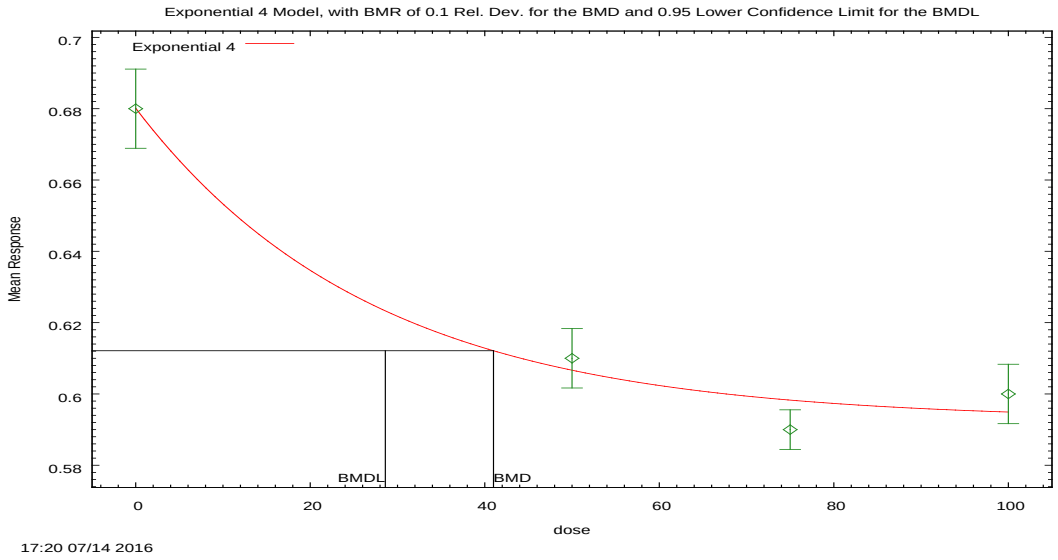


Figure E-13. Plot of mean ovarian weight by dose, with fitted curve for Exponential (M4) model with constant variance for female F344 rats exposed to benzo[a]pyrene for 14 days prior to mating (Archibong et al., 2012); BMR = 10% relative deviation from control mean; dose in µg/m³.

Table E-18. Summary of BMD modeling results for ovulation rate (ovulated oocytes/dam) in female F344 rats following inhalation exposure to benzo[a]pyrene for 14 days (Archibong et al., 2012); BMR = 1 or 10% relative deviation from control mean

Model[a]	Goodness of fit		BMD_{1RD} (µg/m³)	$BMDL_{1RD}$ (µg/m³)	BMD_{10RD} (µg/m³)	$BMDL_{10RD}$ (µg/m³)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.423	87.885	2.22	1.24	23.3	13.0	Among adequately fitting models at BMR=10% (omitting Exponential M4[b]), the BMDLs fell within a 3-fold range. Although Polynomial 2° had the lowest AIC, the 16-fold range of BMD_{1RD} estimates indicates substantial model uncertainty for low-dose extrapolation. No model was selected.
Exponential (M3)	0.721	88.291	36.9	1.49	65.8	15.6	
Exponential (M4)[b]	0.423	87.885	2.22	0.011	23.3	0.190	
Exponential (M5)	0.721	88.291	36.9	1.20	65.8	13.5	
Hill	NA	90.263	32.3	1.82	64.1	18.3	
Power	0.753	88.262	32.2	1.84	64.1	18.4	
Polynomial 3°	0.742	88.271	26.3	1.84	60.2	18.4	
Polynomial 2°	0.845	86.500	15.1	1.80	47.9	39.0	
Linear	0.500	87.551	2.56	1.67	25.6	16.7	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.148, BMDS Test 3 *p*-value = 0.148), no model was selected as a best-fitting model.
[b]Exponential (M4) parameters were identical to Exponential (M2), but the $BMDL_{10RD}$ was >100-fold was lower than the BMD_{10RD}.
NA = No available degrees of freedom to calculate a goodness-of-fit-value.

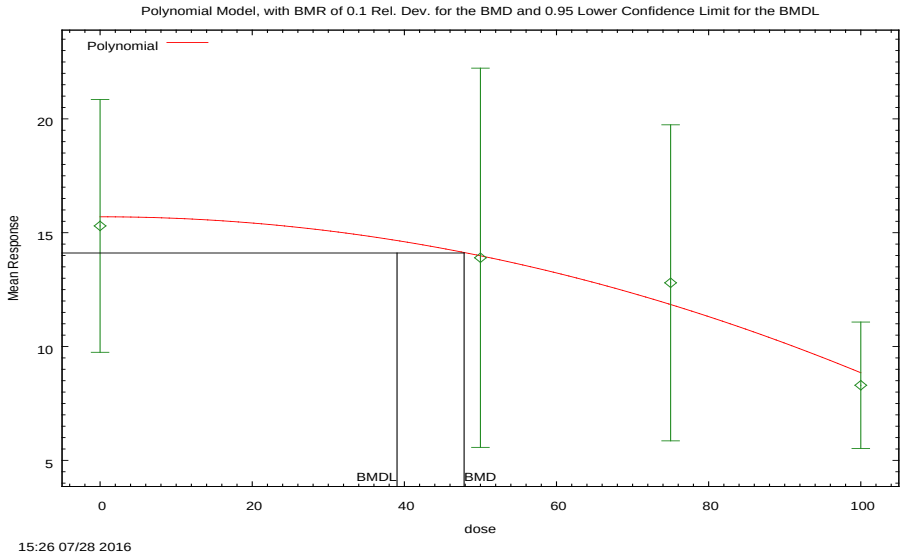


Figure E-14. Plot of mean ovulation rate by dose, with fitted curve for Polynomial 2° model with constant variance, for female F344 rats following inhalation exposure to benzo[a]pyrene for 14 days (Archibong et al., 2012); BMR = 10% relative deviation from control mean; dose in µg/m³.

Polynomial Model (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + …
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% Relative deviation
BMD = 47.8549
BMDL at the 95% confidence level = 39.0362

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	20.5942	25.3125
rho	N/A	0
beta_0	15.6769	15.2145
beta_1	-1.37569×10^{-24}	0
beta_2	−0.000684553	−0.00101091

Table of Data and Estimated Values of Interest

Dose	N	Oberved mean	Estimated mean	Observed SD	Estimated SD	Scaled Residual
0	5	15.3	15.7	4.47	4.54	−0.186
50	5	13.9	14	6.71	4.54	−0.0323
75	5	12.8	11.8	5.59	4.54	0.48
100	5	8.3	8.83	2.24	4.54	−0.262

Likelihoods of Interest

Model	Log(likelihood)	Number of parameters	AIC
A1	−40.081548	5	90.163096
A2	−37.403195	8	90.806389
A3	−40.081548	5	90.163096
fitted	−40.250096	3	86.500193
R	−43.005249	2	90.010499

Tests of Interest

Test	−2*log(likelihood ratio)	Test df	*p*-value
Test 1	11.2041	6	0.08227
Test 2	5.35671	3	0.1475
Test 3	5.35671	3	0.1475
Test 4	0.337097	2	0.8449

E.1.3. Dosimetry Modeling for Estimation of Human Equivalent Concentrations for Reference Concentration (RfC)

As discussed in Section 2.2.2, the human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by a dosimetric adjustment factor (DAF), which, in this case, was the regional deposited dose ratio ($RDDR_{ER}$) for extrarespiratory (i.e., systemic) effects. The observed developmental effects are considered systemic in nature (i.e., extrarespiratory) and the normalizing factor for extrarespiratory effects of particles is body weight. The $RDDR_{ER}$ was calculated as follows:

$$RDDR_{ER} = \frac{BW_H}{BW_A} \times \frac{(V_E)_A}{(V_E)_H} \times \frac{(F_{TOT})_A}{(F_{TOT})_H}$$

where:

BW = body weight (kg)

V_E = ventilation rate (L/minute)

F_{TOT} = total fractional deposition

The total fractional deposition includes particle deposition in the nasal-pharyngeal, tracheobronchial, and pulmonary regions. F_{TOT} for both animals and humans was calculated using the Multi-Path Particle Dosimetry (MPPD) model, a computational model used for estimating human and rat airway particle deposition and clearance (MPPD; Version 2.0 © 2006, publicly available through the Hamner Institute). See Figure E-15 and E-16 and model output below.

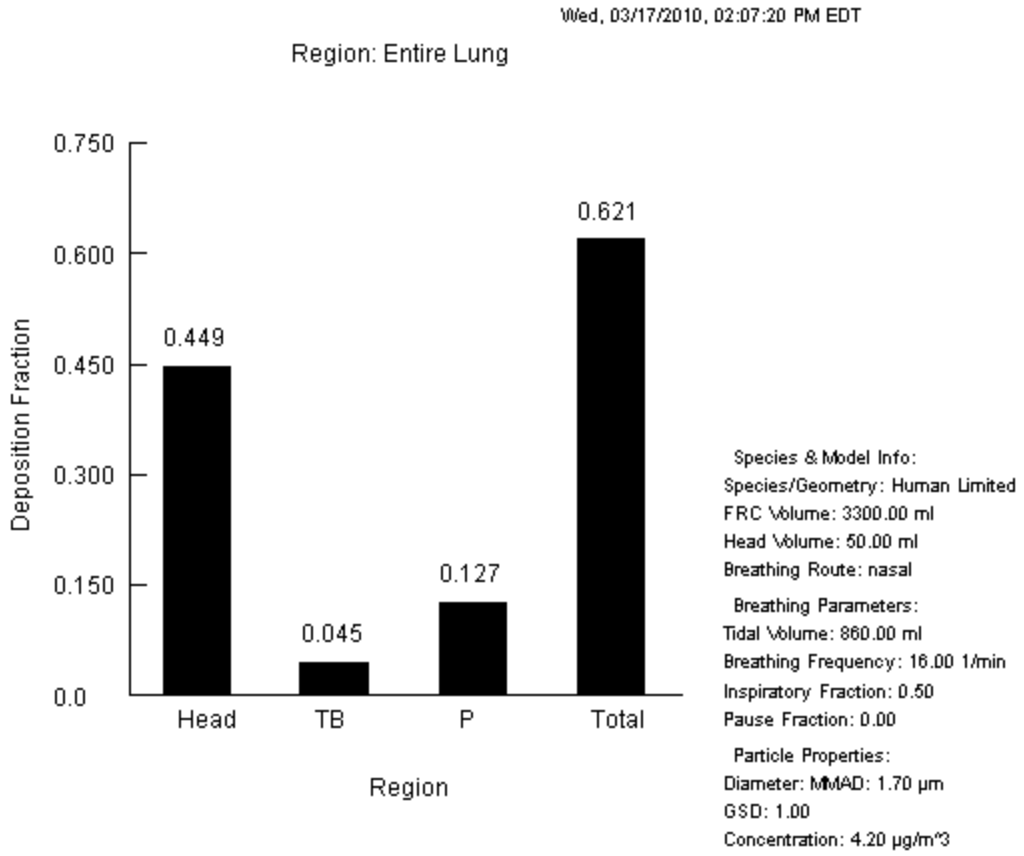


Figure E-15. Human fractional deposition.

```
Species = humanlimited
FRC = 3300.0
Head volume = 50.0
Density = 1.0
Number of particles calculated = single
Diameter = 1.7000000000000002 µm  MMAD
Inhalability =   yes
GSD = 1.0
Breathing interval: One single breath
Concentration = 4.2
Breathing Frequency = 16.0
Tidal Volume = 860.0
Inspiratory Fraction = 0.5
Pause Fraction = 0.0
Breathing Route = nasal

Region: Entire Lung
Region: Entire Lung
Region  Deposition Fraction
--      --
Head    0.449
TB      0.045
 P      0.127
Total   0.621
```

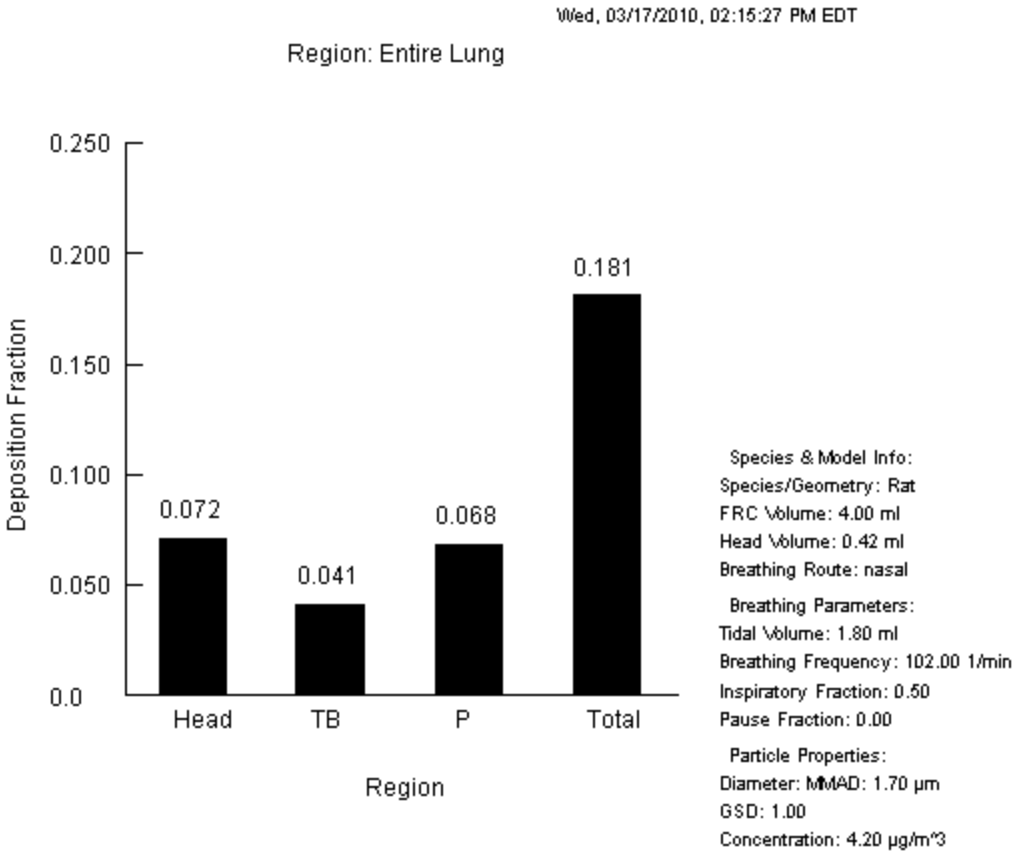


Figure E-16. Rat fractional deposition.

```
Species = rat
FRC = 4.0
Head volume = 0.42
Density = 1.0
Number of particles calculated = single
Diameter = 1.7000000000000002 µm  MMAD
Inhalability =   yes
GSD = 1.0
Breathing interval: One single breath
Concentration = 4.2
Breathing Frequency = 102.0
Tidal Volume = 1.8
Inspiratory Fraction = 0.5
Pause Fraction = 0.0
Breathing Route = nasal

Region: Entire Lung
Region: Entire Lung
Region  Deposition Fraction
--      --
Head    0.072
TB      0.041
 P      0.068
Total   0.181
```

E.2. Cancer Endpoints

E.2.1. Dose-Response Modeling for the Oral Slope Factor

E.2.1.1. *Dose-Response Models*

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of the high-dose group in the oral carcinogenicity studies (see Appendix D for study details), methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. EPA has generally used a model that incorporates the time at which death-with-tumor occurred as well as the dose; the multistage-Weibull model is multistage in dose and Weibull in time, and has the form:

$$P(d, t) = 1 - exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t \pm t_0)^c],$$

where *P(d, t)* represents the lifetime risk (probability) of cancer at dose d (i.e., human equivalent exposure in this case) and age t (in bioassay weeks); parameters $q_i \geq 0$, for $i = 0, 1, ..., k$; t is the time at which the tumor was observed; and c is a parameter which characterizes the change in response with age. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death, and is generally set to 0 either when all tumors are considered incidental or because of a lack of data to estimate the time reliably. The dose-response analyses were conducted using the computer software program MultiStage-Weibull (U.S. EPA, 2010b), which is based on Weibull models drawn from Krewski et al. (1983). Parameters were estimated using the method of maximum likelihood. From specific model fits using stages up to n −1, where n is the number of dose groups, the model fit with the lowest AIC was selected.

E.2.1.2. *Data Adjustments Prior to Modeling*

Two general characteristics of the observed tumor types were considered prior to modeling: allowance for different, although unidentified modes of action, and allowance for relative severity of tumor types. First, etiologically different tumor types were not combined across sites prior to modeling (i.e., overall counts of tumor-bearing animals were not tabulated) in order to allow for the possibility that different tumor types could have different dose-response relationships due to different underlying mechanisms or factors, such as latency. Consequently, all of the tumor types were also modeled separately.

Additionally, the multistage-Weibull model can address relative severity of tumor types to some extent by distinguishing between tumors as being either fatal or incidental to the death of an animal in order to adjust partially for competing risks. In contrast to fatal tumors, incidental tumors are those tumors thought not to have caused the death of an animal. Cause-of-death information for most early animal deaths was provided by the investigators of both bioassays. In the rat study of Kroese et al. (2001), tumors of the forestomach or liver were the principal cause of

death for most animals dying or sacrificed (due to moribundity) before the end of the study, while tumors of the forestomach were the most common cause of early deaths in the mouse study of Beland and Culp (1998). The incidence data modeled are listed in Tables E-19 (male rats), E-20 (female rats), and E-21 (female mice).

Consistent with EPA's *Recommended use of body weight $^{3/4}$ as the default method in derivation of the oral reference dose*, human-equivalent dose (HED) estimates used for dose-response modeling were based on scaling by body weight$^{3/4}$, as there were no pharmacokinetic models or data to inform another approach (U.S. EPA, 2011). The dose estimates are provided in Tables E-22 (Kroese et al., 2001) and E-23 (Beland and Culp, 1998).

E.2.1.3. *Evaluation of Model Fit and Model Selection*

Each model was examined for adequacy of fit in the low-dose region and in the vicinity of the BMR of 10% extra risk. In general, the model fit with the lowest AIC was selected, except when model fit near the BMR and in the low-dose region was improved by including an additional stage (parameter) in the model.

PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk, where extra risk is defined as [P(d) − P(0)]/ [1 − P(0)]. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit (UCL), represents a plausible upper bound on the true risk.

E.2.1.4. *Overall Risk*

Although the time-to-tumor modeling helps account for competing risks associated with decreased survival times and other tumors, considering the tumor sites individually still does not convey the total amount of risk potentially arising from the sensitivity of multiple sites (i.e., the risk of developing any combination of the increased tumor types, not just the risk of developing all simultaneously). One approach suggested in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used this approach until the National Research Council (NRC) document *Science and Judgment in Risk Assessment* (NRC, 1994) made a case that this approach would tend to underestimate overall risk when tumor types occur in a statistically independent manner. In addition, application of one model to a composite data set does not accommodate biologically relevant information that may vary across sites or may only be available for a subset of sites. For instance, the time courses of the multiple tumor types evaluated varied, as is suggested by the variation in estimates of *c*, from 1.5 (e.g., male rat skin or mammary gland basal cell tumors), indicating relatively little effect of age on tumor incidence, to 3.7 (e.g., male mouse alimentary tract tumors), indicating a more rapidly increasing response with increasing age (in addition to exposure level). The result of fitting a model with parameters that can reflect underlying mechanisms, such as *z* in the multistage-Weibull

model, would be difficult to interpret with composite data (i.e., counts of tumor-bearing animals). A simpler model, such as the multistage model, could be used for the composite data, but relevant biological information would then be ignored.

Following the recommendations of the NRC (1994) regarding combining risk estimates, statistical methods that can accommodate the underlying distribution of slope factors are optimal, such as through maximum likelihood estimation or through bootstrapping or Bayesian analysis. However, these methods have not yet been extended to models such as the multistage-Weibull model. A method involving the assumption that the variability in the slope factors could be characterized by a normal distribution is detailed below (U.S. EPA, 2010b). Using the results in female rats to illustrate, the overall risk estimate involved the following steps:

1) It was assumed that the tumor groupings modeled above were statistically independent (i.e., that the occurrence of a liver tumor was not dependent upon whether there was a forestomach tumor). This assumption cannot currently be verified, and if not correct, could lead to an overestimate of risk from summing across tumor sites. However, NRC (1994) argued that a general assumption of statistical independence of tumor-type occurrences within animals was not likely to introduce substantial error in assessing carcinogenic potency from rodent bioassay data.

2) The models previously fitted to estimate the BMDs and BMDLs were used to extrapolate to a lower level of risk (R), in order to reach the region of each estimated dose-response function where the slope was reasonably constant and upper bound estimation was still numerically stable. For these data, a 10^{-3} risk was generally the lowest risk necessary. The oral slope factor for each site was then estimated by $R/BMDL_R$, as for the estimates for each tumor site above.

3) The maximum likelihood estimates (MLE) of unit potency (i.e., risk per unit of exposure) estimated by R/BMD_R, were summed across the alimentary tract, liver, and jejunum/ duodenum in female rats.

