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Toxicological Review of Benzo[a]pyrene

(CASRN 50-32-8)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

Supplemental Information

August 2013

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National Center for Environmental Assessment
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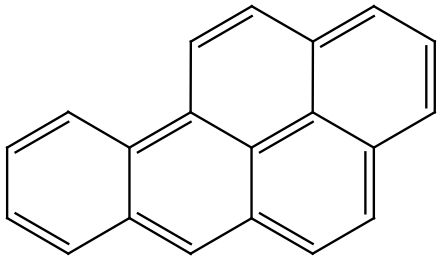
ABBREVIATIONS

1-OH-Py	1-hydroxypyrene
AchE	acetylcholine esterase
Ah	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
AhR	Ah receptor
AIC	Akaike's Information Criterion
AMI	acute myocardial infarction
ANOVA	analysis of variance
ARNT	Ah receptor nuclear translocator
AST	aspartate transaminase
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
BrdU	bromodeoxyuridine
BSM	benzene-soluble matter
BUN	blood urea nitrogen
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CI	confidence interval
CYP	cytochrome
CYP450	cytochrome P450
dbcAMP	dibutyl cyclic adenosine monophosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC	European Commission
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EROD	7-ethoxyresorufin-O-deethylase
ETS	environmental tobacco smoke
Fe_2O_3	ferrous oxide
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
HED	human equivalent dose
HFC	high-frequency cells
HPLC	high-performance liquid chromatography
hprt	hypoxanthine guanine phosphoribosyl transferase
HR	hazard ratio
Hsp90	heat shock protein 90
Ig	immunoglobulin
IHD	ischemic heart disease
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MAP	mitogen-activated protein
MLE	maximum likelihood estimate
MMAD	mass median aerodynamic diameter
MN	micronucleus
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NCE	normochromatic erythrocyte
NK	natural-killer
NMDA	N-methyl-D-aspartate
nNOS	neuronal nitric oxide system
NOAEL	no-observed-adverse-effect level
NQO	NADPH:quinone oxidoreductase
NRC	National Research Council
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PBMC	peripheral blood mononuclear cell
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
PCR	polymerase chain reaction
PND	postnatal day
POD	point of departure
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
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
SOD	superoxide dismutase
SSB	single strand break
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TK	thymidine kinase

TPA	12-O-tetradecanoylphorbol-13-acetate
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
TWA	time-weighted average
UCL	upper confidence limit
WESPOC	water escape pole climbing
WT	wild type
WTC	World Trade Center
XPA	xeroderma pigmentosum group A

APPENDIX A. CHEMICAL PROPERTIES AND EXPOSURE INFORMATION

Benzo[a]pyrene is a five-ring polycyclic aromatic hydrocarbon (PAH) (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 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 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). It 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, cigarettes, and various industrial combustion processes (ATSDR, 1995). Benzo[a]pyrene is also found in soot and coal tars. Mahler et al. (2005) 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. 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, 1973). 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).

Inhalation. 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 non-smokers. Naumova et al. (2002) measured PAHs in 55 non-smoking 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	Year	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 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 mean intake via inhalation for an adult non-smoker was estimated as 20 ng/day. Naumova et al. (2002) focused on non-smoking 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. 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). 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).

Dermal. 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 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
Non-cancer: oral value	
Cal/EPA (2010)	The concentration of 4 µg/L (Acceptable Daily Dose = **1.7×10^{-3} mg/kg-day**) for benzo[a]pyrene in water for noncarcinogenic effects was derived from a lowest-observed-adverse-effect level (LOAEL) of 5 mg/kg-day for renal toxicity from Knuckles et al. (2001), an uncertainty factor of 3,000.
Non-cancer: inhalation value	
WHO (2003, 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-day)$^{-1}$** derived from Neal and Rigdon (1967) and a lifetime excess cancer risk of 10^{-5}.
Health Canada (2010, 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, 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}.)
Cancer: oral value	
Cal/EPA (2010)	Cancer slope factor of **2.9 (mg/kg-day)$^{-1}$** derived from Culp et al. (1998). This includes an age sensitivity factor of 1.7.
Cancer: inhalation value	
WHO (2000, 1997)	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.
Cal/EPA (1994)	The inhalation unit risk of **1.1 (mg/m^3)$^{-1}$** was derived based on Thyssen et al. (1981).
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.)
Cal/EPA (2010)	"Sufficient reason for concern regarding the carcinogenic potential of this toxicant in humans."
Health Canada (1998)	Probably carcinogenic to man.

CalEPA = California 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 KEYWORDS

Table C-1. Literature search strategy keywords for benzo[a]pyrene

Database	Set #	Terms	Hits
Initial Strategy			
PubMed Date range: 1950's to 2/14/2012 Search date: 2/14/2012	1A	("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 "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	5,184

		"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 Date range: 1960's-2/14/2012 Search date: 2/14/2012	1B	(((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 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	25,621

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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 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 were 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 find 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 fourfold 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

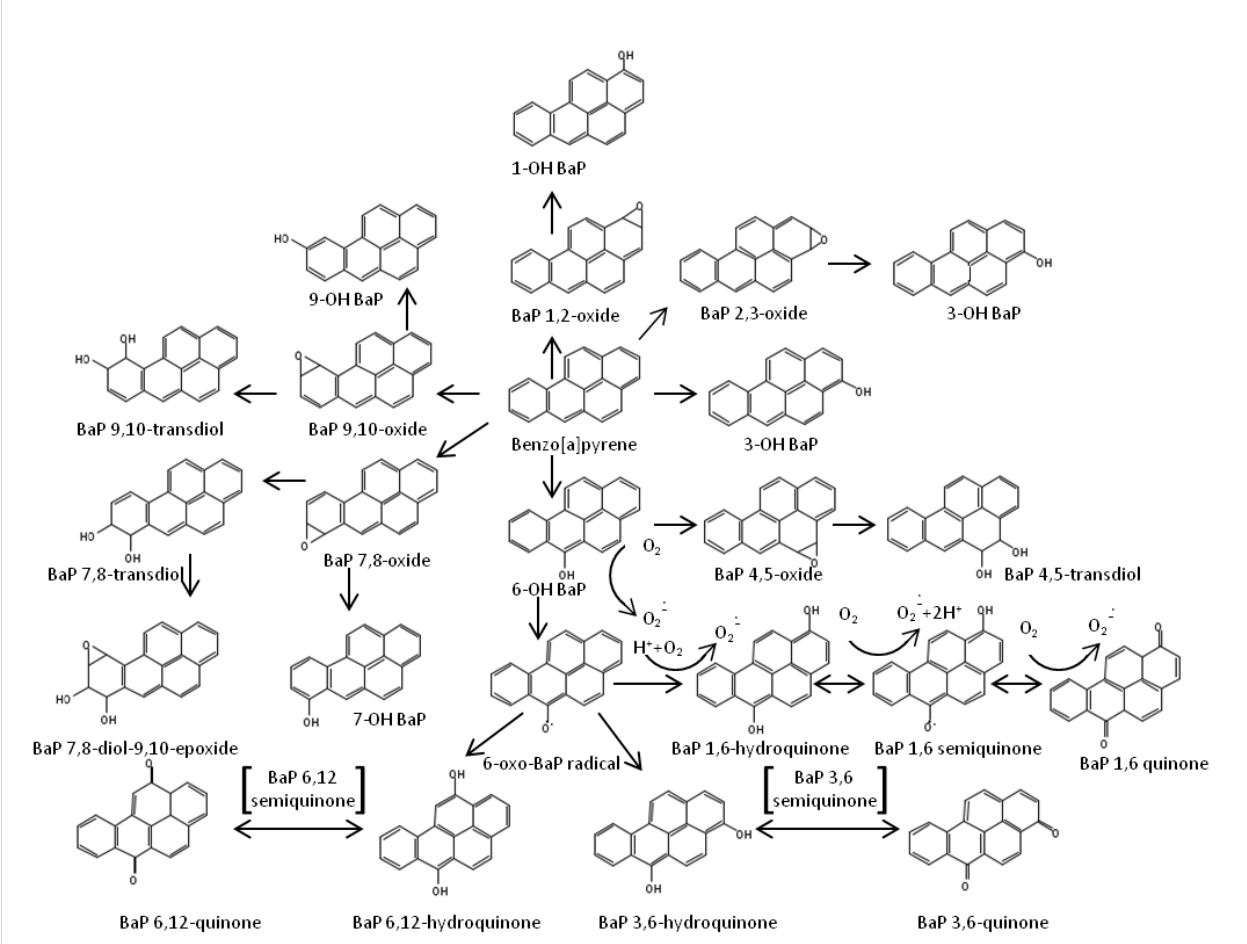
No adequate quantitative studies of benzo[a]pyrene tissue distribution in exposed humans were identified. Obana et al. (1981) observed low levels of benzo[a]pyrene in liver and fat tissues from autopsy samples. However, prior exposure histories were not available for the donors. Nevertheless, the identification 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). 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). 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).

D.1.4. Metabolism

The metabolic pathways of benzo[a]pyrene (Figure D-1) and variation in species, strains, 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).

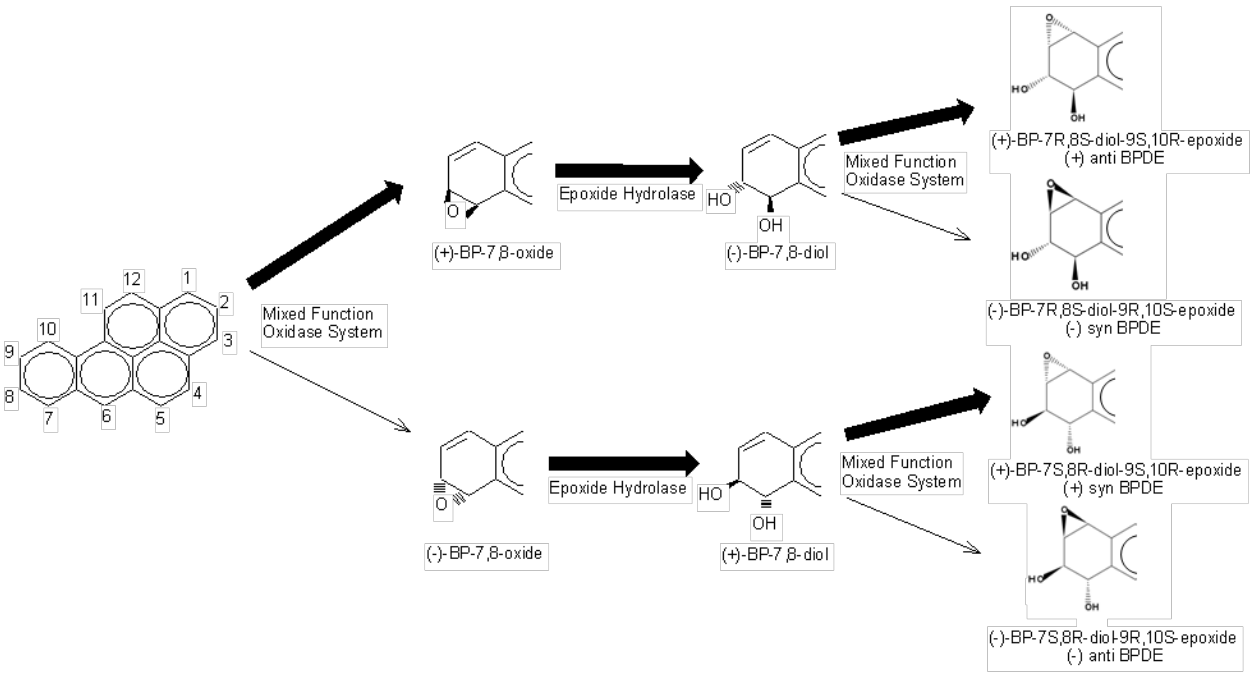
Figure D-1. Metabolic pathways for benzo[a]pyrene.

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). The 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. Also, the 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, or (3) the 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 epoxide hydrolase (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-benzo[a]pyrene-7,8-diol-9,10-epoxide (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 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 reactive oxygen species (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 glutathione (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 formation of DNA adducts (Hemminki et al., 1997) or blood protein adducts (Pastorelli et al., 1998).

Conjugation with uridine diphosphate-glucuronide catalyzed by UDP-glucuronosyltransferase (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 are metabolites (Ramesh et al., 2004; Ramesh et al., 2001b; Yamazaki and Kakiuchi, 1989). In rats administered a 100 nmol dose, greater than 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 and found that benzo[a]pyrene is extensively metabolized by the intestinal Caco-2 cells and 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, phenolas, 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, rat, mouse, rabbit and marmoset (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 are 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. 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 rat, 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. If 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). 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 (1983a, b) 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 (3MC) to induce benzo[a]pyrene metabolism. In microsomal preparations from control and 3MC 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 3MC-pretreated rats the clearance in lungs and livers were of similar magnitude. The PBPK simulations model based on this 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. Non-Cancer 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 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 relative risk (RR) attributed to cigarette smoking in former and current smokers was 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; 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 non-smokers/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/m^3-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/m^3)**					***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
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 non-malignant 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 non-malignant diseases combined in male exposed workers versus external referents. Using a continuous variable, the authors calculated that the risk of death from IHD to be 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 IHC 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

	Categorical Cumulative Exposure with a 5-yr Lag (μg/m^3-yr)					*p*-value for Trend[a]	Continuous[b]
Effect Measured	**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
Acute myocardial infarction (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 non-smoking 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 non-smoking 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 non-smoking 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., non-smoker with a partner who smoked), and 10 non-smokers 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 nondectable levels of serum benzo[a]pyrene was highest in the non-smoking group (60.0, 14.3, and 21.0% below detection limit in non-smoking, 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 detection limit in non-smoking, 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 non-smoking (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 non-smoking, 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 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 chloine 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, personal habits, 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 (1-OH-Py). 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 determined by hydroxyamine chromometry, and acetylcholine esterase (AchE) levels 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-OH-Py 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-OH-Py 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 control group; when stratified by urinary metabolite level, scores significantly decreased with increasing 1-OH-Py 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-OH-Py 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-OH-Py levels, although this relationship was statistically significant. Acetylcholine levels were fourfold 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-OH-Py. 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-OH-Py	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-OH-Py 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
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, 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" 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-OH-Py. 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 twofold) 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-OH-Py levels and years of coke oven operation were positively associated with increased early and late PMBC 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-OH-Py 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/m^3; 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-OH-Py	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 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-OH-Py 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-OH-Py 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).

Cohort Cancer 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).

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-6). 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-6). 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-6).

Table D-6. 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 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 fourfold 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-7). In both sexes, liver weight was statistically significantly increased only at 50 mg/kg-day by about 18% (Table D-7).

Table D-7. 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-8). At 10 mg/kg-day, thymus weight in males was decreased by 15%, but the decrease did not reach statistical significance.

Table D-8. 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 two- and three–fourfold 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, 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-9) (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-9). 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-9). 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-9). 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-9). 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-9). Gross examination revealed auditory canal tumors only in the high-dose group.

Table D-9. 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-9). 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) were associated with increased incidences of forestomach and liver tumors at these dose levels. The authors of this study note 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-10 [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-10). 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-10). 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-10 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-10. 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
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 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 intakes 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 group, 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-11). 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 at ≥25 ppm and hyperkeratosis at ≥25 ppm (Table D-11). The esophagus was the only other examined tissue showing elevated incidence of a nonneoplastic lesion (basal cell hyperplasia, see Table D-11). Tumors (papillomas and carcinomas) were also significantly elevated in the esophagus and tongue at 100 ppm (Table D-11). Esophogeal 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-11).

Table D-11. 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

[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 (U.S. EPA, 1988) and resulting doses were averaged between males and females.

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 incidence 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.

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-12 for references).

Table D-12. 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 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 9th day, every 3rd day, or 5 times/week.	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)

Species/Strain	Exposure	Results	Comments	Reference
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)
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 Carcinoma Dose (mg) 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 20–30 animals (8 weeks old) were exposed by nose-only inhalation to NaCl aerosols (controls; 240 µg NaCl/m^3) or benzo[a]pyrene condensed onto NaCl 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). Each group initially consisted of 24 hamsters; final group sizes were larger as animals dying during the first 12 months of the study were replaced.

Survival was similar in the control, low-dose, and mid-dose groups, but was significantly decreased in the high-dose group. Average survival times in the control, low-, mid-, and high-dose groups were 96.4 ± 27.6, 95.2 ± 29.1, 96.4 ± 27.8, and 59.5 ± 15.2 weeks, respectively. After the 60th week, body weights decreased and mortality increased steeply in the highest dose group. Histologic examination of organs [a complete list of organs examined histologically was not reported by Thyssen et al. (1981)] revealed a dose-related increase in tumors in the upper respiratory tract, including the nasal cavity, pharynx, larynx, and trachea, and in the digestive tract in the mid- and high-dose groups (Table D-13). A statistical analysis was not included in the Thyssen et al. (1981) report. No lung tumors were observed. Squamous cell tumors in the esophagus and forestomach were also observed in the high-dose group, presumably as a consequence of mucociliary particle clearance. Tumors were detected in other sites, but none of these appeared to be related to exposure. The results indicated that the pharynx and larynx, including the epiglottis, were the main cancer targets (Table D-13).

Table D-13. Incidence of respiratory and upper digestive tract tumors in male hamsters treated for life with benzo[a]pyrene by inhalation

Tumor site	Reported benzo[a]pyrene concentration (mg/m^3)			
	0[a]	2[b]	10	50
	Tumor incidence (latency in wks[c])			
Nasal cavity	0	0	3/26 (116 ± 1.5)	1/25 (79)
Larynx	0	0	8/26 (107.1 ± 15.5)	13/25 (67.6 ± 12.1)
Trachea	0	0	1/26 (115)	3/25 (63.3 ± 33.3)
Lung	0	0	0	0
Pharynx	0	0	6/26 (97.2 ± 16.9)	14/25 (67.5 ± 12.2)
Esophagus	0	0	0	2/25 (70, 79)
Forestomach	0	0	1/26 (119)	1/25 (72)

[a]Effective number of animals in control group: n = 27.
[b]Effective number of animals in 2 mg/m^3 dose group: n = 27.
[c]Mean ± SD.

Source: Thyssen et al. (1981).

Under contract to the U.S. EPA, Clement Associates obtained the individual animal data (including individual animal pathology reports, time-to-death data, and exposure chamber monitoring data) collected by Thyssen et al. (U.S. EPA, 1990a). Review of the original data 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 three week period to as many as five measurements in one week. Individual measurements (in mg/m^3) ranged from 0.2 to 4.52, 1.16 to 19.2, and 0.96 to 118.6 in the 2, 10, and 50 mg/m^3 target concentration groups, respectively. Overall, weekly average exposure concentrations varied two- to fivefold 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%. Because different animals were started at different times, 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 original individual animal data to calculate average continuous lifetime exposures for each individual hamster. Group averages of individual average continuous lifetime exposure concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3 for the control through high-exposure groups. These averages were approximately 10% lower than the averages

across the entire duration of the study, with 95% confidence limits for the average exposure level among animals in each group varying within 2%.

The individual animal pathology reports prepared by Thyssen et al. (1981) were examined to assess the joint incidence of tumors in the larynx and pharynx in each group and the relative incidences of malignant tumors (U.S. EPA (1990a). Table D-14 presents the number of animals with tumors in the larynx and pharynx and the numbers of animals in each exposure group. Numbers of animals with either laryngeal or pharyngeal tumors are also noted in Table D-14, since these two types of tumors arise in close anatomical proximity from similar cell types. Examination of the individual animal pathology reports also showed that all of the nasal, forestomach, esophageal, and tracheal tumors occurred in animals that also had either laryngeal or pharyngeal tumors, except for two animals in the mid-dose group that displayed nasal tumors (one malignant and one benign) without displaying tumors in the pharynx or larynx.

Table D-14. Number of animals with pharynx and larynx tumors in male hamsters exposed by inhalation to benzo[a]pyrene for life

Average Continuous Benzo[a]pyrene Concentration[a] (mg/m^3)	Number of Hamsters in Group[b]	Larynx[b]		Pharynx[b]		Larynx or Pharynx, Combined[c]	
		Malignant	All	Malignant	All	Malignant	All
Control	27	0	0	0	0	0	0
0.25	27	0	0	0	0	0	0
1.01	26	8	11	7	9	11	16
4.29	34	9	12	17	18	17	18

[a]As calculated by Clement Associates (U.S. EPA, 1990a) from air monitoring data measured by Thyssen et al. (1981).
[b]As counted from information in Table E-16 in Appendix E which was obtained from examination of individual animal pathology reports prepared by Thyssen and colleagues and obtained by Clement Associates.
[c]As counted from information in Table E-16 in Appendix E. Nasal, forestomach, esophageal, and tracheal tumors occurred in hamsters that also had tumors in the larynx or pharynx, except for two animals in the mid-dose group that displayed nasal tumors (one malignant and one benign) without displaying tumors in the pharynx or larynx.

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, 1960, 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 of 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 (1960) 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 (1960) 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

	SWR Mice		C3HeB Mice		A/He Mice	
Dose (µg)[a]	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 (1960).

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 in Weeks
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 Ah-receptor-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 DBP 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 (enzyme-linked immunosorbent assays [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 (~twofold 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. (2011b) examined effects of benzo[a]pyrene exposure via on cervical cell morphology. 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., 2011b). 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. Cervical 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 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. (2011b).

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 twofold higher than untreated or vehicle controls at the low dose, to ~sixfold 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, 17B-estradiol blood level, and ovarian follicle populations (including primordial, primary, secondary, atretic, and corpora leutea). 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 radioimmunassay 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 benzo[a]pyrene by gavage in peanut oil to pregnant Long-Evans rats (n = 5 or 6) on gestational 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 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 group at PND 12, in the 0.02 and 2 mg/kg groups at PND 14, in only the high-dose group at PND 16, and 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 benzo[a]pyrene, which was also present in the 2 mg/kg 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 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 groups, while rearing was significantly increased in only the 2 mg/kg 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 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 treatment groups; these groups also spent significantly more time in open maze arms, along with the 0.02 mg/kg female group. At PND 70, the 2 mg/kg males, along with the 0.2 and 2 mg/kg 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 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 benzo[a]pyrene at both PNDs 39 and 74, and were also significantly elevated in 0.2 mg/kg 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 rats of both sexes at both PNDs 40 and 75. PND 75 females treated with 0.2 mg/kg benzo[a]pyrene also showed significant decreases in both performance metrics, while PND 75 0.2 mg/kg 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 evaluated for each treatment group. Pups from 4 to 5 individual litters were analyzed for each endpoint, which was independently repeated for a total of 3 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 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, trended between the 150 and 600 µg/kg 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 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 benzo[a]pyrene group, while the average heart rate of the 1,200 μg/kg 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: *water escape pole climbing (WESPOC) test*, administered at PND 20, in which the time to find the pole, time to climb the pole, and the time to reach the safety platform were reported; *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 *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 group and a 10–12% weight gain in pups from the 20 mg/kg 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 group was continuously higher than either the 2 mg/kg 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 PND 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 treatment group demonstrated significantly increased negative geotaxis latency, which was twofold greater than controls at PNDs 5, 7, and 9, but returned to control levels at PND 11. Interestingly, mice in the

20 mg/kg 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 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 WESPOC test data: female pups were not significantly affected using any metric, while males in the 20 mg/kg group demonstrated a statistically significantly longer pole-grasping latency (threefold), 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 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 treatment group entered into closed arms 20% less frequently than controls, mice in the 20 mg/kg group were not significantly different. Likewise, mice nursing from mothers treated with 2 mg/kg 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 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. (2001) 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% were observed at doses ≥75 μg/m^3 (14 and 16% decreases at 75 and 100 μg/m^3, respectively, $p < 0.05$). Exposure to benzo[a]pyrene did not affect 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

Parameter[a]	Administered Concentration of Benzo[a]pyrene (μg/m^3)				
	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) 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). Dams were maintained to term and pups were weaned on PND 30. Benzo[a]pyrene reduced the number of live pups to one-third of control values without affecting the number of implantation sites. 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. In this study, confirmed-pregnant F344 rats 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 (GDs 11–21). Control animals either were exposed to carbon black (sham) to control for inert carrier effects or remained untreated. Each benzo[a]pyrene concentration had its own set of controls (carbon black and untreated). 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). Results indicated a dose-related decrease in the ratio of the total number of pups born per litter to the total number of implantation

sites per litter. The number of resorptions at 75 and 100 µg/m^3, but not at 25 µg/m^3, was statistically significantly increased compared with controls.

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 regarding the genotoxicity of benzo[a]pyrene in in vitro and in vivo systems is presented in Tables D-31, D-32, and D-33.

