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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

September 2014

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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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ABBREVIATIONS

1-OH-Py	1-hydroxypyrene	ETS	environmental tobacco smoke
AchE	acetylcholine esterase	EU	European Union
ADAF	age-dependent adjustment factor	Fe ₂ O ₃	ferrous oxide
Ah	aryl hydrocarbon	FSH	follicle stimulating hormone
AHH	aryl hydrocarbon hydroxylase	GABA	gamma-aminobutyric acid
AhR	aryl hydrocarbon receptor	GD	gestational day
AIC	Akaike's Information Criterion	GI	gastrointestinal
AKR	aldo-keto reductase	GJIC	gap junctional intercellular communication
AMI	acute myocardial infarction	GSH	reduced glutathione
ANOVA	analysis of variance	GST	glutathione-S-transferase
ARNT	Ah receptor nuclear translocator	GSTM1	glutathione-S-transferase M1
AST	aspartate transaminase	hCG	human chorionic gonadotropin
ATSDR	Agency for Toxic Substances and Disease Registry	HEC	human equivalent concentration
BMC	benchmark concentration	HED	human equivalent dose
BMCL	benchmark concentration lower confidence limit	HERO	Health and Environmental Research Online
BMD	benchmark dose	HFC	high-frequency cell
BMDL	benchmark dose, 95% lower bound	HPLC	high-performance liquid chromatography
BMDS	Benchmark Dose Software	hppt	hypoxanthine guanine phosphoribosyl transferase
BMR	benchmark response	HR	hazard ratio
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide	Hsp90	heat shock protein 90
BPQ	benzo[a]pyrene semiquinone	i.p.	intraperitoneal
BrdU	bromodeoxyuridine	i.v.	intravenous
BSM	benzene-soluble matter	Ig	immunoglobulin
BUN	blood urea nitrogen	IHD	ischemic heart disease
BW	body weight	IRIS	Integrated Risk Information System
CA	chromosomal aberration	LDH	lactate dehydrogenase
CAL/EPA	California Environmental Protection Agency	LH	luteinizing hormone
CASRN	Chemical Abstracts Service Registry Number	LOAEL	lowest-observed-adverse-effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act	MAP	mitogen-activated protein
CHO	Chinese hamster ovary	MCL	Maximum Contaminant Level
CI	confidence interval	MCLG	Maximum Contaminant Level Goal
CYP	cytochrome	MIAME	Minimum Information About a Microarray Experiment
CYP450	cytochrome P450	MLE	maximum likelihood estimate
DAF	dosimetric adjustment factor	MMAD	mass median aerodynamic diameter
dbcAMP	dibutyl cyclic adenosine monophosphate	MN	micronucleus
DMSO	dimethyl sulfoxide	MPPD	Multi-Path Particle Deposition
DNA	deoxyribonucleic acid	mRNA	messenger ribonucleic acid
EC	European Commission	MS	mass spectrometry
EH	epoxide hydrolase	NCE	normochromatic erythrocyte
ELISA	enzyme-linked immunosorbent assay	NCEA	National Center for Environmental Assessment
EPA	Environmental Protection Agency	NIOSH	National Institute for Occupational Safety and Health
EROD	7-ethoxyresorufin-O-deethylase	NK	natural-killer

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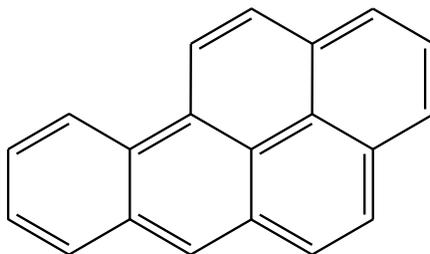
Supplemental Information—Benzo[a]pyrene