4) An estimate of the 95% (one-sided) upper bound on the summed oral slope factor was calculated by assuming a normal distribution for the individual risk estimates, and deriving the variance of the risk estimate for each tumor site from its 95% UCL according to the formula:

$$95\%\ UCL = MLE + 1.645 \times SD,$$

rearranged to:

$$SD = (UCL - MLE) / 1.645,$$

where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval (CI) and >120 degrees of freedom, and the SD is the square root of the variance of the MLE. The variances (variance = SD^2) for each site-specific estimate were summed across tumor sites to obtain the variance of the sum of the MLEs. The 95% UCL on the sum of MLEs was calculated from the expression above for the UCL, using the variance of the sum of the MLE to obtain the relevant SD (SD = $variance^{1/2}$).

Table E-19. Tumor incidence data, with time to death with tumor for male Wistar rats exposed by gavage to benzo[a]pyrene for 104 weeks (Kroese et al., 2001)

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:							
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors	Skin or mammary gland		Kidney urothelial carcinoma
								Basal cell tumors	Squamous cell tumors	
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental	Incidental	Incidental	Incidental
0	44	1	0	0	0	0	0	1	0	0
	80	1	0	0	0	0	0	0	0	0
	82	1	0	0	0	0	0	0	0	0
	84	1	0	0	0	0	0	0	0	0
	89	1	0	0	0	0	0	0	0	0
	90	3	0	0	0	0	0	0	0	0
	91	1	0	0	0	0	0	0	0	0
	92	1	0	0	0	0	0	0	0	0
	93	1	0	0	0	0	0	0	0	0
	94	1	0	0	0	0	0	0	0	0
	95	2	0	0	0	0	0	0	0	0
	96	2	0	0	0	0	0	0	0	0
	97	1	0	0	0	0	0	0	0	0
	98	1	0	0	0	0	0	0	0	0
	100	3	0	0	0	0	0	1	0	0
	104	1	0	0	0	0	0	0	0	0
	105	1	0	0	0	0	0	0	0	0
	108	7	0	0	0	0	0	0	0	0
	109	22	0	0	0	0	0	0	0	0
3	29	1	0	0	0	0	0	0	0	0
	40	1	1	0	0	0	0	0	0	0
	74	1	0	0	0	0	0	0	0	0
	76	1	0	0	0	0	0	0	0	0
	79	1	0	0	0	0	0	0	0	0
	82	1	0	0	0	0	0	0	0	0
	92	2	0	0	0	0	0	0	0	0
	93	1	0	0	0	0	0	0	0	0
	94	1	0	0	0	0	0	0	0	0
	95	2	0	0	0	0	0	0	0	0
	98	1	0	0	0	0	0	0	0	0
	107	10	4	0	1	0	0	0	0	0
	108	15	2	0	3	0	0	1	1	0
	109	14	1	0	0	0	0	0	0	0

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:							
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors	Skin or mammary gland		Kidney urothelial carcinoma
								Basal cell tumors	Squamous cell tumors	
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental	Incidental	Incidental	Incidental
10	39	1	0	0	0	0	0	0	0	0
	47	2	0	0	0	0	0	0	0	0
	63	1	1	0	0	0	0	0	0	0
	68	2	2	0	0	0	0	0	0	0
	69	1	1	0	0	0	0	0	0	0
	77	1	0	0	1	0	0	0	0	0
	80	1	0	0	1	0	0	0	0	0
	81	1	1	0	0	0	1	0	0	0
	84	1	1	0	0	1	0	0	0	0
	86	1	0	0	1	0	0	0	0	0
	90	1	1	0	0	0	0	0	0	0
	95	3	3	0	2	0	0	0	0	0
	97	1	1	0	0	1	0	0	0	0
	100	1	1	0	1	0	0	0	0	0
	102	1	1	0	1	0	0	0	0	0
	103	1	1	0	1	0	0	0	0	0
	104	3	3	0	3	0	0	0	0	0
	107	12	12	0	11	0	0	0	1	0
	108	11	11	0	11	0	0	1	0	0
	109	6	5	0	3	0	0	0	0	0

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:							
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors	Skin or mammary gland		Kidney urothelial carcinoma
								Basal cell tumors	Squamous cell tumors	
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental	Incidental	Incidental	Incidental
30	32	1	1	0	0	0	0	0	0	0
	35	1	1	0	1	0	0	0	0	0
	37	1	1	0	0	0	0	0	0	0
	44	1	0	1	1	0	0	0	0	0
	45	2	2	0	2	0	0	0	0	0
	47	1	1	0	1	0	0	0	0	0
	48	1	1	0	1	0	0	0	0	0
	49	1	1	0	1	0	0	0	0	0
	50	1	1	0	1	0	0	0	0	0
	51	1	1	0	1	0	1	0	0	0
	52	4	3	1	3	1	0	1	1	0
	53	1	1	0	1	0	0	1	0	0
	56	2	1	1	1	1	0	0	0	0
	58	2	2	0	2	0	0	1	0	0
	59	2	2	0	2	0	0	0	0	0
	60	2	1	1	1	1	1	0	0	0
	61	3	2	1	1	2	1	0	0	0
	62	5	5	0	0	4	3	0	0	0
	63	5	5	0	4	1	1	2	1	2
	64	2	2	0	1	1	0	0	0	1
	65	3	2	1	1	2	0	3	2	0
	66	1	1	0	0	1	0	0	0	0
	67	3	1	2	2	1	1	1	1	0
	68	1	1	0	1	0	0	0	0	0
	70	2	2	0	1	1	1	1	0	0
	71	1	1	0	1	0	0	1	1	0
	73	1	0	1	1	0	0	1	0	0
	76	1	1	0	0	1	0	1	0	0

[a]"Incidental" denotes presence of tumors not known to have caused death of particular animals. "Fatal" denotes incidence of tumors reported by the study investigators to have caused death of particular animals.

Table E-20. Tumor incidence data, with time to death with tumor for female Wistar rats exposed by gavage to benzo[a]pyrene for 104 weeks (Kroese et al., 2001)

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:				
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental
0	64	1	0	0	0	0	0
	69	1	0	0	0	0	0
	75	1	0	0	0	0	0
	104	1	0	0	0	0	0
	106	4	0	0	0	0	0
	107	7	0	0	0	0	0
	108	7	0	0	0	0	0
	109	30	1	0	0	0	0
3	8	1	0	0	0	0	0
	47	1	0	0	0	0	0
	52	1	0	0	0	0	0
	60	1	0	0	0	0	0
	65	1	0	0	0	0	0
	76	1	0	0	0	0	0
	77	1	0	0	0	0	0
	83	2	0	0	0	0	0
	85	1	0	0	0	0	0
	86	1	0	0	0	0	0
	88	1	0	0	0	0	0
	93	2	0	0	0	0	0
	94	1	0	0	0	0	0
	97	1	1	0	0	0	0
	107	6	2	0	1	0	0
	108	9	2	0	0	0	0
	109	21	1	0	0	0	0

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:				
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental
10	42	1	0	0	0	0	0
	43	1	0	0	0	0	0
	44	1	0	0	0	0	0
	45	1	0	0	0	0	0
	48	1	0	0	0	0	0
	55	1	0	0	1	0	0
	59	1	0	0	0	0	0
	75	1	0	0	1	0	0
	76	2	0	0	1	0	0
	77	2	0	0	0	0	0
	80	1	1	0	1	0	0
	81	1	1	0	0	1	0
	82	1	1	0	1	0	0
	83	1	0	0	1	0	0
	85	2	1	0	1	1	0
	86	1	1	0	0	1	0
	87	1	0	0	1	0	0
	88	2	1	0	1	1	0
	89	1	1	0	0	1	0
	91	1	0	0	0	1	0
	95	1	0	0	0	0	0
	96	1	0	0	0	0	0
	98	2	2	0	1	1	0
	99	3	3	0	1	2	0
	102	1	1	0	0	1	0
	104	1	1	0	1	0	0
	105	2	1	0	1	1	0
	106	1	1	0	0	1	0
	107	5	5	0	5	0	0
	108	7	7	0	7	0	0
	109	4	2	0	2	0	0

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:				
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental
30	26	1	0	0	0	0	0
	44	4	4	0	3	1	0
	47	3	3	0	2	1	0
	48	1	1	0	0	1	0
	54	1	0	0	1	0	0
	55	3	3	0	1	2	0
	56	2	2	0	0	2	0
	57	2	2	0	2	0	0
	58	4	3	1	0	4	0
	59	2	1	1	0	2	0
	60	1	0	1	1	0	0
	61	2	2	0	0	2	0
	62	2	2	0	1	1	0
	63	3	3	0	0	3	0
	64	5	5	0	0	5	3
	66	3	3	0	0	3	0
	67	2	1	1	0	2	0
	68	1	1	0	0	1	0
	69	4	3	1	1	3	1
	71	4	3	1	1	3	0
	72	2	1	1	0	2	0

[a]"Incidental" denotes presence of tumors not known to have caused death of particular animals. "Fatal" denotes incidence of tumors indicated by the study investigators to have caused death of particular animals.

Table E-21. Tumor incidence, with time to death with tumor; B6C3F$_1$ female mice exposed to benzo[a]pyrene via diet for 2 years (Beland and Culp, 1998)

Dose group (ppm in diet)	Wk of death	Total examined	Number of animals with alimentary tract squamous cell tumors		Dose group (ppm in diet)	Wk of death	Total examined	Number of animals with alimentary tract squamous cell tumors	
			Fatal[a]	Incidental				Fatal[a]	Incidental
0	31	1	0	0	5	25	1	0	0
	74	1	0	0		55	1	0	0
	89	2	0	0		83	1	0	0
	91	1	0	0		86	1	0	0
	93	2	0	0		87	2	0	0
	94	2	0	0		88	2	0	0
	97	2	0	0		90	1	0	0
	98	2	0	0		94	1	0	0
	99	1	0	0		95	2	0	0
	100	2	0	0		96	1	0	0
	101	2	0	0		97	2	0	0
	104	1	0	0		98	2	0	0
	105	29	0	1		101	2	0	0
						102	2	0	0
						105	27	0	3
25	44	1	1	0	100	39	1	1	0
	47	1	0	0		40	1	1	0
	64	1[b]	0	0		42	1	1	0
	70	1	1	0		47	2	2	0
	77	1	1	0		49	1	0	0
	80	1	0	0		50	1	1	0
	81	1	1	0		53	1[b]	0	0
	84	2	1	1		55	3	3	0
	85	1	1	0		56	1	1	0
	86	1	1	0		57	1	1	0
	88	1	1	0		58	1	1	0
	89	1	0	0		59	3	3	0
	90	4	4	0		60	1	1	0
	93	3	2	1		61	3	3	0
	94	2	2	0		62	5	5	0
	96	3	0	2		63	4	4	0
	97	1	1	0		64	3	3	0
	98	1	1	0		65	2	2	0
	99	2	1	1		66	3	3	0
	100	1	1	0		68	1	1	0
	101	1	0	0		69	2	2	0
	102	2	2	0		70	2	2	0
	104	1	1	0		71	1	1	0
	105	13	0	10		72	1	1	0
						73	1	1	0
						74	1	1	0
						79	1	1	0

[a]"Incidental" denotes presence of tumors not known to have caused death of particular animals. "Fatal" denotes incidence of tumors indicated by the study investigators to have caused death of particular animals.

Table E-22. Derivation of HEDs to use for BMD modeling of Wistar rat tumor incidence data from Kroese et al. (2001)

Benzo[a]pyrene dose (mg/kg-d)	TWA body weight (kg)	Interspecies scaling factor[a]	HED[b] (mg/kg-d)
Male			
3	0.349	0.27	0.54
10	0.349	0.27	1.81
30	0.288	0.25	5.17
Female			
3	0.222	0.24	0.49
10	0.222	0.24	1.62
30	0.222	0.24	4.85

[a]Scaling factors were calculated using U.S. EPA (1988) reference body weights for humans (70 kg), and the TWA body weights for each dose group: rat-to-human = $(\text{TWA body weight}/70)^{0.25}$ = scaling factor.
[b]HED = administered dose × scaling factor.

Table E-23. Derivation of HEDs for dose-response modeling of B6C3F$_1$ female mouse tumor incidence data from Beland and Culp (1998)

Benzo[a]pyrene dose in diet (ppm)	Intake (µg/d)	TWA body weight average (kg)	Administered dose[a] (mg/kg-d)	Scaling factor[b]	HED[c] (mg/kg-d)
5	21	0.032	0.7	0.15	0.10
25	104	0.032	3.3	0.15	0.48
100	430	0.027	16.5	0.14	2.32

[a]Administered doses in mg/kg-day were calculated from dietary concentrations of benzo[a]pyrene using the TWA body weight and reported food intakes for mice.
[b]Scaling factors were calculated using U.S. EPA (1988) reference body weights for humans (70 kg), and the TWA body weights for each dose group: mouse-to-human = $(\text{TWA body weight}/70)^{0.25}$ = scaling factor.
[c]HED = administered dose × scaling factor.

E.2.1.5. *Sensitivity Analyses*

Alternative dose-response models were also considered, limited to the most sensitive sites for male and female rats (alimentary system tumors) and the overall incidence of alimentary system tumors for female mice. Tumor incidences were adjusted for early mortality using the poly-3 procedure (Bailer and Portier, 1988); the adjusted incidences are provided in Tables E-25 (male rats), E-26 (female rats), and E-29 (female mice). Adjusted incidences were fit using dichotomous models in BMDS (see Section E.1.2 for model fitting methods).

E.2.1.6. *Dose-Response Modeling Results*

Tables E-24 (male and female rats) and E-28 (female mice) summarize the multistage-Weibull modeling results supporting the oral slope factor for benzo[a]pyrene. The model outputs and graphs following each of these tables (male rats: Figures E-17 through E-22; female rats: Figures E-23 through E-25; female mice: Figure E-26) provide more details for the best-fitting models in each case.

Derivations of overall risk estimates for male and female rats are summarized in Table E-27.

Alternative dose-response modeling results are provided in Tables E-25 (male rats), E-26 (female rats), and E-29 (female mice).

Table E-24. Summary of BMD modeling results for best-fitting multistage-Weibull models, using time-to-tumor data for Wistar rats exposed to benzo[a]pyrene via gavage for 104 weeks (Kroese et al., 2001); BMR = 10% extra risk

	Endpoints	Model stages	AIC	BMD_{10}	$BMDL_{10}$ – $BMDU_{10}$	Basis for model selection
Male rats	Oral cavity and forestomach: squamous cell tumors	1 2 **3**	577.8 407.6 **229.0**	0.104 0.678 **0.453**	**0.281–0.612**	**Lowest AIC, best fit to low dose data**
	Hepatocellular tumors	1 2 **3**	367.3 301.5 **289.1**	0.181 0.472 **0.651**	**0.449–0.772**	**Lowest AIC, best fit to low dose data**
	Duodenum and jejunum tumors	1 2 **3**	69.6 65.9 **66.9**	2.64 3.04 **3.03**	**2.38–3.87**	**Best fit to data**
	Kidney: uroethelial carcinoma	1 2 **3**	31.9 31.7 **32.8**	9.16 5.71 **4.65**	**2.50–9.01**	**Best fit to data**
	Skin and mammary gland: basal cell tumors	1 2 **3**	110.6 105.1 **104.7**	1.88 2.58 **2.86**	**2.35–3.62**	**Lowest AIC, best fit to low dose data**
	Skin and mammary gland: squamous cell tumors	**1** 2 3	63.5 64.3 **65.3**	3.36 2.75 **2.64**	**1.77–4.42**	**Best fit to low dose data**
Female rats	Oral cavity and forestomach: squamous cell tumors	1 2 3	277.1 211.6 **201.0**	0.245 0.428 **0.539**	**0.328–0.717**	**Lowest AIC, best fit to low dose data**
	Hepatocellular tumors	1 2 3	595.5 774.9 **468.3**	0.146 0.370 **0.575**	**0.507–0.630**	**Lowest AIC, best fit to low dose data**
	Duodenum and jejunum tumors	1 2 **3**	37.9 37.0 **37.8**	6.00 4.33 **3.43**	**1.95–5.70**	**Best fit to low dose data**

Male rat (Kroese et al., 2001): Squamous cell papilloma or carcinoma in oral cavity or forestomach

```
================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: OralForstKroeseM3.(d)
 ================================================================
   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =          3.6
                        t_0    =      39.1111
                        beta_0 =            0
                        beta_1 =  8.8911e-009
                        beta_2 = 1.60475e-031
                        beta_3 = 1.95818e-008


          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -beta_0    -beta_2
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                c            t_0          beta_1       beta_3

    c                1        -0.53        -0.93        -0.99

    t_0           -0.53            1         0.47         0.57

    beta_1        -0.93         0.47            1          0.9

    beta_3        -0.99         0.57          0.9            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.74559         0.447309            2.86888             4.6223
         t_0            41.4581          2.14975            37.2447            45.6716
         beta_0               0               NA
         beta_1     4.37816e-009    1.07528e-008        -1.6697e-008        2.54533e-008
         beta_2               0               NA
         beta_3     1.01904e-008    1.94164e-008       -2.78651e-008        4.82458e-008

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


               Log(likelihood)   # Param            AIC
   Fitted Model        -108.512         6         229.024
```

```
                Data Summary
                    CONTEXT
            C      F      I      U  Total  Expected Response
    DOSE
        0     52      0      0      0     52     0.00
     0.54     44      0      8      0     52     6.77
      1.8      7      0     45      0     52    41.69
      5.2      0      9     43      0     52    49.97

    Minimum observation time for F tumor context =         44


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


        Specified effect =  0.1           0.01           0.001
                     BMD =  0.453471      0.0633681      0.00636659
                    BMDL =  0.281044      0.0286649      0.00285563
                    BMDU =  0.612462      0.248377       > 0.0509326
```

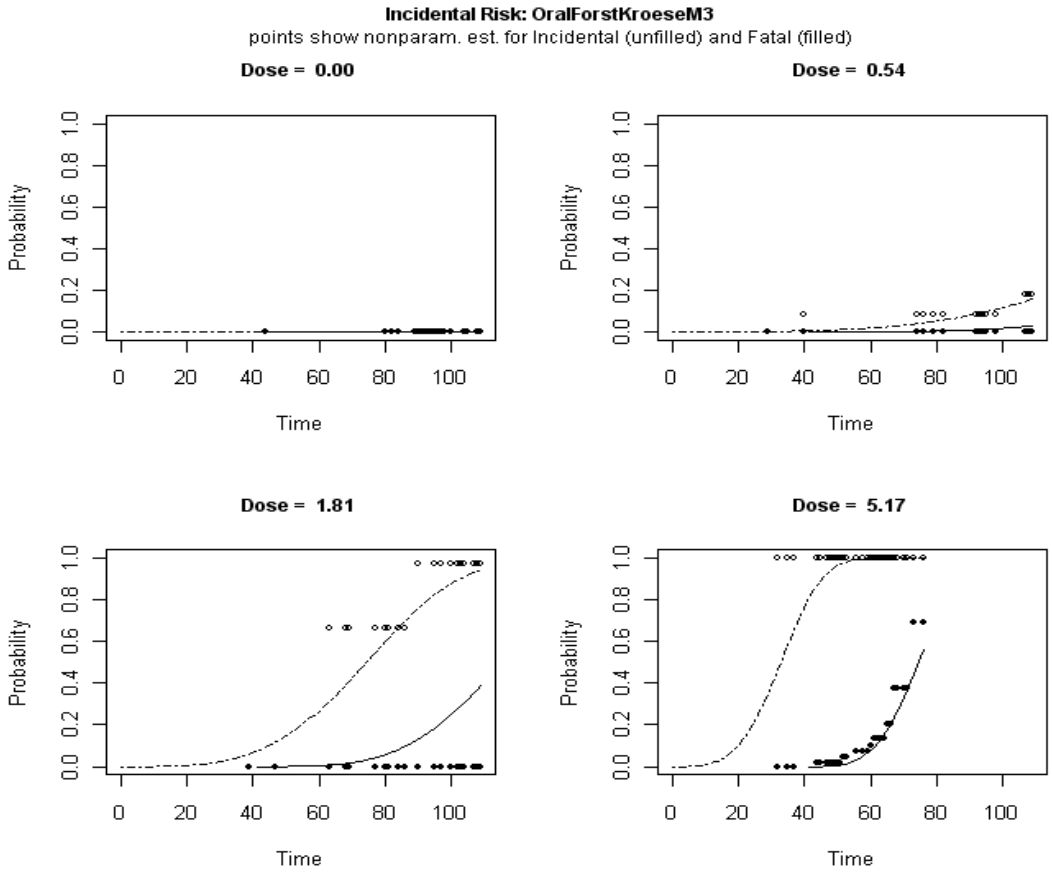


Figure E-17. Fit of multistage Weibull model to squamous cell papillomas or carcinomas in oral cavity or forestomach of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Table E-25. Summary of alternate BMD modeling results for squamous cell papillomas or carcinomas in oral cavity or forestomach of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001); poly-3 incidences[a]

Model	Goodness of fit		BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	Comments
	p-value	AIC			
Multistage 3°[b]	1.000	62.662	0.406	0.200	Among multistage models, two-stage model provided the most parsimonious fit.
Multistage 2°	**0.861**	**61.467**	**0.349**	**0.243**	
Quantal-Linear	0.0012	79.862	0.106	0.0838	
Gamma	1.000	62.662	0.439	0.323	Other dichotomous models yielded BMD_{10} values ranging from 0.412 to 0.470 and $BMDL_{10}$ values ranging from 0.287 to 0.367.
Dichotomous-Hill LogLogistic	0.980	62.741	0.455	0.364	
Logistic	0.539	64.750	0.470	0.357	
Probit	0.657	64.053	0.454	0.343	
LogProbit	0.999	62.665	0.459	0.367	
Weibull	1.000	62.662	0.412	0.287	

[a]Doses: 0 mg/kg-day 0/43
0.54 mg/kg-day 8/45
1.8 mg/kg-day 45/47
5.2 mg/kg-day 52/52

[b]Coefficients b_0 and b_1 fit at boundary of permitted values (0).