Table D-31. 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			

	Result		Reference
	+S9	–S9	
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)
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			

	Result		Reference
	+S9	–S9	
Escherichia coli/pol A	+	–	Rosenkranz and Poirier (1979)
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-32. 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)
Chinese hamster V79 cells (co-cultured with irradiated HepG2 cells)	+	ND	Diamond et al. (1980)

Assay/Test System	Result		Reference
	+S9	–S9	
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)
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)

Assay/Test System	Result		Reference
	+S9	–S9	
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. (1983a, b)
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)
Human peripheral blood lymphocytes	+	ND	Lo Jacono et al. (1992)
Chinese hamster Don-6 cells	ND	+	Abe et al. (1983a, b)
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)

Assay/Test System	Result		Reference
	+S9	–S9	
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. (1983a, b)
Rat esophageal tumor R1	ND	+	Abe et al. (1983a, b)
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 (FSHL) 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)
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)

Assay/Test System	Result		Reference
	+S9	–S9	
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-33. In vivo genotoxicity studies of benzo[a]pyrene

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Human, blood T lymphocytes (smokers and non-smokers); hprt locus mutation assay	T-cells of lung cancer patients (smokers and non-smokers from lung cancer patients and population controls with known smoking status) analyzed for hprt locus mutations.	+	Smokers and non-smokers	Splicing mutations, base-pair substitutions, frameshift, and deletion mutations observed. Smokers and non-smokers 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, 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 from 9 to 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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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).	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)
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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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)
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^{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 *lacZ* mutation frequency.	+	25, 50, and 75 mg/kg-day	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^{TM}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-day	Highest mutation frequency observed in colon followed by ileum > forestomach > bone marrow = spleen > glandular stomach > liver = lung > kidney = heart.	Hakura et al. (1998)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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)
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.	$+^{Sk}$ or $-^{Li,Lu}$	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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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)
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 PCR 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	Human, WBCs	96 people occupationally or medically exposed to PAH mixtures (psoriatic patients, coke oven workers, chimney sweeps, and aluminum anode plant workers); adducts measured by HPLC/fluorescence analysis.	+		Percentages of subjects with adduct levels greater than the 95th percentile control value were 47% (7/15), 21% (4/19), and 3% (1/34) in coke oven workers, chimney sweeps, and controls, respectively.	Pavanello et al. (1999)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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 NER capacity.	Pavanello et al. (2005)
BPDE-DNA adducts	Human, peripheral lymphocytes	585 Caucasian municipal workers (52% males, 20–62 years old) from northeast Italy environmentally exposed to PAH mixtures were screened for adducts measured by HPLC/fluorescence analysis.	+		Forty-two percent of the participants had elevated anti-BPDE-DNA adduct levels, defined as >0.5 adducts/108 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, maternal and umbilical cord blood	Maternal and umbilical cord blood obtained following normal delivery from 329 non-smoking pregnant women exposed to emissions from fires during the 4 weeks following the collapse of the WTC building in New York City on 09/11/2001.	+		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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	Workers were exposed 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 of benzo[a]pyrene	PAH exposure, CYP1A1 status and smoking significantly affected DNA adduct levels, i.e., *CYP1A1*(**1/*2* or **2A/*2*a) > *CYP1A1*1/*1*; occupational > environmental exposure; smokers > non-smokers; 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 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 non-smokers; no correlation with air benzo[a]pyrene levels and adduct levels.	Mensing et al. (2005)
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, and 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 fourfold 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) and 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 threefold 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 and 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 day 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)
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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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 twofold 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 BaP-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 hours 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, or1 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, b)
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, and 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, and 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-Köcher et al. (1979)
SCEs	Hamster	Animals implanted subcutaneously 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 increased with low dose of phorone significantly.	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 and 125 mg/kg	Produced doubling of SCE frequency.	Pereira et al. (1982)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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)
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 BaP-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 BaP-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	Threefold 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

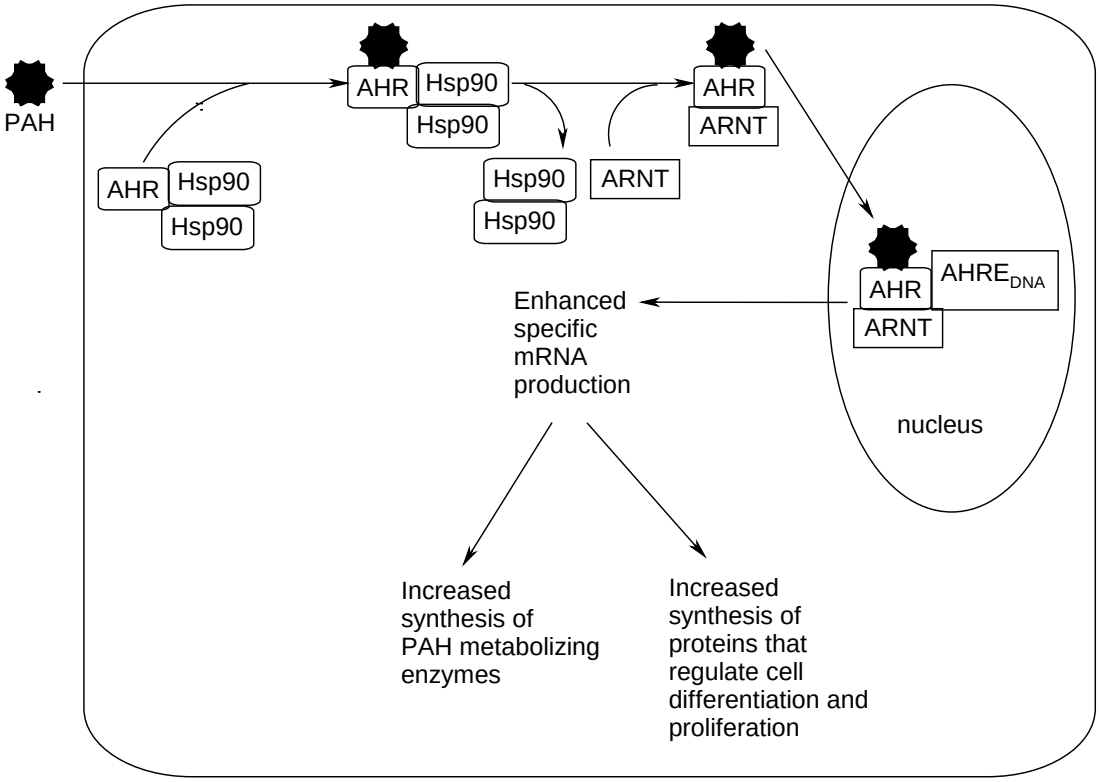
Cytotoxicity and Inflammatory Response

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 Ah receptor 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 = Ah receptor nuclear translocator; Hsp90 = heat shock protein 90

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 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 PCR, 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 [^{32}P]-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 Ah receptor 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-34. Many of the search terms included terms for specific polycyclic aromatic hydrocarbon mixtures (PAH), 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-34. 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, 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, 75 mg/kg benzo[a]pyrene or olive oil vehicle for 28 days by oral gavage. The second study(Yauk et al., 2011), GSE18789, exposed 27–30 day old male B6C3F_1 mice to 150 mg/kg benzo[a]pyrene by oral gavage for 3 days and sacrificed 4 hours 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. Principal Components Analysis is commonly used for cluster analysis based on the variance within the dataset. The PCA algorithm (in this case we used singular value decomposition) 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 (DEG) 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 MOA 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-35. 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 hr after final dose)	Yauk et al. (2011)
3	150 mg/kg	3 d exposure (sacrificed 24 hr 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)

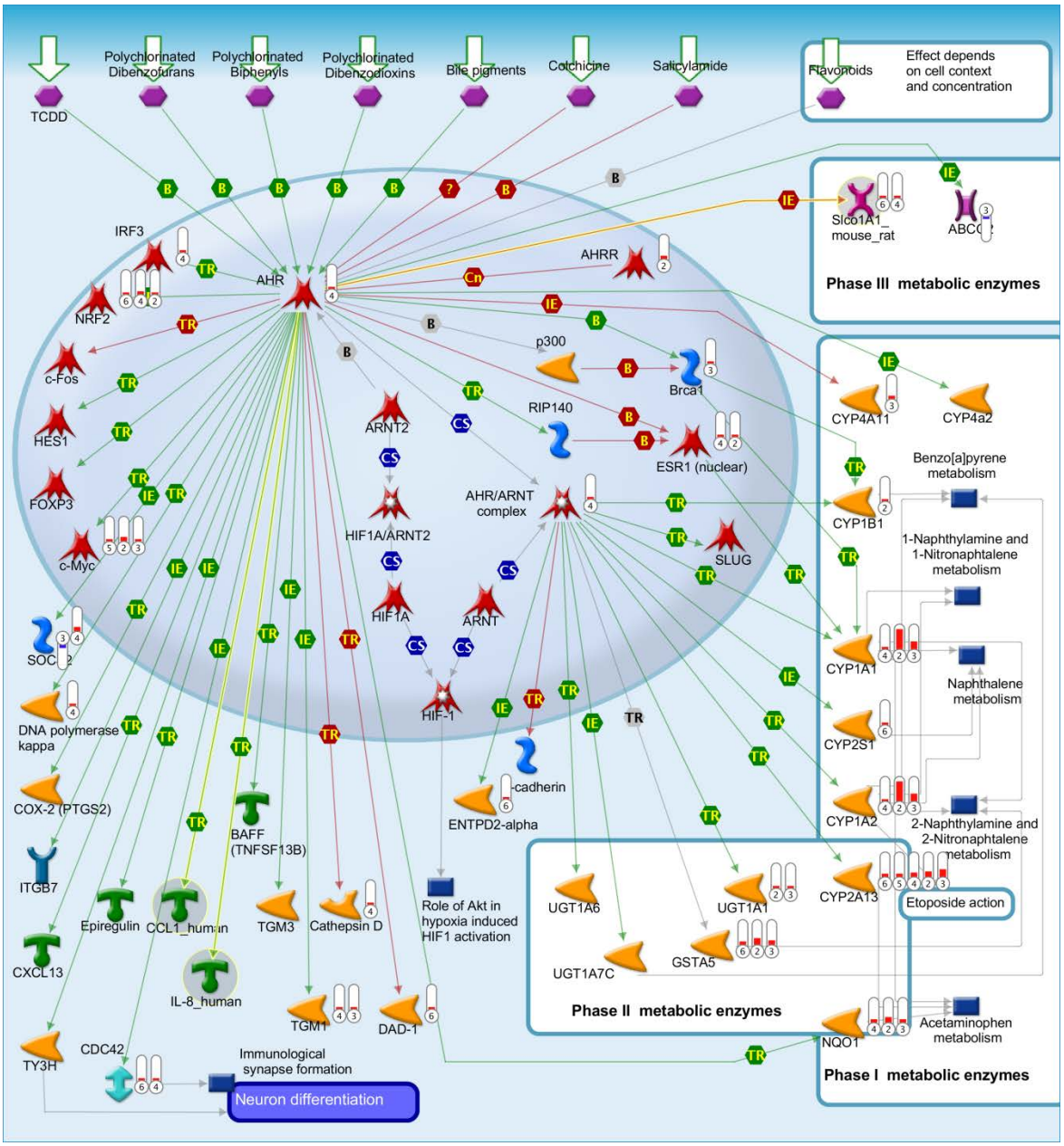


Figure D-4. Aryl hydrocarbon receptor pathway. For Figures D-4–D-6, the "thermometers" display the fold change gene expression. The numbers under the thermometer represent the group within the two studies (see Table D-35). For instance, NRF2 is upregulated in the 25 mg/kg.

Aryl Hydrocarbon receptor 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

mg/kg and 75 mg/kg doses. c-Myc has been shown to be upregulated following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin, 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).

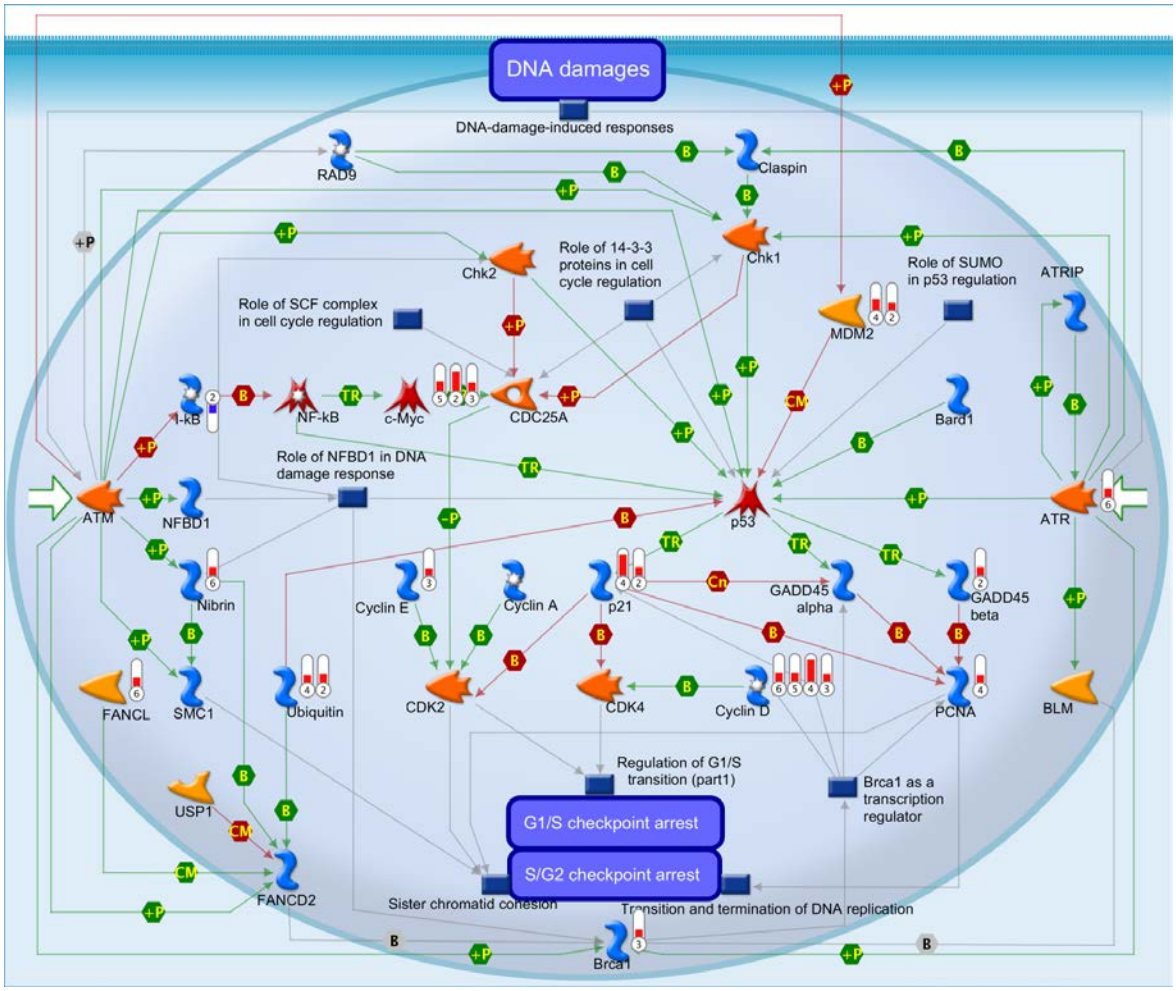


Figure D-5. DNA Damage pathway. Activation of transcriptional targets of p53, including p21 and GADD45, and upregulation of the downstream transcriptional target, PCNA, suggests that p53 is activated.

DNA Damage Signaling

The strong upregulation of p21 and MDM2 at 4 hours and 75 mg/kg 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 in mice (Figure D-5). MDM2 is a target gene of p53, and also negatively feedback inhibits p53 signaling through ubiquitination. Ubiquitin is also upregulated at 4 hour and 75 mg/kg, further suggesting that that p53 may initially be upregulated at times prior to 4 hour and prior to sacrifice in the 75 mg/kg 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 (among other conditions), this suggests a pro-mitotic shift may be occurring which could lead to cellular proliferation in the liver in the mice exposed to 75 mg/kg per day.

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 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 substaniated by the lack of upregulation of anti-oxidant genes at 75 mg/kg, with the exception of GCL cat.

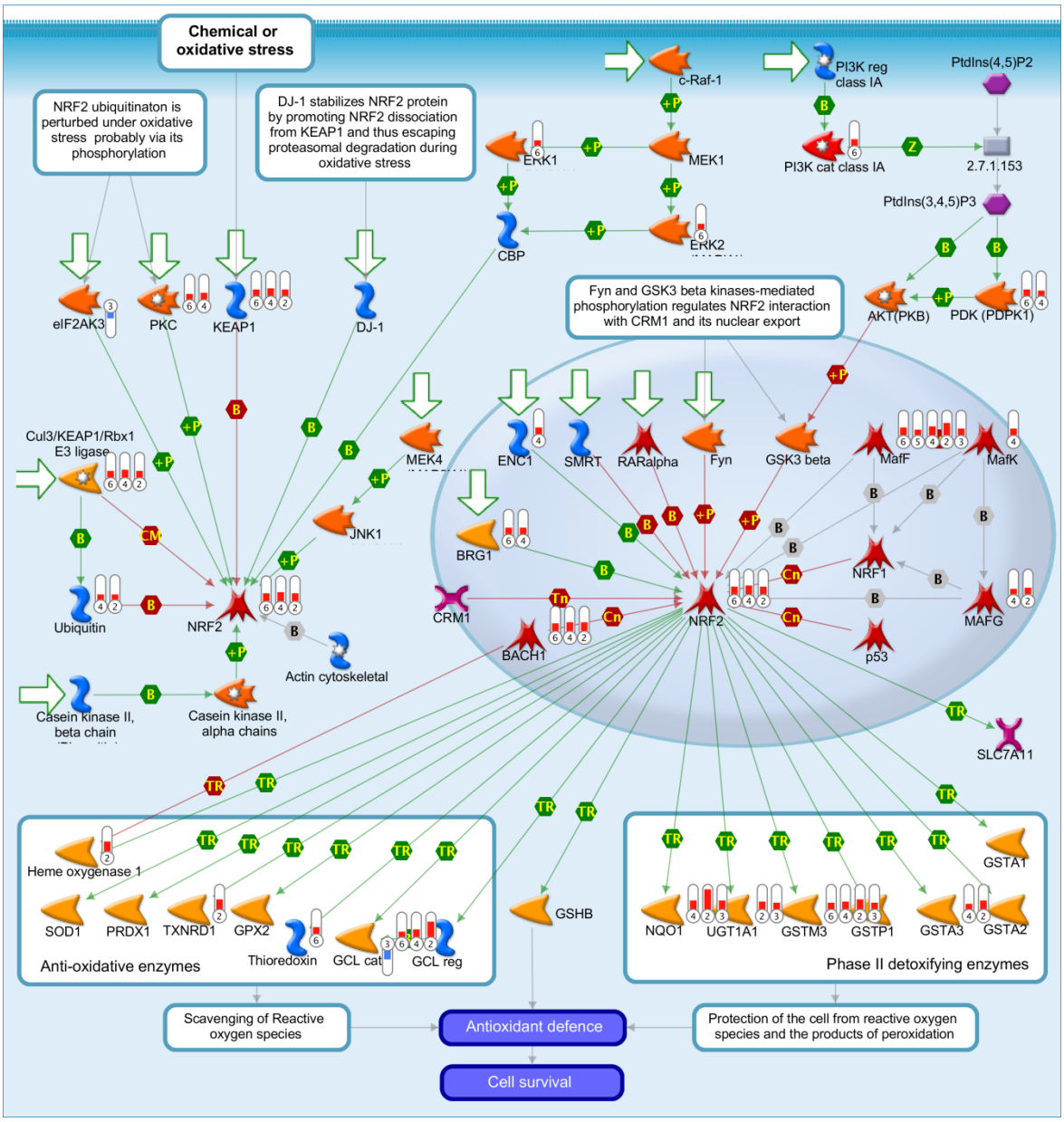


Figure D-6. Nrf2 pathway. Nrf2 is upregulated by benzo[a]pyrene exposure, which results in the upregulation of Phase II detoxifying enzymes. This appears to be a compensatory response due to increased oxidative status within cells.

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 MOA in the liver to support an overall MOA.

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 (POD) 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. EPA's Benchmark Dose Software [BMDS; (U.S. EPA, 2012a); version 2.0 or later]. The preambles for the cancer and non-cancer 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. NON-CANCER ENDPOINTS

E.1.1. Reference Dose (RfD)

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. For each model, 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), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), 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.

Model Selection

For each endpoint selected for modeling (see Table E-1), 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 threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

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

Study	Endpoint	Species / Sex	Doses and Effect Data				
Kroese et al. (2001)	Thymus weight (mg)	Rat (Wistar) / Male	Dose (mg/kg-d)	0	3	10	30
			Mean ± SD[a]	380 ± 60	380 ± 110	330 ± 60	270 ± 40*
Kroese et al. (2001)	Thymus weight (mg)	Rat (Wistar) / Female	Dose (mg/kg-d)	0	3	10	30
			Mean ± SD[a]	320 ± 60	310 ± 50	300 ± 40	230 ± 30*
Xu et al. (2010)	Ovary weight (mg)	Sprague-Dawley / Female	Dose (mg/kg-d)[b]	0	2.5	5	
			Mean ± SD	0.160 ± 0.0146	0.143 ± 0.0098**	0.136 ± 0.0098**	
Chen et al. (2012)	Morris water maze	Sprague-Dawley / Male and Female	Dose (mg/kg-d)	0	0.02	0.2	2.0
			Escape latency (sec); mean ± SD	9.89 ± 5.76	12.5 ± 5.10	19.1 ± 5.85	33.5 ± 9.93
			Time spent in target quadrant (sec); mean ± SD	33.6 ± 8.92	31.9 ± 8.63	16.6 ± 5.74	11.1 ± 5.12
	Elevated plus maze	Sprague-Dawley / Female	Number of open arm entries	10.24 ± 1.905	10.36 ± 3.048	12.89 ± 2.667	16.39 ± 3.048
Gao et al. (2011b)	Cervical epithelial hyperplasia	ICR / Female	Dose (mg/kg-d)[c]	0	0.71	1.4	2.9
			Incidence	0/26	4/26	6/25	7/24

*Significantly (p < 0.05) different from control mean; student t-test (unpaired, two-tailed); n = 10/sex/group.
**Statistically different (p < 0.05) from controls using one-way ANOVA.

[a]Reported as SE, but judged to be SD (and confirmed by study authors).
[b]TWA doses over the 60-day study period.
[c]Doses converted to mg/kg-d after adjustment for equivalent continuous dosing (2/7 d/wk).

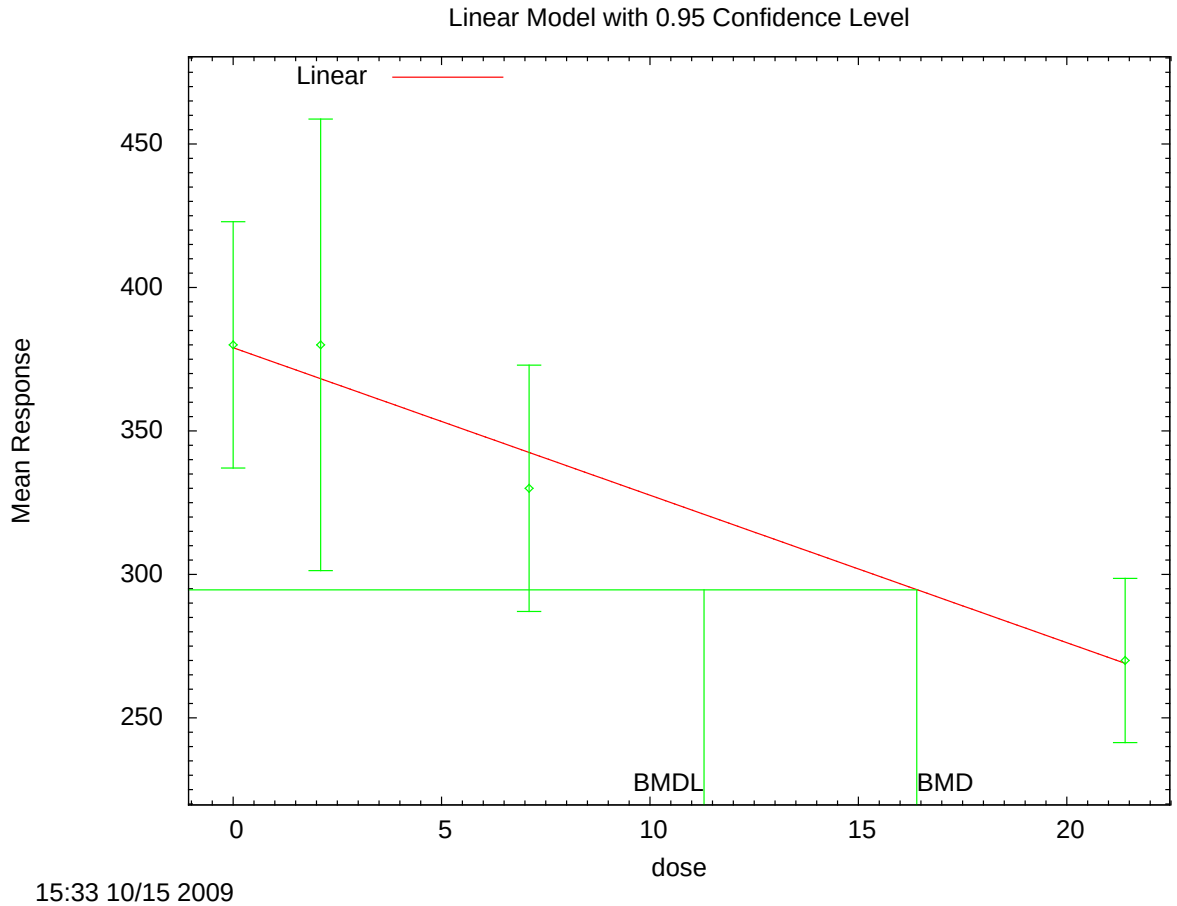
Modeling Results

Below are tables and figures summarizing the modeling results for the non-cancer endpoints modeled (see Tables E-2 through E-8 and Figures E-1 through E-7).

Table E-2. 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[a]	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[b]	Insufficient degrees of freedom				
Linear, Polynomial (2-degree), Power[c]	**0.30**	**0.23**	**380.71**	**16.40**	**11.30**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.



BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.

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).

```
================================================================
         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
```

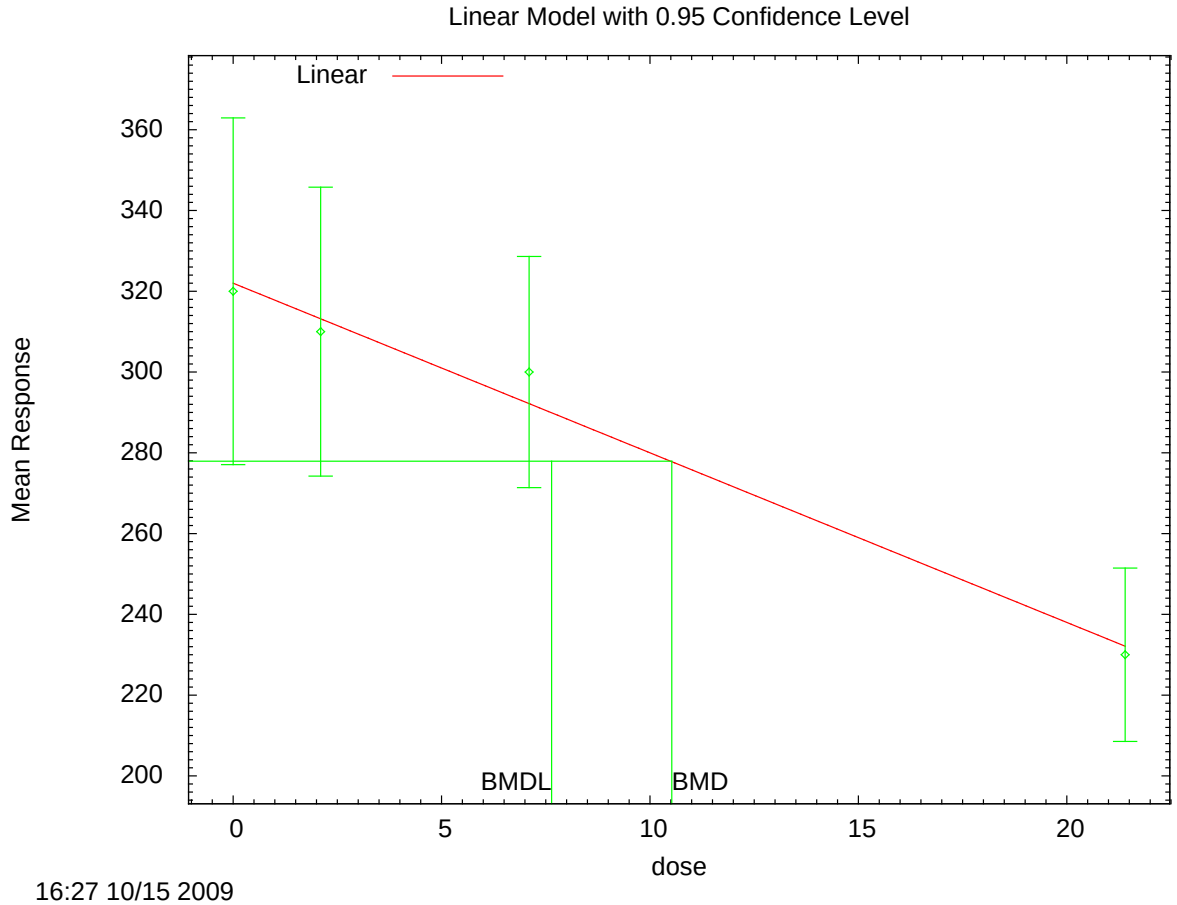
```
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

              BMD =        16.4008


              BMDL =        11.2965

```

Table E-3. 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[a]	Mean *p*-value[a]	AIC		
Hill[b]	NA				
Linear[c]	**0.17**	**0.81**	**349.12**	**10.52**	**7.64**
Polynomial (2-degree)[c,d]	0.17	0.77	350.80	13.29	7.77
Power[b]	NA				

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Coefficients restricted to be negative.
[d]Lowest degree polynomial with an adequate fit is reported.

BMD/BMC = maximum likelihood estimate (MLE) of the dose/concentration associated with the selected BMR;
NA = not applicable; model failed to generate.



16:27 10/15 2009

BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.