NMDA	N-methyl-D-aspartate	SHE	Syrian hamster embryo
NOAEL	no-observed-adverse-effect level	SIR	standardized incidence ratio
NPL	National Priorities List	SMR	standardized mortality ratio
NQO	NADPH:quinone oxidoreductase	SOAR	Systematic Omics Analysis Review
NRC	National Research Council	SOD	superoxide dismutase
NTP	National Toxicology Program	SRBC	sheep red blood cells
OECD	Organisation for Economic Co-operation and Development	SSB	single-strand break
OR	odds ratio	TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
ORD	Office of Research and Development	TK	thymidine kinase
PAH	polycyclic aromatic hydrocarbon	ToxR	Toxicological Reliability Assessment
PBMC	peripheral blood mononuclear cell	TPA	12-O-tetradecanoylphorbol-13-acetate
PBPK	physiologically based pharmacokinetic	TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
PCA	Principal Components Analysis	TWA	time-weighted average
PCE	polychromatic erythrocyte	UCL	upper confidence limit
PCNA	proliferating cell nuclear antigen	UDP-UGT	uridine diphosphate- glucuronosyltransferase
PND	postnatal day	UDS	unscheduled DNA synthesis
POD	point of departure	UF	uncertainty factor
PUVA	psoralen plus ultraviolet-A	UF _A	interspecies uncertainty factor
RBC	red blood cell	UF _D	database deficiencies uncertainty factor
RDDR _{ER}	regional deposited dose ratio for extrarespiratory effects	UF _H	intraspecies uncertainty factor
RfC	inhalation reference concentration	UF _L	LOAEL-to-NOAEL uncertainty factor
RfD	oral reference dose	UF _S	subchronic-to-chronic uncertainty factor
RNA	ribonucleic acid	UVA	ultraviolet-A
ROS	reactive oxygen species	UVB	ultraviolet-B
RR	relative risk	WBC	white blood cell
s.c.	subcutaneous	WESPOC	water escape pole climbing
SCC	squamous cell carcinoma	WT	wild type
SCE	sister chromatid exchange	WTC	World Trade Center
SCSA	sperm chromatin structure assay	XPA	xeroderma pigmentosum group A
SD	standard deviation		
SE	standard error		
SEM	standard error of the mean		

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 wet and dry deposition to land or water. In air, benzo[a]pyrene is predominantly adsorbed to particulates, but may also exist as a vapor at high temperatures ([HSDB, 2012](#)). The half-lives for degradation of benzo[a]pyrene in soil, air, water, and sediment are 229–309, 0.02–7, 39–71, and 196–2293 days, respectively ([HSDB, 2012](#); [GLC, 2007](#)).

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



Benzo[a]pyrene

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

1 **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	$1.6\text{--}2.3 \times 10^{-3}$ 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)

2
3 ^aCalculated based on the ideal gas law, $PV = nRT$ at 25°C: $\text{ppm} = \text{mg/m}^3 \times 24.45 \div \text{molecular weight}$.

4
5 No reference to any commercial use for purified benzo[a]pyrene, other than for research
6 purposes, was found. The earliest research reference for benzo[a]pyrene was related to the
7 identification of coal tar constituents associated with human skin tumors ([Phillips, 1983](#); [Cook et](#)
8 [al., 1933](#)). It is found ubiquitously in the environment, primarily as a result of incomplete
9 combustion emissions ([Boström et al., 2002](#)). It is released to the environment via both natural
10 sources (such as forest fires) and anthropogenic sources including stoves/furnaces burning fossil
11 fuels (especially wood and coal), motor vehicle exhaust, cigarette smoke, and various industrial
12 combustion processes ([ATSDR, 1995](#)). Benzo[a]pyrene is also found in soot and coal tars. Studies
13 have reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar
14 emulsion seal could account for the majority of PAHs in many watersheds ([Rowe and O'Connor,](#)
15 [2011](#); [Van Metre and Mahler, 2010](#); [Mahler et al., 2005](#)). Occupational exposure to PAHs occurs
16 primarily through inhalation and skin contact during the production and use of coal tar and coal-
17 tar-derived products, such as roofing tars, creosote, and asphalt ([IARC, 1973](#)). Chimney sweeping
18 can result in exposure to benzo[a]pyrene-contaminated soot ([ATSDR, 1995](#)). Workers involved in
19 the production of aluminum, coke, graphite, and silicon carbide may also be exposed to
20 benzo[a]pyrene (see Table A-2).