Male rat (Kroese et al., 2001): Hepatocellular adenoma or carcinoma

```
=======================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: LiverKroeseM3.(d)
=======================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =          3.6
                        t_0    =      34.6667
                        beta_0 =            0
                        beta_1 = 2.73535e-009
                        beta_2 =   8.116e-028
                        beta_3 = 1.43532e-008


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -beta_0    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            t_0          beta_1       beta_3

    c                 1        -0.84        -0.88           -1

    t_0           -0.84            1         0.71         0.86

    beta_1        -0.88         0.71            1         0.86

    beta_3           -1         0.86         0.86            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.49582         0.629257            2.26249             4.72914
         t_0            40.2211          5.65421            29.1391             51.3032
         beta_0               0               NA
         beta_1     4.43906e-009    1.76051e-008       -3.00664e-008       3.89445e-008
         beta_2               0               NA
         beta_3     2.35065e-008    6.47999e-008       -1.03499e-007       1.50512e-007

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

               Log(likelihood)   # Param            AIC
   Fitted Model        -138.544         6        289.088


                    Data Summary
```

```
                    CONTEXT
             C      F      I      U  Total  Expected Response
    DOSE
        0     52      0      0      0     52     0.00
     0.54     48      0      4      0     52     3.38
      1.8     14      2     36      0     52    36.81
      5.2      3     17     32      0     52    49.55

    Minimum observation time for F tumor context =         52

   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


        Specified effect =  0.1             0.01          0.001
                     BMD =  0.6507          0.173556      0.0199908
                    BMDL =  0.44868         0.0530469     0.00530386
                    BMDU =  0.772467        0.352684      > 0.159927
```

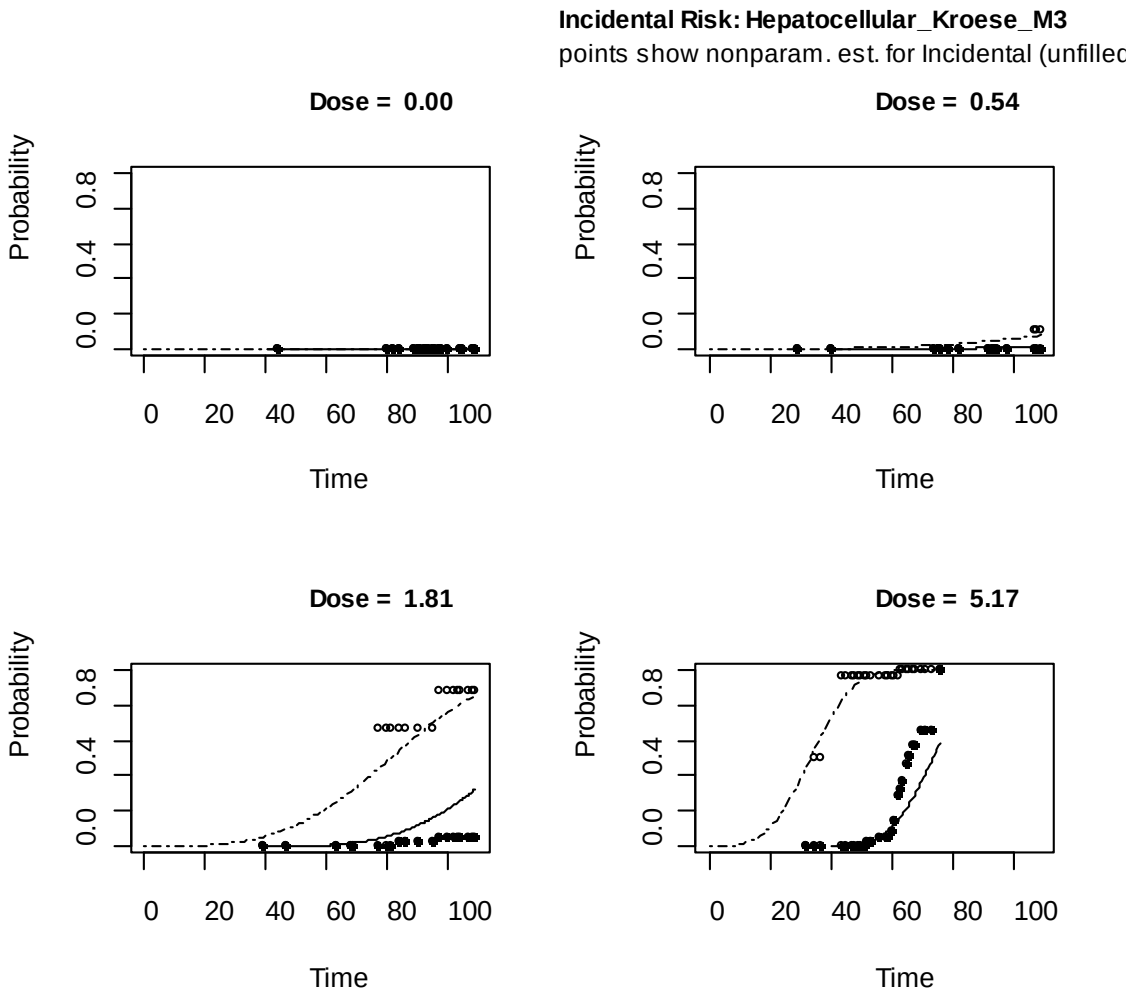


Figure E-18. Fit of multistage Weibull model to hepatocellular adenomas or carcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Male rat (Kroese et al., 2001): Duodenum or jejunum adenocarcinoma

```
=======================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: DuoJejKroeseM3.(d)
=======================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =      1.63636
                        t_0    =            0   Specified
                        beta_0 = 4.31119e-027
                        beta_1 = 2.96347e-025
                        beta_2 =            0
                        beta_3 = 1.76198e-006


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              1.77722          2.03042           -2.20233             5.75677
         beta_0               0               NA
         beta_1               0               NA
         beta_2               0               NA
         beta_3     9.82635e-007     8.29355e-006       -1.52724e-005        1.72377e-005

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


               Log(likelihood)   # Param            AIC
   Fitted Model        -28.4387         5        66.8773
```

Data Summary

DOSE	CONTEXT C	F	I	U	Total	Expected	Response
0	52	0	0	0	52	0.00	
0.54	52	0	0	0	52	0.03	
1.8	51	0	1	0	52	1.04	
5.2	43	0	9	0	52	8.96	

Benchmark Dose Computation

Risk Response	= Incidental
Risk Type	= Extra
Specified effect	= 0.1
Confidence level	= 0.9
Time	= 104

Specified effect =	0.1	0.01	0.001
BMD =	3.03291	1.38578	0.642252
BMDL =	2.37782	0.418285	0.0420835
BMDU =	3.87183	1.76166	0.811476

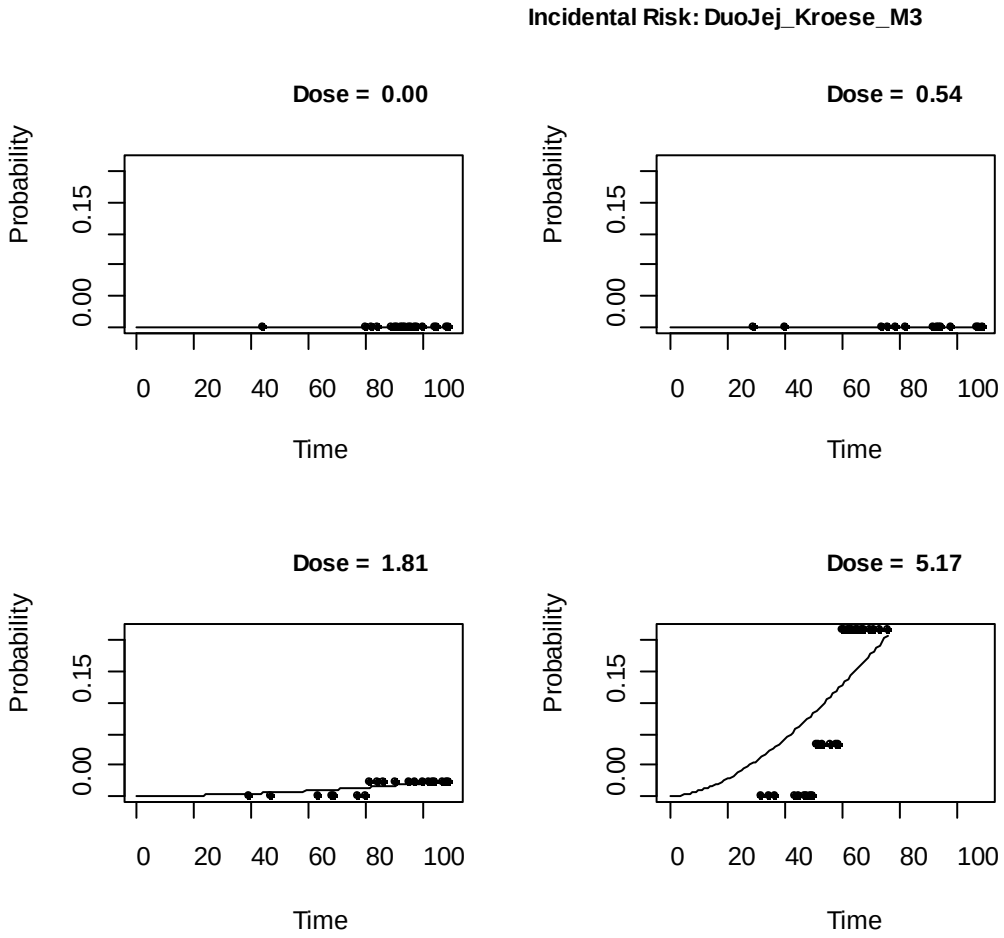


Figure E-19. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Male rat (Kroese et al., 2001): Skin or mammary gland basal cell tumors

```
=======================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: SKinMamBasalKroeseM3.(d)
=======================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =      1.38462
                        t_0    =            0   Specified
                        beta_0 = 3.84298e-005
                        beta_1 = 1.06194e-028
                        beta_2 =            0
                        beta_3 = 6.84718e-006


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_3

    c                 1           -1           -1

    beta_0           -1            1         0.99

    beta_3           -1         0.99            1


                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              1.47227          1.76686            -1.9907             4.93525
         beta_0     2.54786e-005      0.000211261        -0.000388585         0.000439542
         beta_1                0                NA
         beta_2                0                NA
         beta_3     4.81611e-006        3.49e-005       -6.35866e-005        7.32188e-005

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


                Log(likelihood)   # Param             AIC
   Fitted Model        -47.3623         5         104.725
```

Data Summary

DOSE	CONTEXT C	F	I	U	Total	Expected	Response
0	50	0	2	0	52	1.18	
0.54	51	0	1	0	52	1.22	
1.8	51	0	1	0	52	2.32	
5.2	39	0	13	0	52	12.54	

Benchmark Dose Computation

Risk Response	=	Incidental	
Risk Type	=	Extra	
Confidence level	=	0.9	
Time	=	104	

Specified effect	=	0.1	0.01	0.001
BMD	=	2.86276	1.30804	0.606222
BMDL	=	2.35118	0.415897	0.0424277
BMDU	=	3.62258	1.69571	0.761447

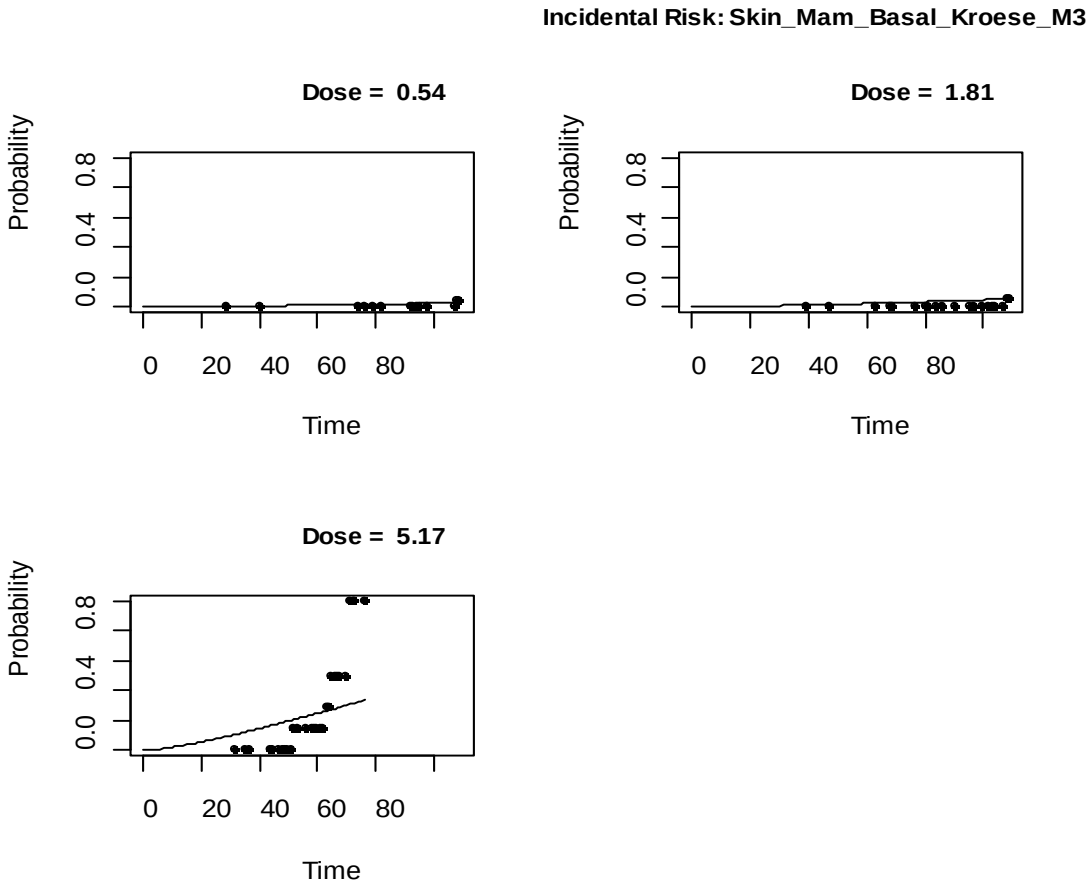


Figure E-20. Fit of multistage Weibull model to skin or mammary gland basal cell tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Male rat (Kroese et al., 2001): Skin or mammary gland squamous cell tumors

```
=================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: SKinMamSCCKroeseM3.(d)
 =================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =            3
                        t_0    =            0   Specified
                        beta_0 =            0
                        beta_1 = 1.25256e-008
                        beta_2 = 1.25627e-030
                        beta_3 = 3.34696e-009


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_1       beta_3

    c                 1        -0.99           -1

    beta_1        -0.99            1         0.99
    beta_3           -1         0.99            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               2.96213            2.591            -2.11613             8.04039
         beta_0                0               NA
         beta_1     1.50104e-008     1.86972e-007       -3.51447e-007        3.81468e-007
         beta_2                0               NA
         beta_3      3.9084e-009     4.15374e-008       -7.75033e-008        8.53201e-008

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


                Log(likelihood)   # Param            AIC
   Fitted Model         -27.652         5          65.304
```

```
                Data Summary
                   CONTEXT
            C      F      I      U  Total  Expected Response
DOSE
    0      52      0      0      0     52     0.00
 0.54      51      0      1      0     52     0.42
  1.8      51      0      1      0     52     2.12
  5.2      46      0      6      0     52     5.51


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


         Specified effect =  0.1          0.01          0.001
                      BMD =  2.6414       0.64109       0.070558
                     BMDL =  1.76931      0.211043      0.0210552
                     BMDU =  4.42145      2.03605       > 0.564463
```

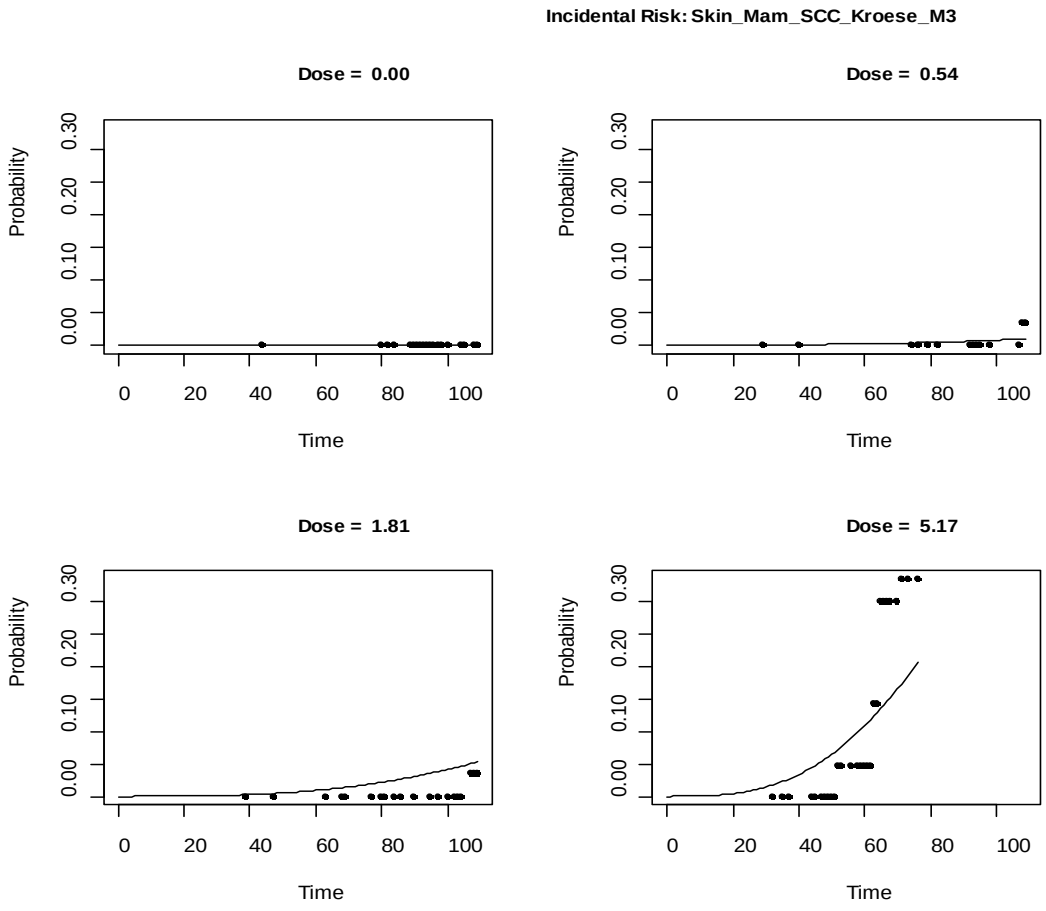


Figure E-21. Fit of multistage Weibull model to skin or mammary gland squamous cell tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Male rat (Kroese et al., 2001): Kidney urothelial carcinomas

```
================================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: KidneyUrothelialCarKroeseM3.(d)
================================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =      1.63636
                        t_0    =            0   Specified
                        beta_0 = 3.78734e-027
                        beta_1 = 1.59278e-027
                        beta_2 =   2.718e-024
                        beta_3 = 4.96063e-007


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               1.74897          3.79403           -5.68719             9.18512
         beta_0                0               NA
         beta_1                0               NA
         beta_2                0               NA
         beta_3     3.11107e-007     4.90313e-006       -9.29885e-006        9.92107e-006

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


                Log(likelihood)   # Param            AIC
   Fitted Model        -11.3978         5         32.7956
```

Data Summary

DOSE	CONTEXT C	F	I	U	Total	Expected	Response
0	52	0	0	0	52	0.00	
0.54	52	0	0	0	52	0.01	
1.8	52	0	0	0	52	0.29	
5.2	49	0	3	0	52	2.71	

Benchmark Dose Computation

```
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104
```

Specified effect =	0.1	0.01	0.001
BMD =	4.64886	2.12413	0.984449
BMDL =	2.49972	0.734665	0.0748097
BMDU =	9.01023	3.49311	1.61892