Figure E-2. Fit of linear model (constant variance) to data on decreased thymus weight in female Wistar rats—90 days (Kroese et al., 2001).

```
=====================================================================
         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
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   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-4. 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[a]			
Linear, Polynomial (1°)	**0.39**	**-138.67**	**2.27**	**1.49**

[a]NA = not applicable; model failed to generate.

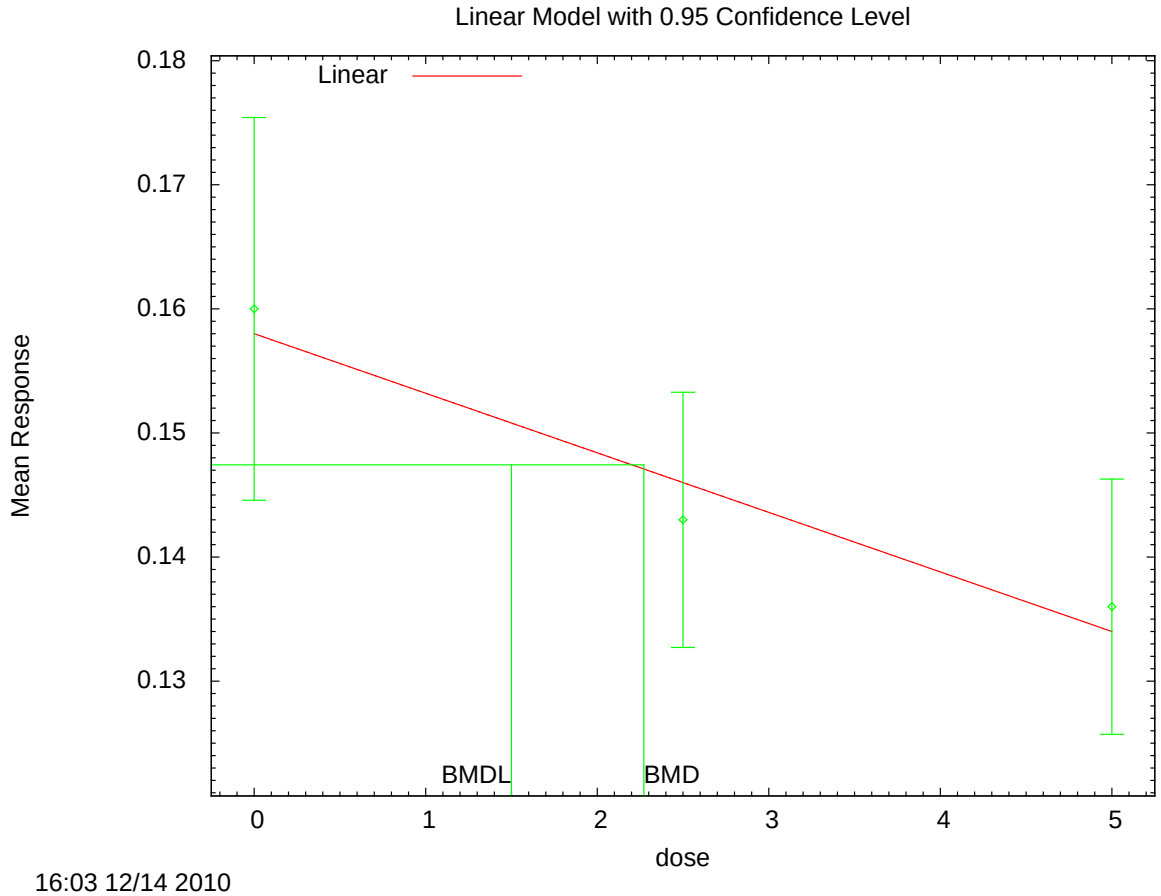

Figure E-3. Fit of linear/polynomial (1°) model to data on decreased ovary weight (Xu et al., 2010).

```
================================================================
         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-5. Summary of BMD modeling results for Morris water maze: escape latency in male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 90 days (Chen et al., 2012); BMR = 1 SD change from the control mean

Model[a]	Goodness of Fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
	p-value	AIC		
Hill[b]	**0.515**	**386.3**	**0.106**	**0.061**
Exponential 4, 5	0.466	386.4	0.115	0.071
Polynomial (2°)	0.423	386.6	0.123	0.083
Linear, Power	0.002	396.7	0.543	0.421
Exponential 2, 3	<0.001	400.3	0.815	0.687

[a]Includes modeling of heterogeneous variances (BMDS Test 3, $p = 0.313$).
[b]Power parameter *n* was estimated to be 1 (boundary of parameter space).

Data from Morris water maze was presented graphically in Chen et al. (2012), but dose group means and SDs were provided upon request by the study authors, which enabled modeling of this endpoint. In addition, the data for male and female rats were combined for dose-response analysis because there was no substantive difference between males and females for each dose group (supported by statistical testing using two-way ANOVA, and allowing for interactions), and because there was no rationale or information available suggesting there would be sex-mediated differences for these neurobehavioral tests.

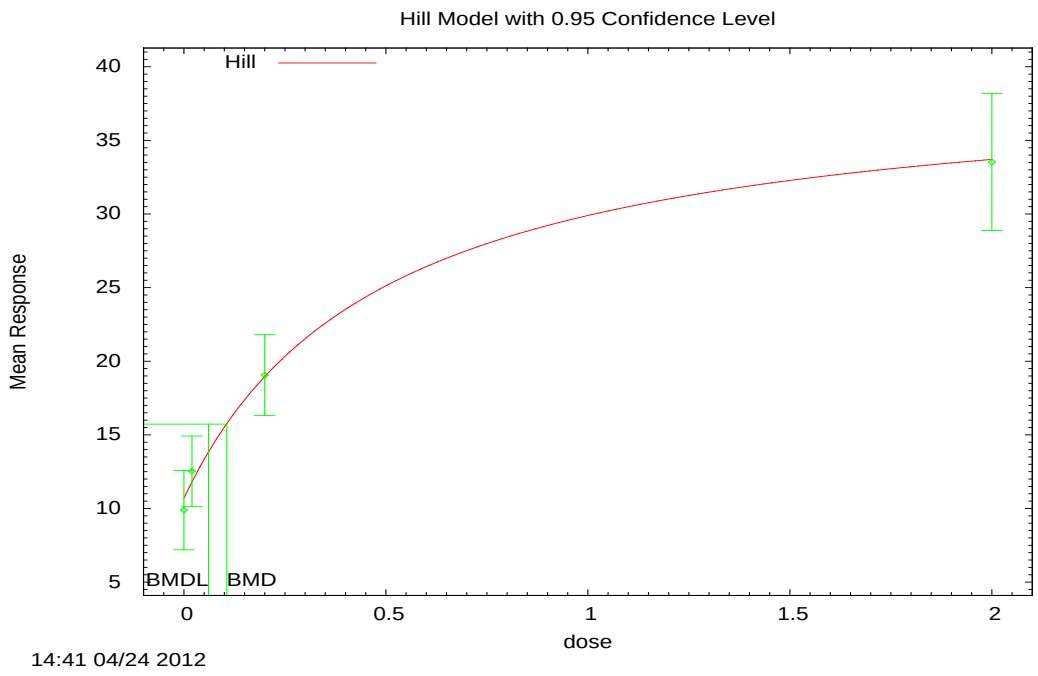

Figure E-4. Fit of Hill model to data on Morris water maze test escape latency (Chen et al., 2012).

```
================================================================
         Hill Model. (Version: 2.16;  Date: 04/06/2011)
         Input  Data  File:  C:\Documents  and  Settings\jfox\My  Documents\_CURRENTWORK\_CAST
plus\BaP\BMDS\hil_Chen.FM.latency_Hil-ModelVariance-BMR1Std-Restrict.(d)
         Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\_CAST
plus\BaP\BMDS\hil_Chen.FM.latency_Hil-ModelVariance-BMR1Std-Restrict.plt
                                                  Tue Apr 24 14:41:26 2012
 ================================================================


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

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   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 =      3.87128
                           rho =            0
                     intercept =        9.888
                             v =      23.6385
                             n =     0.187055
```

k = 3.47082

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	intercept	v	k
lalpha	1	-0.99	-0.27	0.062	-0.11
rho	-0.99	1	0.24	-0.063	0.12
intercept	-0.27	0.24	1	0.017	0.47
v	0.062	-0.063	0.017	1	0.73
k	-0.11	0.12	0.47	0.73	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	0.88775	0.974841	-1.0229	2.7984
rho	0.998033	0.338845	0.33391	1.66216
intercept	10.6545	0.914127	8.86283	12.4461
v	28.7081	3.94381	20.9783	36.4378
n	1	NA		
k	0.494812	0.213359	0.0766351	0.912988

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	20	9.89	10.7	5.76	5.08	-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	0.0952
2	20	33.5	33.7	9.93	9.01	-0.0706

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	-192.799518	5	395.599036
A2	-186.795503	8	389.591006
A3	-187.957975	6	387.915949
fitted	-188.169983	5	386.339965
R	-234.549118	2	473.098237

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	95.5072	6	<.0001
Test 2	12.008	3	0.007356
Test 3	2.32494	2	0.3127
Test 4	0.424016	1	0.5149

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 = 0.106284

BMDL = 0.0609511

Table E-6. Summary of BMD modeling results for Morris water maze: time spent in quadrant for in male and female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 90 days (Chen et al., 2012); BMR = 1 SD change from the control mean

Model[a]	Goodness of Fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
	p-value	AIC		
Exponential 4	**0.576**	**395.4**	**0.065**	**0.043**
Exponential 5	NA[b]	397.1	0.084	0.044
Hill	NA[b]	397.1	0.071	0.038
Linear, Power, Polynomial (1°, 2°, 3°)	<0.001	433.1	1.23	0.98

[a]Includes modeling of heterogenous variances (BMDS Test 3, $p = 0.919$).
[b]NA: insufficient degrees of freedom to evaluate χ^2.

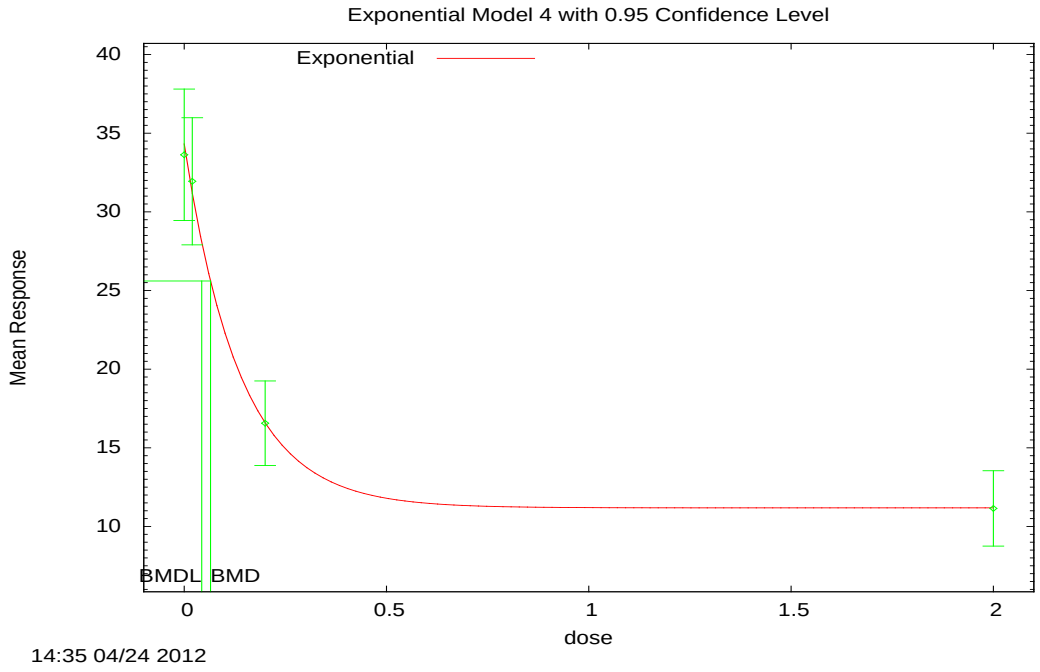

Figure E-5. Fit of Exponential 4 model to data on Morris water maze time spent in target quadrant (Chen et al., 2012).

```
================================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input  Data  File:  C:\Documents  and  Settings\...\exp_Chen.FM.target_Exp-ModelVariance-
BMR1Std-Down.(d)
 ================================================================================

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

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp$$sign * b * dose}
      Model 3:     Y[dose] = a * exp$$sign * (b * dose)^d}
```

```
  Model 4:     Y[dose] = a * [c-(c-1) * exp$$-b * dose}]
  Model 5:     Y[dose] = a * [c-(c-1) * exp$$-(b * dose)^d}]

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

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


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
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

MLE solution provided: Exact


              Initial Parameter Values

              Variable          Model 4
              --------          --------
                lnalpha            0.666712
                    rho             1.04799
                      a             35.3094
                      b             1.97191
                      c            0.300675
                      d                   1



                Parameter Estimates

               Variable          Model 4
               --------          -------
                lnalpha           0.601192
                    rho            1.05452
                      a            34.3199
                      b            7.26795
                      c           0.325841
                      d                  1

 NC = No Convergence


       Table of Stats From Input Data

 Dose      N         Obs Mean     Obs Std Dev
 -----    ---       ----------   -------------
     0     20        33.63        8.924
  0.02     20        31.94        8.633
   0.2     20        16.56        5.744
     2     20        11.15        5.117


             Estimated Values of Interest

  Dose      Est Mean      Est Std     Scaled Residual
------    ----------    ---------    ----------------
     0         34.32        8.713          -0.3551
  0.02         31.19        8.285           0.4069
   0.2         16.59        5.939         -0.02044
```

```
      2         11.18        4.824         -0.03277


Other models for which likelihoods are calculated:

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

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

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

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


                            Likelihoods of Interest

                 Model      Log(likelihood)      DF         AIC
                -------    -----------------    ----   ------------
                    A1       -197.0118           5      404.0235
                    A2        -192.448           8       400.896
                    A3       -192.5331           6      397.0662
                     R       -238.8696           2      481.7393
                     4       -192.6894           5      395.3787


Additive constant for all log-likelihoods =     -73.52.  This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.


                             Explanation of Tests

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

Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

  Test          -2*log(Likelihood Ratio)       D. F.         p-value
--------        ------------------------      ------     --------------
  Test 1                       92.84            6            < 0.0001
  Test 2                       9.127            3             0.02764
  Test 3                      0.1701            2               0.9185
 Test 6a                      0.3126            1               0.5761


  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 6a is greater than .1.  Model 4 seems
  to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 1.000000
```

```
        Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

             BMD =    0.0650194

            BMDL =    0.0432761
```

Table E-7. Summary of BMD modeling results for elevated plus maze: open arm entries for females at PND 70 (Chen et al., 2012); BMR = 1 SD change from the control mean

Model[a]	Goodness of fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
	p-value[b]	AIC		
Exponential (M2) Exponential (M3)	0.107	125.93	1.086	0.845
Exponential (M4)	**0.840**	**123.51**	**0.184**	**0.086**
Exponential (M5)	NA	125.47	0.194	0.087
Hill	NA	125.47	0.193	0.066
Polynomial 1° Polynomial 2° Polynomial 3° Power	0.129	125.57	0.964	0.713

[a] Constant variance models are presented (BMDS Test 2 *p*-value = 0.46), with the selected model in bold. Scaled residuals for selected model for doses 0, 0.02, 0.2, and 2 mg/kg-d were 0.13, –0.15, 0.03, and –0.003, respectively.
For exponential model M3, parameter d = 1, reducing it to M2.
For the power model, the power parameter estimate was 1 (boundary of parameter space). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary of parameter space). Consequently, these three models all reduced to the polynomial 1° model.
[b] Exponential M5 and Hill model required four parameters and there are four dose groups, leaving no d.f. for the goodness-of-fit test. Therefore these were not considered for model selection

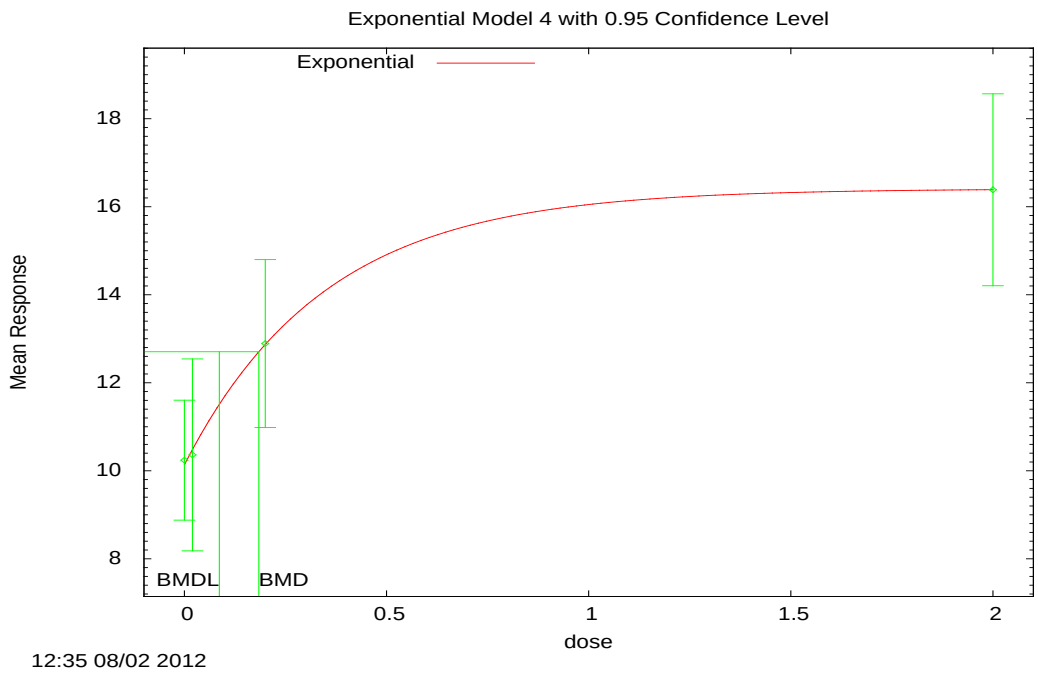

Figure E-6. Fit of exponential model (4) to data on elevated plus maze open arm maze entries (Chen et al., 2012).

```
 ================================================================
         Exponential Model. (Version: 1.7;  Date: 12/10/2009)
         Input  Data  File:  C:\Documents  and  Settings\jfox\My  Documents\_CURRENTWORK\_CAST
plus\BaP\BMDS\exp_ChenFO70_Exp-ConstantVariance-BMR1Std-Up.(d)
         Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\_CAST
plus\BaP\BMDS\exp_ChenFO70_Exp-ConstantVariance-BMR1Std-Up.plt
                                                  Thu Aug 02 12:35:33 2012
 ================================================================

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

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

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

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


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

   Total number of dose groups = 4
   Total number of records with missing values = 0
```

```
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact


               Initial Parameter Values

               Variable          Model 4
               --------          --------
                 lnalpha             1.88669
                     rho(S)                0
                       a             9.72892
                       b             1.12212
                       c             1.76842
                       d                   1

  (S) = Specified


                 Parameter Estimates

                Variable          Model 4
                --------          -------
                 lnalpha             1.8877
                     rho                  0
                       a              10.136
                       b             2.86365
                       c             1.61881
                       d                   1


        Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0     10        10.24        1.905
   0.02     10        10.36        3.048
    0.2     10        12.89        2.667
      2     10        16.39        3.048


             Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         10.14         2.57           0.1292
   0.02         10.49         2.57          -0.1521
    0.2         12.87         2.57          0.02563
      2         16.39         2.57        -0.002716


Other models for which likelihoods are calculated:

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

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

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

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


                            Likelihoods of Interest
```

Model	Log(likelihood)	DF	AIC
A1	-57.73371	5	125.4674
A2	-56.43655	8	128.8731
A3	-57.73371	5	125.4674
R	-71.03323	2	146.0665
4	-57.75397	4	123.5079

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

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

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	29.19	6	< 0.0001
Test 2	2.594	3	0.4585
Test 3	2.594	3	0.4585
Test 6a	0.04053	1	0.8404

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 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 0.184087

BMDL = 0.0864691

Table E-8. 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., 2011b); 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		
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

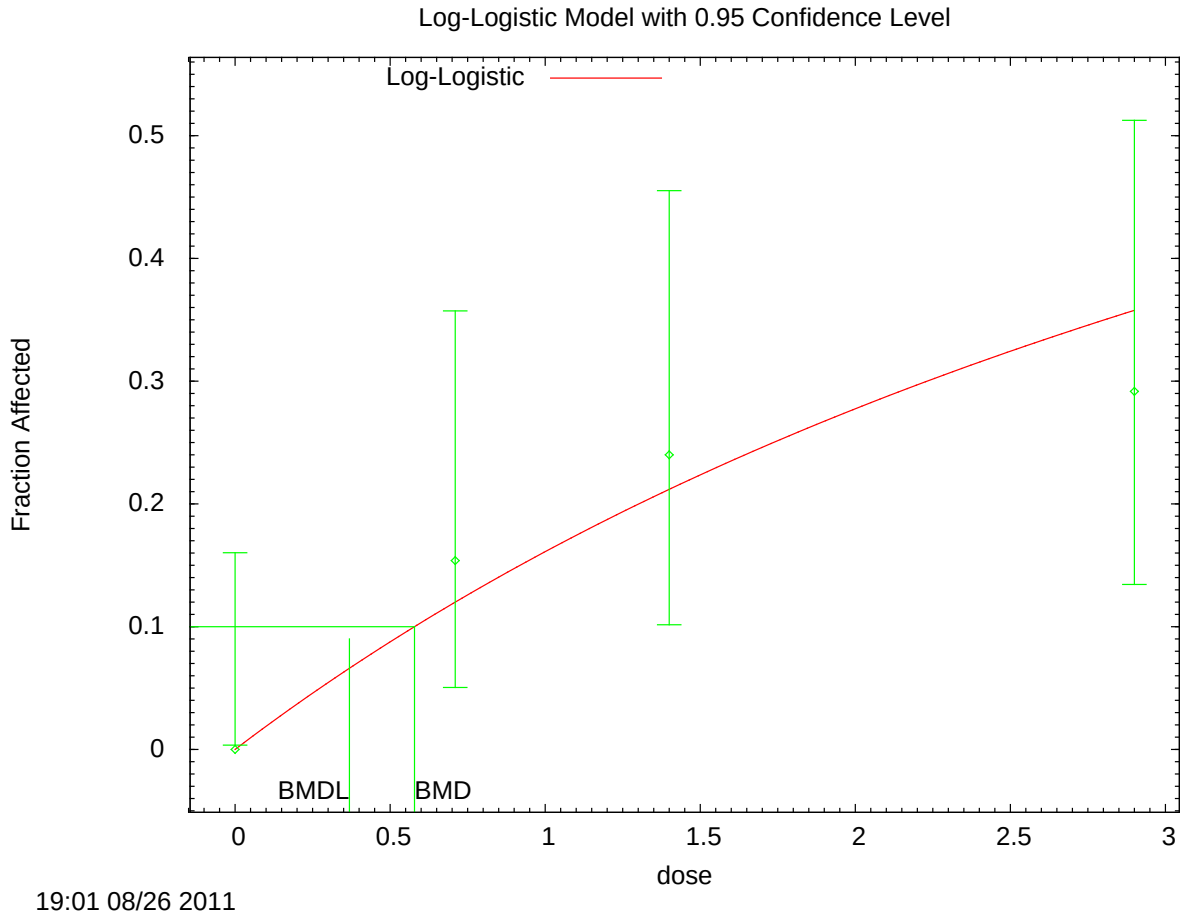

Figure E-7. Fit of log-logistic model to data on cervical epithelial hyperplasia (Gao et al., 2011b)

==========

```
          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
================================================================

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

   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
```

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

Reference Concentration (RfC)

Candidate studies for the development of the RfC were not amenable to BMD modeling.

DosimetryModeling for Estimation of Human Equivalent Concentrations

As discussed in Section 2.2.2, the human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by a 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 model, a computational model used for estimating human and rat airway particle deposition and clearance (Multi-Path Particle Dosimetry [MPPD]; Version 2.0 © 2006, publicly available through the Hamner Institute). See model output below.

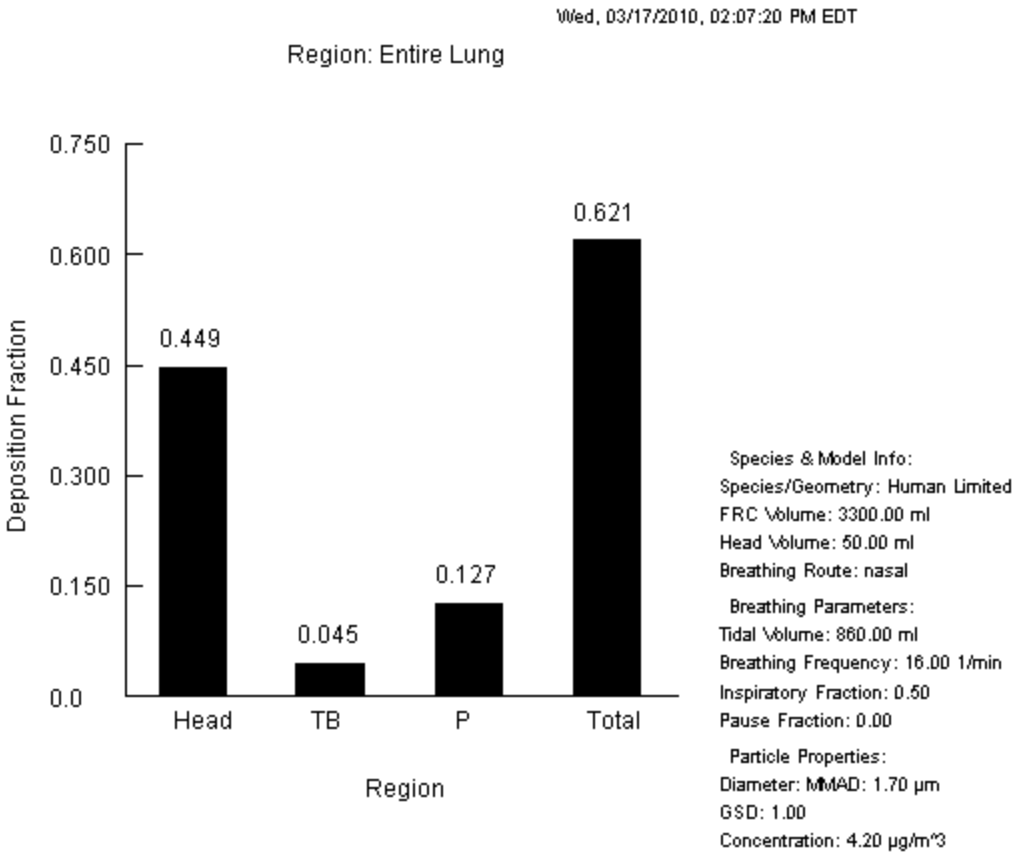

Figure E-8. Human fractional deposition.

```
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
```