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

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

4 *Inhalation Exposure.* The Agency for Toxic Substances and Disease Registry ([ATSDR, 1995](#))
5 reported average indoor concentrations of benzo[a]pyrene of 0.37–1.7 ng/m³ for smokers and
6 0.27–0.58 ng/m³ for nonsmokers. [Naumova et al. \(2002\)](#) measured PAHs in 55 nonsmoking
7 residences in three urban areas during June 1999–May 2000. Mean indoor benzo[a]pyrene levels
8 ranged from 0.02 to 0.078 ng/m³; outdoor levels were 0.025–0.14 ng/m³. The authors concluded
9 that indoor levels of the 5–7-ring PAHs (such as benzo[a]pyrene) were dominated by outdoor
10 sources and observed an average indoor/outdoor ratio of approximately 0.7 ([Naumova et al.](#)
11 [2002](#)). [Mitra and Wilson \(1992\)](#) measured benzo[a]pyrene air levels in Columbus, Ohio, and found
12 elevated indoor levels in homes with smokers. The measured average concentration was
13 1.38 ng/m³ for outdoor air; indoor concentrations were 0.07 ng/m³ for homes with electrical
14 utilities, 0.91 ng/m³ for homes with gas utilities, 0.80 ng/m³ for homes with gas utilities and a
15 fireplace, 2.75 ng/m³ for homes with gas utilities and smokers, and 1.82 ng/m³ for homes with gas
16 utilities, smokers, and a fireplace ([Mitra and Wilson, 1992](#)). [Mitra and Ray \(1995\)](#) evaluated data
17 on benzo[a]pyrene air levels in Columbus, Ohio, and reported average concentrations of 0.77 ng/m³
18 inside homes and 0.23 ng/m³ outdoors. [Park et al. \(2001\)](#) measured an average ambient level of
19 benzo[a]pyrene in Seabrook, Texas during 1995–1996 of 0.05 ng/m³ (vapor plus particulate). [Park](#)
20 [et al. \(2001\)](#) also reported average ambient air levels from earlier studies as 1.0 ng/m³ for Chicago,
21 0.19 ng/m³ for Lake Michigan, 0.01 ng/m³ for Chesapeake Bay, and 0.02 ng/m³ for Corpus Christie,
22 Texas. [Petry et al. \(1996\)](#) conducted personal air sampling during 1992 at five workplaces in
23 Switzerland: carbon anode production, graphite production, silicon carbide production, bitumen
24 paving work, and metal recycling. Table A-2 summarizes the benzo[a]pyrene air concentration
25 data from the previous studies.

26

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

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

2
3 NA = not available.

4
5 [Santodonato et al. \(1981\)](#) estimated the adult daily intake from inhalation as 9–43 ng/day.
6 The European Commission ([EC, 2002](#)) reported benzo[a]pyrene air levels in Europe during the
7 1990s as 0.1–1 ng/m³ in rural areas and 0.5–3 ng/m³ in urban areas. The amount of
8 benzo[a]pyrene is reported to be 5–80 ng per cigarette in mainstream cigarette smoke, but
9 significantly higher, 25–200 ng per cigarette in sidestream smoke. Concentrations of
10 400–760,000 ng/m³ have been reported in a cigarette smoke-polluted environment ([Cal/EPA,](#)

1 [2010](#)). The mean intake via inhalation for an adult nonsmoker was estimated as 20 ng/day.
 2 [Naumova et al. \(2002\)](#) focused on nonsmoking residences and suggested that typical air exposures
 3 are <0.14 ng/m³, which would result in an intake of <3 ng/day assuming an inhalation rate of
 4 20 m³/day.

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

13 [Kazerouni et al. \(2001\)](#) measured benzo[a]pyrene in a variety of commonly consumed foods
 14 collected from grocery stores and restaurants in Maryland (analyzed as a composite from
 15 4–6 samples of each food type). The foods were tested after various methods of cooking; the
 16 results are reported in Table A-3. The concentrations were combined with food consumption data
 17 to estimate intake. The intakes of the 228 subjects ranged from approximately 10 to 160 ng/day,
 18 with about 30% in the 40–60 ng/day range. The largest contributions to total intake were reported
 19 as bread, cereal, and grain (29%) and grilled/barbecued meats (21%).