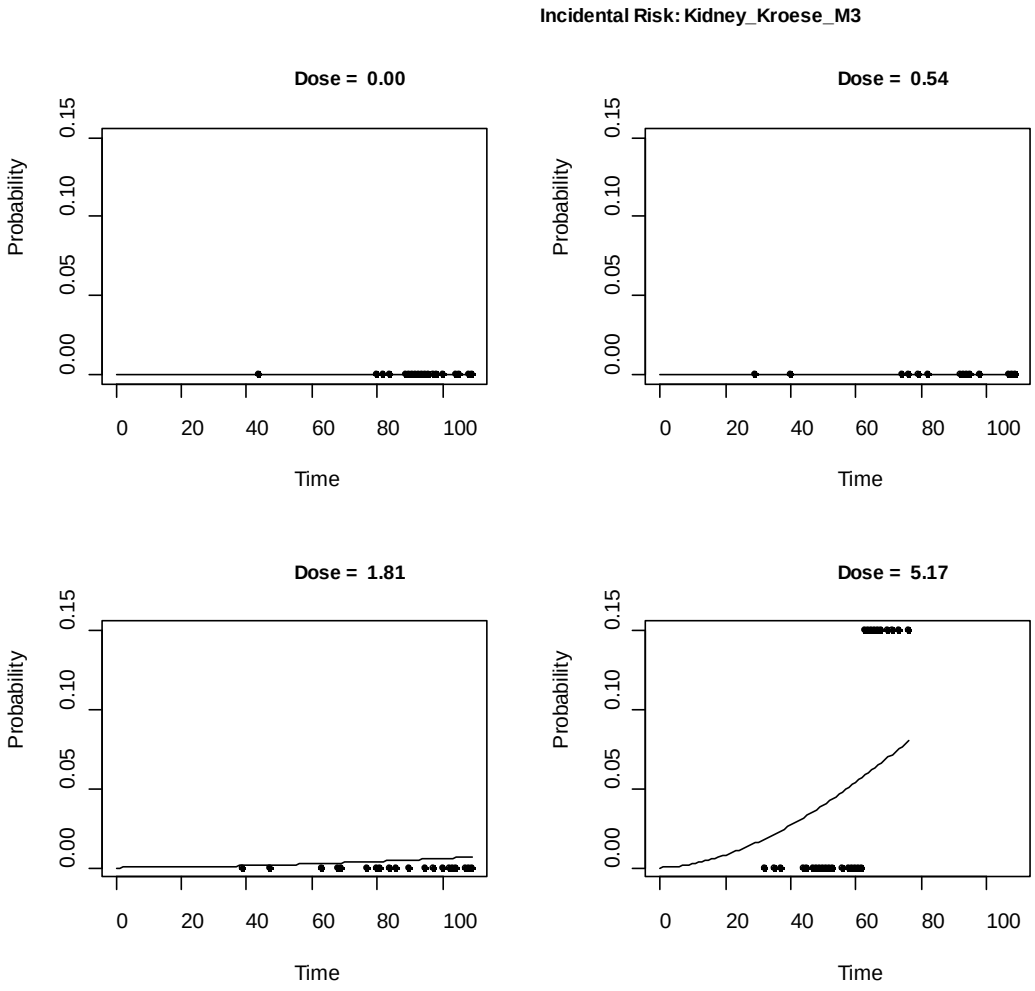


Figure E-22. Fit of multistage Weibull model to kidney urothelial tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Female rat (Kroese et al., 2001): Oral cavity or forestomach, squamous cell papilloma or carcinoma

```
=============================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: OralForstKroeseF3.(d)
=============================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =          3.6
                        t_0    =      45.1111
                        beta_0 = 1.11645e-009
                        beta_1 = 4.85388e-009
                        beta_2 =            0
                        beta_3 = 1.95655e-008

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            t_0          beta_0       beta_1       beta_3

    c                 1        -0.79        -0.92        -0.93           -1

    t_0           -0.79            1         0.73         0.72          0.8

    beta_0        -0.92         0.73            1         0.79         0.92

    beta_1        -0.93         0.72         0.79            1         0.91

    beta_3           -1          0.8         0.92         0.91            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.52871         0.701117            2.15454             4.90287
         t_0             46.553          5.93306            34.9244             58.1816
         beta_0     1.53589e-009     5.40523e-009       -9.05817e-009        1.21299e-008
         beta_1     7.57004e-009      2.9647e-008       -5.05369e-008         6.5677e-008
         beta_2                0               NA
         beta_3     2.53126e-008     7.66404e-008         -1.249e-007        1.75525e-007

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


                Log(likelihood)   # Param            AIC
   Fitted Model        -94.5119         6         201.024
```

```
                  Data Summary
                      CONTEXT
             C      F      I      U  Total  Expected Response
    DOSE
        0     51      0      1      0     52     1.14
     0.49     46      0      6      0     52     4.90
      1.6     22      0     30      0     52    31.81
      4.6      2      7     43      0     52    49.43

    Minimum observation time for F tumor context =         58

   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


   Specified effect =  0.1                0.01                 0.001
                BMD =  0.538801           0.0981283            0.0100797
               BMDL =  0.328135           0.0345104            0.00344714
               BMDU =  0.717127           0.325909             > 0.0806373
```

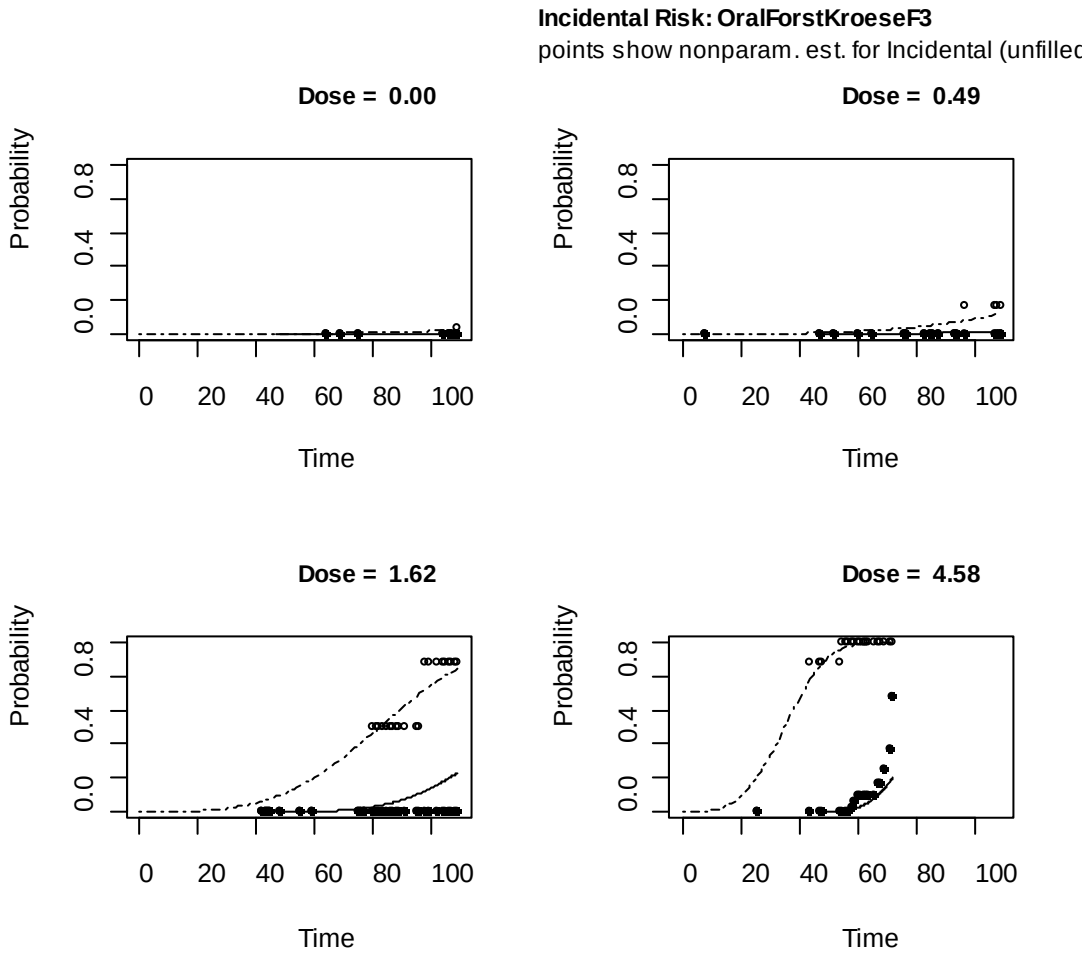


Figure E-23. Fit of multistage Weibull model to squamous cell papillomas or carcinomas in oral cavity or forestomach of female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Table E-26. Summary of alternative BMD modeling results for squamous cell papillomas or carcinomas in oral cavity or forestomach of female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001): poly-3 adjusted incidences[a]

Model	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Comments
	p-value	AIC			
Multistage 3°	0	63,912	4.92×10^{-7}	4.92×10^{-7}	Among multistage models, only the two-stage model provided an acceptable fit.
Multistage 2°	**0.991**	**92.349**	**0.435**	**0.228**	
Quantal-Linear	0.0174	100.65	0.139	0.110	
Gamma	0.873	92.397	0.446	0.279	Among other dichotomous models, BMD_{10} values ranged from 0.435 to 0.516 and $BMDL_{10}$ values ranged from 0.258 to 0.395.
Dichotomous-Hill LogLogistic	0.369	93.694	0.474	0.333	
Logistic	0.804	90.817	0.516	0.395	
Probit	0.938	90.482	0.471	0.364	
LogProbit	0.559	92.913	0.466	0.338	
Weibull	0.991	92.349	0.435	0.258	

[a]Doses: 0 mg/kg-day 1/49
0.49 mg/kg-day 6/42
1.6 mg/kg-day 30/39
4.6 mg/kg-day 50/50

Female rat (Kroese et al., 2001): Hepatocellular adenoma or carcinoma

```
=================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: LiverKroeseF3.(d)
         Fri Apr 16 09:08:03 2010
 =================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                        c      =          3.6
                        t_0    =      31.7778
                        beta_0 =            0
                        beta_1 =  4.9104e-031
                        beta_2 = 5.45766e-030
                        beta_3 = 3.44704e-008


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -beta_0    -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            t_0          beta_3

    c                 1         -0.9           -1

    t_0            -0.9            1         0.92

    beta_3           -1         0.92            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.11076         0.549208            2.03434             4.18719
         t_0            38.6965          5.21028            28.4846             48.9085
         beta_0               0               NA
         beta_1               0               NA
         beta_2               0               NA
         beta_3     2.94354e-007    7.19418e-007       -1.11568e-006       1.70439e-006

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


              Log(likelihood)   # Param            AIC
   Fitted Model         -228.17         6         468.34
```

```
                Data Summary
                    CONTEXT
             C      F      I      U  Total  Expected Response
  DOSE
      0     52      0      0      0     52     0.00
   0.49     51      0      1      0     52     3.02
    1.6     13     12     27      0     52    38.36
    4.6      1     38     13      0     52    51.36

  Minimum observation time for F tumor context =         44


 Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


 Specified effect =  0.1           0.01           0.001
              BMD =  0.575127      0.262783       0.12179
             BMDL =  0.506633      0.134213       0.0152934
             BMDU =  0.629806      0.287232       0.133064
```

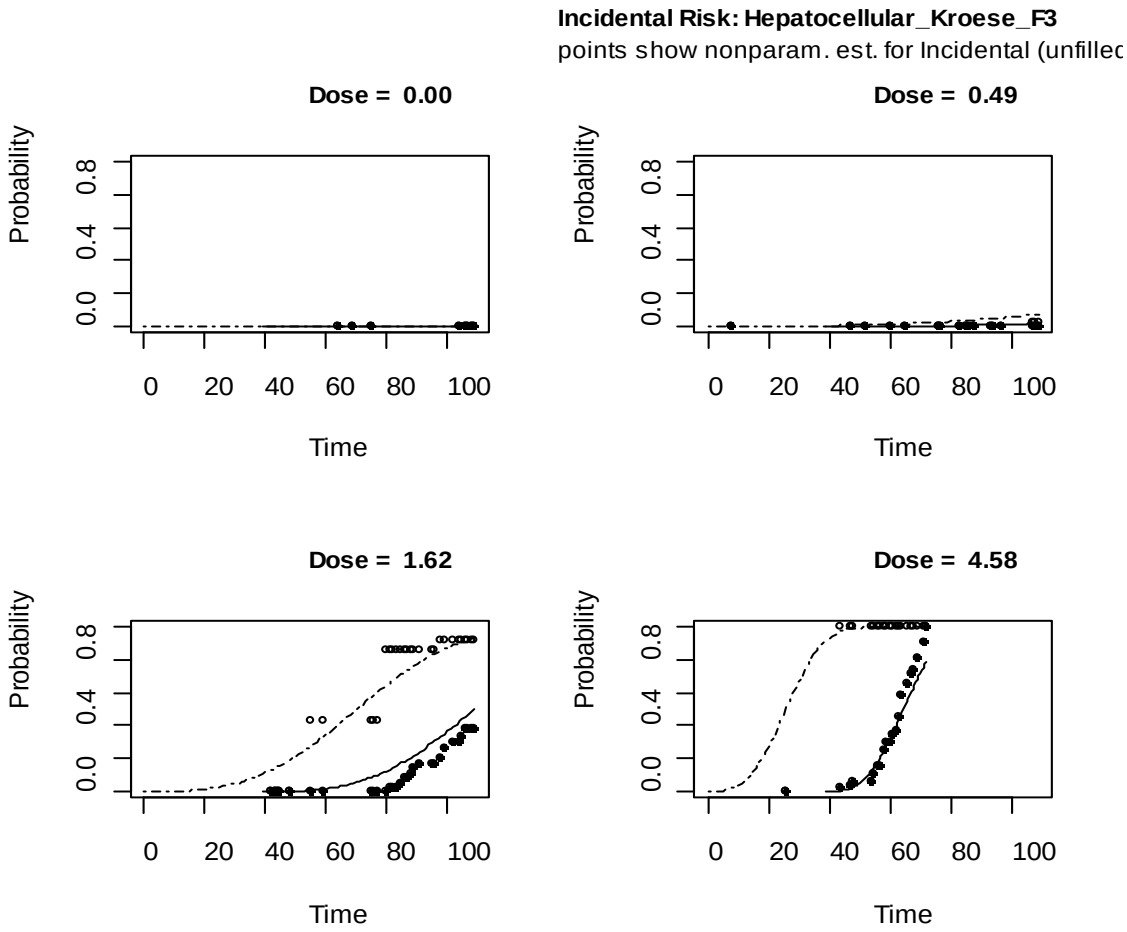


Figure E-24. Fit of multistage Weibull model to hepatocellular adenomas or carcinomas in female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Female rat (Kroese et al., 2001): Duodenum or jejunum adenocarcinoma

```
=================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: DuoJejKroeseF3.(d)
 =================================================================
   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 208
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3


   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                         c      =         2.25
                         t_0    =            0   Specified
                         beta_0 =            0
                         beta_1 =            0
                         beta_2 =            0
                         beta_3 =   7.289e-008


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_1    -beta_2
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               2.32531          3.58729           -4.70565            9.35626
         beta_0                0               NA
         beta_1                0               NA
         beta_2                0               NA
         beta_3     5.32209e-008     7.98487e-007       -1.51178e-006        1.61823e-006

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


               Log(likelihood)   # Param            AIC
   Fitted Model        -13.8784         5         37.7569


                   Data Summary
                       CONTEXT
```

DOSE	C	F	I	U	Total	Expected Response
0	52	0	0	0	52	0.00
0.49	52	0	0	0	52	0.01
1.6	52	0	0	0	52	0.44
4.6	48	0	4	0	52	3.57

Benchmark Dose Computation

Risk Response	= Incidental
Risk Type	= Extra
Confidence level	= 0.9
Time	= 104

Specified effect	=	0.1	0.01	0.001
BMD	=	3.43129	1.56781	0.726615
BMDL	=	1.94745	0.560867	0.0584891
BMDU	=	5.70108	2.61447	1.21046

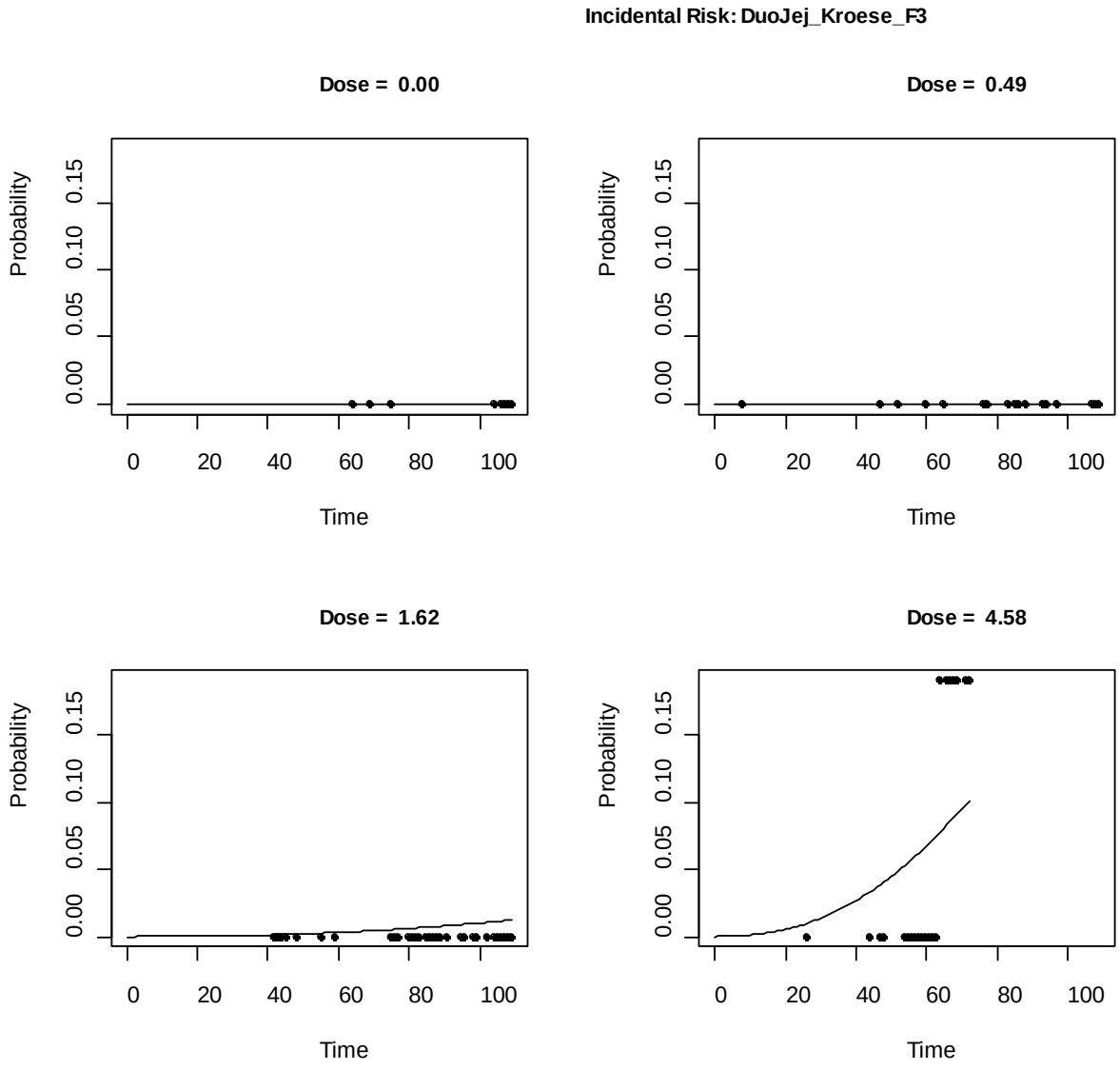


Figure E-25. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Table E-27. Summary of human equivalent overall oral slope factors, based on tumor incidence in male and female Wistar rats exposed to benzo[a]pyrene by gavage for 104 weeks (Kroese et al., 2001)

Data set	Tumor site	BMD_{001}	$BMDL_{001}$	Risk value[a] at BMD_{001}	Risk value[a] at $BMDL_{001}$	SD	SD^2	Proportion of total variance
Males	Oral cavity/ forestomach	6.37×10^{-3}	2.86×10^{-3}	1.57×10^{-1}	3.50×10^{-1}	1.17×10^{-1}	1.38×10^{-2}	0.64
	Liver	2.00×10^{-2}	5.30×10^{-3}	5.00×10^{-2}	1.89×10^{-1}	8.42×10^{-2}	7.09×10^{-3}	0.33
	Duodenum/ jejunum	6.42×10^{-1}	4.21×10^{-2}	1.56×10^{-3}	2.38×10^{-2}	1.35×10^{-2}	1.82×10^{-4}	0.01
	Skin/mammary gland: basal cell	6.06×10^{-1}	4.24×10^{-2}	1.65×10^{-3}	2.36×10^{-2}	1.33×10^{-2}	1.78×10^{-4}	0.01
	Skin/mammary gland: squamous cell	7.06×10^{-2}	2.11×10^{-2}	1.42×10^{-2}	4.75×10^{-2}	2.03×10^{-2}	4.10×10^{-4}	0.02
	Kidney	9.84×10^{-1}	7.48×10^{-2}	1.02×10^{-3}	1.34×10^{-2}	7.51×10^{-3}	5.64×10^{-5}	0.00
	Sum, risk values at BMD_{001}:			2.25×10^{-1}	Sum, SD^2:		2.17×10^{-2}	
	Overall SD[b]:						1.47×10^{-1}	
	Upper bound on sum of risk estimates[c]:				4.68×10^{-1}			
Females	Oral cavity/ forestomach	3.45×10^{-3}	1.01×10^{-2}	2.90×10^{-1}	9.92×10^{-2}	1.16×10^{-1}	1.35×10^{-2}	0.91
	Liver	1.53×10^{-2}	1.22×10^{-1}	6.54×10^{-2}	8.21×10^{-3}	3.48×10^{-2}	1.21×10^{-3}	0.08
	Duodenum/ jejunum	5.85×10^{-2}	7.27×10^{-1}	1.71×10^{-2}	1.38×10^{-3}	9.56×10^{-3}	9.13×10^{-5}	0.01
	Sum, risk values at BMD_{001}:			1.09×10^{-1}	Sum, SD^2:		1.48×10^{-2}	
	Overall SD:						1.22×10^{-1}	
	Upper bound on sum of risk estimates[c]:				3.09×10^{-1}			

[a]Risk value = $0.001/BMDL_{001}$.
[b]Overall SD = $(\text{sum, } SD^2)^{0.5}$.
[c]Upper bound on the overall risk estimate = sum of BMD_{001} risk values + 1.645 × overall SD.