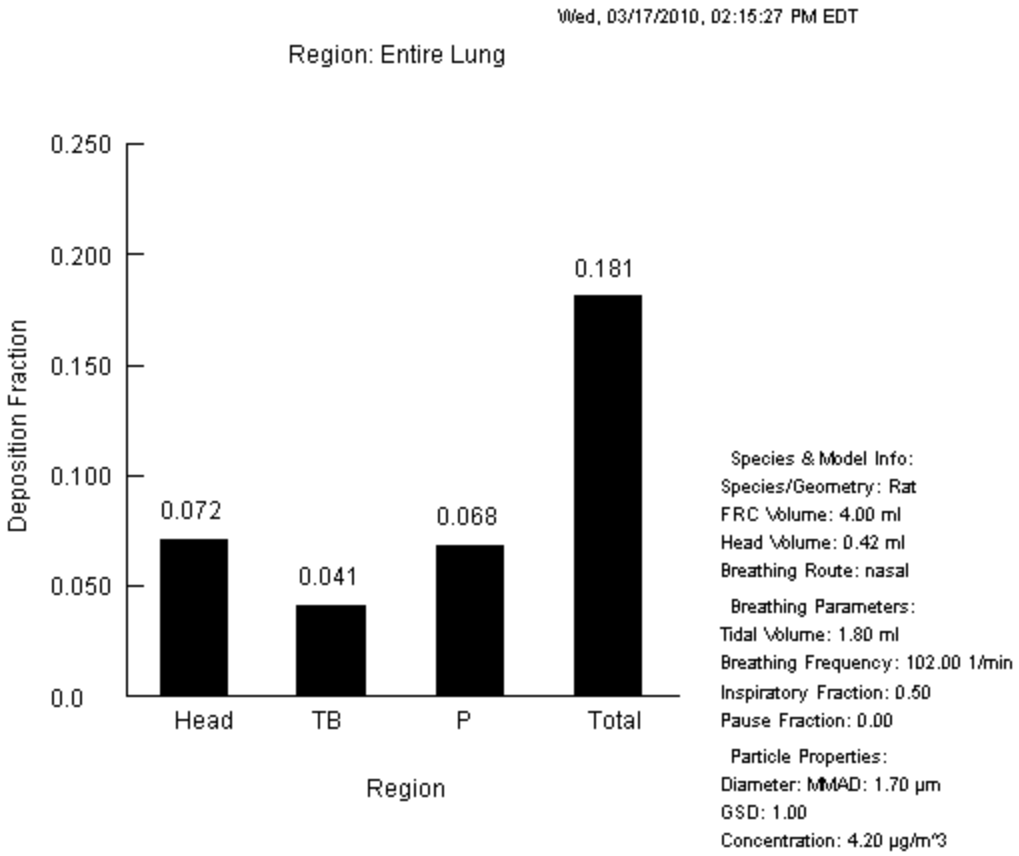

Figure E-9. Rat fractional deposition.

```
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

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, 2010), 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.2.2. 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 (that is, 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 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-9 (male rats), E-10 (female rats), and E-11 (female mice).

Human-equivalent dose 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. The dose estimates are provided in Table E-12 (Kroese et al., 2001) and E-13 (Beland and Culp, 1998).

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 benchmark response (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.

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, 2005) 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, 2010). 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% 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$^{1/2}$).

Dose-Response Modeling for the Oral Slope Factor

Table E-9. 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)	Week 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

Dose (mg/kg-d)	Week 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
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
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)	Week 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-10. 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)	Week 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)	Week 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)	Week 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-11. 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)	Week of Death	Total Examined	Number of Animals with Alimentary Tract Squamous Cell Tumors	
			Fatal[a]	Incidental
0	31	1	0	0
	74	1	0	0
	89	2	0	0
	91	1	0	0
	93	2	0	0
	94	2	0	0
	97	2	0	0
	98	2	0	0
	99	1	0	0
	100	2	0	0
	101	2	0	0
	104	1	0	0
	105	29	0	1

Dose Group (ppm in Diet)	Week of Death	Total Examined	Number of Animals with Alimentary Tract Squamous Cell Tumors	
			Fatal[a]	Incidental
5	25	1	0	0
	55	1	0	0
	83	1	0	0
	86	1	0	0
	87	2	0	0
	88	2	0	0
	90	1	0	0
	94	1	0	0
	95	2	0	0
	96	1	0	0
	97	2	0	0
	98	2	0	0
	101	2	0	0
	102	2	0	0
	105	27	0	3
25	44	1	1	0
	47	1	0	0
	64	1	0	0
	70	1	1	0
	77	1	1	0
	80	1	0	0
	81	1	1	0
	84	2	1	1
	85	1	1	0
	86	1	1	0
	88	1	1	0
	89	1	0	0
	90	4	4	0
	93	3	2	1
	94	2	2	0
	96	3	0	2
	97	1	1	0
	98	1	1	0
	99	2	1	1
	100	1	1	0
	101	1	0	0
	102	2	2	0
	104	1	1	0
	105	13	0	10

Dose Group (ppm in Diet)	Week of Death	Total Examined	Number of Animals with Alimentary Tract Squamous Cell Tumors	
			Fatal[a]	Incidental
100	39	1	1	0
	40	1	1	0
	42	1	1	0
	47	2	2	0
	49	1	0	0
	50	1	1	0
	53	1	0	0
	55	3	3	0
	56	1	1	0
	57	1	1	0
	58	1	1	0
	59	3	3	0
	60	1	1	0
	61	3	3	0
	62	5	5	0
	63	4	4	0
	64	3	3	0
	65	2	2	0
	66	3	3	0
	68	1	1	0
	69	2	2	0
	70	2	2	0
	71	1	1	0
	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-12. 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]Human equivalent dose (HED) = administered dose × scaling factor.

Table E-13. 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.

Modeling Results

Tables E-14 (male and female rats) and E-16 (female mice) summarize the modeling results supporting the oral slope factor for BaP. The model outputs and graphs following each of these tables (Figures E-10 through E-19) provide more details for the best-fitting models in each case.

Table E-14. 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
```

DOSE	C	F	I	U	Total	Expected	Response
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

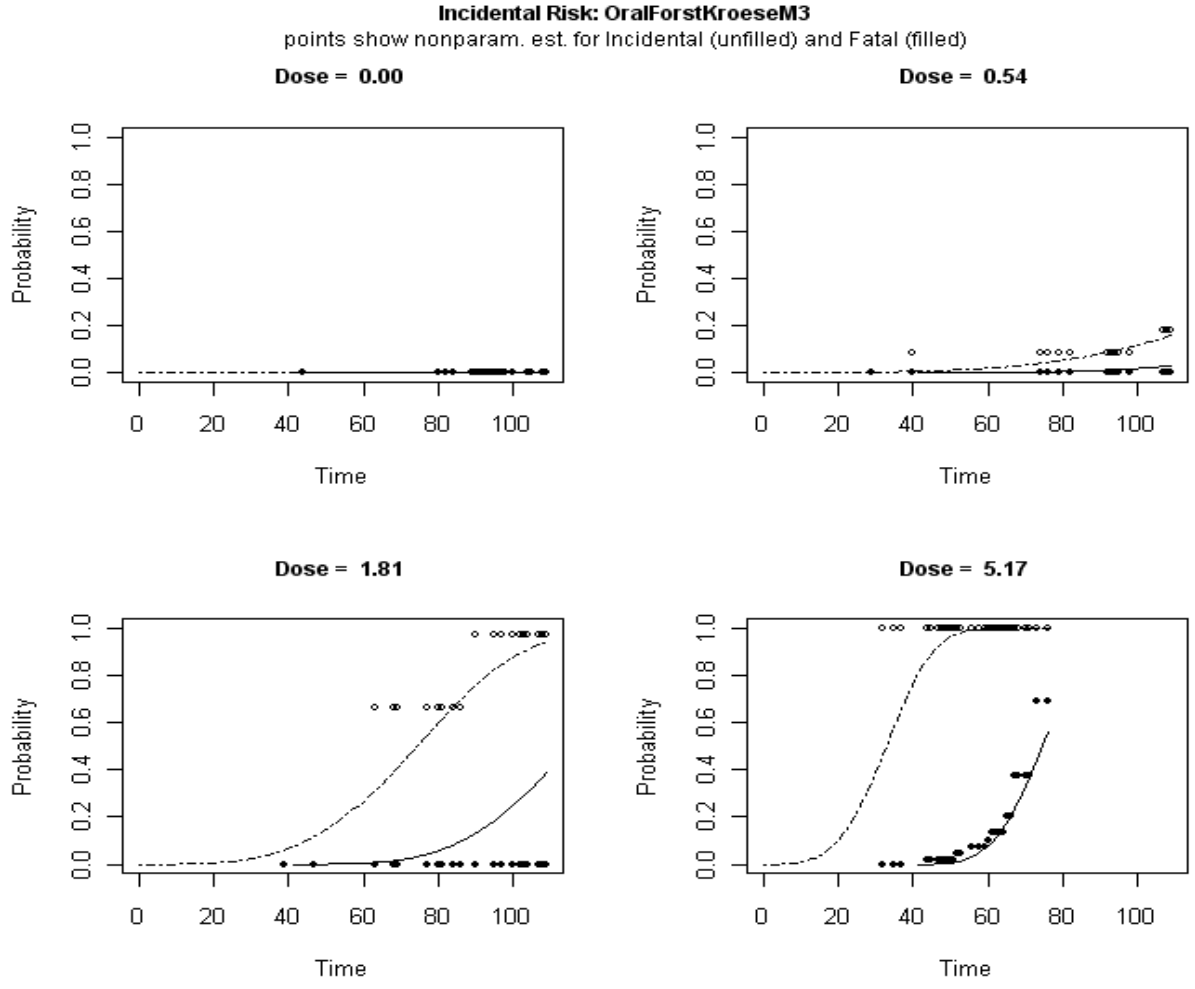

Figure E-10. Fit of multistage Weibull model to squamous cell papillomas or carcinomas in oral cavity or forestomach of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
```

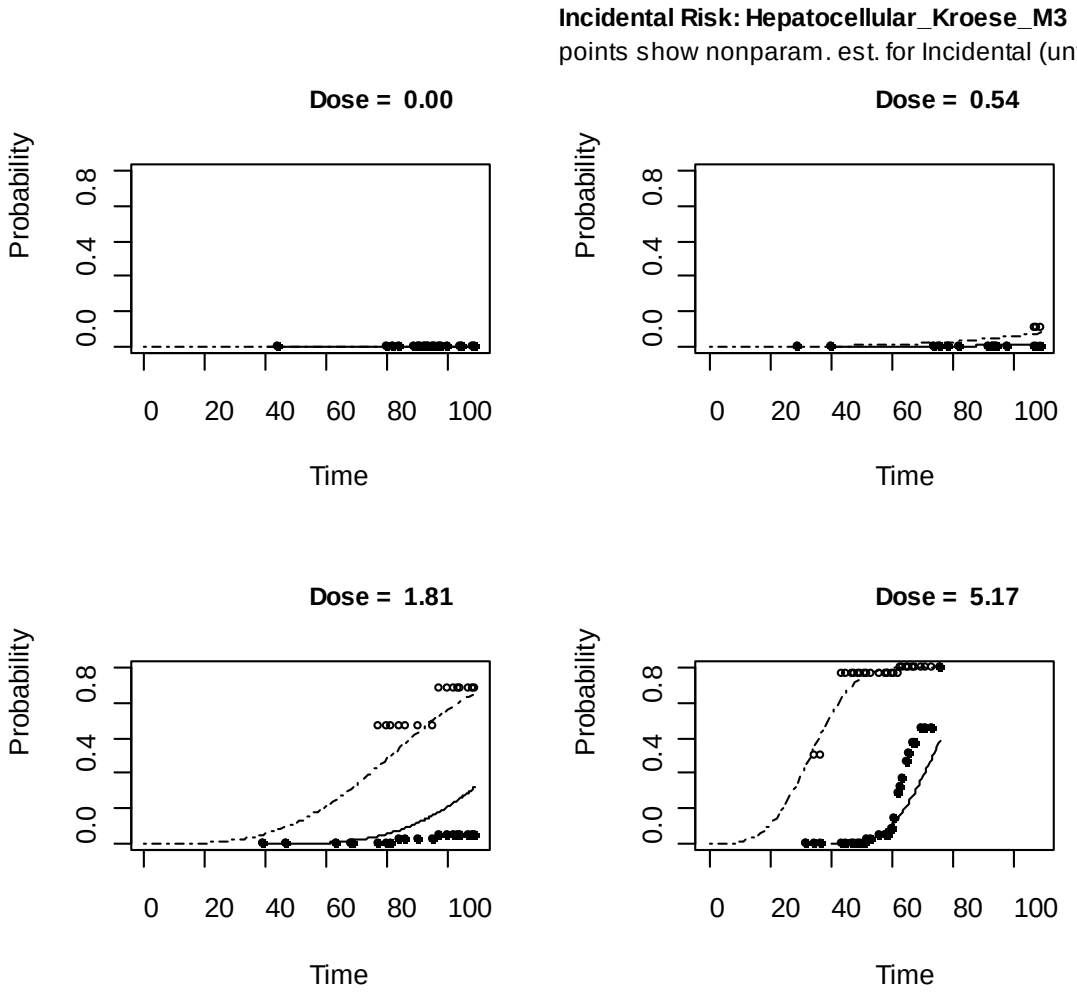

Figure E-11. Fit of multistage Weibull model to hepatocellular adenomas or carcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
                       CONTEXT
              C      F      I      U  Total  Expected Response
    DOSE
```

```
          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
```

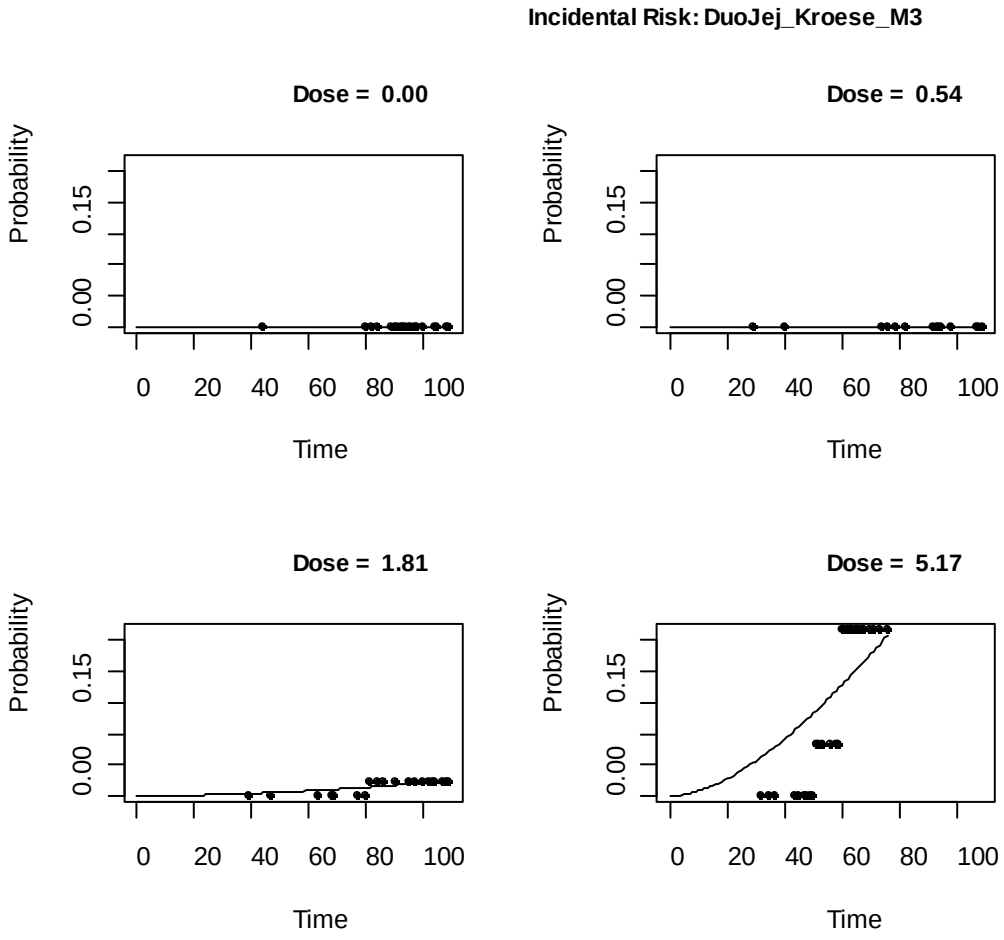

Figure E-12. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
                         CONTEXT
```

DOSE	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

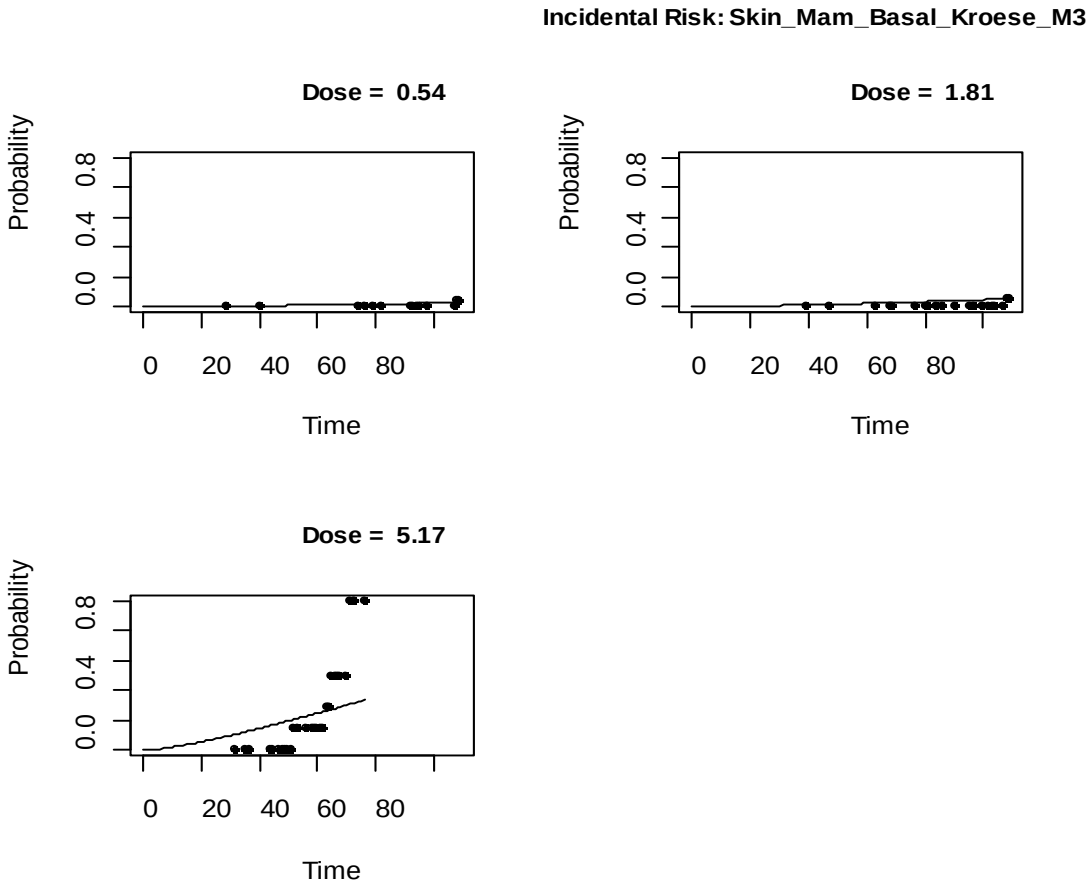

Figure E-13. Fit of multistage Weibull model to skin or mammary gland basal cell tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
```

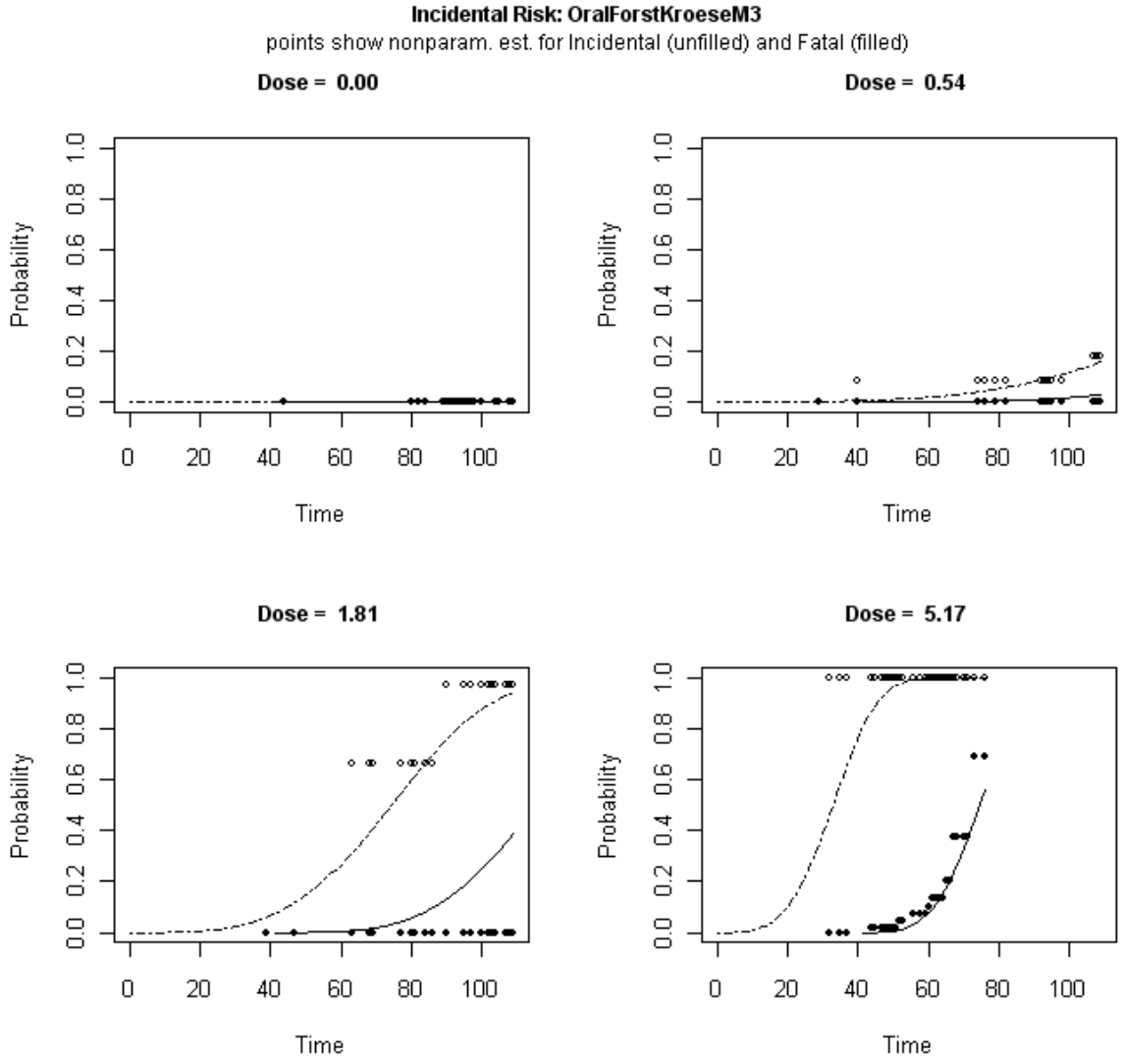

Figure E-14. Fit of multistage Weibull model to skin or mammary gland squamous cell tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
                       CONTEXT
              C      F      I      U  Total  Expected Response
```

```
      DOSE
         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
```

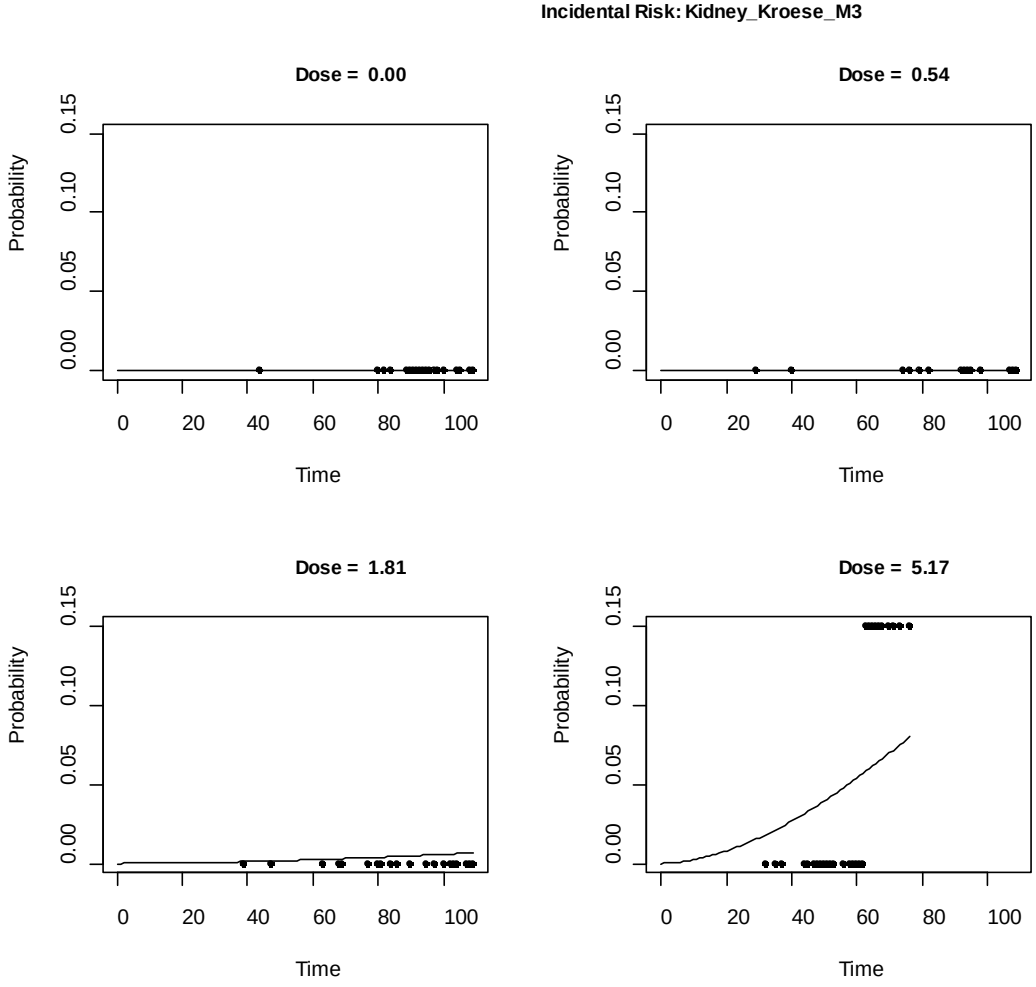

Figure E-15. Fit of multistage Weibull model to kidney urothelial tumors of male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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

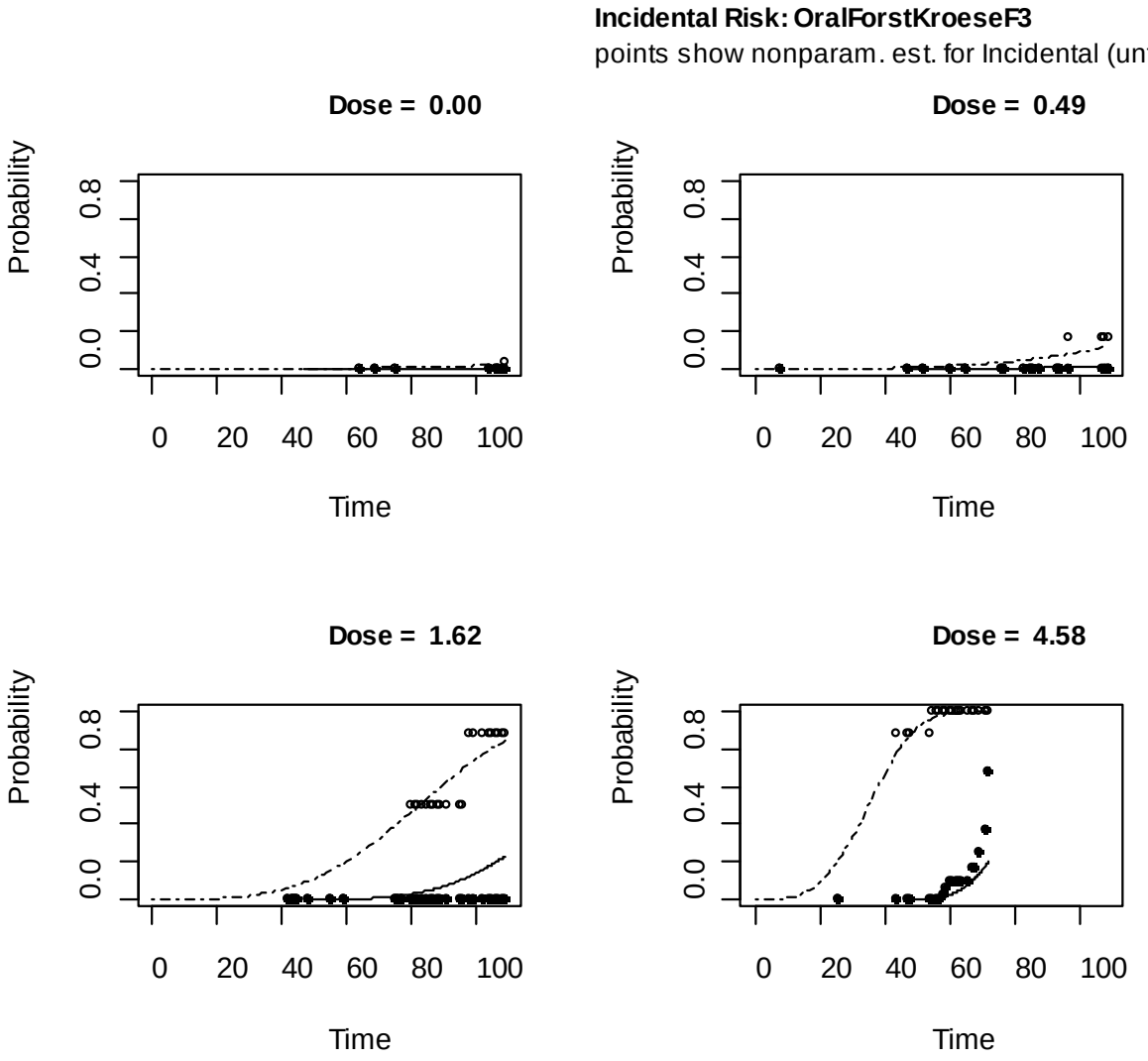

Figure E-16. Fit of multistage Weibull model to squamous cell papillomas or carcinomas in oral cavity or forestomach of female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
 =====================================================================

 Timer to Tumor Model, Liver Hepatocellular Tumors, Kroese et al, Female

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

DOSE	CONTEXT C	F	I	U	Total	Expected	Response
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

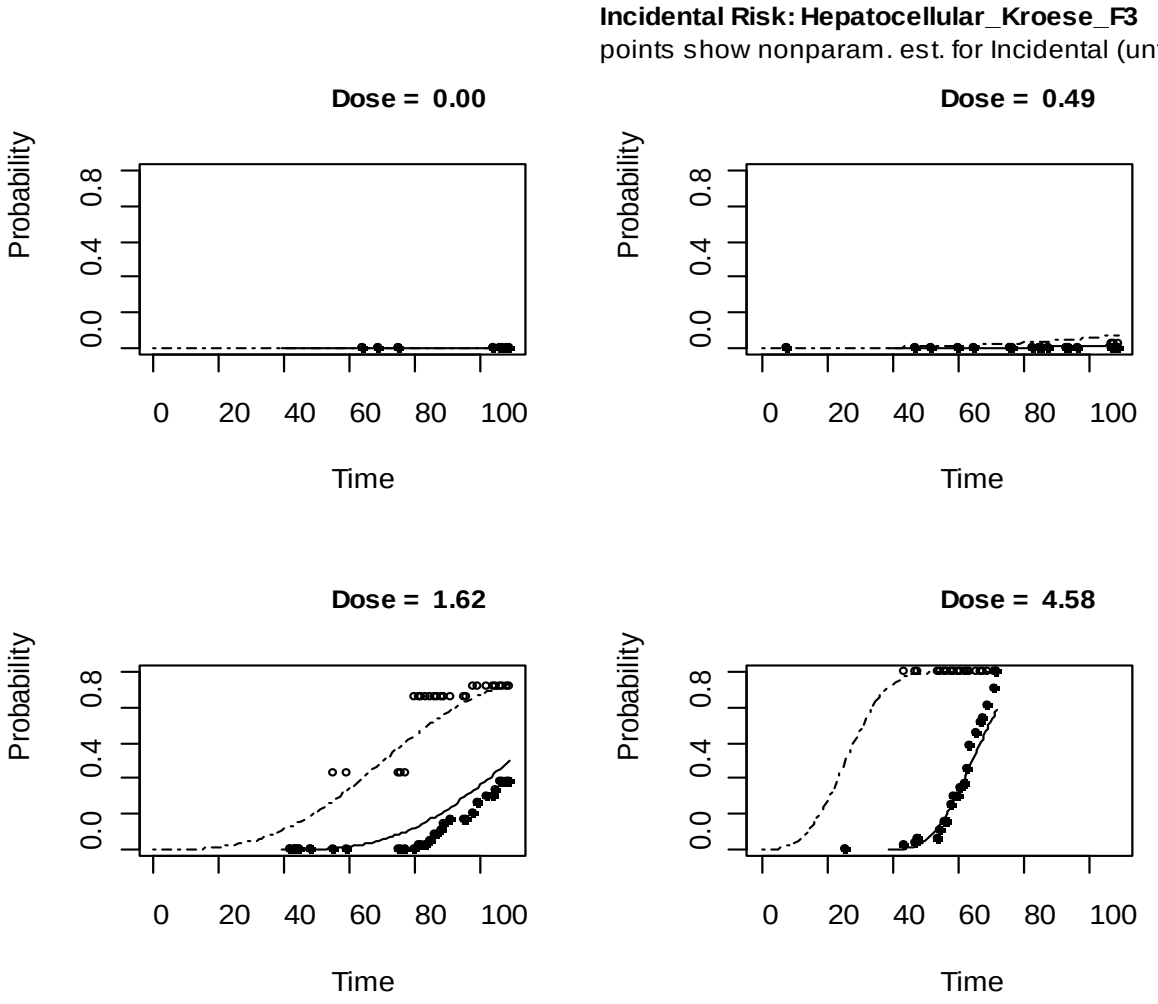

Figure E-17. Fit of multistage Weibull model to hepatocellular adenomas or carcinomas in female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

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
               C      F      I      U  Total  Expected Response
    DOSE
        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
```