20 **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

Food	Concentration (ng/g)
Milk	0.02
Fruit	0.01–0.17

1
2 Source: [Kazerouni et al. \(2001\)](#).

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

7 From the surveys conducted in six EU countries, the mean or national-averaged dietary
8 intake of benzo[a]pyrene for an adult person was estimated in the range of 0.05–0.29 µg/day ([EC,
9 2002](#)). Children may be subject to higher oral intake of benzo[a]pyrene. In a Spanish study in
10 which benzo[a]pyrene was detected in foods, children ages 4–9 years old were found to have the
11 highest estimated daily intake, as compared to adults and adolescents ([Falco et al., 2003](#)). In the
12 United Kingdom, average intakes on a ng kg⁻¹ day⁻¹ basis were estimated for the following age
13 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,
14 3.1–3.8. The major contributors were the oils and fats group (50%), cereals (30%), and vegetables
15 (8%) ([EC, 2002](#)). The contribution from grilled foods appeared less important in Europe than in the
16 United States because grilled foods are consumed less often ([EC, 2002](#)). In the United States, the
17 ingested dose of benzo[a]pyrene may be much higher than the amount inhaled. A study in New
18 Jersey estimated a daily median total ingested dose of 176 ng based on a urinary biomarker study
19 of 14 adult volunteers over 14 consecutive days, which exceeded the winter inhalation dose
20 (11 ng/day) by 16-fold and the summer/fall inhalation dose (2.3 ng/day) by 122-fold ([Buckley et
21 al., 1995](#)).

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

26 PAHs are commonly found in all types of soils. [ATSDR \(1995\)](#) reported benzo[a]pyrene
27 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
28 urban areas, and 14,000–159,000 µg/kg at contaminated sites (before remediation). The soil levels
29 for all land uses appear highly variable. The levels are affected by proximity to roads/combustion
30 sources, use of sewage-sludge-derived amendments on agricultural lands, particle size, and organic
31 carbon content. [Weinberg et al. \(1989\)](#) reported that PAH levels in soils generally increased during
32 the 1900s and that sediment studies suggest that some declines may have occurred since the 1970s.
33 An illustration of benzo[a]pyrene levels in soil is presented in Table A-4.

34

1

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

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

APPENDIX B. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

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

Organization	Toxicity value or determination
Oral value	
(WHO (2003), 1996)	The guideline value for benzo[a]pyrene in drinking water of 0.7 µg/L was based on a cancer slope factor of 0.46 (mg/kg-d)⁻¹ derived from Neal and Rigdon (1967) and a lifetime excess cancer risk of 10 ⁻⁵ .
(Health Canada (2010), 1998)	The Maximum Acceptable Concentration for benzo[a]pyrene in drinking water of 0.01 µg/L was derived from Neal and Rigdon (1967) using a drinking water consumption rate of 1.5 L/day, a body weight of 70 kg, and a lifetime cancer risk of 5 × 10 ⁻⁷ . (The concentrations of 2, 0.2, and 0.02 µg/L benzo[a]pyrene correspond to lifetime excess cancer risks of 10 ⁻⁴ , 10 ⁻⁵ , and 10 ⁻⁶ .)
Inhalation value	
(WHO (2000), 1997)	Does not recommend specific guideline values for polycyclic aromatic hydrocarbons (PAHs) in air. A unit risk of 87 (mg/m³)⁻¹ for benzo[a]pyrene, as an indicator a PAH mixtures, was derived from U.S. EPA's inhalation unit risk from coke oven emissions.
EU (2005)	Target value of 1 ng/m³ benzo[a]pyrene (averaged over 1 calendar year) as a marker of PAH carcinogenic risk. Does not include information for how target value was derived.
Cancer characterization	
IARC (2010)	Carcinogenic to humans (Group 1) (based on mechanistic data).
NTP (2011)	Reasonably anticipated to be a human carcinogen. (First classified in 1981.)
Health Canada (1998)	Probably carcinogenic to man.