Table E-28. Summary of BMD model selection among multistage-Weibull models fit to alimentary tract tumor data for female B6C3F$_1$ mice exposed to benzo[a]pyrene for 2 years (Beland and Culp, 1998)

Model stages	AIC	BMD_{10}[a]	$BMDL_{10}$–$BMDU_{10}$[a]	Basis for model selection
1	688.5	0.104		
2	629.2	0.102		
3	**624.5**	**0.127**	**0.071–0.179**	**Lowest AIC, best fit to low dose data**

[a]Corresponding to lifetime exposure (104 weeks).

Female mouse (Beland and Culp, 1998): Alimentary tract squamous cell tumors

```
=============================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: C:\msw10-09\benzo[a]pyrene_FemaleSquamF3i.(d)
=============================================================================

   The form of the probability function is:
   P[response] = 1-EXP$$-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = Class
   Independent variables = Dose, time

 Total number of observations = 191
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                User Inputs Initial Parameter Values
                        c      =            2
                        t_0    =           15
                        beta_0 =     1.6e-014
                        beta_1 =            0
                        beta_2 =     5.5e-012
                        beta_3 =     4.4e-012


          Asymptotic Correlation Matrix of Parameter Estimates
                c            t_0          beta_0       beta_1       beta_2       beta_3

    c                1        -0.78        -0.97        -0.42        -0.99        -0.99

    t_0           -0.78            1         0.76         0.39         0.74         0.84

    beta_0        -0.97         0.76            1         0.33         0.97         0.96

    beta_1        -0.42         0.39         0.33            1         0.31         0.46

    beta_2        -0.99         0.74         0.97         0.31            1         0.97

    beta_3        -0.99         0.84         0.96         0.46         0.97            1


                                Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              6.92317          1.33874            4.29929             9.54705
         t_0            13.9429          4.96646            4.20881              23.677
         beta_0     2.46916e-016     1.47619e-015       -2.64636e-015       3.14019e-015
         beta_1               0     1.30525e-014       -2.55825e-014
         beta_2     5.85452e-014     3.75144e-013       -6.76723e-013       7.93813e-013
         beta_3     9.76542e-014     5.62017e-013       -1.00388e-012       1.19919e-012


              Log(likelihood)   # Param            AIC
  Fitted Model        -306.265         6          624.53
```

```
                 Data Summary
                     Class
            C      F      I      U  Total  Expected Response
    Dose
        0     47      0      1      0     48     0.93
      0.1     45      0      3      0     48     3.21
     0.48      8     23     15      1     47    30.82
      2.3      1     46      0      1     48    41.91

    Minimum observation time for F tumor context =         39


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Specified effect =            0.1
Confidence level =            0.9

Time             =            104

             BMD =       0.126983
            BMDL =      0.0706103
            BMDU =       0.179419
```

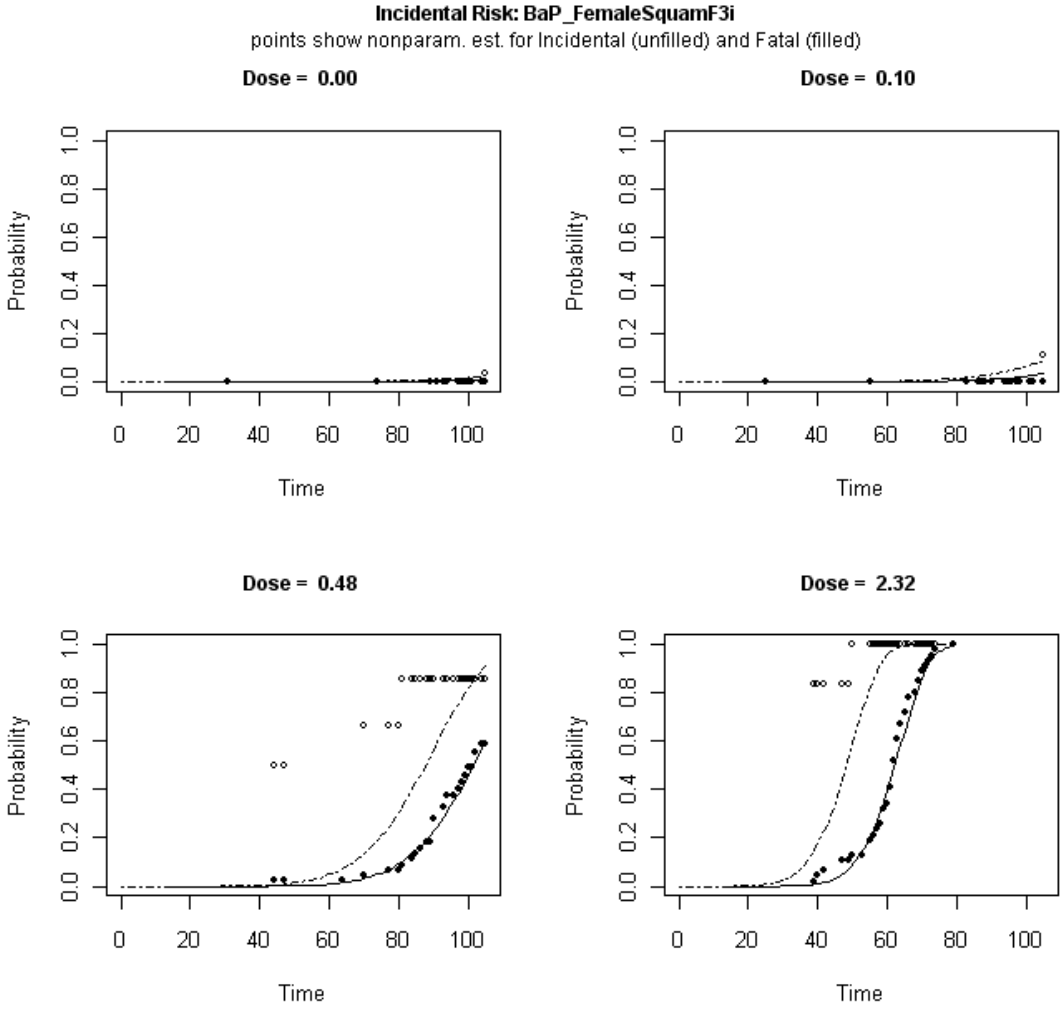


Figure E-26. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001).

Table E-29. Summary of alternative BMD modeling results for alimentary tract squamous cell tumors in female B6C3F$_1$ mice exposed to benzo[a]pyrene for 2 years (Beland and Culp, 1998): poly-3 adjusted incidences[a]

Model[a]	Goodness of fit		BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	Comments
	p-value	AIC			
Multistage 3°	1.000	72.015	0.138	0.0712	Among multistage models, 2-stage model provided most parsimonious fit
Multistage 2°	**0.845**	**70.371**	**0.113**	**0.0730**	
Quantal-Linear	0.0049	83.200	0.0358	0.0274	
Gamma	1.000	72.015	0.129	0.0815	Other dichotomous models provided BMD_{10} values ranging from 0.123 to 0.150, and $BMDL_{10}$ values ranging from 0.079 to 0.110.
Dichotomous-Hill LogLogistic	0.803	72.133	0.129	0.0857	
Logistic	0.999	70.016	0.150	0.110	
Probit	0.972	70.070	0.134	0.101	
LogProbit	0.956	72.021	0.123	0.0859	
Weibull	1.000	72.015	0.135	0.0793	

[a]Doses: 0 mg/kg-day 1/43
0.1 mg/kg-dat 3/41
0.48 mg/kg-day 38/44
2.3 mg/kg-day 46/46

E.2.2. Dose-Response Modeling for the Inhalation Unit Risk

E.2.2.1. *Modeling Methods*

As with the tumor data used for the oral slope factor (see Section E.2.1, *Dose Response-modeling for the Oral Slope Factor*), there was earlier occurrence of tumors with increasing exposure, and early termination of the high-dose group (Thyssen et al., 1981; see Appendix D for study details). The software program Multistage Weibull (U.S. EPA, 2010b) was used as described in the analysis of the oral carcinogenicity data. See Section E.2.1 for details of the modeling methods. A previous time-to-tumor analysis (U.S. EPA, 1990a) was not used because of several discrepancies between the summarized dose-response data and the individual pathology reports, because the use of age at necropsy rather than the time since first exposure, and because multistage Weibull provides a corrected estimate of the confidence bounds on the BMD.

E.2.2.2. *Data Adjustments Prior to Modeling*

As with the oral slope factor (see Section E.2.1, *Dose Response-modeling for the Oral Slope Factor*), etiologically similar tumor types (i.e., benign and malignant tumors of the same cell type) were combined for dose-response modeling. Here the benign tumors (papillomas, polyps, and papillary polyps) were judged to be of the same cell type as the squamous cell carcinomas (SCCs). As described in Section 2.4.2, the overall incidences of benign or malignant tumors in the respiratory tract (larynx, trachea, and nasal cavity) and pharynx were used for dose-response modeling.

Thyssen et al. (1981) did not determine cause of death for any of the animals. Although the (U.S. EPA, 1990a) analysis made use of judgments from an independent toxicologist about the likely causes of death for each animal, these judgments were not available for this assessment. Since the investigators for the oral bioassays considered the same tumor types to be fatal at least some of the time, bounding estimates for the Thyssen et al. (1981) data were developed by treating the tumors alternately as either all incidental or all fatal. In either case, therefore, an estimate of t_0 (the time between a tumor first becoming observable and causing death) could not be estimated and was set to 0. The data analyzed are summarized in Table E-30. Animals without confirmation of one or more of the pharynx or respiratory tract tissues being examined were not included in the incidences, unless a tumor was diagnosed in those that were examined. Group average TWA continuous exposures, based on chamber air monitoring data and individual hamsters' time on study, of 0, 0.25, 1.01, and 4.29 mg/m^3 corresponded to the 0, 2, 10, and 50 mg/m^3 nominal study concentrations, respectively (U.S. EPA, 1990a).

Table E-30. Individual pathology and tumor incidence data for male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime—Thyssen et al. (1981)[a]

Exposure concentration: target (lifetime average continuous exposure)[b], mg/m³	Time of tumor observed (wk)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach	
0	16	0	—[c]	0	0	0	0	—
(0)	39	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0
	79	0	0	0	0	0	0	0
	82	0	0	0	—	0	0	—
	85	0	—	0	0	0	0	—
	85	0	0	0	0	0	0	0
	87	0	0	0	0	0	0	0
	87	0	0	0	0	0	0	0
	88	0	0	0	0	0	0	0
	88	0	0	0	0	0	0	0
	89	0	0	0	0	0	0	0
	101	0	0	0	0	0	0	0
	101	0	0	0	0	0	0	0
	103	0	0	0	0	0	0	0
	106	0	0	0	0	0	0	0
	107	0	0	0	0	0	0	0
	109	0	0	0	0	0	0	0
	111	0	0	0	0	0	0	0
	114	0	0	0	0	0	0	0
	115	0	—	0	0	0	0	—
	121	0	0	0	0	0	0	0
	122	0	0	0	0	0	0	0
	124	—	0	0	0	0	0	—
	124	0	0	0	0	0	0	0
	126	0	—	0	0	0	0	—
	131	0	0	0	0	0	0	0
2	13	—	—	—	0	0	0	—
(0.25)	35	0	0	0	0	0	0	0
	53	0	0	0	0	0	0	0
	58	0	0	0	0	0	0	0
	70	0	0	0	0	0	0	0
	77	0	0	0	0	0	0	0
	79	0	0	0	0	0	0	0
	84	0	0	0	0	0	0	0
	87	0	0	0	0	0	0	0
	93	0	0	0	0	0	0	0
	97	0	—	0	0	0	0	—

Exposure concentration: target (lifetime average continuous exposure)[b], mg/m³	Time of tumor observed (wk)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach	
	99	0	0	0	0	0	0	0
	102	0	0	0	0	0	0	0
	102	0	0	0	0	0	0	0
	108	0	0	0	0	0	0	0
	113	0	0	0	0	0	0	0
	114	0	0	0	0	0	0	0
	115	0	0	0	0	0	0	0
	115	0	0	0	0	0	0	0
	119	0	0	0	0	0	0	0
	121	—	—	0	0	0	0	—
	132	0	0	0	0	0	0	0
10 (1.01)	30	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0
	51	0	0	0	0	0	0	0
	66	0	0	0	0	0	0	0
	73	0	0	0	0	0	0	0
	76	0	1 (1)	0	0	0	0	1
	76	0	1 (1)	0	0	0	0	1
	80	1 (1)[d]	0	0	0	0	0	0
	85	0	0	0	0	0	0	0
	93	1 (1)	0	0	0	0	0	1
	99	0	0	0	0	0	0	0
	102	0	1 (0)	0	0	0	0	1
	105	1 (1)	1 (1)	0	0	0	0	1
	110	0	1 (1)	0	0	0	0	1
	113	0	1 (0)	0	0	0	0	1
	114	1 (1)	1 (1)	0	0	0	0	1
	115	1 (1)	—	1 (0)	1 (0)	0	0	1
	115	0	—	1 (0)	1 (1)[e]	0	0	1
	116	1 (0)	—	0	0	0	0	1
	117	1 (1)	1 (1)	0	0	0	0	1
	118	1 (0)	0	0	0	0	0	1
	118	0	—	0	0	0	0	—
	118	1 (1)	0	0	1 (0)	0	1 (1)	1
	121	1 (0)	0	0	0	0	0	1
	124	1 (1)	1 (1)	0	0	0	0	1
	124	0	0	0	1 (0)	0	0	1
50 (4.29)	21	—	—	—	0	0	0	—
	22	—	—	—	0	0	0	—
	25	—	—	—	0	0	0	—
	30	—	—	—	0	0	0	—

Exposure concentration: target (lifetime average continuous exposure)[b], mg/m³	Time of tumor observed (wk)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach	
	30	0	—	0	0	0	0	—
	30	—	—	—	0	0	0	—
	35	—	—	—	0	0	0	—
	36	0	0	0	0	0	0	0
	36	—	—	—	0	0	0	—
	38	—	—	—	0	0	0	0
	40	0[f]	1 (1)	1 (0)	0	0	0	1
	41	0	0	0	0	0	0	0
	41	—	—	—	0	0	0	—
	42	0	0	0	0	0	0	0
	42	0	0	0	0	0	0	0
	46	1 (1)	1 (1)	0	0	0	0	1
	47	0	1 (1)	0	0	0	0	1
	53	0	—	0	0	0	0	—
	55	1 (1)	1 (1)	0	0	0	0	1
	56	0	1 (1)	0	0	0	0	1
	60	0	1 (1)	0	0	0	0	1
	62	0	0	0	0	0	0	0
	63	0	1 (0)	0	0	0	1 (0)	1
	66	1 (1)	1 (1)	0	0	—	—	1
	67	0	1 (1)	0	0	0	0	1
	69	1 (0)	1 (1)	0	0	1 (0)	0	1
	71	1 (1)	1 (1)	1 (1)	0	0	0	1
	71	1 (0)	1 (1)	0	0	0	1 (0)	1
	72	1 (1)	1 (1)	0	0	0	0	1
	72	1 (1)	1 (1)	0	0	0	0	1
	82	0	1 (1)	0	0	0	0	1
	82	1 (1)	1 (1)	0	0	1 (0)	0	1
	83	1 (0)	1 (1)	0	0	0	0	1
	102[g]	1 (1)	1 (1)	1 (0)	1 (0)	0	0	1

[a]Histopathology incidence from U.S. EPA (1990a); Clement Associates (1990).
[b]See Section D.4.2.
[c]Tissue was not examined.
[d]In situ carcinoma; not included in overall tumor incidence.
[e]Adenocarcinoma; not included in overall tumor incidence.
[f]Metastasis from pharynx not shown.
[g]Necropsy occurred 24 weeks after 79 weeks of exposure.

E.2.2.3. *Sensitivity Analyses*

Alternative dose-response models and alternative assumptions regarding missing observations and latency estimates were also conducted. First, alternative dose-response models were considered through applying dichotomous models in BMDS to summary incidence data for each exposure group, adjusted for early mortality using the poly-3 technique (Bailer and Portier, 1988).

E.2.2.4. *Dose-Response Modeling Results*

Table E-31 summarizes the modeling results supporting the derivation of an inhalation unit risk value for benzo[a]pyrene. The model outputs and graphs (Figures E-27 and E-28) following Table E-31 provide more details for the best-fitting models under the conditions of taking all tumors to be incidental to the cause of death, or to be the cause of death, respectively.

The sensitivity analyses of Section E.2.2.3 are summarized in Table E-32.

Table E-31. Summary of BMD model selection among multistage-Weibull models fit to tumor data for male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime (Thyssen et al., 1981)

Tumor context	Model stages	AIC	BMD_{10}[a]	$BMDL_{10}$[a]	Basis for model selection
All tumors considered incidental to cause of death	1 **2**	50.5 **40.4**	0.076 **0.254**	0.052 **0.163**	**Lowest AIC, best fit to data ($BMDU_{10}$ = 0.324)**
All tumors considered to be cause of death	1 **2**	315.0 **302.9**	0.135 **0.468**	0.104 **0.256**	**Lowest AIC; best fit to data ($BMDU_{10}$ = 0.544)**

[a]Corresponding to lifetime exposure (104 weeks).

Output for squamous cell neoplasia following inhalation exposure to benzo[a]pyrene: all tumors considered incidental to cause of death

```
================================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: ThyssenI2sL104noUw.(d)
         Fri Oct 14 10:23:57 2016
 ================================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 88
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                         c      =          4.5
                         t_0    =            0   Specified
                         beta_0 = 8.02969e-034
                         beta_1 = 5.12551e-032
                         beta_2 = 1.30309e-009


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_1
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_2

    c                 1           -1

    beta_2           -1            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               4.70606         0.953708            2.83682             6.57529
         beta_0                0               NA
         beta_1                0               NA
         beta_2     5.29609e-010     2.21617e-009       -3.81401e-009        4.87323e-009

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


               Log(likelihood)   # Param            AIC
   Fitted Model          -16.18         4          40.36


                    Data Summary
                        CONTEXT
```

```
            C      F      I      U  Total
DOSE
     0     21      0      0      0     21
  0.25     19      0      0      0     19
     1      8      0     17      0     25
   4.3      5      0     18      0     23


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Specified effect =            0.1
Confidence level =            0.9

Time             =            104

             BMD =       0.253061
            BMDL =       0.163183
            BMDU =       0.318982
```

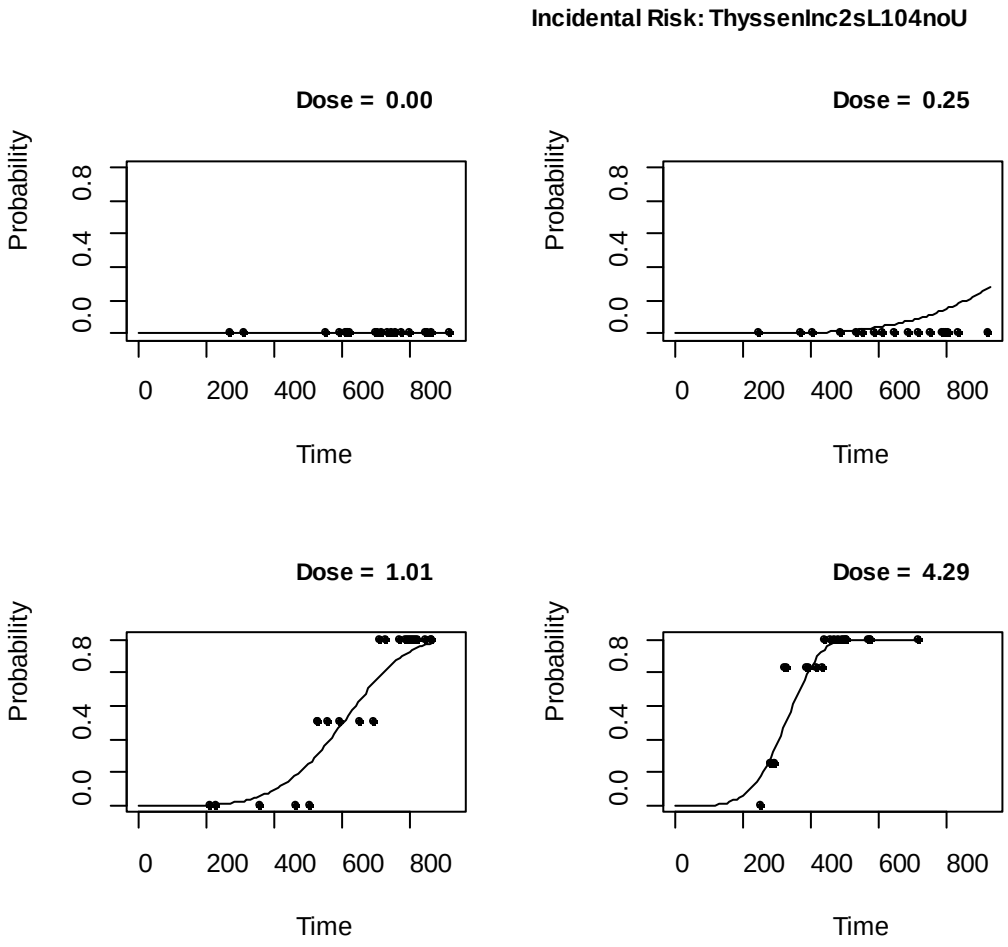


Figure E-27. Fit of multistage Weibull model to respiratory tract tumors in male hamsters exposed via inhalation to benzo[a]pyrene (Thyssen et al., 1981); tumors treated as incidental to death.