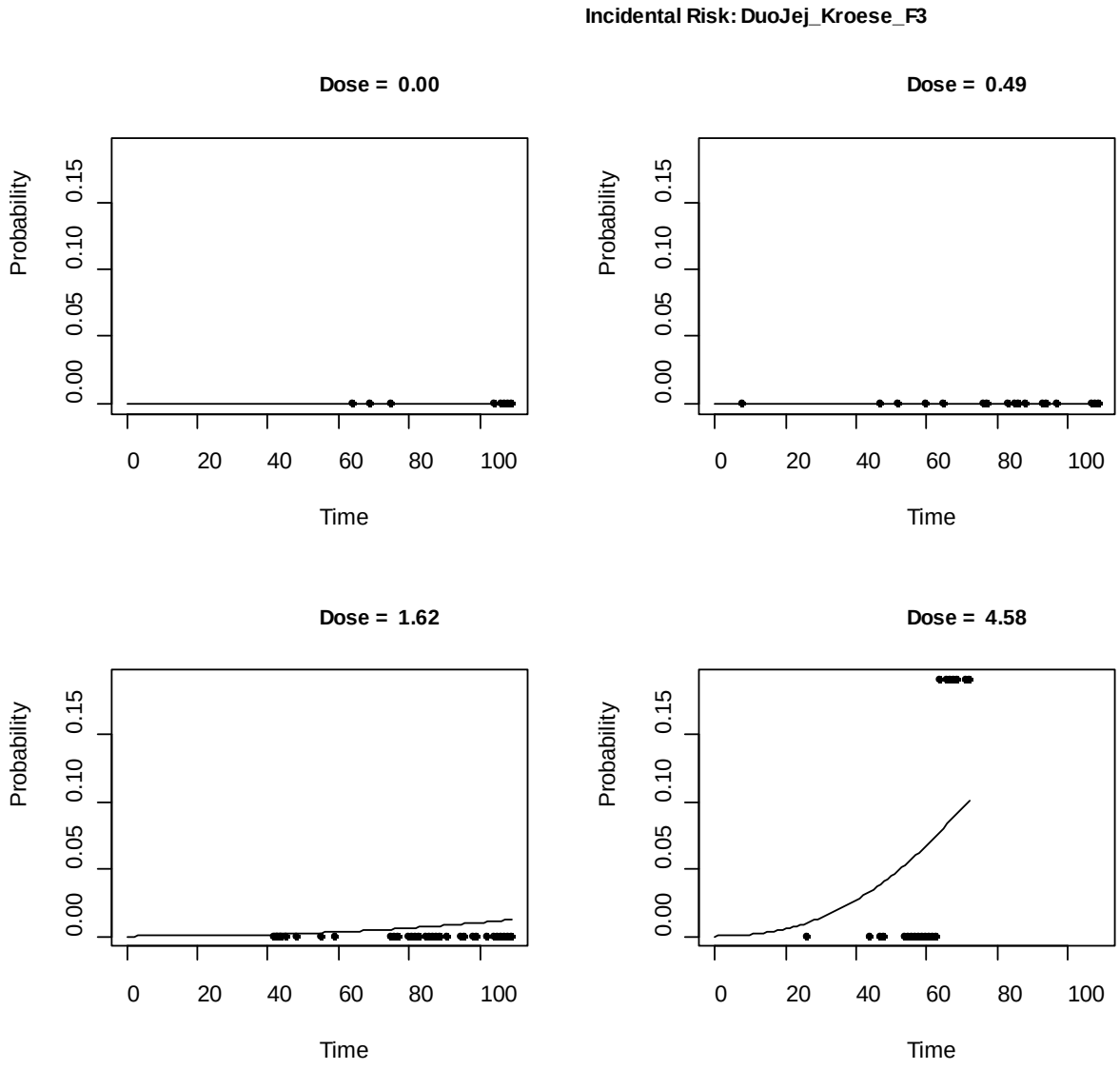

Figure E-18. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in female rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

Table E-15. 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: squam. 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-16. 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}	$BMDL_{10}$ – $BMDU_{10}$	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**

Female mice (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       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
```

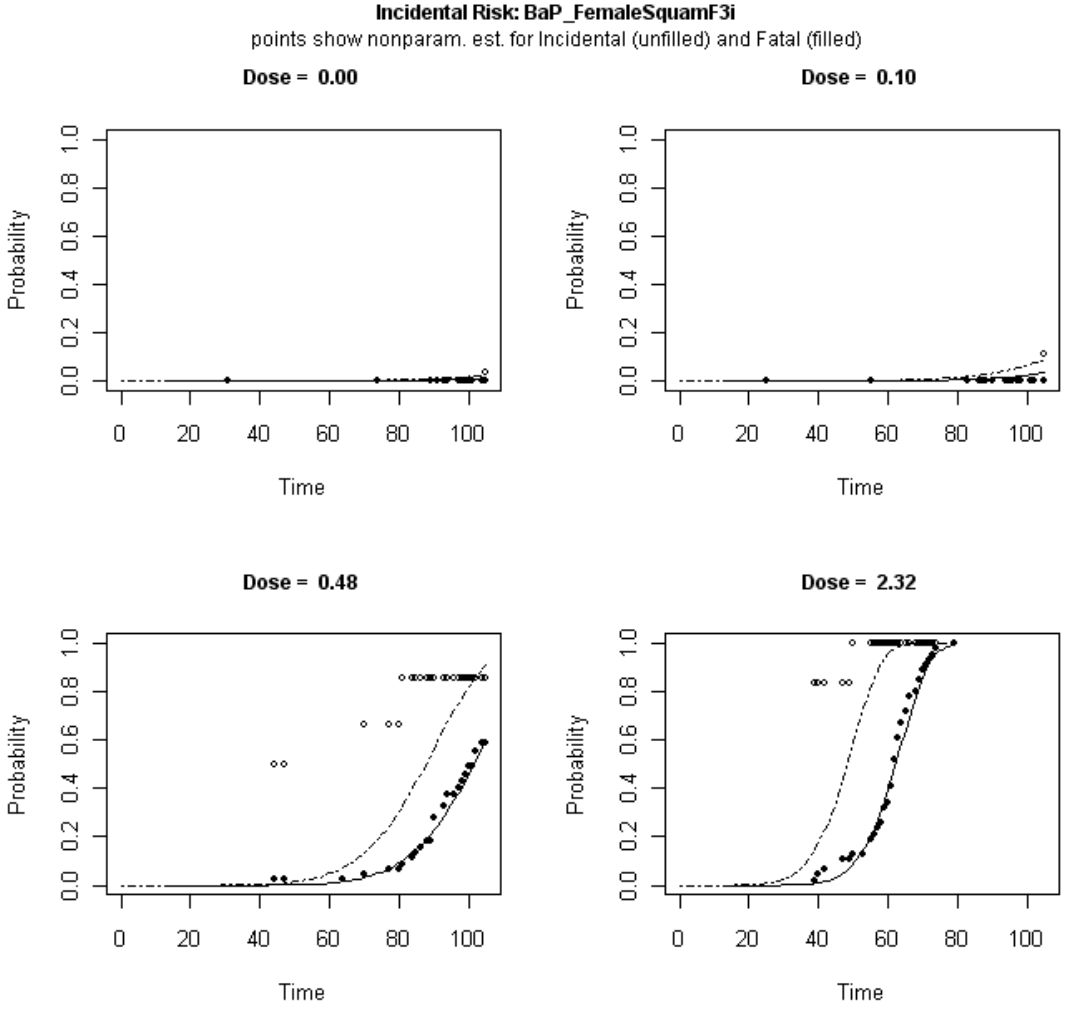

Figure E-19. Fit of multistage Weibull model to duodenum or jejunum adenocarcinomas in male rats exposed orally to benzo[a]pyrene (Kroese et al., 2001)

E.2.3. Dose-Response Modeling for the Inhalation Unit Risk

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 computer software program Multistage Weibull (U.S. EPA, 2010) was used as described in the analysis of the oral carcinogenicity data. See Section E.2.1 for details of the modeling methods.

Data adjustments prior to modeling

Thyssen et al. (1981) did not determine cause of death for any of the animals. Since the investigators for the oral bioassays considered the same tumors 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. The data analyzed are summarized in Table E-17. 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-17. Individual pathology and tumor occurrence data for male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime—Thyssen et al. (1981)[a]

Nominal Exposure Concentration (mg/m^3)	Time on Study	Number Examined	Papillomas, Polyps, Papillary Polyps, Squamous Cell Carcinomas					
			Larynx	Pharynx	Trachea	Esophagus	Forestomach	Nasal Cavity
0	17	1	0	0[b]	0	0	0	0
	39	1	0	0	0	0	0	0
	45	1	0	0	0	0	0	0
	79	1	0	0	0	0	0	0
	83	1	0	0	0	0	0	0
	85	1	0	0[b]	0	0	0	0
	86	1	0	0	0	0	0	0
	88	2	0	0	0	0	0	0
	89	2	0	0	0	0	0	0
	90	1	0	0	0	0	0	0
	101	1	0	0	0	0	0	0
	102	1	0	0	0	0	0	0
	103	1	0	0	0	0	0	0
	106	1	0	0	0	0	0	0
	108	1	0	0	0	0	0	0
	109	1	0	0	0	0	0	0
	112	1	0	0	0	0	0	0
	115	1	0	0	0	0	0	0
	116	1	0	0[b]	0	0	0	0
	122	1	0	0	0	0	0	0
	123	1	0	0	0	0	0	0
	124	1	0[b]	0	0	0	0	0
	125	1	0	0	0	0	0	0
	127	1	0	0[b]	0	0	0	0
	132	1	0	0	0	0	0	0
2	14	1	0[b]	0[b]	0	0	0	0
	35	1	0	0	0	0	0	0
	53	1	0	0	0	0	0	0
	59	1	0	0	0	0	0	0
	71	1	0	0	0	0	0	0
	78	1	0	0	0	0	0	0
	80	1	0	0	0	0	0	0
	85	1	0	0	0	0	0	0
	87	1	0	0	0	0	0	0
	88	1	0	0	0	0	0	0
	93	1	0	0	0	0	0	0
	98	1	0	0[b]	0	0	0	0
	99	1	0	0	0	0	0	0
	102	1	0	0	0	0	0	0
	103	1	0	0	0	0	0	0
	108	1	0	0	0	0	0	0
	111	1	0	0	0	0	0	0
	113	1	0	0	0	0	0	0
	114	1	0	0	0	0	0	0
	115	1	0	0	0	0	0	0
	116	1	0	0	0	0	0	0

Nominal Exposure Concentration (mg/m^3)	Time on Study	Number Examined	Papillomas, Polyps, Papillary Polyps, Squamous Cell Carcinomas					
			Larynx	Pharynx	Trachea	Esophagus	Forestomach	Nasal Cavity
	117	1	0	0	0	0	0	0
	120	1	0	0	0	0	0	0
	122	2	0[b]	0[b]	0	0	0	0
	133	2	0	0	0	0	0	0
10	31	1	0	0	0	0	0	0
	32	1	0	0	0	0	0	0
	52	1	0	0	0	0	0	0
	67	1	0	0	0	0	0	0
	73	1	0	0	0	0	0	0
	76	2	0	2	0	0	0	0
	80	1	1	0	0	0	0	0
	85	1	0	0	0	0	0	0
	94	1	1	0	0	0	0	0
	100	1	0	0	0	0	0	0
	102	1	0	1	0	0	0	0
	105	1	1	1	0	0	0	0
	111	1	0	1	0	0	0	0[d]
	113	1	0	1	0	0	0	0
	114	1	1	1	0	0	0	0
	115	1	1	0[b]	1	0	0	1
	116	1	0	0	1	0	0	1
	117	1	1	0	0	0	0	0
	118	4	3	1[c]	0	0	1	1
	122	1	1	0	0	0	0	0
	124	1	1	1	0	0	0	0
	125	1	0	0	0	0	0	1
50	20	1	0[b]	0[b]	0[b]	0	0	0
	21	1	0[b]	0[b]	0[b]	0	0	0
	25	2	0[b]	0[b]	0[b]	0	0	0
	29	1	0[b]	0[b]	0[b]	0	0	0
	30	1	0[b]	0[b]	0[b]	0	0	0
	34	1	0[b]	0[b]	0[b]	0	0	0
	36	2	0[b]	0[b]	0[b]	0	0	0
	37	1	0[b]	0[b]	0[b]	0	0	0
	40	2	1[b]	1[b]	1[b]	0	0	0
	41	1	0	0	0	0	0	0
	43	1	0	0	0	0	0	0
	47	1	1	1	0	0	0	0
	48	1	0	1	0	0	0	0
	51	1	0	0[b]	0	0	0	0
	56	1	1	1	0	0	0	0
	57	1	0	1	0	0	0	0
	60	1	0	1	0	0	0	0
	63	1	0	0	0	0	0	0
	64	1	0	1	0	0	1	0
	66	1	1	1	0	0	0	0
	68	1	0	1	0	0	0	0
	70	1	1	1	0	1	0	0
	71	1	1	1	1	0	0	0
	72	1	1	1	0	0	0	0

Nominal Exposure Concentration (mg/m^3)	Time on Study	Number Examined	Papillomas, Polyps, Papillary Polyps, Squamous Cell Carcinomas					
			Larynx	Pharynx	Trachea	Esophagus	Forestomach	Nasal Cavity
	73	2	2	2	0	0	0	0
	79	4	3	4	1	1	0	1

[a] Histopathology incidence from U.S. EPA (1990a)
[b]Tissue was not examined for one animal of total examined.
[c]Tissue was not examined for two animals of total examined.
[d]An adenocarcinoma was observed in this tissue, but not included in the dose-response analysis because it was of a different cell type than the other tumors listed. It was judged to be an isolated finding not clearly associated with exposure.

Modeling Results

Table E-18 summarizes the modeling results supporting the derivation of an inhalation unit risk value for BaP. The model outputs and graphs (Figures E-20 and E-21) following Table E-18 provide more details for the best-fitting models.

Table E-18. 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}	$BMDL_{10}$	Basis for Model Selection
All tumors considered incidental to cause of death	1	58.0	0.090	0.064	
	2	**47.9**	**0.285**	**0.198**	**Lowest AIC, best fit to data ($BMDU_{10}$ = 0.350)**
All tumors considered to be cause of death	1	327.3	0.136	0.104	
	2	302.9	0.421	0.343	
	3	**299.0**	**0.648**	**0.461**	**Lowest AIC; best fit to data ($BMDU1_0$ = 0.719)**

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: C:\msw\benzo[a]pyrene-Thyssen_inc2st.(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)}

   The parameter betas are restricted to be positive

   Dependent variable = Class
   Independent variables = Conc, Time

 Total number of observations = 96
 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 = 32
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        c      =          3.6
                        t_0    =            0   Specified
                        beta_0 = 1.18657e-031
                        beta_1 =    1.49e-030
                        beta_2 = 6.10362e-008


           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               4.21938         0.840997             2.57105              5.8677
         beta_0                0               NA
         beta_1                0               NA
         beta_2     4.00402e-009       1.495e-008       -2.52974e-008        3.33054e-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         -19.967         4         47.9339


                   Data Summary
                       Class
              C      F      I      U  Total  Expected Response
    Conc
        0     23      0      0      0     23     0.00
     0.25     24      0      0      0     24     1.92
        1      8      0     18      0     26    16.04
      4.3      5      0     18      0     23    18.22


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Specified effect =            0.1
Confidence level =            0.9

Time             =            104

             BMD =       0.284958
            BMDL =       0.197807
            BMDU =       0.350247
```

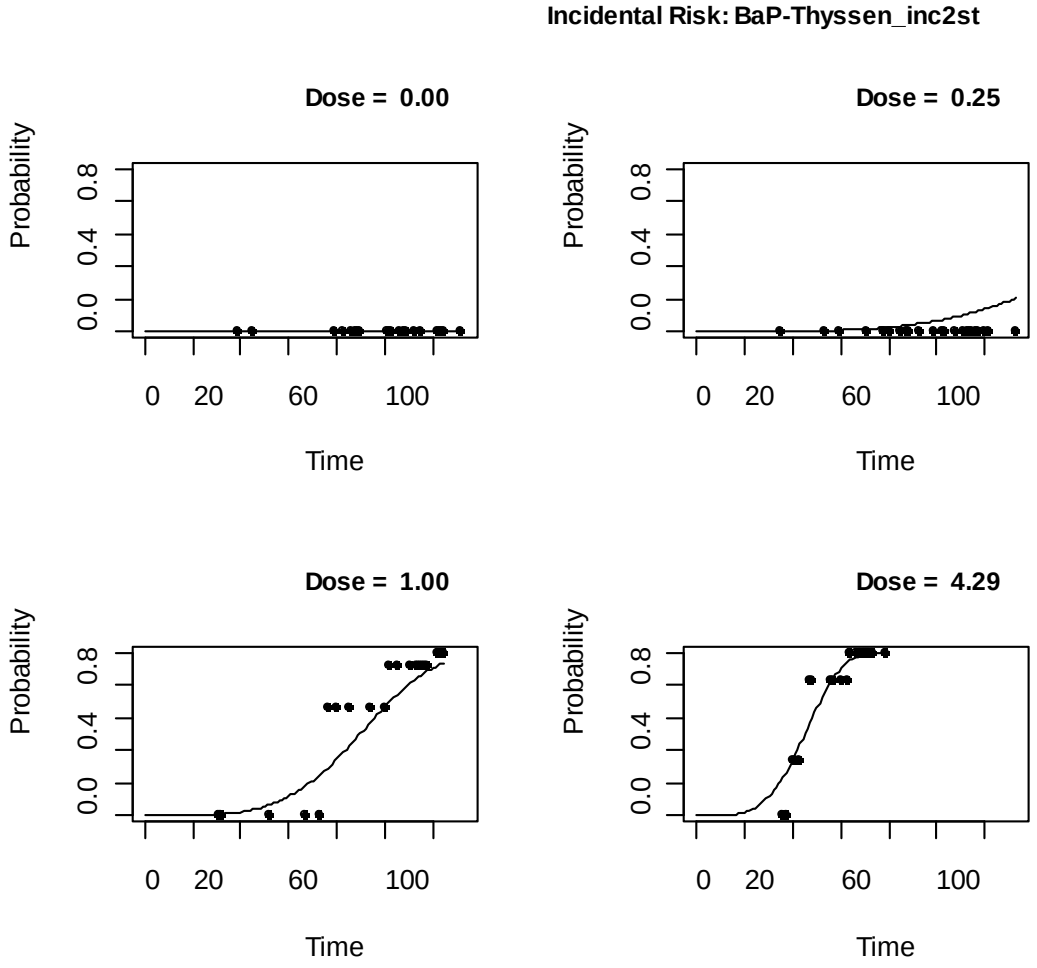

Figure E-20. Fit of multistage Weibull model to respiratory tract tumors in male hamsters exposed via inhalation to benzo[a]pyrene Thyssen et al. (1981); tumors treated as incidental to death.