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
<i>Initial strategy</i>			
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 Benzopyrene[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	5,184

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Database	Set #	Terms	Hits
		<p>Terms] OR animals[MeSH Terms])))) OR (("Benzo a pyrene"[tw] OR "Benzo d, e, f chrysene"[tw] OR "Benzo def chrysene"[tw] OR "3,4-Benzopyrene"[tw] OR "1,2-Benzpyrene"[tw] OR "3,4-BP"[tw] OR "Benz(a)pyrene"[tw] OR "3,4-Benzpyren"[tw] OR "3,4-Benzpyrene"[tw] OR "4,5-Benzpyrene"[tw] OR "6,7-Benzopyrene"[tw] OR Benzopirene[tw] OR "benzo[alpha]pyrene"[tw] OR ("B(a)P"[tw] OR BaP[tw]) AND (pyrene*[tw] OR benzopyrene*[tw] OR pah[tw] OR pahs[tw] OR polycyclic aromatic hydrocarbon[tw] OR polycyclic aromatic hydrocarbons[tw]))AND ("Benzopyrenes/adverse effects"[MeSH Terms] OR "Benzopyrenes/antagonists and inhibitors"[MeSH Terms] OR "Benzopyrenes/blood"[MeSH Terms] OR "Benzopyrenes/pharmacokinetics"[MeSH Terms] OR "Benzopyrenes/poisoning"[MeSH Terms] OR "Benzopyrenes/toxicity"[MeSH Terms] OR "Benzopyrenes/urine"[MeSH Terms] OR ("benzopyrenes"[MeSH Terms] AND ("chemically induced"[Subheading] OR "environmental exposure"[MeSH Terms])) OR "benzopyrenes/metabolism"[Mesh Terms]) AND 1966[PDAT] : 1984[PDAT])) AND (cancer[sb] OR "genes"[MeSH Terms] OR "genetic processes"[MeSH Terms] OR "mutagenicity tests"[MeSH Terms] OR "mutagenesis"[MeSH Terms] OR "mutagens"[MeSH Terms] OR "mutation"[MeSH Terms] OR "neurotoxicity syndromes"[MeSH Terms] OR "nervous system"[MeSH Terms] OR "nervous system diseases"[MeSH Terms] OR "immune system"[MeSH Terms] OR "immune system diseases"[MeSH Terms] OR "immunologic factors"[MeSH Terms] OR "reproductive physiological phenomena"[MeSH Terms] OR ("growth and development"[Subheading] OR "urogenital system"[MeSH Terms] OR "congenital, hereditary, and neonatal diseases and abnormalities"[MeSH Terms] OR "teratogens"[MeSH Terms]))</p>	
<p>ToxLine Date range: 1960's- 2/14/2012 Search date: 2/14/2012</p>	<p>1B</p>	<p>((((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</p>	<p>25,621</p>

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Database	Set #	Terms	Hits
		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 prenataally OR reproduction OR reproductive OR sperm OR spermatid OR spermatids OR spermatocidal OR spermatocyte OR spermatocytes OR spermatogenesis OR spermatogonia OR spermatozoa OR sterile OR sterility OR teratogen OR teratogenesis OR teratogenic OR teratogenicities OR teratogenicity OR teratogens OR weaned OR weaning OR weanling OR weanlings OR zygote OR zygotes) OR (ames OR aneuploid OR aneuploidy OR chromosomal OR chromosome OR chromosomes OR clastogen OR clastogenesis OR clastogenic OR clastogenicities OR clastogenicity OR clastogens OR cytogenesis OR cytogenetic OR cytogenetics OR dna OR dominant lethal OR gene OR genes OR genetic OR genotoxic OR genotoxicities OR genotoxicity OR genotoxin OR genotoxins OR hyperploid OR hyperploidy OR micronuclei OR micronucleus OR mitotic OR mutagen OR mutagenesis OR mutagenicities OR mutagenicity OR mutagens OR mutate OR mutated OR mutating OR mutation OR mutations OR recessive lethal OR sister chromatid) OR (cancer OR cancerous OR cancers OR carcinogen OR carcinogenesis OR carcinogenic OR carcinogenicities OR carcinogenicity OR carcinogens OR carcinoma OR carcinomas OR cocarcinogen OR cocarcinogenesis OR cocarcinogenic OR cocarcinogens OR lymphoma OR lymphomas OR neoplasm OR neoplasms OR neoplastic OR oncogene OR oncogenes OR oncogenic OR precancerous OR tumor OR tumorigenesis OR tumorigenic OR tumorigenicities OR tumorigenicity OR tumors OR tumour OR tumourigenesis OR tumourigenic OR tumourigenicity OR tumours))	
TSCATS, TSCATS2, TSCA recent notices Date range: TSCATS2 2000-2/14/2012; TSCATS, TSCA notices no limit Search date: 2/14/2012	1C	50-32-8	62 TSCATS (health effects) 0 TSCATS2 1 recent notices
Toxcenter Date range: 2000-2/14/2012 Search date:	1D1	((50-32-8 NOT (patent/dt OR tscats/fs)) AND (py>2007 OR ed>20080930) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR	4,344