Output for respiratory tract tumors: All tumors considered to be cause of death

```
=====================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: ThyssenF2sL104noU.(d)
         Thu Mar 13 14:30:45 2014
 =====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 88
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008


                  Default Initial Parameter Values
                         c      =            6
                         t_0    =            0   Specified
                         beta_0 =  2.0496e-036
                         beta_1 = 4.12988e-014
                         beta_2 = 3.37033e-013


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_1
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 c            beta_2

    c                 1           -1

    beta_2           -1            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               6.61992         0.915036             4.82649             8.41336
         beta_0                0               NA
         beta_1                0               NA
         beta_2     2.13816e-014     8.96466e-014       -1.54323e-013        1.97086e-013

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


                Log(likelihood)   # Param            AIC
   Fitted Model         -147.66         4         303.319
```

```
                  Data Summary
                      CONTEXT
             C      F      I      U  Total
    DOSE
        0    21      0      0      0     21
     0.25    19      0      0      0     19
        1     8     17      0      0     25
      4.3     5     18      0      0     23

    Minimum observation time for F tumor context =         40

   Benchmark Dose Computation
Risk Response    =          Fatal
Risk Type        =          Extra
Specified effect =            0.1
Confidence level =            0.9

Time             =            104

             BMD =       0.467752
            BMDL =       0.256206
            BMDU =       0.543965
```

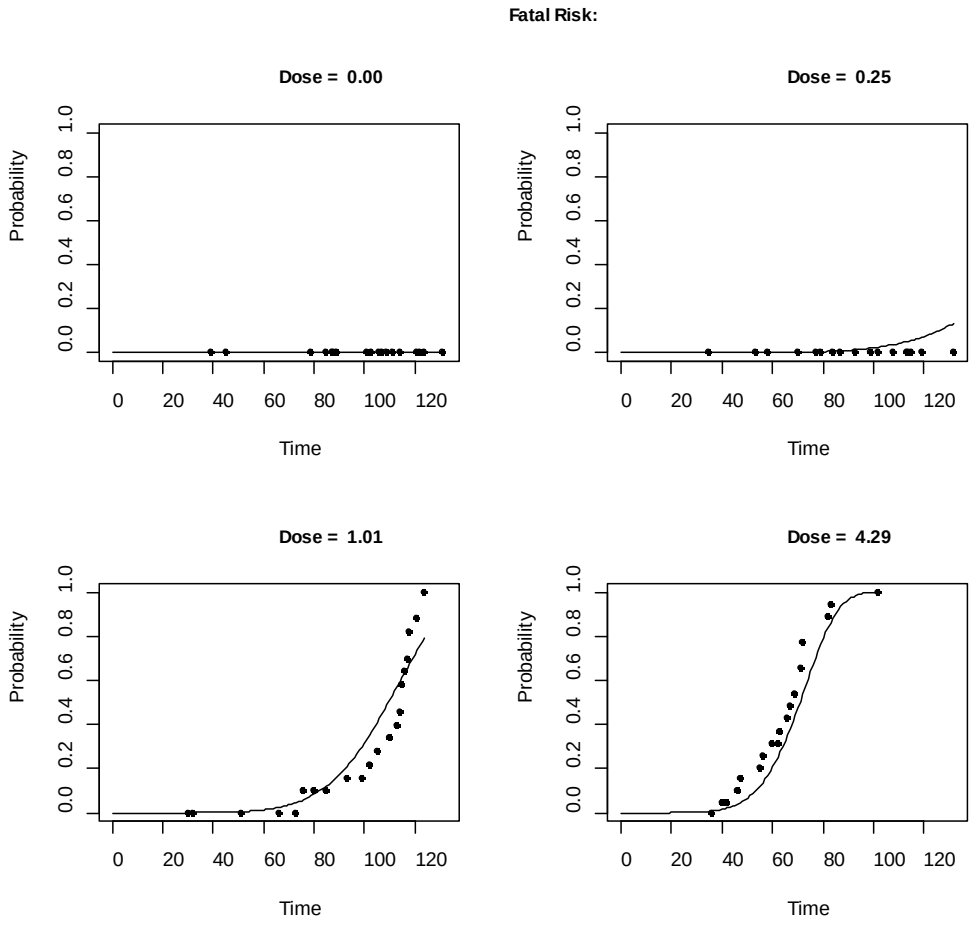


Figure E-28. Fit of multistage Weibull model to respiratory tract tumors in male hamsters exposed via inhalation to benzo[a]pyrene (Thyssen et al., 1981); tumors treated as cause of death.

Table E-32. Summary of alternative dose-response modeling results for respiratory tumors in male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime (Thyssen et al., 1981)

Model	Goodness of fit		BMD_{10} (mg/m^3)	$BMDL_{10}$ (mg/m^3)	Comments
	p-value	AIC			
Dichotomous models applied to poly-3 adjusted group incidences[a]					
Gamma	0.0057	52.800	0.175	0.0990	Excepting the dichotomous Hill model, no models fit adequately. The dichotomous
Dichotomous-Hill	1.000	42.606	0.784	0.635	
Logistic	0	69.973	0.395	0.278	

Model	Goodness of fit		BMD_{10} (mg/m^3)	$BMDL_{10}$ (mg/m^3)	Comments		
	p-value	AIC					
LogLogistic	0.0374	46.943	0.291	0.149	Hill model fit 4 parameters to the observed data, with the exponent set at the lower allowable limit (1), and yielded an implausible shape in the region near the BMR (≤10% extra risk).		
Probit	0	71.796	0.465	0.343			
LogProbit	0.0334	47.964	0.276	0.142			
Weibull[b], Multistage 2°, 1°	0.0246	50.980	0.136	0.0979			
Multistage 3°[c]	0.0246	50.980	0.136	0.0979			
Multistage 2°: highest dose group dropped	0.449	29.98	0.290	0.186	Adequate fit		
Multistage Weibull model, with alternative assumptions					**Parameter estimates[d]**		
					t0 (wks)	**c**	**b2**
Benign tumors->incidental, malignant tumors->fatal	N/A[e]	281.108	0.337	0.198	14.5[f]	5.4	1.4×10^{-11}
All tumors fatal[g]	N/A	302.9	0.468	0.256	0	6.6	2.1×10^{-14}
All tumors considered incidental, latency fixed[e]	N/A	191.29	0.431	0.245	5	6.5	5.4×10^{-14}
	N/A	115.39	0.364	0.238	15	6.2	2.3×10^{-13}
	N/A	51.62	0.261	0.184	45	5.4	1.8×10^{-11}
	N/A	40.45	0.252	0.164	90	4.7	4.8×10^{-10}
Incidental tumors; missing diagnoses assumed negative	N/A	50.68	0.286	0.190	0	4.8	2.3×10^{-14}
All tumors incidental[g]	N/A	40.36	0.254	0.163	0	4.7	5.2×10^{-14}

[a]Exposure (mg/m^3) Poly-3 adjusted Incidence (denominators address all animals with missing diagnoses)
0 0/24
0.25 0/18
1.01 17/23
4.29 18/20

[b]For the Weibull model, the power parameter estimate was 1 (boundary of parameter space). The models in this row all reduced to the Quantal-Linear model.

[c]The multistage 3° model differs from the models in the line just above (Weibull, etc.) in additional digits not displayed in the table.

[d]Models estimated b0 and b1 at 0; latency (t0) fixed at listed values unless noted otherwise.

[e]N/A: Goodness-of-fit tests are not available for the multistage Weibull model.

[f]Maximum likelihood estimate.

[g]Repeated from Table E-31, for comparison.

APPENDIX F. SUMMARY OF SAB PEER REVIEW COMMENTS AND EPA'S DISPOSITION

The draft *Toxicological Review of Benzo[a]pyrene,* dated September 2014, underwent a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2006). This peer review was conducted by the Chemical Assessment Advisory Committee (CAAC) augmented for the Integrated Risk Information System (IRIS) benzo[a]pyrene assessment (CAAC benzo[a]pyrene panel) of EPA's Science Advisory Board (SAB). An external peer review workshop was held on April 15–17, 2015. Public teleconferences of the SAB-CAAC benzo[a]pyrene panel were held on March 4, August 21, and September 2, 2015. The SAB held a public teleconference on January 26, 2016 to conduct a quality review of the draft peer review report. The final report of the SAB was released on April 5, 2016.

The SAB was tasked with providing feedback in response to charge questions that addressed scientific issues related to the hazard identification and dose-response assessment of benzo[a]pyrene, as well as EPA's disposition of major public comments. A summary of major recommendations of the SAB and EPA's responses to these recommendations, organized by charge question, follow.

Charge Question 1. The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection and Evaluation section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.

Comment: The EPA should specify whether the literature search strategy included a review of the references in the primary and secondary literature as a means to identify potentially relevant articles not identified through the systematic searching and manual screening processes, and EPA should conduct secondary literature searches as evidence for additional effects (e.g., cardio) or specific data gaps (e.g., mechanistic, in vitro studies) that emerged.

Response: Comprehensive literature searches of several databases were performed for benzo[a]pyrene in 2008 and 2012 (see Table LS-1 in the Toxicological Review). In addition to EPA's search of online databases, secondary references, primarily assessments by other health agencies, were consulted to ensure that critical studies were not missed by the literature search.

The database literature searches performed for benzo[a]pyrene were designed to search for all possible health outcomes of benzo[a]pyrene exposure, and as such, did not include terms for specific organs or endpoints. Instead, the literature search strategy used for benzo[a]pyrene was designed to use fewer, more comprehensive terms that capture many health outcomes, such as "benzo[a]pyrene", "toxicity" and "adverse effect." The use of these broad terms captures the vast majority of studies, likely more than would be identified with a more targeted literature search.

Many of the cardiovascular studies identified by the SAB as missing from the assessment were identified in early literature searches. However, these studies were not included because the assessment focused on endpoints that were established in subchronic or chronic oral and inhalation studies, rather than in vitro studies, studies using less environmentally-relevant routes of exposure, and studies in genetically modified animals or non-mammalian species.

In addition to the comprehensive search, iterative literature searches were conducted during the draft development process. For example, specialized searches were conducted during draft development to provide additional context for potential mechanisms of hazards identified from in vivo subchronic, chronic, and developmental studies. These additional searches of PubMed were conducted to fill data gaps and to help address peer review comments.

The assessment section entitled "Literature Search Strategy/Study Selection" has been updated to clarify these aspects of the literature search strategy.

Comment: The EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the point of departure (POD) assessment while the handbook that will outline the tools and processes is being developed.

Response: General considerations for the identification of pertinent studies, credible health hazards, and informative studies for dose-response analysis are discussed in the IRIS Preamble, which is included in the front matter of the IRIS Toxicological Review of Benzo[a]pyrene. Sections especially pertinent to the SAB comment include: Section 3, Identifying and Selecting Pertinent Studies; Section 4, Evaluating Study Methods and Quality; Section 6, Selecting Studies for Derivation of Toxicity Values; and Section 7, Deriving Toxicity Values.

Rationales specific to the benzo[a]pyrene database, which lead to the selection of key studies and the PODs, are discussed throughout the document starting with considerations for literature screening and evaluation in the Literature Search Strategy/Study Selection section of the document. Considerations for the selection of studies for dose-response analysis specific to the

benzo[a]pyrene database are discussed in Sections 2.1.1 (for the oral database) and 2.2.1 (for the inhalation database) of the Toxicological Review.

Charge Question 2a. The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?

The SAB concurred that the available human studies support the conclusion that benzo[a]pyrene exposure contributes to human developmental toxicity and that the available animal studies support this conclusion. The SAB subdivided this Charge Question into two parts: developmental neurotoxicity and developmental toxicity other than neurodevelopment. The SAB had the following specific recommendations:

Developmental neurotoxicity

Comment: The SAB recommended that rather than relying only on the elevated plus maze data and dismissing the Morris water maze data, all of the data in Chen et al. (2012) should be considered collectively, and viewed in their totality as evidence of a developmental neurobehavioral effect of neonatal benzo[a]pyrene exposure. The SAB also commented that the Least Significant Difference test may have been inappropriate for establishing the weight of evidence for developmental neurobehavioral effects.

Response: EPA agrees with this recommendation, and the revised assessment gives further consideration to all of the behavioral outcomes reported in Chen et al. (2012) for use in hazard identification and dose-response analyses. Specifically, text within Section 1.1.1 (e.g., "Neurodevelopmental Effects" and "Summary of Developmental Effects") of the revised assessment provides increased consideration of the following endpoints as collectively providing evidence of a neurodevelopmental effect: surface righting, negative geotaxis, open field activity, elevated plus maze, and Morris water maze.

Concerning the Least Significant Difference test, EPA agrees that this test can over-emphasize differences as significant that may not be (i.e., by underestimating p-values). Statistical significance testing was one of several factors in evaluating the weight of evidence, including evaluating magnitudes of effect, overall biological significance across the various time points evaluated, and consistency of the effects across similar protocols. Clarification to this effect was added to Table 1-4.

Ultimately, the revised assessment emphasized the totality of the evidence for behavioral effects assessed by Chen et al. (2012) for dose-response analyses. (See also response to Charge Question 3a.)

Comment: EPA should consider the significant exposure gaps in brain development in existing studies in the overall evaluation of benzo[a]pyrene developmental neurotoxicity.

Response: The EPA agrees that this is an important point. In the revised assessment, a figure arraying the exposure paradigms used across the available studies evaluating developmental neurotoxicity has been added to Section 1.1.1. This figure provides a visual representation of exposure gaps across the available developmental neurotoxicity studies.

Neurodevelopmental exposure gaps identified are now summarized in the Summary of Section 1.1.1 and considered more carefully in Section 2. Overall, the exposure gaps indicate that the available benzo[a]pyrene studies do not comprehensively cover the exposure periods pertinent to assessing the potential vulnerability of the developing nervous system to toxic insult, namely from implantation through adolescence.

Furthermore, since developmental neurotoxicity can be expressed differentially depending on both the timing of exposure and the endpoint measures assessed (noting that the mode of action for benzo[a]pyrene-induced neurotoxicity remains unknown), and because many studies in the database did not evaluate multiple parameters of nervous system structure and function, it is likely that at least some of the exposure periods examined were not adequately assessed. However, the available studies include a detailed evaluation of exposure during several developmental ages known to be sensitive for detecting developmental neurotoxicity. These include late gestation and the early neonatal period (although exposures combining these periods were not evaluated), during which substantial brain region-specific changes in proliferation, synaptogenesis, and perhaps most noticeably, growth, occur. These exposure gaps during sensitive periods of brain development were considered in the application of a database uncertainty factor (UF_D) to help address residual uncertainty associated with the potential for neurodevelopmental effects at lower doses (see also response to Charge Question 3a).

Developmental toxicity other than neurodevelopment

Comment: The SAB recommended that EPA conduct a more complete literature search on developmental toxicity of benzo[a]pyrene to characterize benzo[a]pyrene-mediated developmental toxicity. Specifically, several older teratology studies were suggested for inclusion (Shum et al., 1979; Nebert et al., 1977; Rigdon and Rennels, 1964). In addition, the SAB recommended consideration of a publication by Thakur et al. (2014), evaluating fetal benzo[a]pyrene-related effects on fetal lung growth and function.

Response: Several teratology studies were suggested for inclusion by the SAB (Shum et al., 1979; Nebert et al., 1977; Rigdon and Rennels, 1964). Two oral, high-dose teratology studies in rats (Rigdon and Rennels, 1964) and mice (Rigdon and Neal, 1965) were identified in the original comprehensive literature search for benzo[a]pyrene and were discussed in the supplementary

material in Appendix D. However, these older, high-dose teratology studies were generally limited in terms of study design, documentation of methods, and reporting of results (see Appendix D for details).

Two additional studies recommended by the SAB (Shum et al., 1979; Nebert et al., 1977), were considered to provide mechanistic information. Shum et al. (1979), a high-dose intraperitoneal (i.p.) study (200 mg/kg), suggests that developmental effects of benzo[a]pyrene may occur via the aryl hydrocarbon receptor (AhR) pathway. Similar developmental findings were reported in Nebert et al. (1977), which looked at developmental toxicity of two polycyclic aromatic hydrocarbons (PAHs) (3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene) in AhR responsive and non-responsive mice. These studies have been included in Section 1.1.1, Mode-of-Action Analysis—Developmental Toxicity and Developmental Neurotoxicity.

Regarding the study by Thakur et al. (2014) (also discussed under Charge Question 2e), this study reported increased susceptibility to lung injury in offspring of rat dams treated with high dose i.p. exposure (25 mg/kg) to benzo[a]pyrene on gestation days (GDs) 18–20 and subsequent challenge with hyperoxic air (85% O_2). However, interpretation of this study is complicated due to the route of exposure and the high doses employed, which were one to two magnitudes greater than doses at which effects were observed in the oral developmental database. Discussion of this study's implications regarding increased susceptibility to oxidative stress subsequent to benzo[a]pyrene exposure has been added to Section 1.1.1, Mode-of-Action Analysis—Developmental Toxicity and Developmental Neurotoxicity.

Comment: Adverse outcomes resulting from benzo[a]pyrene exposure should take into context the susceptible window of exposure (i.e., whether exposure occurs in early gestation, late gestation [GDs 6–12/15], or postnatal exposure).

Response: EPA agrees that the timing of developmental exposures can be a critical determinant of health effects observed in a particular study. The available developmental studies in the benzo[a]pyrene database often exposed animals during different windows of development. Specific durations of exposure are listed in the relevant evidence tables (see Tables 1-2 and 1-4) and discussed in the text. However, conclusions regarding the windows of development most relevant to benzo[a]pyrene-induced developmental effects cannot be made due to varying study design across studies. Increased discussion regarding the exposure timing of developmental studies has been added to the document in Sections 1.1.1 and 2.1.3.

Comment: The EPA should consider including mechanistic studies that provide perspectives on the likely mode of action leading to benzo[a]pyrene-related developmental toxicity. Specifically, the SAB recommended the addition of studies investigating the role of mechanisms such as genotoxicity and oxidative stress.

Response: Mechanistic information potentially informative of benzo[a]pyrene-related developmental effects is included in Section 1.1.1, Developmental Toxicity under the subsection Mode-of-Action Analysis—Developmental Toxicity and Neurodevelopmental Toxicity. Additional consideration has been given to the studies suggested for consideration. This section has been expanded to acknowledge potential developmental effects subsequent to genotoxic and mutagenic mechanisms in germline and fetal cells, as well as changes in oxidative stress as a possible contributing mechanism to developmental toxicity. Mechanistic references suggested by the peer reviewers have been considered and incorporated where relevant.

Comment: Toxicokinetic information regarding fetal exposures and lactational transfer should be included in the consideration of developmental hazard.

Response: Information regarding the potential for lactational transfer of benzo[a]pyrene has been added to the toxicokinetic information in Section D.1 of the Supplemental Information. In addition, a concise discussion of this information, as well as information on fetal distribution has been added to Section 1.1.1, Developmental Toxicity.

Charge Question 2b. The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

The SAB agreed that the data support the conclusion that benzo[a]pyrene is a male and female reproductive toxicant, with rodent data demonstrating convincingly that benzo[a]pyrene affects fertility and fecundity. The SAB had the following specific recommendations:

Comment: The SAB recommended that the EPA consider additional female reproductive endpoints for POD/benchmark dose (BMD) analyses and reference dose (RfD) derivation. The SAB suggested that decreased follicular counts be considered as well as uterine hyperplasia and inflammation observed in the Gao et al. (2011) study. The SAB recommended that EPA either include these endpoints or provide appropriate justification as to why they are not suitable for RfD determination.

Response: In response to the SAB recommendation to consider ovarian follicular counts further, decreased primordial follicles reported by Xu et al. (2010) were considered supportive of reproductive toxicity, as a depletion of follicles can result in shortening of a woman's reproductive lifespan (U.S. EPA, 1996). Means and standard deviations (SDs) were obtained from this graphically reported endpoint, modeled, and included for candidate value derivation in Section 2 (also see Appendix E.1).