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: C:\msw\benzo[a]pyrene-Thyssen_allfatal_noU_3st.(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 = Conc, Time

 Total number of observations = 96
 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 = 32
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        c      =          4.5
                        t_0    =            0   Specified
                        beta_0 =            0
                        beta_1 = 1.37501e-010
                        beta_2 = 2.84027e-010
                        beta_3 = 1.44668e-037


           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               8.95016         0.896607             7.19284             10.7075
         beta_0                0               NA
         beta_1                0               NA
         beta_2                0               NA
         beta_3     3.43452e-019     1.39727e-018       -2.39515e-018        3.08205e-018

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        -144.522         5         299.043


                   Data Summary
                       Class
              C      F      I      U  Total
    Conc
        0     23      0      0      0     23
     0.25     24      0      0      0     24
        1      8     18      0      0     26
      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 =       0.647659
            BMDL =       0.461415
            BMDU =       0.719325
```

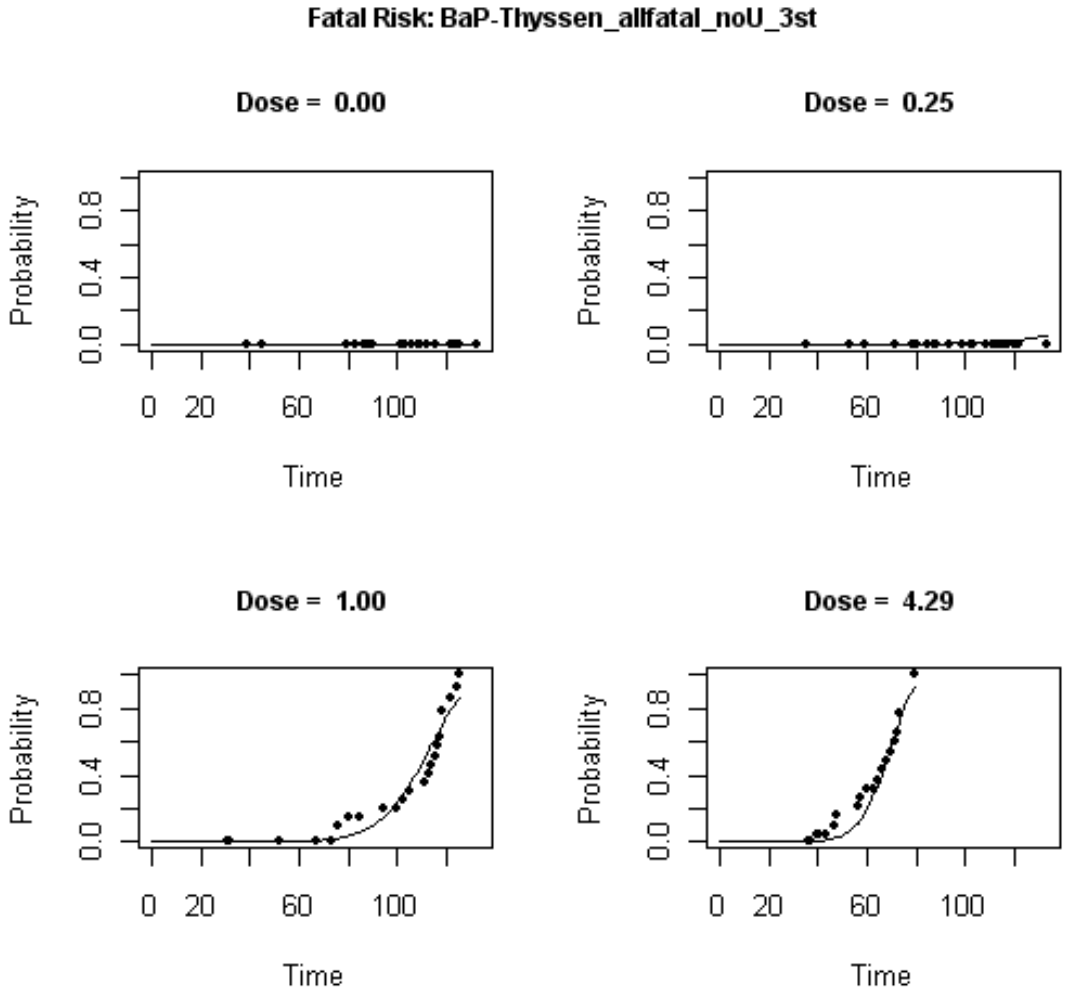

Figure E-21. Fit of multistage Weibull model to respiratory tract tumors in male hamsters exposed via inhalation to benzo[a]pyrene Thyssen et al. (1981); tumors treated as cause of death.

E.2.4. Dose-Response Modeling for the Dermal Slope Factor

Modeling methods

For each endpoint, multistage models [BMDS; (U.S. EPA, 2012a); v 2.1] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.05 indicates lack of fit). Other factors were 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 BMR. The BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. The data modeled are summarized in Tables E-19 through E-22.

Data adjustments prior to modeling

Roe et al. (1970) applied benzo[a]pyrene dermally for 93 weeks or until natural death; with the exception of the highest dose group, each group still had approximately 20 animals at 86 weeks (see Table E-19). The tumors were first observed in the lowest and highest dose groups during the interval of weeks 29–43. Mice that died before week 29 were likely not at risk of tumor development. However, because tumor incidence and mortality were reported in 100-day intervals, mice that had not been on study long enough to develop tumors were not easily identifiable. Incidence denominators reflect the number of animals alive at week 29, and may thus tend to lead to underestimates of tumor risk if the number of animals at risk has been overestimated.

Schmidt et al. (1973) did not report survival information; instead, the authors provided incidences based on the numbers of mice initially included in each dose group at the start of the study. Overall latency was reported for the two high-dose groups in each series, but these data only describe the survival of mice with tumors (animals were removed from study when a tumor appeared). It is not clear how long exposures lasted overall in each dose group, or whether some mice may have died on study from other causes before tumors appeared. While it is possible that no mice died during the study, all of the other studies considered here demonstrate mortality. However, the data were modeled as reported, recognizing the possibility of underestimating risk associated with incidences reported and lack of duration of exposure (see Table E-19).

Schmähl et al. (1977) reported that reduced numbers of animals at risk (77–88 mice per dose group compared with the initial group sizes of 100) resulted from varying rates of autolysis. No other survival or latency information was provided, so all exposures were assumed to have lasted for 104 weeks and were modeled as reported. Given the results of the other studies, it seems possible that the numbers at risk in each group may be overestimated, which could lead to an underestimate of lifetime risk (see Table E-19).

Habs et al. (1980) reported age-standardized skin tumor incidence rates, indicating earlier mortality in the two highest dose groups (2.8 and 4.6 µg/application). These rates were used to estimate the number at risk in the dose-response modeling, by dividing the number of mice with tumors by the age-standardized rates. Exposure lasted longer than 104 weeks in the two lower exposure groups, at about 120 and 112 weeks, and until about 88 weeks in the highest exposure group. Incidence in the two lower exposure groups may be higher than if the exposure had lasted just 104 weeks. There was mortality in the first 52 weeks of exposure, about 10–15% in the three exposure groups, but because there was no information concerning when tumors first appeared, it is not possible to determine how much the early mortality may have impacted the number of mice at risk in each group (see Table E-19).

Habs et al. (1984) reported mean survival times (with 95% CIs) for each dose group. The CIs supported the judgment that the control and lower dose groups were treated for 104 weeks. The higher dose group (4 µg/application) was probably treated for <104 weeks, because the upper 95% confidence limit for the mean survival was approximately 79 weeks. However, since it was

not possible to estimate a more realistic duration for this group, an estimate of 104 weeks was used (see Table E-19).

The studies by Poel (1960, 1959) were conducted in male mice and used toluene as the vehicle. In addition to a control group, the 1959 study included nine dose groups of one mouse strain (C57L) and the 1960 study included seven dose groups of three other mouse strains. Both studies demonstrated high mortality and tumor incidence at higher exposure levels. All C57L mice in dose groups with >3.8 µg/application died by week 44 of the study (Poel, 1959). Therefore, these five dose groups were omitted prior to dose-response modeling because of the relatively large uncertainty in extrapolating cancer risk as a result of lifetime exposure. Four dose groups in addition to control remained. Among these groups, mice survived and were exposed until weeks 83–103. According to the lifespan ranges provided, at least one mouse in each dose group died before the first appearance of tumor, but insufficient information was available to determine how many; consequently, the incidence denominators were not adjusted. The dose-response data are summarized in Table E-20.

For the Poel (1960) studies, all tumors in the highest three dose groups for each of the three mouse strains had occurred by week 40. While these observations support concern for cancer risk, as noted above such results are relatively uncertain for estimating lifetime cancer risk. In addition, there was no information indicating duration of exposure for the mice without tumors; although exposure was for lifetime, it might have been as short as for the mice with tumors. Overall, these datasets did not provide sufficient information to estimate the extent of exposure associated with the observed tumor incidence. Consequently, the experiments reported by Poel (1960) were not used for dose-response modeling.

Grimmer et al. (1984); Grimmer et al. (1983), studied female CFLP mice, using acetone:DMSO (1:3) as the vehicle. Mean or median latency times were reported (as well as measures of variability), but no information concerning overall length of exposure or survival was included in the results. The total of tumor-bearing mice and the reported percentages of mice with any skin tumors was reported and varied, at most, one animal from the number of animals initially placed on study. The decreasing latency and variability and increasing tumor incidence with increasing benzo[a]pyrene exposure suggests that exposure probably did not last for 104 weeks in at least the high-dose group, but the available information did not provide duration of exposure. The data reported were modeled under the assumption that at least some animals in each group were treated and survived until week 104 (see Table E-21).

Sivak et al. (1997) exposed male C3H/HeJ mice dermally to benzo[a]pyrene in cyclohexanone/acetone (1:1) for 24 months, and reported mean survival times for each group. All high-dose mice died before the final sacrifice. From the information provided, it is apparent that the animals in the control and lower two dose groups survived until study termination at week 104. The study authors did not report how long treatment in the highest dose group lasted, but estimation of the figure from the publication suggest that exposure duration was 74 weeks (see Table E-22).

Table E-19. Skin tumor incidence, benign or malignant in female Swiss or NMRI mice dermally exposed to benzo[a]pyrene; data from Roe et al. (1970), Schmidt et al. (1973), Schmähl et al. (1977), Habs et al. (1980), Habs et al. (1984)

Study	Mouse Strain	Dose (µg)	Average Daily Dose (µg/d)	First Appearance of Tumor (wks)	Length of Exposure (wks)	Lifetime Average Daily Dose (µg/d)	Skin Tumor Incidence (All Types)
Roe et al. (1970)[a,b]	Swiss	0 (acetone)	0	–	93	0.00	0/49 (0%)
		0.1	0.04	29–43	93	0.03	1/45 (2%)
		0.3	0.13	–	93	0.09	0/46 (0%)
		1	0.43	57–71	93	0.31	1/48 (2%)
		3	1.29	43–57	93	0.92	8/47 (20%)
		9	3.86	29–43	93	2.76	34/46 (74%)
Schmidt et al. (1973)[c]	NMRI	0 (acetone)	0	–	*104*[d]	0	0/100 (0%)
		0.05	0.01	–	*104*	0.01	0/100 (0%)
		0.2	0.06	–	*104*	0.06	0/100 (0%)
		0.8	0.23	53[e]	*104*	0.23	2/100 (2%)
		2	0.57	76[e]	*104*	0.57	30/100 (30%)
	Swiss	0 (acetone)	0	–	*104*	0	0/80 (0%)
		0.05	0.01	–	*104*	0.01	0/80 (0%)
		0.2	0.06	–	*104*	0.06	0/80 (0%)
		0.8	0.23	58[e]	*104*	0.23	5/80 (6%)
		2	0.57	61[e]	*104*	0.57	45/80 (56%)
Schmähl et al. (1977)[c]	NMRI	0 (acetone)	0	–	*104*	0	1/81 (1%)
		1	0.29	NR	*104*	0.29	11/77 (14%)
		1.7	0.49	NR	*104*	0.49	25/88 (28%)
		3	0.86	NR	*104*	0.86	45/81 (56%)
Habs et al. (1980)[c,f]	NMRI	0 (acetone)	0	–	128	0	0/35 (0%)
		1.7	0.49	NR	120	0.49	8/34 (24.8%)
		2.6	0.74	NR	112	0.74	24/27 (89.3%)
		4.6	1.31	NR	88	0.80	22/24 91.7%)
Habs et al. (1984)[c]	NMRI	0 (acetone)	0	–	104	0	0/20 (0%)
		2	0.57	NR	104	0.57	9/20 (45%)
		4	1.14	NR	104	1.14	17/20 (85%)

[a]Doses were applied 3 times/wk for up to 93 weeks to shaved dorsal skin.
[b]Numerator: number of mice detected with a skin tumor. Tumors were thought to be malignant based on invasion or penetration of the panniculus carnosus muscle. Denominator: number of mice surviving to 29 weeks (200 days).
[c]Doses were applied 2 times/wk to shaved skin of the back. Mice were exposed until natural death or until they developed a carcinoma at the site of application.
[d]Exposure periods not reported were assumed to be 104 weeks; indicated in italics.
[e]Central tendency estimates; range or other variability measure not reported.
[f]The percentages were reported by the authors as age-standardized incidences of animals with local tumors, derived using mortality data from the entire study population. The incidences reflect reported counts of tumor-bearing animals and denominators estimated from the reported age-standardized rates. The authors did not report the percentages of local tumors which were carcinomas or papillomas.

NR = not reported.

Table E-20. Skin tumor incidence, benign or malignant, in C57L male mice dermally exposed to benzo[a]pyrene; data from Poel (1959)

Study	Mouse Strain	Dose (µg)[a]	Average Daily Dose (µg/d)	First Appearance of Tumor (wks)	Length of Exposure (wks)	Lifetime Average Daily Dose[b]	Skin Tumor Incidence (All Types)[c]
Poel (1959)	C57L	0 (toluene)	0	–	92	0.00	0/33 (0%)
		0.15	0.06	42	98	0.05	5/55 (9%)
		0.38	0.16	24	103	0.16	11/55 (20%)
		0.75	0.32	36	94	0.24	7/56 (13%)
		3.8	1.63	21–25	82	0.80	41/49 (84%)

[a]Doses were applied to interscapular skin 3 times/wk for up to 103 wks or until time of appearance of a grossly detected skin tumor. See Table E-15 for data of five highest dose groups (19–752 µg) in which all mice died by wk 44. These groups were not considered for dose-response modeling.
[b]See Section 2.5.2 of Toxicological Review for discussion of extrapolation to lifetime average daily doses.
[c]Tumors were histologically confirmed as epidermoid carcinomas.

Table E-21. Skin tumor incidence, benign or malignant, in female CFLP mice dermally exposed to benzo[a]pyrene; data from Grimmer et al. (1983), Grimmer et al. (1984)

Study	Dose (µg)[a]	Average Daily Dose (µg/d)	Mean or Median Time of Tumor Appearance (wks)	Length of Exposure (wks)[b]	Lifetime Average Daily Dose (µg/d)	Skin Tumor Incidence (All Types)[c]
Grimmer et al. (1983)	0 (1:3 acetone:DMSO)	0	—	*104*	0	0/80 (0%)
	3.9	1.1	74.6 ± 16.8[d]	*104*	1.1	22/65 (34%)
	7.7	2.2	60.9 ± 13.9	*104*	2.2	39/64 (61%)
	15.4	4.4	44.1 ± 7.7	*104*	4.4	56/64 (88%)
Grimmer et al. (1984)	0 (1:3 acetone:DMSO)	0	—	*104*	0	0/80 (0%)
	3.4	0.97	61 (53–65)[e]	*104*	0.97	43/64 (67%)
	6.7	1.9	47 (43–50)	*104*	1.9	53/65 (82%)
	13.5	3.9	35 (32–36)	*104*	3.9	57/65 (88%)

[a] Indicated doses were applied twice/week to shaved skin of the back for up to 104 weeks.
[b] Assumed exposure period is indicated in italics.
[c] Incidence denominators were calculated from reported tumor-bearing animals and reported percentages.
[d] Mean ± SD.
[e] Median and 95% confidence limit.

Table E-22. Skin tumor incidence, benign or malignant, in male C3H/HeJ mice dermally exposed to benzo[a]pyrene; data from Sivak et al. (1997)

Dose (µg)[a]	Average Daily Dose (µg/d)	First Appearance of Tumor (wks)	Length of Exposure (wks)[b]	Lifetime Average Daily Dose (µg/d)	Skin Tumor Incidence (All Types)[b]
0 (1:1 cyclohexanone/acetone)	0	–	104	0.0	0/30 (0%)
0.05	0.01	–	104	0.01	0/30 (0%)
0.5	0.14	NR	104	0.14	5/30 (17%)
5.0	1.4	~43	*74*	0.51	27/30 (90%)

[a]Indicated doses were applied twice/week to shaved dorsal skin.
[b]Number of skin tumor-bearing mice.

NR = not reported.

Modeling Results

The modeling results are summarized in Table E-23. The modeling details are provided with Figures E-22 through E-33.

Table E-23. Summary of BMD model selection and modeling results for best-fitting multistage models, for multiple data sets of skin tumors in mice following dermal benzo[a]pyrene exposure

Data set	Model	Goodness-of-Fit		BMD_{10} (µg/d)	$BMDL_{10}$ (µg/d)	Basis for Model Selection[a]	Figure Number
		p-value	AIC				
Poel (1959) Male C57L	Multistage 1°	0.011	191.5	0.070	0.057		
	Multistage 2°	0.027	188.6	0.134	0.078		
	Multistage 3°	**0.053**	**186.9**	**0.127**	**0.078**	**No significant improvement in model fit with higher stage**	E-22
	Multistage 4°	0.068	186.2	0.123	0.077		
Roe et al. (1970) Female Swiss	Multistage 1°	0.110	131.1	0.318	0.249		
	Multistage 2°	**0.485**	**123.6**	**0.748**	**0.480**	**No significant improvement in model fit with higher stages**	E-23
	Multistage 3°	0.485	123.6	0.748	0.480		
Schmidt et al. (1973) Female NMRI	Multistage 1°	0.008	162.7	0.256	0.194		
	Multistage 2°	**0.609**	**147.4**	**0.329**	**0.287**	**No significant improvement in model fit with higher stages**	E-24
	Multistage 3°	0.999	143.9	0.381	0.326		
Schmidt et al. (1973) Female Swiss	Multistage 1°	<0.01	178.0	0.116	0.093		
	Multistage 2°	0.514	153.3	0.216	0.192		
	Multistage 3°	**0.983**	**151.3**	**0.282**	**0.223**	**No significant improvement in model fit with higher stage**	E-25
	Multistage 4°	0.983	151.3	0.282	0.223		
Schmähl et al. (1977) Female NMRI	Multistage 1°	0.136	298.4	0.140	0.117		
	Multistage 2°	**0.939**	296.3	**0.233**	**0.149**	**No significant improvement in model fit with higher stage**	E-26
	Multistage 3°	**0.939**	296.3	0.233	0.143		
Habs et al. (1980) Female NMRI	Multistage 1°	0.0	96.5	0.063	0.050		
	Multistage 2°	0.009	84.4	0.198	0.143		
	Multistage 3°	**0.207**	**76.7**	**0.294**	**0.215**	**Only model with adequate fit**	E-27
Habs et al. (1984) Female NMRI	**Multistage 1°**	**0.577**	**48.4**	**0.078**	**0.056**	**No significant improvement in model fit with higher stage**	E-28
	Multistage 2°	1.000	47.6	0.171	0.060		
Grimmer et al. (1983) Female CFLP	**Multistage 1°**	**0.850**	**219.9**	**0.245**	**0.208**	**No significant improvement in model fit with higher stages**	E-29
	Multistage 2°	0.972	221.1	0.292	0.213		
	Multistage 3°	0.972	221.1	0.292	0.213		
Grimmer et al. (1984)[b] Female CFLP	Multistage 1°	0.003	205.3	0.132	0.113	(Higher stages did not provide better fit)	E-30
	LogLogistic	**0.919**	**195.8**	**1.07**	**0.479**	**Lowest AIC among adequately fitting models.**	**E-31**
	Dichotomous-Hill	1.000	197.7	0.902	0.533		
	LogProbit	0.047	200.2	1.33	1.11		
	Gamma,	0.003	205.3	0.132	0.113	(Same as Multistage 1°)	
	Weibull	0.0	250.5	2.03	1.76		
	Logistic	0.0	255.4	2.29	2.03		
	Probit						
	Multistage 1°, high dose dropped	**0.499**	—	**1.21**	**1.01**		E-32
Sivak et al. (1997) Male CeH/HeJ	Multistage 1°	0.059	57.8	0.036	0.026		
	Multistage 2°	**0.998**	**48.6**	**0.109**	**0.058**	**No significant improvement in model fit with higher stage**	E-33
	Multistage 3°	0.998	48.6	0.109	0.052		

[a]Adequate fit: goodness-of-fit $p > 0.05$, scaled residuals <2.0, good fit near BMR, lack of extreme curvature not reflected in the observed data.

[b]The POD for Grimmer et al. (1984), using a BMR of 70% (near response at the lowest dose), was based on the LogLogistic model. For comparison purposes, the multistage model was it fit to the Grimmer et al. (1984) data with the highest dose dropped (AIC not provided because it is not comparable to fits of the full dataset).

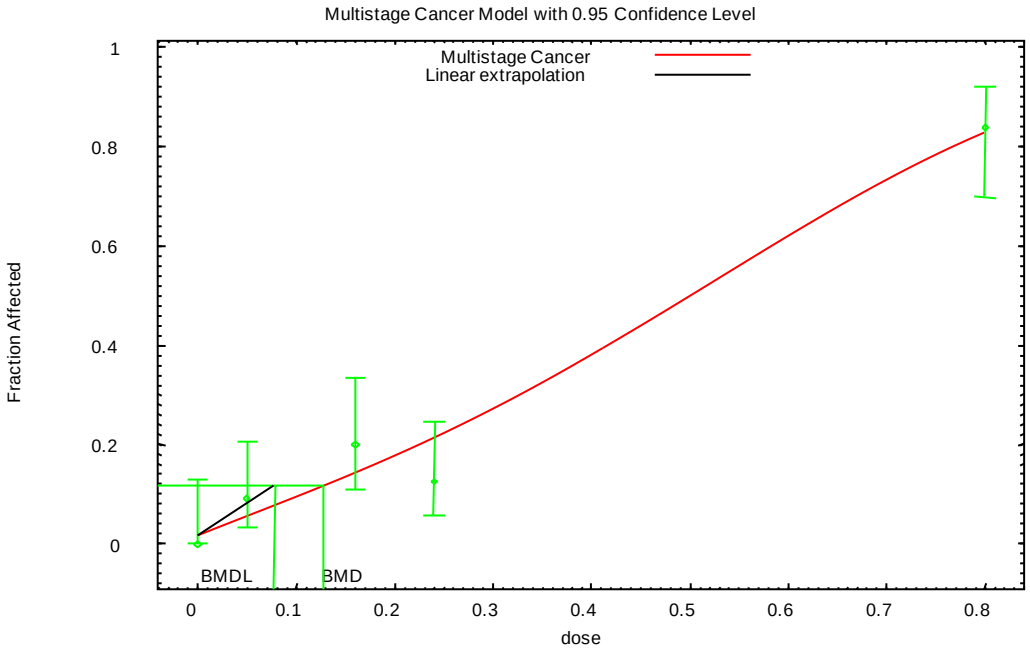

Figure E-22. Fit of multistage model to skin tumors in C57L mice exposed dermally to benzo[a]pyrene (Poel, 1959).

```
=============================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Poel_1959_MultiCanc3_0.1.(d)
          Gnuplot                                    Plotting                                    File:
C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Poel_1959_MultiCanc3_0.1.plt

 =============================================================================


    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

    The parameter betas are restricted to be positive


    Dependent variable = NumAff
    Independent variable = LADD

  Total number of observations = 5
  Total number of records with missing values = 0
  Total number of parameters in model = 4
  Total number of specified parameters = 0
  Degree of polynomial = 3


  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
                       Background =    0.0449589
                          Beta(1) =     0.490451
                          Beta(2) =            0
                          Beta(3) =      2.68146
```

```
            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 )

              Background      Beta(1)      Beta(3)

Background            1        -0.87         0.74

   Beta(1)        -0.87            1        -0.92

   Beta(3)         0.74        -0.92            1


                                Parameter Estimates

                                                                95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0176699            *                *                   *
        Beta(1)          0.79766            *                *                   *
        Beta(2)                0            *                *                   *
        Beta(3)          2.17146            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -87.1835         5
   Fitted model        -90.4265         3       6.48606      2         0.03905
  Reduced model        -141.614         1        108.86      4         <.0001

            AIC:         186.853


                                  Goodness  of  Fit
                                                                      Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.0177         0.583     0.000          33       -0.770
     0.0500     0.0563         3.098     5.000          55        1.112
     0.1600     0.1430         7.866    11.000          55        1.207
     0.2400     0.2128        11.917     7.000          56       -1.605
     0.8000     0.8293        40.635    41.000          49        0.139

 Chi^2 = 5.88      d.f. = 2        P-value = 0.0528


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =       0.126567

             BMDL =      0.0777875

             BMDU =       0.272961

Taken together, (0.0777875, 0.272961) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =       1.28555
```

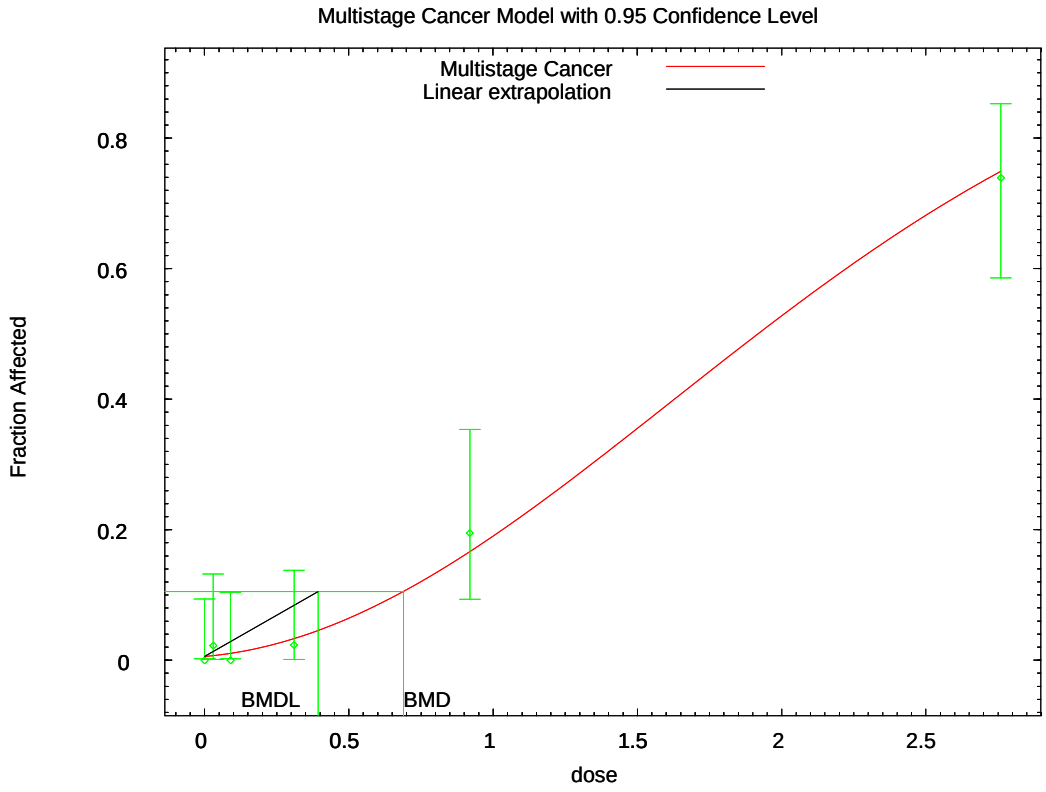

Figure E-23. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Roe et al., 1970).