This document is a draft for review purposes only and does not constitute Agency policy.

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Database	Set #	Terms	Hits
2/14/2012		(toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (biosis/fs OR (caplus/fs AND (rat OR rats OR mouse OR mice OR guinea pig OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem? OR human? OR hominidae OR mammal? OR subject? OR patient? OR genetox? OR mutat? OR mutag?))) OR (((50-32-8 NOT (patent/dt OR tscats/fs)) NOT (py>2007 OR ed>20080930)) AND py>1999 AND (((caplus/fs OR biosis/fs) AND (cancer? OR carcinog? OR carcinom? OR cocarcinog? OR lymphoma? OR neoplas? OR oncogen? OR precancer? OR tumor? OR tumour?)/ti,ct,st,it OR (ames assay OR ames test OR aneuploid? OR chromosom? OR clastogen? OR cytogen? OR dna OR dominant lethal OR genetic OR gene? OR genetox? OR hyperploid? OR micronucle? OR mitotic OR mutagen? OR mutat? OR recessive lethal OR sister chromatid)/ti,ct,st,it OR (brain OR cerebral OR cognition OR cognitive OR encephal? OR nerve? OR nervous OR neural OR neurolog? OR neuron? OR neurop? OR neurotox? OR spinal cord)/ti,ct,st,it OR (antibod? OR antigen? OR autoimmun? OR cytokine? OR granulocyte? OR immun? OR inflamm? OR interferon? OR interleukin? OR leukocyte? OR lymph? OR lymphocyt? OR monocyt?)/ti,ct,st,it OR (abnormal? OR abort? OR cleft? OR development OR developmental OR embryo? OR endocrine OR fertil? OR fetal? OR fetus? OR foetal? OR foetus? OR gestation? OR infertil? OR malform? OR neonat? OR newborn? OR ova OR ovaries OR ovary OR ovum)/ti,ct,st,it OR (perinatal? OR placenta? OR postnatal? OR pregnan? OR prenatal? OR reproduc? OR sperm? OR steril? OR teratogen? OR wean? OR zygote?)/ti,ct,st,it) OR ((chronic OR immunotox? OR neurotox? OR	