A single study (Gao et al., 2011) reported increased inflammatory cells in the uterine cervix as well as hyperplasia at higher doses. Effects in the uterus were not evaluated in other noncancer or cancer bioassays in the database, except perhaps grossly in the cancer bioassay by Kroese et al. (2001). Furthermore, it is unclear that the observed histological changes in the cervix are associated with impaired reproductive function. This study also observed a depression of body weight (10, 15, and 30%) and elevated mortality in the two higher dose groups (4 and 8%), suggesting general systemic toxicity. Overall, benzo[a]pyrene-related effects in the uterus are less supported than ovarian/oocyte effects reported in subchronic and gestational studies (Xu et al., 2010; Kristensen et al., 1995; Mackenzie and Angevine, 1981) and supported by a large body of studies by other routes of exposure (i.p.) as well as in vitro mechanistic data (see Section 1.1.2, Mode-of-Action Analysis—Female Reproductive Effects). Therefore, although uterine hyperplasia reported by Gao et al. (2011) was modeled and considered as a candidate toxicity value, ovotoxicity, including decreased ovarian weight and decreased ovarian follicles, was deemed to be more representative of the current body of evidence regarding benzo[a]pyrene-induced female reproductive toxicity.

Sections 1.1.2 and 2.1 have been clarified to reflect the above considerations.

Comment: The SAB recommended that the EPA consider other male reproductive endpoints in addition to the classical reproductive hazard endpoints included in the draft assessment. The SAB specifically recommended considering germline mutagenesis as an endpoint.

Response: Studies that evaluated germ cell mutagenesis in experimental animals following oral exposure were not identified in the benzo[a]pyrene database. However, discussion of increased male germ cell mutation in transgenic lac I mice treated with high dose i.p. doses (Xu et al., 2014) has been added to the document in Section 1.1.2, Mode-of-Action Analysis—Male Reproductive Effects). The derivation of candidate toxicity values based on other effects in male germ cells, such as decreased sperm count and mobility, which may be related to genotoxic mechanisms of benzo[a]pyrene (also reviewed in Section 1.1.2, Mode-of-Action Analysis—Male Reproductive Effects) were considered in the assessment (see Section 2.1.1). The stronger of the available studies reporting these sperm endpoints, Mohamed et al. (2010), still involved too much overall uncertainty as reflected in the composite uncertainty factor (see Table 2-2); therefore, a candidate value to represent effects on male germ cells could not be derived.

Comment: For male reproductive studies, the SAB recommended considering the recovery time after treatment prior to endpoint measurement since the testis is proliferative and new rounds of spermatogenesis could change the outcome. The SAB also noted that because the testis matures after birth, additional consideration be given to the lifestage at which the animals are exposed to benzo[a]pyrene. The SAB specifically recommended consideration of studies demonstrating that

exposure at different lifestages (e.g., pre-adult versus adult) can have differential effects on reproductive health.

Response: The discussion of studies that evaluated reproductive endpoints in male rodents has been clarified to note the age of the animals at treatment (see Table 1-5). For the male reproductive studies evaluated in Section 1.1.2, all but two of the studies (Mohamed et al., 2010; Archibong et al., 2008) evaluated endpoints directly following the exposure period. A footnote has been added to the evidence table to clarify that endpoints were assessed directly following the exposure period unless otherwise indicated.

Furthermore, additional discussion of studies indicating differential effects on male reproductive endpoints following early life exposure (Xu et al., 2014; Liang et al., 2012) has been added to Section 1.1.2 in the subsection Susceptible Populations and Lifestages.

Comment: The SAB recommended that genotoxic and mutagenic aspects of reproductive hazard be addressed, especially as they provide perspective on likely mode of action.

Response: Additional discussion of genotoxic and mutagenic properties of benzo[a]pyrene and the corresponding endpoint of germline mutagenesis and its potential impact on reproductive hazard has been added to the mode-of-action analysis sections for male and female reproductive effects (see Section 1.1.2).

Comment: Several publications were recommended regarding inform sperm effects (Jeng et al., 2013), ovarian effects (Kummer et al., 2013; Sadeu and Foster, 2011; Mattison and Nightingale, 1980; Mattison, 1980), and mode of action for female reproductive effects (Young et al., 2014; Sadeu and Foster, 2013).

Response: Two of these studies were already discussed in the assessment (Sadeu and Foster, 2011; Mattison and Nightingale, 1980). Of the other suggested studies, Jeng et al. (2013), was identified as a new subchronic study. The sperm effects observed in this study were supportive of the existing characterization of benzo[a]pyrene as a male reproductive hazard, but were seen at higher doses than other studies investigating sperm parameters. This study has been added to the text and evidence table informing male reproductive effects (see Section 1.1.2 and Table 1-5). The additional studies informing potential mechanisms of ovarian follicle toxicity studies suggested by the SAB (Kummer et al., 2013; Sadeu and Foster, 2011; Mattison, 1980) support the hazard conclusions in the assessment and support suspected mechanistic pathways of benzo[a]pyrene and have been added to Section 1.1.2 Mode-of-Action Analysis—Female Reproductive Effects. Young et al. (2014) was not considered, as it was available as an abstract only.

Charge Question 2c. The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal, and mechanistic studies support this conclusion?

The SAB agreed that the available immunotoxicity data from animal models and humans exposed to complex PAH mixtures exposures support the claim that benzo[a]pyrene is a human hazard for the immune system. The SAB listed several recommendations.

Comment: The SAB noted concerns that sensitive immune function endpoints (e.g., functional immune tests) are not available to permit proper evaluation of benzo[a]pyrene immunotoxicity in animal models, especially in developing animals. In addition, potential gender differences in immunotoxicity were not addressed. The SAB recommended that these data gaps be acknowledged in the draft assessment.

Response: The available benzo[a]pyrene animal and mechanistic studies, as well as supportive data from PAH mixture exposures in humans, indicate that immune toxicity is a hazard of benzo[a]pyrene exposure. However as pointed out by the SAB, data gaps exist in the assessment of immune hazard from benzo[a]pyrene. Discussion of the lack of functional endpoints to assess immunotoxicity of benzo[a]pyrene following subchronic or chronic exposure has been added in the assessment in Sections 1.1.3 and 2.1.1. In addition, the lack of studies evaluating functional changes in the immune system following developmental exposure is discussed in Section 1.1.3 of the Toxicological Review under Susceptible Populations and Lifestages.

Scarce data are available to inform gender differences in immunotoxicity of benzo[a]pyrene. However, increased discussion of the available studies has been added in Section 1.1.3 under Susceptible Populations and Lifestages.

Comment: The SAB recommended that the EPA consider developing guidelines for immunotoxicity risk assessment, as has been done by the WHO (2012).

Response: The development of EPA immunotoxicity guidelines would be helpful in the consideration of immunotoxicity data; however, such an effort is outside of the scope of the benzo[a]pyrene IRIS Toxicological Review.

Comment: The SAB recommended the consideration of additional studies including in vitro studies in human peripheral blood mononuclear cells (no specific references were suggested) that may inform mode of action, as well as three epidemiological studies that investigated the association of benzo[a]pyrene-adducts and immune endpoints (Jung et al., 2015; Tang et al., 2012; Jedrychowski et al., 2011).

Response: Additional literature was considered and incorporated into the assessment where relevant.

Charge Question 2d. The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

The SAB concurred that the EPA has demonstrated that benzo[a]pyrene is a human carcinogen in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The SAB had the following specific recommendations.

Comment: The Supplemental Material document contains only six papers in which DNA adduct formation has been measured in humans. There are many more such papers in the literature and this draft assessment would be more balanced if at least 20 of the most significant papers could be included.

Response: In Section D.5.1 (Genotoxicity Information) of the Supplemental Information document, Table D-33 (In vivo genotoxicity studies of benzo[a]pyrene) has been split into two tables: Table D-34, Studies of benzo[a]pyrene-induced genotoxicity in humans exposed to PAHs, and Table D-35, Non-human in vivo genotoxicity studies of benzo[a]pyrene. The previous table contained a selection of studies in humans; the new Table D-34 contains all studies measuring BPDE-DNA adduct formation in humans exposed to PAHs, along with the methods used, that are cited in the mode of action for carcinogenicity in Section 1.1.5.

Comment: The current version of the draft assessment does not make a clear case for the pathway of benzo[a]pyrene biotransformation that results in a mutagenic mode of action. A series of the classical critical papers, and their findings, have been listed as bullet points (under the discussion of EPA Criterion 2), and this material should be included in the final benzo[a]pyrene document.

Response: EPA has revised the Mode of Action Analysis—Carcinogenicity text in Section 1.1.5 to more clearly describe the sequence of key events leading to cancer following benzo[a]pyrene exposure and to include the following references suggested in the bullet points in the SAB comments on EPA Criterion 2 (Hussain et al., 2001; Sticha et al., 2000; Beland and Culp, 1998; Culp et al., 1998; Wei et al., 1995; Manchester et al., 1988; Marshall et al., 1984; Grover et al., 1976; Jeffrey et al., 1976; King et al., 1976; Osborne et al., 1976; Daudel et al., 1975; Sims et al., 1974). Two references were not added: Boysen and Hecht (2003) is a review of methods for analyzing DNA adducts, and Pratt et al. (2011) utilized an immunoassay for detecting PAH-DNA adducts that was not specific to benzo[a]pyrene.

Comment: There is evidence of a strong association (Relative Risk or Odds Ratio) between increased human cancer risk in particular organs, such as lung (Tang et al., 1995) and colon (Gunter et al., 2007) and high levels of benzo[a]pyrene-7,8-diol-9,10-epoxide-N2-deoxyguanosine (BPdG) or PAH-DNA adduct formation in human nucleated blood cells. It would be useful to have these mentioned in a paragraph.

Response: EPA recognizes the importance of correlations between levels of PAH-DNA adducts in humans and cancer risk. However, for the assessment of benzo[a]pyrene, more weight has been given to studies specifically detecting BPDE-DNA adducts (and primarily BPdG adducts) that strengthen the causal relationship between benzo[a]pyrene exposure and cancer risk. Tang et al. (1995) and Gunter et al. (2007) utilized methods of detecting PAH-DNA adducts that were not specific to benzo[a]pyrene. Therefore, these studies were not added to the mode-of-action discussion.

Comment: A table describing the nomenclature, characteristics, specificity, sensitivity range, and detection limit for the various methodologies used for human BPdG and PAH-DNA adduct measurements could be easily assembled.

Response: EPA added Table D-31 to Section D.5.1 Genotoxicity Information in the Supplemental Information document, which summarizes the nomenclature, characteristics, specificity, sensitivity range, and detection limit on the various methodologies for adduct detection.

Charge Question 2e. The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

With respect to the health endpoints discussed in Section 1.1.4, Other Toxicity, the SAB concurs with the conclusion that the evidence presented does not support liver, kidney, or hematological effects as human hazards. However, the SAB requested additional clarification on the hazard conclusions regarding additional endpoints (such as forestomach toxicity, cardiovascular toxicity, and adult nervous system effects) as discussed in the following comments.

Comment: The EPA should evaluate the missing references identified by the SAB on cardiovascular, pulmonary, and kidney toxicity of benzo[a]pyrene. The SAB suggested several specific references and opined that the literature search and study selection process may not have been sufficiently comprehensive to identify all potential hazards credibly associated with benzo[a]pyrene exposure.

Response: The literature search performed for benzo[a]pyrene was designed to search for all possible health outcomes of benzo[a]pyrene exposure, and as such, did not include individual terms

for all organs or endpoints. (See discussion under Charge Question 1, Literature Search, Study Selection and Evaluation.) For example, hazard identification for chronic health effects, such as cardiovascular toxicity, gave preference to studies that examined animal models translatable to humans (e.g., Jules et al., 2012), rather than on studies of genetically modified animals with heightened disease susceptibility (Knaapen et al., 2007; Curfs et al., 2005; Curfs et al., 2004) or non-mammalian species (Hough et al., 1993; Albert et al., 1977).

In other comments made by the SAB in response to this charge question, adult and developmental pulmonary toxicity were proposed as additional noncancer endpoints potentially associated with benzo[a]pyrene exposure. However, little data exist to evaluate noncancer pulmonary effects in adult or developing animals. As noted in the assessment, a 4-week inhalation study in adult rats is available that investigated, but did not detect, lung injury (Wolff et al., 1989). Regarding pulmonary effects in developing animals, a recent developmental study (highlighted for consideration by the SAB) suggests pulmonary effects with high dose (25 mg/kg), i.p. exposure to benzo[a]pyrene and subsequent challenge with hyperoxic air (Thakur et al., 2014). However, interpretation of this study is complicated due to the route of exposure and the high doses employed, which were 1–2 orders of magnitudes greater than doses at which effects were observed in the oral developmental database. Therefore, the evidence available for pulmonary noncancer effects was judged too sparse to make a hazard determination. The document has been clarified to reflect these points in Sections 1.1.4 and 1.2.1.

Additional references suggested by the SAB regarding cardiovascular, pulmonary, and kidney toxicity of benzo[a]pyrene were reviewed and incorporated in the document where relevant.

Comment: The EPA should be explicit as to the rationale for concluding that the available evidence either does or does not support adult nervous system effects as a potential human hazard. The SAB also stated that the basis for arriving at the hazard conclusions for the other endpoints identified in Section 1.1.4, Other Toxicities be expanded (e.g., for hematological toxicity, liver toxicity, kidney toxicity, and cardiovascular toxicity). The SAB stated that the current text does not provide an adequate rationale for the characterization (in Section 1.2.1) that the evidence does not support these noncancer effects as potential human hazards. The SAB suggested additional clarification be provided as to whether this conclusion is due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not sensitive endpoints.

Response: The characterizations of hazard summarized in Section 1.2.1, Weight of Evidence for Effects Other than Cancer have been expanded for organ/systems discussed in Section 1.1.4 to further clarify the overall hazard characterization.

- Specifically regarding the potential for benzo[a]pyrene exposure to cause adult nervous system toxicity, this evidence is now more explicitly considered in the context of the totality

of the evidence available for potential nervous system effects of benzo[a]pyrene exposure in Sections 1.1.4 and 1.2.1. As a result, while the adult neurotoxicity data are discussed as consistent with the developmental neurotoxicity endpoints and indicated as suggestive of a potential hazard in themselves, these data were comparably less robust than the studies and data supporting developmental neurotoxicity as a hazard, and additional studies are needed to draw a stronger conclusion regarding the identification of adult neurotoxicity as a human hazard.

- Regarding the hazard characterization of forestomach toxicity (specifically forestomach hyperplasia), EPA agrees with the SAB that forestomach toxicity in animal models is credibly associated with benzo[a]pyrene exposure and that it likely reflects early events in benzo[a]pyrene-induced carcinogenicity. As benzo[a]pyrene-induced forestomach hyperplasia was determined to be a preneoplastic lesion, it was relocated from Section 1.1.4, Other Toxicities to the discussion of forestomach tumors and related lesions in Section 1.1.5, Carcinogenicity.

- Regarding the hazard characterization for cardiovascular effects of benzo[a]pyrene, the interpretation of hazard is complicated by issues of co-exposure in human studies of cardiovascular effects in populations highly exposed to benzo[a]pyrene as a component of a complex PAH mixtures as well as the paucity of studies examining cardiovascular endpoints in wild-type (WT) laboratory animals exposed by environmentally relevant routes for subchronic or chronic durations. Short-duration animal studies and studies by other routes of exposure (e.g., i.p. and intratracheal instillation), as well as studies in genetically modified, highly susceptible animal strains (e.g., ApoE−/− mice), contribute to the plausibility of cardiovascular effects providing suggestive evidence of cardiovascular toxicity due to benzo[a]pyrene exposure. The cardiovascular endpoints were not considered for dose-response due to the relatively lower confidence in this hazard. The discussion of cardiovascular hazard in Sections 1.1.4 and1.2.1 has been expanded and clarified.

- In addition, as hematological effects can inform the weight of evidence for immunotoxicity (WHO, 2012), the tables and discussion regarding hematological effects observed in subchronic and chronic studies have been relocated from Section 1.1.4, Other Toxicity to Section 1.1.3, Immune Toxicity. Hematological changes are therefore considered within the context of the overall body of immune system changes.

Charge Question 3a. The draft assessment proposes an overall reference dose of $3x10^{-4}$ mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

Comment: The EPA should specifically consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012), including plus maze, reflex, locomotor activity, and water maze to justify and support the choice of the critical endpoint. In

particular, the SAB suggested that the EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze.

Response: As summarized in EPA's response to Charge Question 2a, EPA has further evaluated the collection of neurodevelopmental behavioral effects reported by Chen et al. (2012), rather than relying on the elevated plus maze alone. In the revised assessment, modeling results in postnatal day (PND) 69–74 rats for open arm entries in the elevated plus maze (female rats), locomotor activity in the open field (both sexes), and escape latency in the Morris water maze (both sexes) are used to define the overall effect on behavior. Together, these results represent the most reliable and persistent behavioral effects of benzo[a]pyrene exposure detected by Chen et al. (2012).

Comment: The EPA should explain how the BMD was calculated for escape latency in the Morris water maze.

Response: The external peer review draft was not clear that escape latency for males and females combined at PND 74 was used to calculate the BMD. Following the SAB's recommendation to consider the overall impact on neurodevelopmental effects, and in considering the lack of differences in escape latency across sexes as a result of changes in learning (as inferred by the EPA and corroborated by the SAB), the revised assessment considers all 4 trial days. A more transparent description of the BMD calculation for escape latency is provided (see Section 2.1.2 and Appendix E.1). Specifically, EPA performed BMD modeling for escape latency at each of the 4 trial days, PNDs 71–74, for males and females combined. EPA interpreted the trial day results to equally represent the observed behavioral effect (although the underlying behavior affected remains unidentified), and the revised assessment presents the ranges of the BMD and 95% lower confidence limit on the BMD (BMDL) values to characterize this effect.

Comment: EPA should consider data on reproductive outcomes, including cervical hyperplasia and inflammation from Gao et al. (2011), and clearly articulate the rationale for a candidate RfD based on an ovarian effect.

Response: See response to Charge Question 2b, which summarizes the evidence for hazard among the reproductive outcomes. The revised assessment provides candidate RfDs for uterine hyperplasia of the cervix, reduced ovarian weight, and reduced ovarian follicle count.

Comment: The EPA should consider application of a $BW^{3/4}$ adjustment for extrapolation from neonatal animal to neonatal human.

Response: The peer review draft benzo[a]pyrene assessment did not perform allometric scaling in the calculation of an RfD based on animals directly dosed on PNDs 5–11 (Chen et al., 2012). This

was due to several areas of uncertainty. The first issue was whether allometric (i.e., $BW^{3/4}$) scaling, originally derived from data in adult animals and adult humans, holds when extrapolating from doses in neonatal animals to neonatal humans. This uncertainty arises because of the absence of quantitative information to characterize the toxicokinetic and toxicodynamic differences between animals and humans in early lifestages (U.S. EPA, 2011). In addition, interspecies extrapolation across early lifestages is complicated by differences in temporal patterns of development across species. U.S. EPA (2011), *Recommended Use of Body Weight 3/4 as the Default Method in Derivation of the Oral Reference Dose,* states that when such an extrapolation is considered, key developmental processes need to be matched in a species-dependent manner, because the temporal pattern of development differs across species. In the study at issue, Chen et al. (2012), neurobehavioral changes were observed in adult rats after dosing on PNDs 5–11. This postnatal period of brain development in rats is believed to be more akin to human brain development occurring in the third trimester of pregnancy (Dobbing and Sands, 1979, 1973), thus challenging the suitability of extrapolating exposure doses from rats directly exposed through gavage on PNDs 5–11 to the equivalent developmental period in third trimester humans (where exposure would occur transplacentally).

Therefore, due to several associated uncertainties, EPA did not apply a $BW^{3/4}$ adjustment for extrapolation from neonatal animal to neonatal human. Additional clarification of these considerations has been added to the assessment in Section 2.1.2.

Comment: The SAB recommended that EPA further justify whether the application of an uncertainty factor (UF) of 3 for database deficiency is adequate. The SAB specifically highlighted endpoints that may qualitatively support a hazard, but lack dose-response data sufficient for developing toxicity values (such as cardiovascular effects and developmental immunotoxicity). The SAB also requested additional consideration of the database UF (UF_D) in the context of potential effects such as miscarriage, birth defects, and genetic disease.

Response: The UF_D is intended to account for the potential for deriving an under-protective reference value as a result of an incomplete characterization of the chemical's toxicity (U.S. EPA, 2002). In addition to identifying toxicity information that is lacking, existing data may also suggest that a lower reference value might result if additional data were available. When applying this UF, both the data lacking and the data available are considered. For benzo[a]pyrene, a UF_D of 3 was applied to account for database deficiencies, including the lack of a standard multigenerational study or extended 1-generation study that includes exposure from premating through lactation. These types of studies would be useful to understanding the full potential for benzo[a]pyrene exposure to cause reproductive and neurodevelopmental effects. Considering that benzo[a]pyrene has been shown to affect fertility in adult male and female animals by multiple routes of exposure and that decreased fertility in adult male and female mice is observed both following premating

exposure and following gestational exposure (see Section 1.1.2), it is plausible that exposure occurring over this more comprehensive period of development or over multiple generations could result in a more sensitive POD, than the POD selected for developmental neurotoxicity.