```
=================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Roe_1970_Setting.(d)
          Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Roe_1970_Setting.plt

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

    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5)]

    The parameter betas are restricted to be positive

    Dependent variable = tumors
    Independent variable = LADD

  Total number of observations = 6
  Total number of records with missing values = 0
  Total number of parameters in model = 6
  Total number of specified parameters = 0
  Degree of polynomial = 5

  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
                     Background =            0
                        Beta(1) =    0.0962491
                        Beta(2) =     0.141689
                        Beta(3) =            0
                        Beta(4) =            0
                        Beta(5) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(3)    -Beta(4)    -Beta(5)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)      Beta(2)

Background            1        -0.57         0.45

   Beta(1)        -0.57            1        -0.94

   Beta(2)         0.45        -0.94            1


                               Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
      Background       0.00584893            *                *                   *
         Beta(1)        0.0379152            *                *                   *
         Beta(2)         0.166839            *                *                   *
         Beta(3)                0            *                *                   *
         Beta(4)                0            *                *                   *
         Beta(5)                0            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -56.1835         6
   Fitted model        -57.5694         3       2.77176      3          0.4282
  Reduced model        -118.948         1       125.529      5         <.0001

           AIC:         121.139

                               Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0058         0.275     0.000          47       -0.526
    0.0300     0.0071         0.321     1.000          45        1.204
    0.0900     0.0106         0.444     0.000          42       -0.670
    0.3100     0.0331         1.423     1.000          43       -0.361
    0.9200     0.1664         6.821     8.000          41        0.494
    2.7600     0.7488        34.444    34.000          46       -0.151

 Chi^2 = 2.57      d.f. = 3        P-value = 0.4626


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.689131
            BMDL =       0.393806
            BMDU =       0.952365

Taken together, (0.393806, 0.952365) is a 90     % two-sided confidence
interval for the BMD
```

```
Multistage Cancer Slope Factor =      0.253932
```

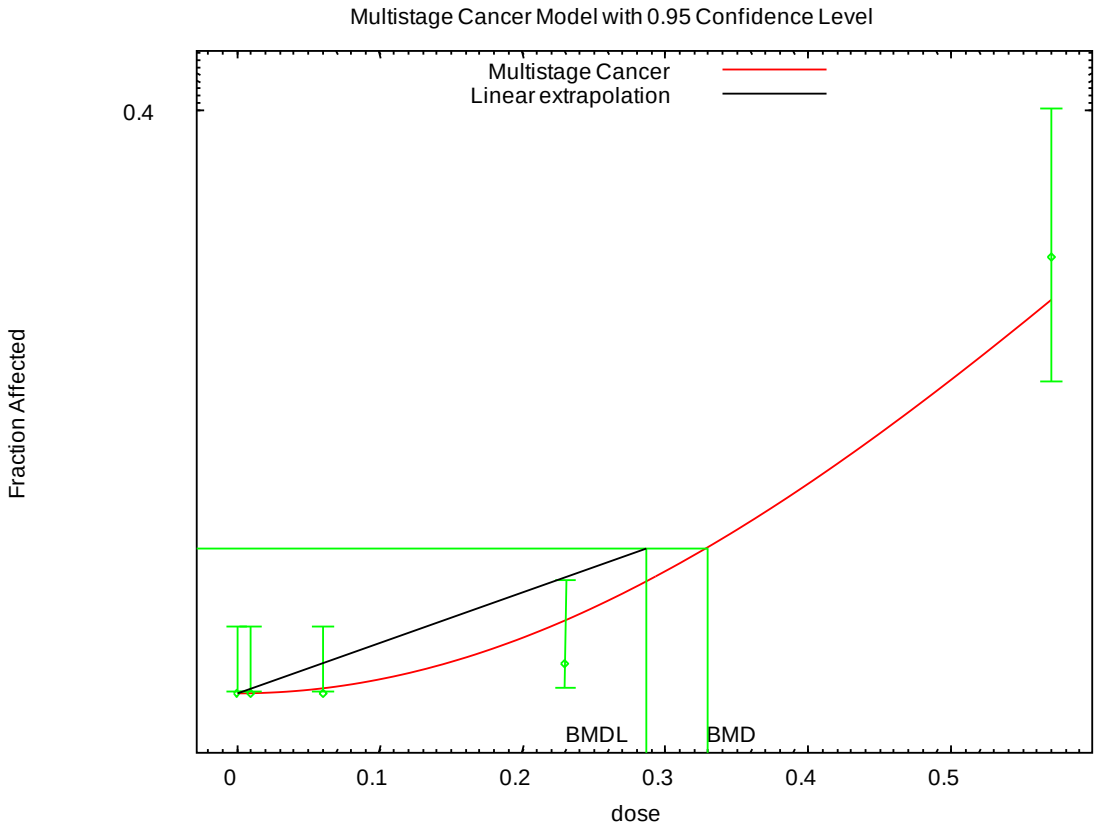

Figure E-24. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Schmidt et al., 1973).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input                                Data                                File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmidt1973femaleNMRI\2MulSchMS_.(d)
        Gnuplot                             Plotting                             File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmidt1973femaleNMRI\2MulSchMS_.plt
 =================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008
```

```
****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =      1.11271


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)           1


                               Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *                *                   *
        Beta(1)                0            *                *                   *
        Beta(2)         0.970648            *                *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -70.8903         5
    Fitted model       -72.6831         1       3.58562      4          0.465
   Reduced model       -118.917         1        96.054      4         <.0001

           AIC:         147.366


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000         100        0.000
     0.0100     0.0001         0.010     0.000         100       -0.099
     0.0600     0.0035         0.349     0.000         100       -0.592
     0.2300     0.0501         5.005     2.000         100       -1.378
     0.5700     0.2705        27.048    30.000         100        0.665

 Chi^2 = 2.70      d.f. = 4        P-value = 0.6091


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =       0.329464

             BMDL =       0.286624

             BMDU =       0.384046

Taken together, (0.286624, 0.384046) is a 90     % two-sided confidence
interval for the BMD
```

```
Multistage Cancer Slope Factor =      0.348889
```

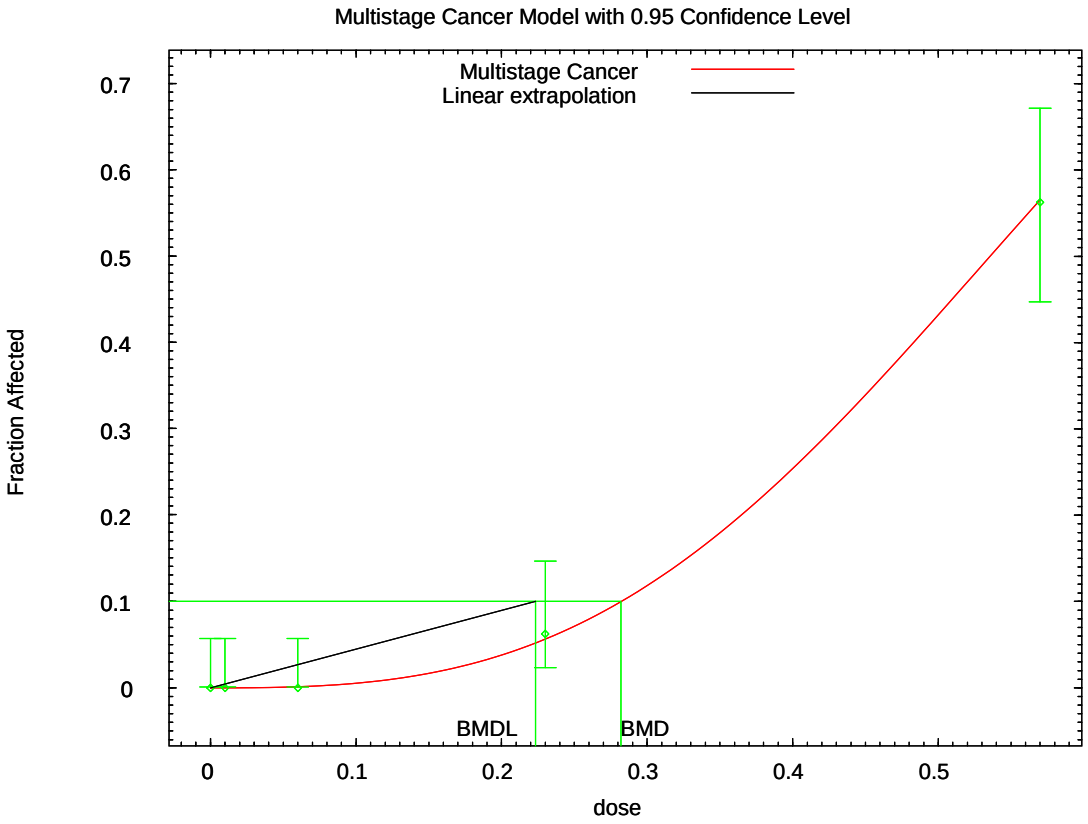

Figure E-25. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Schmidt et al., 1973).

```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input                                    Data                                    File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmidt1973swissmice\3MulSchMS_.(d)
         Gnuplot                                  Plotting                                File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmidt1973swissmice\3MulSchMS_.plt
=================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive

   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008
```

```
****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =     0.338951
                        Beta(3) =       3.8728


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                Beta(2)      Beta(3)

   Beta(2)            1        -0.99

   Beta(3)        -0.99            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *                *                   *
        Beta(1)                0            *                *                   *
        Beta(2)         0.108125            *                *                   *
        Beta(3)          4.31441            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -73.5285         5
   Fitted model         -73.6628         2      0.268637      3          0.9658
  Reduced model         -150.708         1       154.359      4         <.0001

           AIC:         151.326

                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          80        0.000
    0.0100     0.0000         0.001     0.000          80       -0.035
    0.0600     0.0013         0.106     0.000          80       -0.325
    0.2300     0.0566         4.524     5.000          80        0.230
    0.5700     0.5657        45.260    45.000          80       -0.059

 Chi^2 = 0.16      d.f. = 3        P-value = 0.9833

   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.282007
            BMDL =       0.223401
            BMDU =       0.309888

Taken together, (0.223401, 0.309888) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.447626
```

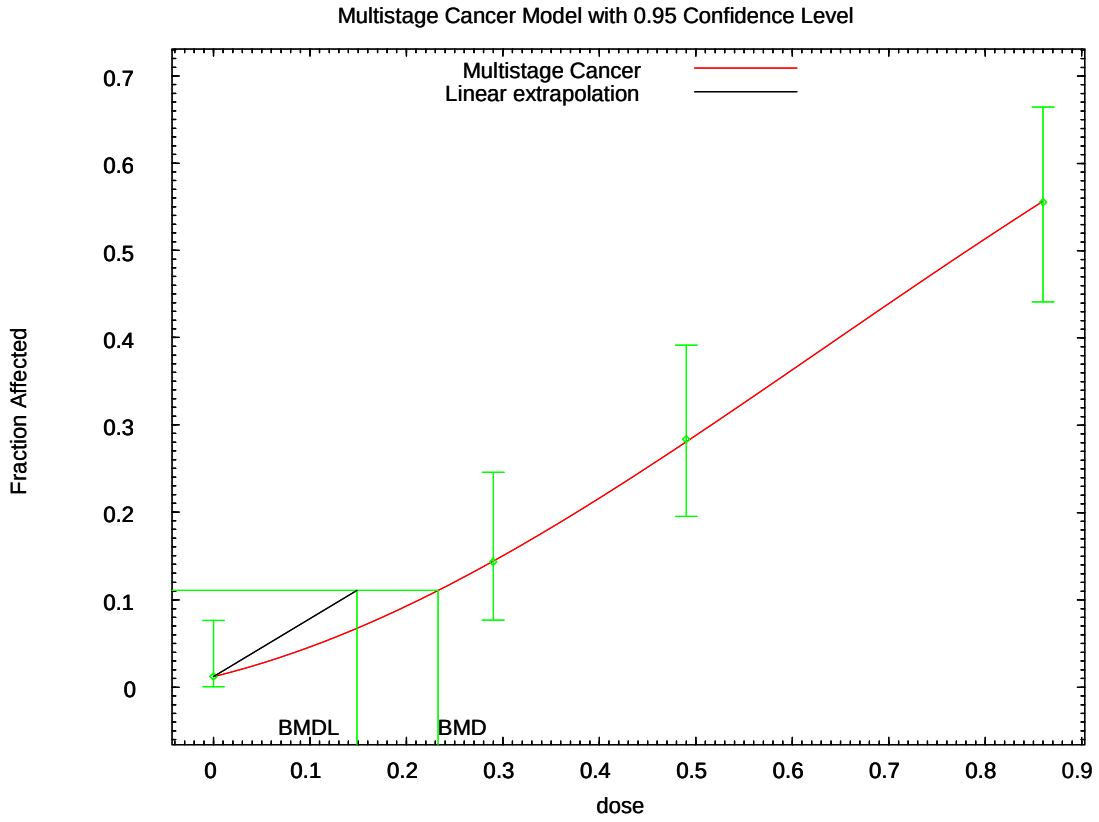

Figure E-26. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Schmähl et al., 1977).

```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input                                  Data                                    File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmahl1977femaleNMRI\2MulschMS_.(d)
         Gnuplot                                Plotting                                File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Schmahl1977femaleNMRI\2MulschMS_.plt
=================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008
```

```
****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =    0.0115034
                        Beta(1) =     0.284955
                        Beta(2) =     0.750235


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)      Beta(2)

Background            1        -0.67         0.47

   Beta(1)        -0.67            1        -0.94

   Beta(2)         0.47        -0.94            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0123066            *                *                   *
        Beta(1)         0.274413            *                *                   *
        Beta(2)         0.764244            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -145.127         4
    Fitted model        -145.13         3    0.00579898      1          0.9393
   Reduced model       -184.158         1       78.0608      3         <.0001

           AIC:         296.261


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0123         0.997     1.000          81        0.003
     0.2900     0.1446        11.137    11.000          77       -0.045
     0.4900     0.2813        24.756    25.000          88        0.058
     0.8600     0.5567        45.096    45.000          81       -0.022

 Chi^2 = 0.01      d.f. = 1        P-value = 0.9393


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.232893
            BMDL =       0.148895
            BMDU =       0.320396

Taken together, (0.148895, 0.320396) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.671616
```

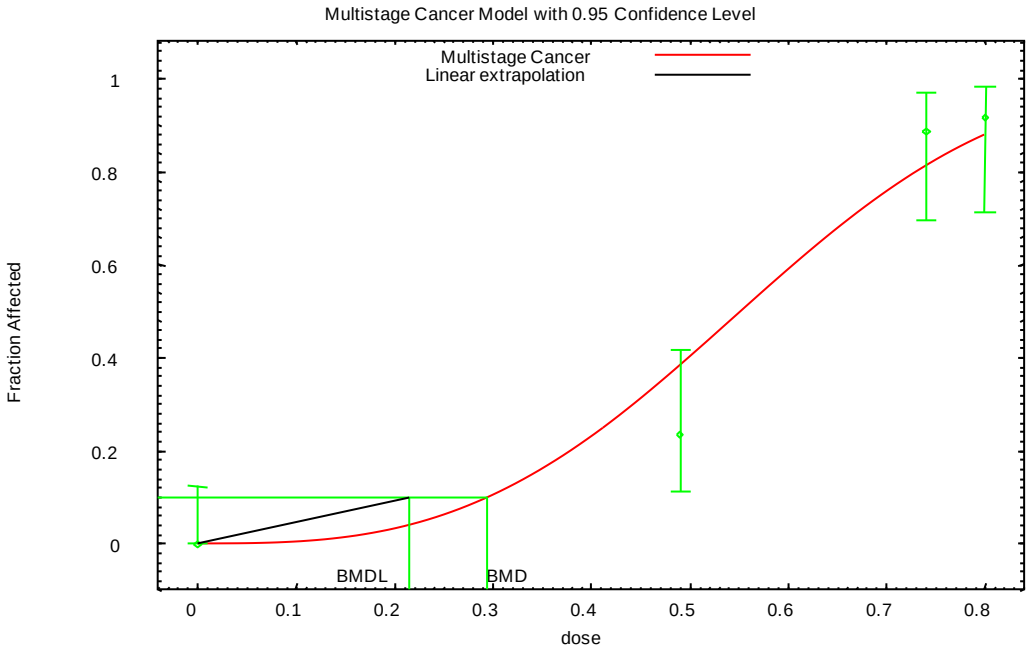

Figure E-27. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Habs et al., 1980).

```
=====================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: M:\_BMDS\msc_BAP_HABS1980_MultiCanc3_0.1.(d)
          Gnuplot Plotting File:  M:\_BMDS\msc_BAP_HABS1980_MultiCanc3_0.1.plt

        =====================================================================

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

    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

    The parameter betas are restricted to be positive


    Dependent variable = NumAff
    Independent variable = LADD

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 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
                      Background =            0
                         Beta(1) =            0
                         Beta(2) =      4.23649
                         Beta(3) =            0
```

```
            Asymptotic Correlation Matrix of Parameter Estimates
            ( *** The model parameter(s)  -Background    -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 )

                 Beta(3)

   Beta(3)           1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
      Background               0            *               *                   *
         Beta(1)               0            *               *                   *
         Beta(2)               0            *               *                   *
         Beta(3)          4.1289            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -34.8527         4
    Fitted model        -37.3373         1       4.96903      3          0.1741
   Reduced model        -82.5767         1       95.4478      3         <.0001

            AIC:         76.6745


                                 Goodness  of  Fit
                                                                      Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          35        0.000
     0.4900     0.3848        13.082     8.000          34       -1.791
     0.7400     0.8123        21.933    24.000          27        1.019
     0.8000     0.8792        21.102    22.000          24        0.563

 Chi^2 = 4.56      d.f. = 3        P-value = 0.2067


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =       0.294407

             BMDL =       0.215151

             BMDU =       0.320955

Taken together, (0.215151, 0.320955) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =       0.46479
```

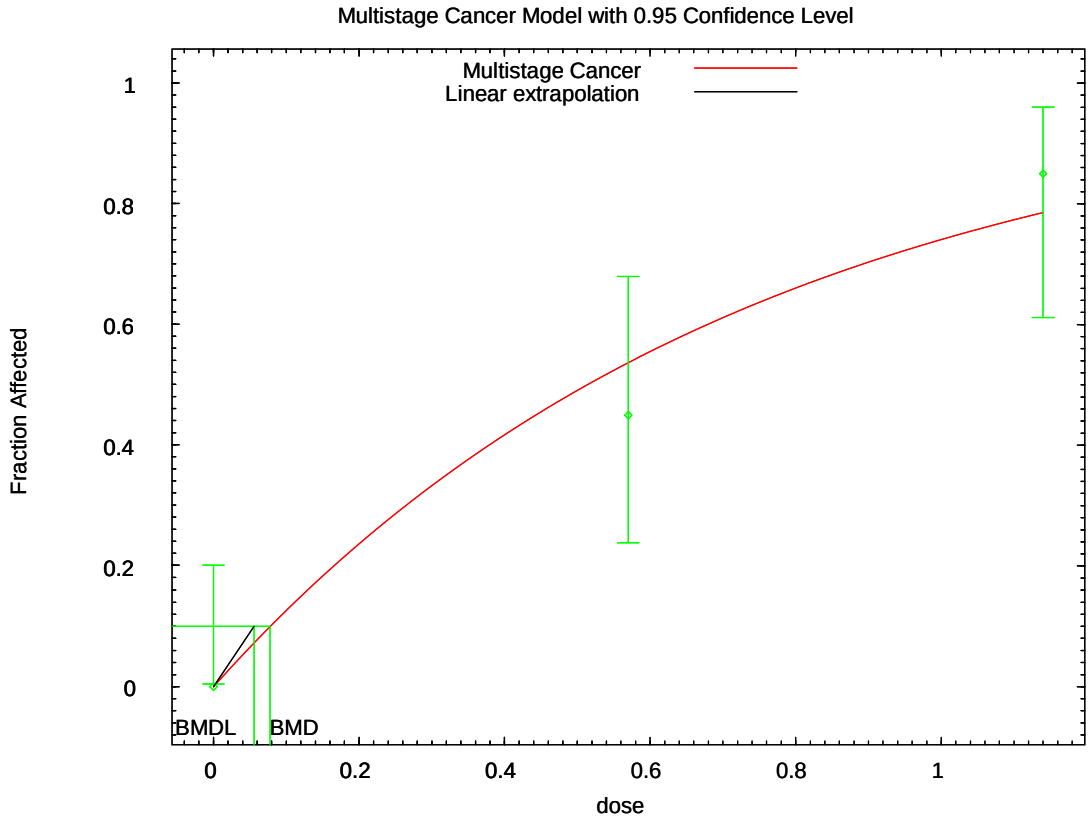

Figure E-28. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Habs et al., 1984).

```
=============================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: C:\Usepa\BMDS21\mscDax_Setting.(d)
          Gnuplot Plotting File:  C:\Usepa\BMDS21\mscDax_Setting.plt

         ============================================================================

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

    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1)]

    The parameter betas are restricted to be positive


    Dependent variable = tumors
    Independent variable = LADD

  Total number of observations = 3
  Total number of records with missing values = 0
  Total number of parameters in model = 2
  Total number of specified parameters = 0
  Degree of polynomial = 1


  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
                     Background =            0
                        Beta(1) =      1.66414


            Asymptotic Correlation Matrix of Parameter Estimates

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

                 Beta(1)

    Beta(1)           1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
      Background                0            *               *                   *
         Beta(1)          1.35264            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -22.217         3
    Fitted model        -22.7878        1       1.14175      2           0.565
   Reduced model        -41.0539        1       37.6739      2         <.0001

            AIC:         47.5757


                                 Goodness  of  Fit
                                                                    Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          20        0.000
     0.5700     0.5375        10.749     9.000          20       -0.784
     1.1400     0.7860        15.721    17.000          20        0.697

 Chi^2 = 1.10      d.f. = 2        P-value = 0.5765


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =      0.0778926

             BMDL =      0.0558466

             BMDU =       0.111853

Taken together, (0.0558466, 0.111853) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =       1.79062
```

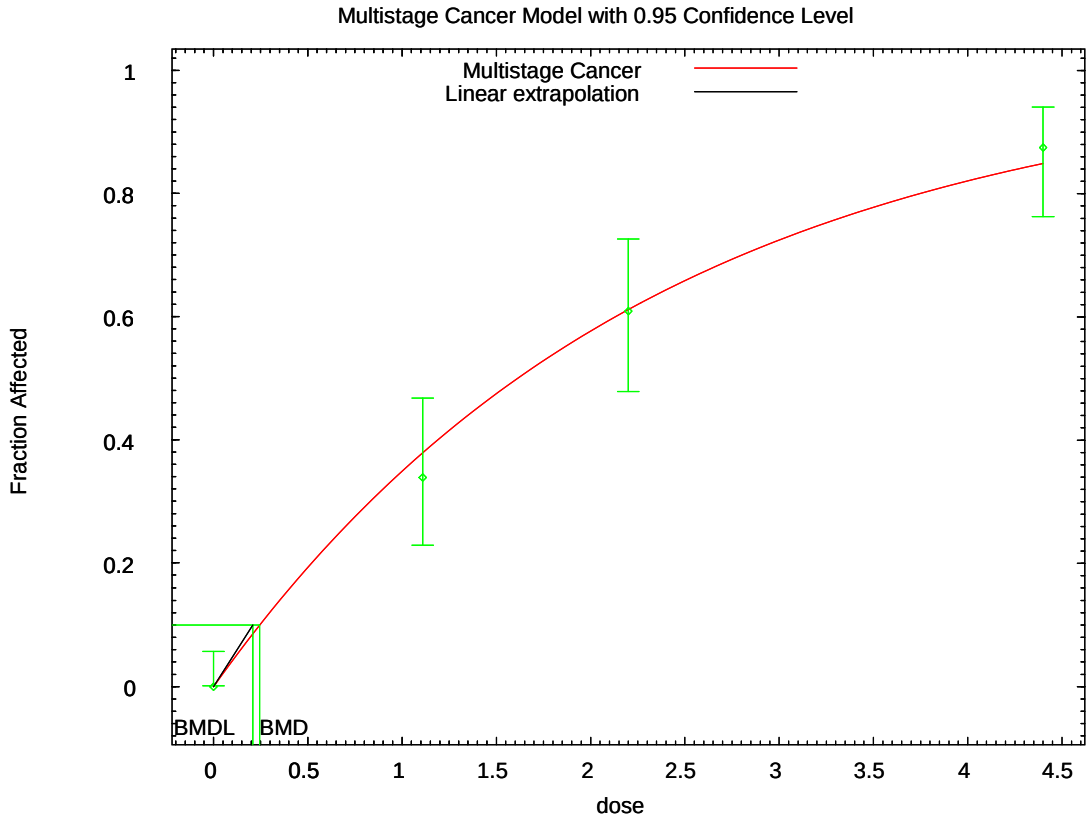

Figure E-29. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1983).

```
=============================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input                                    Data                                    File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Grimmer1983CFLPmice\1MulGriMS_.(d)
         Gnuplot                                  Plotting                                File:
C:\USEPA\IRIS\benzo[a]pyrene\dermalslopefactor\Grimmer1983CFLPmice\1MulGriMS_.plt
 =============================================================================

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

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive

   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008
```

```
****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****


                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =     0.478645


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)           1


                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background               0            *                *                   *
        Beta(1)         0.430366            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -108.532         4
    Fitted model       -108.943         1      0.823537      3          0.8438
   Reduced model       -186.434         1       155.805      3         <.0001

           AIC:         219.887


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          80       -0.000
     1.1100     0.3798        24.687    22.000          65       -0.687
     2.2000     0.6120        39.169    39.000          64       -0.043
     4.4000     0.8495        54.366    56.000          64        0.571

 Chi^2 = 0.80      d.f. = 3        P-value = 0.8496


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =       0.244816

             BMDL =       0.208269

             BMDU =       0.289606

Taken together, (0.208269, 0.289606) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.480148
```

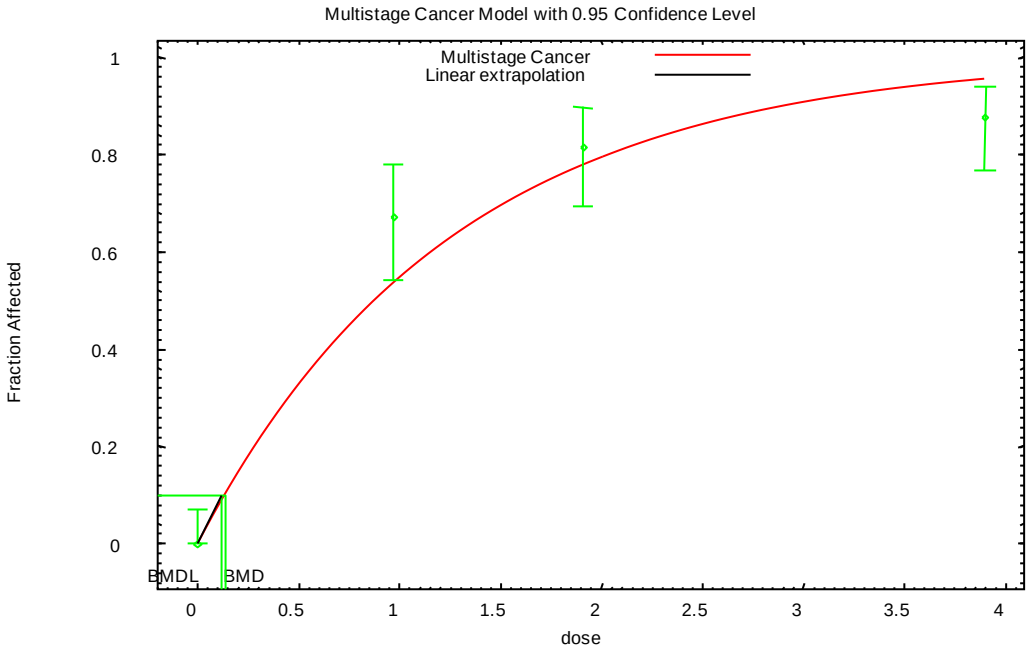

Figure E-30. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984).

```
================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Grimmer1984_MultiCanc1_0.1.(d)
          Gnuplot                                    Plotting                                    File:
C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Grimmer1984_MultiCanc1_0.1.plt
                                                     Wed Apr 27 17:11:28 2011

 [add notes here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1)]

    The parameter betas are restricted to be positive


    Dependent variable = NumAff
    Independent variable = LADD

  Total number of observations = 4
  Total number of records with missing values = 0
  Total number of parameters in model = 2
  Total number of specified parameters = 0
  Degree of polynomial = 1


  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
                      Background =     0.311241
                         Beta(1) =     0.502556
```

```
            Asymptotic Correlation Matrix of Parameter Estimates

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

                 Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0            *               *                   *
        Beta(1)         0.796546            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -95.8385         4
    Fitted model       -101.643         1         11.61      3        0.008846
   Reduced model       -175.237         1       158.797      3         <.0001

            AIC:         205.287


                                 Goodness  of  Fit
                                                                      Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------------
     0.0000     0.0000         0.000     0.000          65        0.000
     0.9700     0.5382        34.446    43.000          64        2.145
     1.9100     0.7816        50.804    53.000          65        0.659
     3.9000     0.9552        62.091    57.000          65       -3.054

 Chi^2 = 14.36     d.f. = 3        P-value = 0.0025


    Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =       0.132272

             BMDL =       0.113427

             BMDU =       0.154848

Taken together, (0.113427, 0.154848) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.881621
```

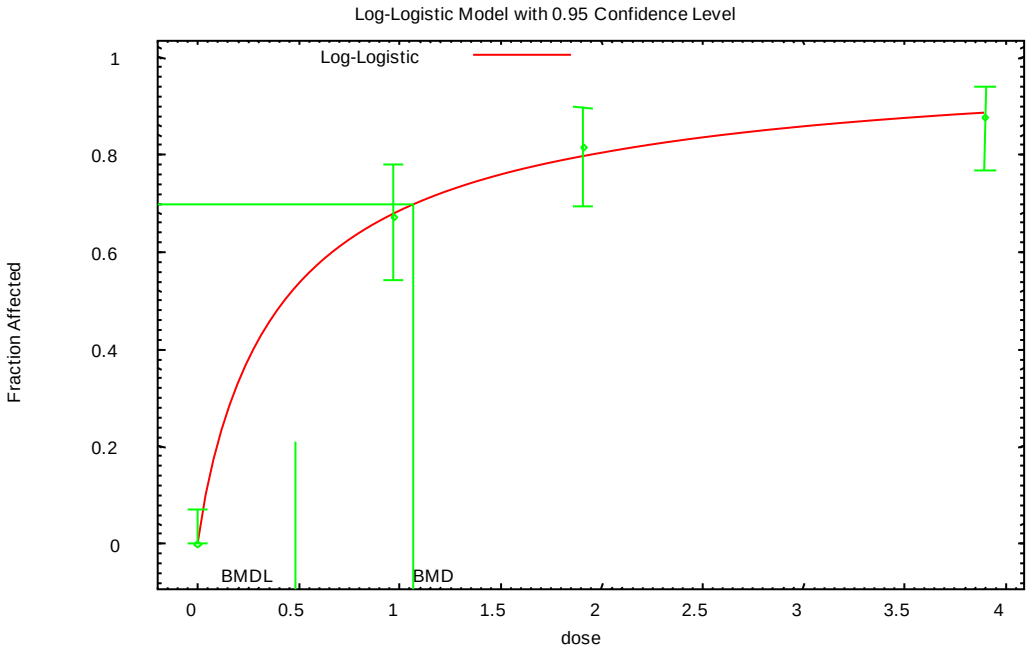

Figure E-31. Fit of log-logistic model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984).

```
================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input                                Data                                File:
C:\Usepa\BMDS21\Data\lnl_benzo[a]pyrene_Grimmer1984_Grimmer1984_0.70u.(d)
         Gnuplot                             Plotting                            File:
C:\Usepa\BMDS21\Data\lnl_benzo[a]pyrene_Grimmer1984_Grimmer1984_0.70u.plt

 ================================================================

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

   The form of the probability function is:

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


   Dependent variable = NumAff
   Independent variable = LADD
   Slope parameter is not restricted

   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 =     0.799142
                        slope =     0.894129


           Asymptotic Correlation Matrix of Parameter Estimates
```

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

              intercept        slope

 intercept            1        -0.68

     slope        -0.68            1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0            *                 *                   *
      intercept         0.783559            *                 *                   *
          slope         0.922655            *                 *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -95.8385         4
   Fitted model         -95.9236         2       0.17031      2          0.9184
  Reduced model         -175.237         1       158.797      3         <.0001

           AIC:         195.847


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          65        0.000
    0.9700     0.6804        43.543    43.000          64       -0.146
    1.9100     0.7991        51.941    53.000          65        0.328
    3.9000     0.8849        57.516    57.000          65       -0.200

 Chi^2 = 0.17      d.f. = 2        P-value = 0.9190


   Benchmark Dose Computation

Specified effect =           0.7

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        1.07152

            BMDL =       0.478669
```

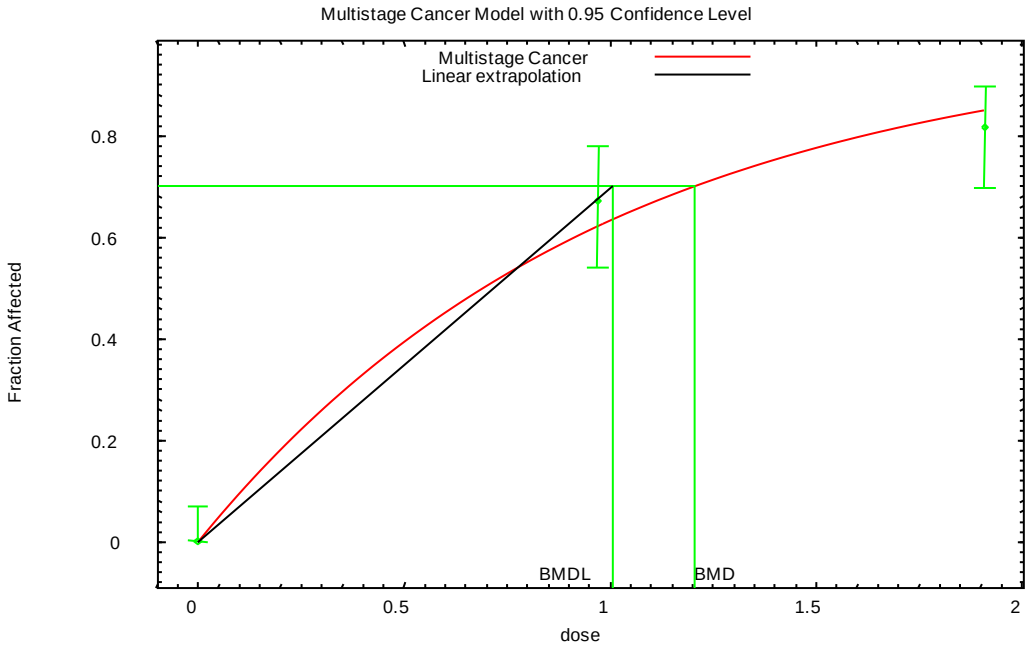

Figure E-32. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984), highest dose dropped.

```
=================================================================
          Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
          Input Data File: C:/Usepa/_BaP/msc_BaP_Grimmer1984_drophidose_MultiCanc1_0.7.(d)
          Gnuplot Plotting File:  C:/Usepa/_BaP/msc_BaP_Grimmer1984_drophidose_MultiCanc1_0.7.plt
 =================================================================

 [add_notes_here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = NumAff
   Independent variable = LADD

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 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
                     Background =    0.0806622
                        Beta(1) =      0.88595
```

```
            Asymptotic Correlation Matrix of Parameter Estimates

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

                 Beta(1)

   Beta(1)            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.997117	*	*	*

```
* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.5928	3			
Fitted model	-72.2756	1	1.36568	2	0.5052
Reduced model	-134.46	1	125.735	2	<.0001

```
            AIC:         146.551
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	65	0.000
0.9700	0.6199	39.671	43.000	64	0.857
1.9100	0.8511	55.322	53.000	65	-0.809

```
 Chi^2 = 1.39      d.f. = 2        P-value = 0.4992


   Benchmark Dose Computation

Specified effect =           0.7

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        1.20745

            BMDL =        1.00734

            BMDU =        1.45789

Taken together, (1.00734, 1.45789) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =        0.6949
```

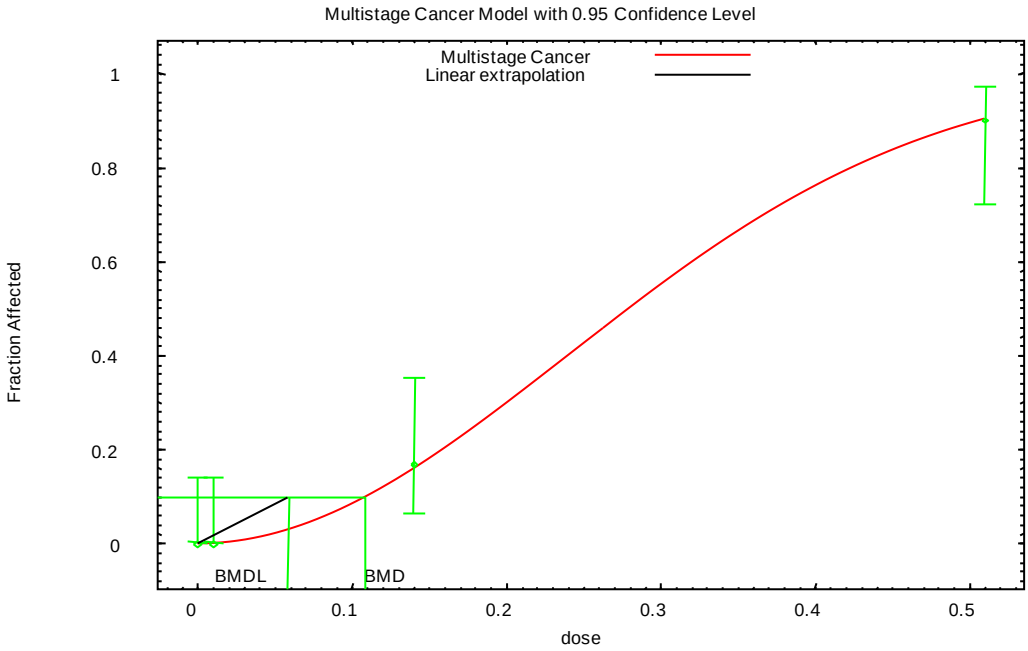

Figure E-33. Fit of multistage model to skin tumors in male CeH/HeJ mice exposed dermally to benzo[a]pyrene (Sivak et al., 1997).

```
=============================================================================
          Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
          Input Data File: C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Sivak1993_MultiCanc2_0.1.(d)
          Gnuplot                                    Plotting                                    File:
C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Sivak1993_MultiCanc2_0.1.plt

 =============================================================================

 [add notes here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the probability function is:

    P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1-beta2*dose^2)]

    The parameter betas are restricted to be positive


    Dependent variable = NumAff
    Independent variable = LADD

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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
                      Background =            0
                         Beta(1) =    0.0936505
                         Beta(2) =      8.67239
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	8.9375	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-23.2693	4			
Fitted model	-23.3009	1	0.0631003	3	0.9959
Reduced model	-69.5898	1	92.641	3	<.0001

AIC: 48.6018

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	30	0.000
0.0100	0.0009	0.027	0.000	30	-0.164
0.1400	0.1607	4.821	5.000	30	0.089
0.5100	0.9022	27.065	27.000	30	-0.040

Chi^2 = 0.04 d.f. = 3 P-value = 0.9982

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.108575

BMDL = 0.058484

BMDU = 0.129641

Taken together, (0.058484, 0.129641) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 1.70987

Alternative Approaches for Cross-Species Scaling of the Dermal Slope Factor

Several publications that develop a dermal slope factor for benzo[a]pyrene are available in the peer-reviewed literature (Knafla et al., 2011; Knafla et al., 2006; Hussain et al., 1998; LaGoy and Quirk, 1994; Sullivan et al., 1991). With the exception of the Knafla et al. (2011), none of these approaches applied quantitative adjustments to account for interspecies differences, though the proposed slope factors were developed to account for human risk. Knafla et al. (2011) qualitatively discuss processes that could affect the extrapolation between mice and humans, including skin metabolic activity adduct formation, stratum corneum thickness, epidermal thickness, etc. Ultimately, the authors apply an adjustment based on the increased epidermal thickness of human skin on the arms and hands compared to mouse interscapular epidermal thickness. They hypothesize that the carcinogenic potential of benzo[a]pyrene may be related to the thickness of the epidermal layer.

Because there is no established methodology for cross-species extrapolation of dermal toxicity, several alternative approaches were evaluated. Each approach begins with the POD of 0.066 µg/day that was based on a 10% extra risk for skin tumors in male mice. Based on the assumptions of each approach, a dermal slope factor for humans is calculated. The discussion of these approaches uses the following abbreviations:

DSF = dermal slope factor
POD_M = point of departure (for 10% extra risk) from mouse bioassay, in µg/day
BW_M = mouse body weight = 0.035 kg (assumed)
BW_H = human body weight = 70 kg (assumed)
SA_H = total human surface area = 19,000 cm^2 (assumed)
SA_M = total mouse surface area = 100 cm^2 (assumed)

Approach 1. No interspecies adjustment to daily applied dose (POD) in mouse model

Under this approach, a given mass of benzo[a]pyrene, applied daily, would pose the same risk in an animal or in humans, regardless of whether it is applied to a small surface area or to a larger surface area at a proportionately lower concentration.

$$DSF = 0.1 / POD_M$$

$$DSF = 0.1/0.068\ \mu g/day = \mathbf{1.5\ (\mu g/day)^{-1}}$$

Assumptions: The same mass of benzo[a]pyrene, applied daily, would have same potency in mice as in human skin regardless of treatment area.

Approach 2. Cross-species adjustment based on whole body surface-area scaling

Under this approach, animals and humans are assumed to have equal lifetime cancer risk with equal average whole body exposures in loading units (µg/cm^2-day). As long as doses are low

enough that risk is proportional to the mass of applied compound, the daily dermal dose of benzo[a]pyrene can be normalized over the total surface area.

$$\text{POD } (\mu g/cm^2\text{-day}) = \text{POD}_{M/SA} \ (\mu g/cm^2\text{-day}) = \text{POD}_M \ (\mu g/day) \ / \ SA_M \ (cm^2)$$
$$\text{POD} = (0.068 \ \mu g/day) \ / \ 100 \ cm^2 = 0.00068 \ \mu g/cm^2\text{-day}$$
$$\text{DSF} = 0.1/(0.00068 \ \mu g/cm^2\text{-day}) \approx \mathbf{147 \ (\mu g/cm^2\text{-day})^{-1}}$$

Assumptions: Mouse and human slope factors are equipotent if total dermal dose is averaged over equal fractions of the entire surface area. Tumor potency of benzo[a]pyrene is assumed to be related to overall dose and not dose per unit area. For example, a human exposed to 0.01 µg/day on 10 cm^2 would be assumed to have the same potential to form a skin tumor as someone treated with 0.01 µg/day over 19,000 cm^2 (assumed human surface area).

Approach 3. Cross-species adjustment based on body weight

Under this approach, a given mass of benzo[a]pyrene is normalized relative to the body weight of the animal or human.

$$\text{POD}_M / \ BW_M = 0.068 \ \mu g/0.035 \ kg\text{-day} = 1.9 \ \mu g/kg\text{-day}$$
$$\text{DSF} = 0.1/1.9 \ \mu g/kg\text{-day} = \mathbf{0.051 \ (\mu g/kg\text{-day})^{-1}}$$

Assumptions: The potency of point of contact skin tumors is related to bodyweight and humans and mice would have an equal likelihood of developing skin tumors based on a dermal dose per kg basis.

Issues: Skin cancer following benzo[a]pyrene exposure is a local effect and not likely dependent on body weight.

Approach 4. Cross-species adjustment based on allometric scaling using body weight to the 3/4 power

Under this approach, rodents and humans exposed to the same daily dose of a carcinogen, adjusted for $BW^{3/4}$, would be expected to have equal lifetime risks of cancer. That is, a lifetime dose expressed as $\mu g/kg^{3/4}$-day would lead to an equal risk in rodents and humans. This scaling reflects the empirically observed phenomena of more rapid distribution, metabolism, and clearance in smaller animals. The metabolism of benzo[a]pyrene to reactive intermediates is a critical step in the carcinogenicity of benzo[a]pyrene, and this metabolism occurs in the skin.

$$POD\ (\mu g/day) = POD_M\ (\mu g/day) \times (BW_H / BW_M)^{3/4}$$
$$POD\ (\mu g/day) = 0.068\ \mu g/day \times (70\ kg / 0.035\ kg)^{3/4} = 20.3\ \mu g/day$$
$$DSF = 0.1/(20.3\ \mu g/day) \approx \mathbf{0.0049\ (\mu g/day)^{-1}}$$

Assumptions: Risk at low doses of benzo[a]pyrene is dependent on absolute dermal dose and not dose per unit of skin, meaning that a higher exposure concentration of benzo[a]pyrene contacting a smaller area of exposed skin could carry the same risk of skin tumors as a lower exposure concentration of benzo[a]pyrene that contacts a larger area of skin.

Issues: It is unclear if scaling of doses based on bodyweight ratios will correspond to differences in metabolic processes in the skin of mice and humans.

Synthesis of the alternative approaches to cross-species scaling

A comparison of the above approaches is provided in Table E-24. The lifetime risk from a nominal human dermal exposure to benzo[a]pyrene over a 5% area of exposed skin (approximately 950 cm^2), estimated at 1×10^{-4} µg/day, is calculated for each of the approaches in order to judge whether the method yields risk estimates that are unrealistically high.

Other potential interspecies adjustments

The above discussion presents several mathematical approaches that result from varying assumptions about what is the relevant dose metric for determining equivalence across species. Biological information (that is not presently comprehensive or detailed enough to develop robust models) that could be used in future biologically based models for cross-species extrapolation include:

a. Quantitative information on interspecies differences in partitioning from exposure medium to the skin and absorption through the skin

b. Thickness of the stratum corneum between anatomical sites and between species

c. Thickness of epidermal layer

d. Skin permeability

e. Metabolic activity of skin

f. Formation of DNA adducts in skin

Table E-24. Alternative approaches to cross-species scaling

Approach	Assumptions	Dose metric	Dermal Slope Factor	Risk at nominal exposure (0.0004 µg/day)[a]
1. Mass-per-day scaling	Equal mass per day (µg/d), if applied to equal areas of skin (cm^2), will affect similar numbers of cells across species. Cancer risk is proportional to the area (cm^2) exposed if the loading rate (µg/cm^2-d) is the same. This approach assumes that risk is proportional to dose expressed as mass per day. This approach implies that any combination of loading rate (µg/cm^2-day) and skin area exposed (cm^2) that have the same product when multiplied, will result in the same risk.	µg/day	1.5 per µg/d	6×10^{-4}
2. Surface-area scaling	Equal mass per day (µg/d), if applied to equal fractions of total skin surface (cm^2) will have similar cancer risks. That is, lifetime exposure normalized over the whole body (e.g., 5%-of-the-body lifetime exposure) at the same loading rate (µg/cm^2-d) gives similar cancer risks across species. This approach assumes that risk is proportional to dose expressed as mass per area per day. This approach implies that risk does not increase with area exposed as long as dose per area remains constant.	µg/cm^2-day	147 per µg/cm^2-d	3×10^{-6}
3. Body-weight scaling	The skin is an organ with thickness and volume; benzo[a]pyrene is distributed within this volume of skin. Cancer risk is proportional to the concentration of benzo[a]pyrene in the exposed volume of skin. Equal mass per day (µg/d), if distributed within equal fractions of total body skin will have similar cancer risks. That is, whole-body lifetime exposure (e.g., 5%-of-the-body lifetime exposure) at the same loading rate (µg/cm^2-d) gives similar cancer risks across species. This approach assumes that risk is proportional to dose expressed as mass per kg body weight per day. This approach implies that any combination of dose (µg/day) and body weight (kg) that have the same result when divided will result in the same risk.	µg/kg-day	0.051 per µg/kg-d	3×10^{-7}
4. Allometric scaling ($BW^{3/4}$)	Same as for body-weight scaling, except that benzo[a]pyrene distribution and metabolism takes place within this volume of skin. Allometric scaling is generally regarded as describing the relative rate of toxicokinetic processes across species. This approach also is used by EPA to scale oral exposures.	µg/day	0.0049 per µg/d	2×10^{-6}

[a]Calculated as a central tendency exposure using an average benzo[a]pyrene soil concentration of 100 ppb, rounded to one significant figure (see Appendix A, Table A-4) and standard exposure assumptions from U.S. EPA (2004).

APPENDIX F. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[2]. The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde[3]. The report language included the following:

> The Agency shall incorporate, as appropriate, based on chemical-specific datasets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process… For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated.

The NRC's recommendations, provided in Chapter 7 of their review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the table below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS benzo[a]pyrene assessment is in Phase 2 and represents a significant advancement in

[2]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.
[3]National Research Council, 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

implementing the NRC recommendations shown in Table F-1 below. The Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table F-2, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced[4] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

Table F-1. The EPA's implementation of the National Research Council's recommendations in the benzo[a]pyrene assessment

National Research Council recommendations that EPA is implementing in the short term	Implementation status
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (p. 152 of the NRC report)	
1. To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Implemented.** The overall document structure has been revised in consideration of this NRC recommendation. The new structure includes a concise Executive Summary and an explanation of the literature review search strategy, study selection criteria, and methods used to develop the assessment. The main body of the assessment has been reorganized into two sections, Hazard Identification and Dose-Response Analysis, to help reduce the volume of text and redundancies that were a part of the previous document structure. Section 1 provides evidence tables and a concise synthesis of hazard information organized by health effect, The Supplemental Information provides more detailed summaries of the most pertinent epidemiology and experimental animal studies (Appendix D), as well as information on chemical and physical properties and toxicokinetics (Appendix D). The main text of the Toxicological Review is approximately 130 pages, which is a major reduction from previous IRIS assessments. Technical and scientific edits were performed to eliminate any redundancies or inconsistencies.

[4]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

National Research Council recommendations that EPA is implementing in the short term	Implementation status
2. Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.	**Implemented.** Chapter 1 has been replaced with a Preamble that describes the application of existing EPA guidance and the methods and criteria used in developing the assessment. The term "Preamble" was chosen to emphasize that these methods and criteria are being applied consistently across IRIS assessments. The new Preamble includes information on identifying and selecting pertinent studies, evaluating the quality of individual studies, weighing the overall evidence of each effect, selecting studies for derivation of toxicity values, and deriving toxicity values. These topics correspond directly to the five steps that the NRC identified in Figure 7-2 of their 2011 report. A new section, Literature Search Strategy and Study Selection, provides detailed information on the search strategy used to identify health effect studies, search outcomes, and selection of studies for hazard identification. This information is chemical-specific and has been designed to provide enough information that an independent literature search would be able to replicate the results. This section also includes information on how studies were selected to be included in the document and provides a link to EPA's Health and Environmental Research Online (HERO) database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited.
3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix.	**Implemented.** In the new document template, standardized evidence tables that present key study findings that support how toxicological hazards are identified for all major health effects are provided in Section 1.1. More detailed summaries of the most pertinent epidemiology and experimental animal studies are provided in the Supplemental Information (Appendix D).
4. All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Partially Implemented.** Information in Section 4 of the Preamble provides an overview of the approach used to evaluate the quality of individual studies. Critical evaluation of the epidemiologic and experimental animal studies is included in the evidence tables in Section 1.1. As more rigorous systematic review processes are developed, they will be utilized in future assessments.
5. The rationales for the selection of the studies that	**Implemented.** The Dose-Response Analysis section of

National Research Council recommendations that EPA is implementing in the short term	Implementation status
are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	the new document structure provides a clear explanation of the rationale used to select and advance studies that were considered for calculating toxicity values. Rationales for the selection of studies advanced for reference value derivation are informed by the weight-of-evidence for hazard identification as discussed in Section 1.2. In support of the derivation of reference values for benzo[a]pyrene, exposure-response arrays are included that compare effect levels for several toxicological effects (Section 2.1, Figure 2-1; Section 2.2., Figure 2.2.). The exposure response array provides a visual representation of points of departure for various effects resulting from exposure to benzo[a]pyrene. The array informs the identification of doses associated with specific effects, and the choice of principal study and critical effects. In the case of the benzo[a]pyrene RfD, the database supported the development of multiple organ/system- specific RfD's. Such values have been developed previously on a case-by-case basis and will be developed in future assessments, where the data allow.
6. Strengthened, more integrative and more transparent discussions of weight-of-evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight-of-evidence, such as consistency.	**Partially implemented.** The new Hazard Identification (Section 1) provides a more strengthened, integrated and transparent discussion of the weight of the available evidence. This section includes standardized evidence tables to present the key study findings that support how potential toxicological hazards are identified and exposure-response arrays for each potential toxicological effect. Summary discussions are provided as a statement of hazard for each major effect (Section 1.1.1.—developmental toxicity, Section 1.1.2.—reproductive toxicity, Section 1.1.3.—immunotoxicity, Section 1.1.4.—other toxicological effects, and Section 1.1.5. —carcinogenicity) as well as a general weight-of-evidence discussion for effects other than cancer (Section 1.2.1.) and cancer (1.2.2.). A more rigorous and formalized approach for characterizing the weight-of-evidence will be developed as a part of Phase 3 of the implementation process.

National Research Council recommendations that EPA is implementing in the short term	Implementation status
Other specific recommendations (p. # in NRC report)	
General Guidance for the Overall Process (p. 164) 7. Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Implemented.** EPA has created Chemical Assessment Support Teams to formalize an internal process to provide additional overall quality control for the development of IRIS assessments. This initiative uses a team approach to making timely, consistent decisions about the development of IRIS assessments across the Program. This team approach has been utilized for the development of the benzo[a]pyrene assessment. Additional objectives of the teams is to help ensure that the necessary disciplinary expertise is available for assessment development and review, to provide a forum for identifying and addressing key issues prior to external peer review, and to monitor progress in implementing the NRC recommendations.
8. Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
9. Assess disciplinary structure of teams needed to conduct the assessments.	
Evidence Identification: Literature Collection and Collation Phase (p. 164) 10. Select outcomes on the basis of available evidence and understanding of mode of action.	**Partially Implemented.** A new section, Literature Search Strategy and Study Selection, contains detailed information on the search strategy used for the benzo[a]pyrene assessment, including key words used to identify relevant health effect studies. Figure LS-1 depicts the study selection strategy and the number of references obtained at each stage of literature screening. This section also includes information on how studies were selected to be included in the document and provides a link to an external database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited. Each citation in the Toxicological Review is linked to HERO such that the public can access the references and abstracts to the scientific studies used in the assessment. The implementation of these NRC recommendations in the benzo[a]pyrene assessment represents a major advance in the standardization and transparency of evidence identification. Section 3 of the Preamble summarizes the standard protocols for evidence identification that are provided in EPA guidance. For each potential toxicological effect identified for benzo[a]pyrene, the available evidence is informed by the mode of action information as discussed in Section 1.1. As more rigorous systematic review processes are developed, they will be utilized in future assessments.
11. Establish standard protocols for evidence identification.	
12. Develop a template for description of the search approach.	
13. Use a database, such as the Health and Environmental Research Online (HERO) database, to capture study information and relevant quantitative data.	
Evidence Evaluation: Hazard Identification and	**Implemented.** Standardized tables have been developed

National Research Council recommendations that EPA is implementing in the short term	Implementation status
Dose-Response Modeling (p. 165) 14. Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight-of-evidence, and utility as a basis for deriving reference values and unit risks.	that provide summaries of key study design information and results by health effect. The inclusion of all positive and negative findings in each health effect-specific evidence table supports a weight-of-evidence analysis. In addition, exposure-response arrays are utilized in the assessment to provide a graphical representation of points of departure for various effects resulting from exposure to benzo[a]pyrene. The exposure-response arrays inform the identification of doses associated with specific effects and the weight-of-evidence for those effects.
15. Develop templates for evidence tables, forest plots, or other displays.	**Implemented.** Templates for evidence tables and exposure-response arrays have been developed and are utilized in Section 1.1.
16. Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Partially Implemented.** General principles for reviewing epidemiologic and experimental animal studies are described in Section 4 of the Preamble. The development of standardized protocols for systematic review of evidence is an ongoing process.
Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165) 17. Establish clear guidelines for study selection.	**Implemented.** EPA guidelines for study selection, including balancing strengths and weaknesses and weighing human vs. experimental evidence are described in the Preamble (Sections 3-6). These guidelines have been applied in Section 2 of the benzo[a]pyrene assessment to evaluate the strengths and weaknesses of individual studies considered for reference value derivation. In the case of benzo[a]pyrene, the database did not support the combination of estimates across studies. In future assessments, combining estimates across studies will be routinely considered.
a) Balance strengths and weaknesses.	
b) Weigh human vs. experimental evidence.	
c) Determine whether combining estimates among studies is warranted.	
Calculation of Reference Values and Unit Risks (pp. 165-166) 18. Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented.** The rationale for the selection of the points of departure for the organ/system specific oral reference values is provided in Section 2.1. The rationale for the selection of the point of departure and the inhalation dosimetry modeling (for the approximation of a human equivalent concentration) for the derivation of the inhalation reference value is transparently described in Section 2.2. The benchmark dose modeling for candidate reference values is transparently described in the Supplemental Information (Appendix E).
19. Provide explanation of the risk-estimation modeling processes (for example, a statistical or	**Implemented.** The risk-estimation modeling processes used to develop cancer risk estimates for benzo[a]pyrene

National Research Council recommendations that EPA is implementing in the short term	Implementation status
biologic model fit to the data) that are used to develop a unit risk estimate.	are described in Section 2 of the Toxicological Review and in the Supplemental Information (Appendix E).
20. Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report, sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided when appropriate.	**Implemented.** The new template structure that has been developed in response to the NRC recommendations provides a clear explanation of the literature search strategy, study selection criteria, and methods used to develop the benzo[a]pyrene assessment. It provides for a clear description of the decisions made in developing the hazard identification and dose-response analysis. Information contained in the Preamble and throughout the document reflects the guidance that has been utilized in developing the assessment. As recommended, supplementary information is provided in the accompanying appendices. Detailed modeling analyses are presented in the appendices.

Table F-2. National Research Council recommendations that the EPA is generally implementing in the long term

National Research Council recommendations that EPA is generally implementing in the long-term (p. # in NRC report)	Implementation status
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (p. 165) 1. Review use of existing weight-of-evidence guidelines. 2. Standardize approach to using weight-of-evidence guidelines. 3. Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. 4. Develop uniform language to describe strength of evidence on noncancer effects. 5. Expand and harmonize the approach for characterizing uncertainty and variability. 6. To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced[5] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA will hold a workshop on August 26, 2013 on issues related to weight-of-evidence.

[5]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

National Research Council recommendations that EPA is generally implementing in the long-term (p. # in NRC report)	Implementation status
Calculation of Reference Values and Unit Risks (pp. 165-166) 7. Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the estimates.	Multiple, endpoint-specific reference values were derived for benzo[a]pyrene (RfD: Table 2-3 and Figure 2-1; RfC: Table 2-5 and Figure 2-2) and demonstrate the sensitivity of the overall reference values depending on the selection of the overall end points.

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APPENDIX G. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND EPA'S DISPOSITION

[placeholder]

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