Some additional uncertainties exist in the benzo[a]pyrene database, including the paucity of sensitive studies evaluating endpoints of immune and cardiovascular toxicity. The lack of developmental immune toxicity studies, especially those examining functional endpoints, is a notable uncertainty in the benzo[a]pyrene database. Some consideration was given to cardiovascular effects through the candidate value derived for developmental effects of the cardiovascular system (Jules et al., 2012), although this candidate value was not as sensitive as the candidate value derived from the neurodevelopmental study selected as the basis of the overall RfD.

As the SAB suggests, genotoxic effects of benzo[a]pyrene could potentially manifest through miscarriage, birth defects, and genetic disease. However, several developmental studies are available that do not report birth defects at doses much higher than the POD used for the RfD (Kristensen et al., 1995; Mackenzie and Angevine, 1981). A decrease in pups per litter/decreased fetal survival was observed in F0 dams at 60 mg/kg-day (Mackenzie and Angevine, 1981), but has not been observed in oral exposure studies at lower doses closer to the POD used for the RfD (see Table 1-2). While consequences of genotoxic aspects of reproductive hazard are plausible due to the genotoxic and mutagenic mode of action of benzo[a]pyrene, endpoints of fetal survival and birth defects, while affected at high doses, have not been detected at studies near the POD for neurodevelopmental changes.

The POD for the overall RfD was based on several sensitive neurobehavioral endpoints observed following treatment during a sensitive period of brain development and were among the lowest effect levels observed in the benzo[a]pyrene database, even among other developmental studies utilizing low doses of benzo[a]pyrene (such as Jules et al., 2012); thus, application of a full UF_D of 10 was not judged to be warranted. However, because studies following a more comprehensive period of developmental exposure (i.e., early gestation through lactation, if not through adolescence) were not available, a UF_D of 3 was applied to address residual uncertainty associated with the potential for effects at lower doses (see also response to Charge Question 2a).

Additional justification of the UF_D has been added to Section 2.1.3.

Charge Question 3b. The draft assessment proposes an overall reference concentration of 2×10^{-6} mg/m^3 based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

Comment: The study used to derive the overall reference concentration (RfC) (Archibong et al., 2002) reported decreases in fetal survival that occurred at all concentrations. EPA reported that the data for this endpoint were not amenable to dose-response modeling; therefore, the POD for this endpoint was derived from the lowest-observed-adverse-effect level (LOAEL) for decreased fetal survival (a 19% response relative to control). The peer reviewers noted that the rationale for not employing BMD modeling was unclear and that the LOAEL provides a weaker basis than a no-observed-adverse-effect level (NOAEL) for the derivation of the RfC.

Response**:** EPA agrees that the rationale for not employing BMD modeling for decreased fetal survival was unclear, and that a LOAEL is a less desirable POD. Since the release of the External Peer Review draft, a recently published approach by Fox et al. (2016) has facilitated the dose-response modeling of the above embryo/fetal survival data using dichotomous Benchmark Dose Software (BMDS) models. In the revised assessment, PODs derived from this newer approach, including derivation of adjustment factors for each exposure group, are provided for comparison. Further details of the modeling calculations are provided in the Supplemental Information (see Appendix E.1.2).

To clarify, in the External Peer Review draft, BMD modeling was attempted by applying continuous dose-response models because the data for this endpoint were reported as means and SDs of litter-specific percentages of fetuses surviving at birth. These models relied on the assumption that a normal approximation for binomial responses was adequate to characterize the underlying (dichotomous) survival incidence data. However, the non-monotonicity of the observed variances, which showed maximum variability near 50% response levels, typical of binomial variability, could not be addressed, and there were no adequate fits. These issues have been clarified in Appendix E.1.2.

Accordingly, a LOAEL was judged to be the only feasible approach for this data set at the time. EPA agrees that a LOAEL is a less desirable POD than a NOAEL, but the study has no NOAEL. However, an approach for approximating the underlying dichotomous data from reported percentages has been developed, thus facilitating application of more relevant dichotomous dose-response models (Fox et al., 2016). The approach, which is included in Appendix E.1.2 for comparative purposes, is based on the work of Rao and Scott (1992), which relies on the incidence among total offspring in each group. While this measure is known to estimate means of effect adequately, it also underestimates variability by overestimating effective sample sizes. Rao and Scott (1992) developed a data transformation that relies on individual litter data to correct for this overestimation through estimating "design effect." Fox et al. (2016) extended this approach for data sets without individual litter data, through analysis of historical data sets of developmental toxicity.

Dose-response modeling of the adjusted data yielded adequate fits to the observed data with all but one of the dichotomous BMDS models. Further details are provided in the

Supplemental Information (see Appendix E.1.2), including derivation of the adjustment factors (design effects) for each exposure group.

Comment: The RfC was based on one outcome in one study (decreased embryo/fetal survival noted following gestational exposure to rat dams). Peer reviewers suggested two additional studies, Wu et al. (2003a) and Archibong et al. (2012), as potentially useful in developing a more comprehensive dose-response relationship for the RfC and suggested consideration of these endpoints for BMD analysis, potentially increasing confidence in the RfC.

Response: The gestational exposure study by Wu et al. (2003a) was clarified to be an extension of the Archibong et al. (2002) study. That is, the pregnancy outcomes reported by Wu et al. (2003a) were those of the dams exposed by Archibong et al. (2002).

The 14-day premating study of F344 rats exposed to 50, 75, and 100 μg/m^3 nose-only inhalation for 4 hours/day (Archibong et al., 2012) showed reductions in ovarian function (ovulation rate), ovarian weight, mean numbers of pups born, and fetal survival with increasing exposure concentration (100 μg/m^3), and has been added to the Table 1-7 pertaining to female reproductive effects. This study covered an exposure period distinct from the developmental period covered in Archibong et al. (2002) (GDs 11–20), and suggests that exposure prior to mating could result in a decreased number of pups per litter distinct from that observed following gestation-only exposure.

The assessment has been clarified to reflect these considerations (see Section 1.1.1 and Appendix D.4.5) and outcomes reported by Archibong et al. (2012) are considered for RfC derivation in Section 2.2.

Comment: The SAB specifically commented on the use of 3 instead of 10 for interspecies extrapolation. The SAB noted that the UF of 3 to address residual uncertainty for interspecies extrapolation in the inhalation reference concentration may be too low as the rat-to-human dosimetric adjustment may not completely account for systemic toxicokinetics leading to a non-respiratory effect of decreased fetal survival following an inhalation exposure. Furthermore, the SAB expressed concern that the dosimetric adjustment used by EPA inadequately accounts for interspecies differences in filtration of the aerosol (based on particle size) by the upper respiratory tract.

Response: EPA agrees that there is uncertainty in the dosimetric adjustment. Since the mode of action leading to decreased fetal survival is not known, it appears reasonable to consider the dose to the entire respiratory tract in either species instead of, for instance, the dose only to the deep lung (in which case, the more efficient filtration of 2.5 μm particles by the rat nose compared to the human nose would have to be accounted for in the extrapolation.) Secondly, data for modeling

species differences in clearance and metabolism of the deposited particles are not available. Therefore, given these uncertainties, EPA assumes the relevant dose metric to be the mass of benzo[a]pyrene deposited per day in the entire respiratory tract normalized by the body weight. This metric would be more accurate than using exposure concentration as the default even if it does not fully account for the toxicokinetics. Accordingly, as per EPA policy, a UF of 3 is used to account for species differences in toxicodynamics and residual differences in toxicokinetics not accounted for in the dosimetric adjustment. Consideration of the above uncertainties in the interspecies adjustment for the RfC has been added to Section 2.2.3, Derivation of Candidate Values.

Comment: The SAB recommended that the EPA include a brief discussion of the rationale for selection of the allometric scaling factor in the context of inhalation exposure to benzo[a]pyrene leading to decreased fetal survival. In particular, the SAB highlighted text from EPA's RfC methodology (U.S. EPA, 1994) that suggests that EPA used BW^{1}-scaling for this outcome, rather than the $BW^{3/4}$-scaling used for the oral toxicity values.

Response: EPA's RfC methodology for estimating human equivalent doses (HEDs) resulting from particle exposure distinguishes between portal-of-entry effects, for which the mass of chemical deposited in the respiratory tract is normalized by the surface area of the affected region, from remote effects, for which body weight is the normalizing factor (analogously to mg/kg-day for oral exposure). The overall dosimetric adjustment factor also involves estimating the mass of particles deposited from minute volume (mL/minute) and the fraction of inhaled dose that is deposited in the respiratory tract, which in turn relies on functional residual capacity and upper respiratory tract volume (mL); all of these considerations incorporate allometric differences between humans and the experimental animals.

The dosimetric adjustment factor for extrarespiratory effects observed in rats was estimated to be 1.1, which is nearly equivalent to assuming that concentrations in air are equipotent across species. Since intakes scale by $BW^{3/4}$, this allometric scaling factor was consistent with the $BW^{3/4}$-scaling used for the oral toxicity values. Additional clarification has been added to the assessment (see Sections 2.2.2 and 2.2.8).

Charge Question 3c. The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

Comment: The SAB noted that if no biological basis exists for concluding that the mouse study is more representative of human response than the rat study, the EPA should consider averaging over both studies to derive the oral slope factor for benzo[a]pyrene.

Response: EPA is not aware of a biological basis for concluding that the mouse study is more representative of human response than the rat study. The three estimated slope factors fall within a 5-fold range (before rounding to one significant digit). Under the assumption that the three data sets have equal relevance for extrapolating to humans, a geometric mean of the three slope factors is 0.60 per mg/kg-day, and a geometric mean that gives equal weight to rats and mice is 0.74 per mg/kg-day, about 50% of the highest slope factor (1.4 per mg/kg-day).

EPA notes that slope factors are intended to provide an upper bound on the cancer risk of a randomly selected individual (U.S. EPA, 2005a). EPA's approach to quantifying low-dose cancer risk relies on a 95% upper bound on the cancer risk that typically addresses only the experimental variability in homogeneous laboratory animals. The NRC (2009) has pointed out that when cancer risk is expected to be linear at low exposures, as with benzo[a]pyrene, EPA's cancer risk values tend not to address human variability and susceptibility adequately. Concern for sensitive subpopulations supports the use of the higher value here as an overall upper bound, 2-fold higher than the geometric mean slope factor.

Comment: The SAB recommended that EPA should compare oral slope factors derived from fitting a range of models to dose-response data.

Response: Given that low-dose linearity is expected for benzo[a]pyrene carcinogenicity due to its mutagenic mode of action, the multistage-Weibull model is preferred because it can incorporate the individual animal data that were available for time and cause of death. For comparison purposes, however, an approximate survival adjustment was applied to summary incidence data using the poly-3 technique (Bailer and Portier, 1988), in order to take into account reductions in animals at risk while using a range of dichotomous models. The incidence data for the alimentary system tumors only were used as basis of comparison across the three data sources. All of the dichotomous BMDS models fit the adjusted summary incidence data well. For each data set, the ranges of BMD_{10} and $BMDL_{10}$ values derived from these models, including models that allow low-dose nonlinearity, were found to include the time-to-tumor estimates and varied less than 1.5-fold from the time-to-tumor estimates. These analyses are summarized in Section 2.3.3 and Appendix E.2.1 (Cancer Endpoints).

Comment: The SAB recommended that the EPA provide an explanation of the rationale for its selection of an allometric scaling factor for the benzo[a]pyrene oral cancer slope factor given what is known about the benzo[a]pyrene mode of action for carcinogenicity, reaction rates, and toxicokinetics, and specifically, how the selection of the allometric scaling factor applies when there is a portal-of-entry effect.

Response: Despite extensive research into benzo[a]pyrene toxicokinetics, very little information directly informs estimates of human-equivalent benzo[a]pyrene doses. It is understood that benzo[a]pyrene carcinogenicity involves a mutagenic mode of action mediated by DNA-reactive metabolites in the tissues where tumors appear, both at the portal of entry and those involving systemic distribution (i.e., liver, kidney, and skin in rats). While the metabolites are highly reactive (likely not limited by processes consistent with $BW^{3/4}$ proportionality), distribution of benzo[a]pyrene to these tissues and formation of the metabolites are limited by processes likely to be consistent with $BW^{3/4}$ proportionality.

EPA guidance (U.S. EPA, 2011) observed that because a "$BW^{3/4}$ relationship exists among species in studies where dose is administered in food, because interspecies food consumption follows a $BW^{3/4}$ relationship … it is reasonable to apply the $BW^{3/4}$ approach for gastrointestinally related, portal-of-entry effects." Beland and Culp (1998) administered benzo[a]pyrene in the diet, while Kroese et al. (2001) administered benzo[a]pyrene via gavage. It is not clear what impact this difference in administration has on estimating HEDs of benzo[a]pyrene. This issue has been clarified in the assessment (see Section 2.3.2).

EPA's guidance also emphasizes that for a portal-of-entry scenario, "the most appropriate dose metric would likely be mass of agent per surface area, e.g., mg/cm^2," but acknowledges that implementation of this approach involves such issues as "the lack of a human anatomical parallel to the rodent forestomach," surface areas of the gastrointestinal (GI) tract in rodents and humans, rates and scenarios of ingestion, and diffusion rates (U.S. EPA, 2011). These considerations have yet to be developed; therefore, EPA has utilized $BW^{3/4}$ for interspecies extrapolation, as recommended by the guidance document. Section 2.3.2 includes clarification of this issue.

Charge Question 3d. The draft assessment proposes an inhalation unit risk of 0.6 per mg/m^3 based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

Comment: The SAB noted that EPA should conduct supplemental sensitivity analyses using other dose-response models, alternative assumptions, and not eliminating from the analysis all animals without confirmed examination of one or more of the pharynx or respiratory tract tissues.

Response: EPA agrees that supplemental sensitivity analyses provide useful perspective for the estimated unit risk and has included a summary of analyses addressing the issues that the SAB identified (see Appendix E.3.2, Table E-32).

First, concerning the incidence of animals without confirmed examination of one or more of the pharynx or respiratory tract tissues, the Multistage Weibull software does not take into account the durations of exposure corresponding to unknown tumor status. An alternative analysis was

conducted to evaluate the impact of assuming that each animal with unknown tumor status had no tumors, a possible underestimate of the true situation; the resulting unit risk was about 20% lower than that based on complete data. The other extreme, assuming that all animals with unknown dispositions actually had tumors, was not considered due to its higher implausibility.

Concerning other dose-response models, BMDS dichotomous dose-response models were applied to poly-3 adjusted incidence data to address intercurrent mortality. These adjusted estimates also considered the length of time on study for the animals with incomplete histopathology. Only one model, a two-degree multistage model applied after dropping the high-exposure group, provided an adequate fit.

Concerning latency in the time-to-tumor model, the lack of information in the scientific literature for respiratory tumors and lack of cause-of-death information in the Thyssen et al. (1981) data set limited the options for sensitivity analyses. One approach involved making assumptions about which tumors were or were not the cause death, and yielded a latency estimate of 14.5 weeks. In another approach, latency was fixed at several values in the range of 2–90 weeks; the best-fitting model, as judged by Akaike's Information Criteria (AIC), assumed a latency of 90 weeks, yet yielded BMD_{10} and $BMDL_{10}$ values very similar to those for the recommended unit risk. These results suggest some insensitivity to latency, or possibly that a constant value for latency across exposure levels is not supported.

Alternatives for cross-species equivalence of exposures were considered independently of additional modeling, because there is no information to suggest that this equivalence changes with exposure level. The recommended unit risk relies on the assumption that the amount inhaled, normalized by body weight, leads to comparable cancer risk across species. The alternatives comprised consideration of scaling inhaled doses, in mg/kg-day units, by $BW^{3/4}$ (highlighting allometric differences in metabolism and clearance rates over their lifetimes) and by $BW^{2/3}$ (highlighting species differences proportional to relative surface areas). Both considerations suggest higher risks to humans than to hamsters at the same exposure level, by about 5- and 8-fold, respectively.

Comment: The SAB recommended that EPA give further consideration to occupational studies, specifically studies of lung cancer with airborne inhalation exposures to PAHs in coke oven and aluminum smelter workers (or meta-analysis of occupational studies), to develop unit risk estimate(s) for inclusion in Table 2-9 alongside the benzo[a]pyrene unit risk estimates calculated from the chronic inhalation cancer bioassay in hamsters (Thyssen et al., 1981). The panel acknowledged that interpretation of the epidemiological evidence is challenging given that exposures are to mixtures of PAHs with poorly understood interactions, but suggest that a model using relative potency factors and an assumption of dose additivity could be considered for adjustment of epidemiological results in estimation of the unit risk attributable to benzo[a]pyrene alone.

Response: EPA agrees that some occupational studies of benzo[a]pyrene-containing PAH mixtures may support development of unit risk estimates, given suitable information to characterize the contributions of other chemicals in the mixtures. Although the SAB suggested a framework for this analysis (U.S. EPA, 1990b), EPA is currently revising its relative potency factor approach for PAH mixtures, and will defer any indirect benzo[a]pyrene unit risk derivation from human studies of PAH mixtures until revised estimates, currently underway, are available.

EPA agrees that studies of cancer in occupations that are highly exposed to PAHs could be used quantitatively to develop unit risk estimates. However, the resulting cancer risk estimate would represent the carcinogenic potential of the entire mixture including a spectrum of PAHs as well as other potentially carcinogenic components, and would not be representative of benzo[a]pyrene alone. The establishment of an inhalation unit risk for benzo[a]pyrene alone is important as it serves as the index chemical for the EPA's relative potency factor approach for assessing the carcinogenic potential of PAH mixtures (U.S. EPA, 1990b), which allows for the estimation of carcinogenic potential of PAH mixtures when unit risk estimates for the whole mixture is not available. While the exercise suggested by the SAB might provide interesting comparisons that could roughly inform the plausibility of the inhalation unit risk calculated from the available animal data, the results would likely be highly uncertain and inconclusive.

Comment: The SAB also suggested the inclusion of an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical environmental exposures (especially for sensitive populations).

Response: Criteria to weigh the confidence in cancer risk values have not yet been developed for IRIS Toxicological Reviews; thus, explicit conclusion statements regarding the overall confidence in cancer risk values were not added to this assessment. However, specific uncertainties in the unit risk value for benzo[a]pyrene are discussed in Section 2.4.4 and outlined in Table 2-10.

Regarding the applicability of the inhalation unit risk to typical environmental exposures including those in early life, statements have been added in Section 2.4.3 to clarify the intended use of this value. Specifically, because cancer risk values calculated for benzo[a]pyrene were derived from adult animal exposures, and because benzo[a]pyrene carcinogenicity occurs via a mutagenic mode of action, exposures that occur during early life would require the application of age-dependent-adjustment-factors (ADAFs) (see Section 2.5). In addition, the inhalation unit risk for benzo[a]pyrene is derived with the intention that it will be paired with EPA's relative potency factors for the assessment of the carcinogenicity of PAH mixtures.

Charge Question 3e. The draft assessment proposes a dermal slope factor of 0.006 per µg/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response

analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

Comment: The SAB commended the EPA's efforts to derive the IRIS Program's first dermal slope factor. However, they noted that the proposed dermal slope factor and the proposed method for cross-species scaling was not sufficiently supported. The SAB did not have a specific recommendation as to the dose metric, except to note that it should be based on absorbed dose. They went on to recommend that in the absence of empirical data, the decision should be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of benzo[a]pyrene in laboratory animal bioassays and anticipated human exposures.

Response: EPA is reviewing the SAB panel's specific advice and is initiating further scientific discussions to gather a broad range of scientific perspectives in order to further refine EPA's approach for deriving a benzo[a]pyrene dermal slope factor. In the interest of timeliness and in consideration of the support for the cancer characterization and the other toxicity values within the benzo[a]pyrene assessment, the continuing efforts to refine the dermal slope factor methodology will be addressed in a separate assessment.

Charge Question 3f. The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo(a)pyrene induces cancer through a mutagenic mode of action. Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo(a)pyrene?

The SAB agreed that the available mechanistic studies in humans and animals support a mutagenic mode of action for benzo[a]pyrene-induced cancers. They also supported the proposed use of ADAFs, as established in EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* (U.S. EPA, 2005b), for the adjustment of tumor risk from childhood exposures to carcinogens with a mutagenic mode of action.

Charge Question 4. Does the executive summary clearly and appropriately present the major conclusions of the assessment?

The SAB found that the major conclusions of the draft assessment were clearly and appropriately presented in the Executive Summary.

Charge Question 5. In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the

public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed by EPA.

The SAB found that most of the scientific issues raised by the public, as described in Appendix G of the peer review draft Supplemental Information document, were adequately addressed by EPA. However, there were some issues for which the SAB provided additional discussion in the report under Charge Questions 2e and 3e, and EPA responded accordingly.

Comment: The SAB recommended that major science issues pointed out by public commenters should be included in the relevant charge questions, allowing the SAB to weigh in on EPA's approach. The SAB recommended that in the future, they should not be asked if EPA has adequately addressed all public comments.

Response: Going forward, EPA will capture major science issues expressed in the public comments within the body of related charge questions.

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