Supplemental Information—Benzo[a]pyrene

Database	Set #	Terms	Hits
		toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (cancer? OR carcinog? OR carcinom? OR cocarcinog? OR lymphoma? OR neoplas? OR oncogen? OR precancer? OR tumor? OR tumour?) OR (ames assay OR ames test OR aneuploid? OR chromosom? OR clastogen? OR cytogen? OR dna OR dominant lethal OR genetic OR gene? OR genotox? OR hyperploid? OR micronucle? OR mitotic OR mutagen? OR mutat? OR recessive lethal OR sister chromatid) OR (brain OR cerebral OR cognition OR cognitive OR encephal? OR nerve? OR nervous OR neural OR neurolog? OR neuron? OR neurop? OR neurotox? OR spinal cord) OR (antibod? OR antigen? OR autoimmun? OR cytokine? OR granulocyte? OR immun? OR inflamm? OR interferon? OR interleukin? OR leukocyte? OR lymph? OR lymphocyt? OR monocyt?) OR (abnormal? OR abort? OR cleft? OR development OR developmental OR embryo? OR endocrine OR fertil? OR fetal? OR fetus? OR foetal? OR foetus? OR gestation? OR infertil? OR malform? OR neonat? OR newborn? OR ova OR ovaries OR ovary OR ovum) OR (perinatal? OR placenta? OR postnatal? OR pregnan? OR prenatal? OR reproduc? OR sperm? OR steril? OR teratogen? OR wean? OR zygote?) AND (medline/fs OR biosis/fs OR (caplus/fs AND (rat OR rats OR mouse OR mice OR guinea pig OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR occupation? OR worker? OR epidem? OR human? OR hominidae OR mammal? OR subject? OR patient? OR genotox? OR mutat? OR mutag?))))))	

Supplemental Information—Benzo[a]pyrene

Database	Set #	Terms	Hits
Combined Reference Set	1	(duplicates eliminated through electronic screen)	20,700
<i>Secondary refinement</i>			
Combined Reference Set with Additional Terms Applied	2	forestomach* OR tongue* OR (auditory AND canal*) OR (ear* AND canal*) OR esophagus* OR esophageal* OR larynx* OR laryngeal* OR pharynx* OR pharyngeal* OR ((lung* OR pulmonary OR skin*) AND (neoplasm* OR tumor* OR tumour* OR papilloma* OR carcinoma*)) OR leukemia* OR leukaemia* OR sperm* OR testic* OR fertilit*OR infertilit* OR testosterone OR ((testis OR testes) AND (weight* OR mass*)) OR epididymis* OR epididymal* OR seminiferous OR ((cervical* OR cervix*) AND hyperplasia*) OR ovary OR ovaries OR ovarian OR primordial OR corpora lutea OR corpus luteum OR estrous* OR estrus* OR thymus* OR spleen* OR spleno* OR immunoglobulin* OR immunoglobulin* OR ((immune OR immun*) AND (suppress* OR immunosuppress*)) OR (functional AND observational AND battery) OR neurobehavioral*OR neurobehavioural* OR rotarod* OR nerve* AND conduction* OR locomotor* OR neuromuscular* OR weight* OR neurodevelopment* OR ((neuro* OR brain*) AND (development* OR developing)) OR intelligence* OR cognition* OR cognitive* OR learn* OR memory OR righting*	6,130

1

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

D.1. TOXICOKINETICS

D.1.1. Overview

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

D.1.2. Absorption

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

Studies of workers occupationally exposed to benzo[a]pyrene have qualitatively demonstrated absorption via inhalation by correlating concentrations of benzo[a]pyrene in the air and benzo[a]pyrene metabolites in the exposed workers' urine. Occupational exposures to benzo[a]pyrene measured with personal air samplers were correlated to urine concentrations of benzo[a]pyrene-9,10-dihydrodiol, a specific metabolite of benzo[a]pyrene, in 24-hour aggregate urine samples by [Grimmer et al. \(1994\)](#). The amount of benzo[a]pyrene extracted from personal air monitoring devices (a surrogate for ambient PAHs) of coke oven workers were correlated with

1 r-7,t-8,9,c 10 tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (trans-anti-benzo[a]pyrene-tetrol, a
2 specific metabolite of benzo[a]pyrene) in the workers' urine by [Wu et al. \(2002\)](#). In both of these
3 studies, only a very small fraction (<1%) of the inhaled benzo[a]pyrene was recovered from urine,
4 consistent with studies in animals that find that urine is not a major route of elimination for
5 benzo[a]pyrene (as described in the excretion section below). These occupational studies cannot
6 be used to quantify absorption through inhalation-only exposure in humans because the
7 persistence of benzo[a]pyrene-contaminated particulate matter on surfaces and food may lead to
8 exposures via additional routes ([Boström et al., 2002](#)). Nevertheless, the observation of
9 benzo[a]pyrene metabolites in excreta of exposed humans provides qualitative evidence for
10 benzo[a]pyrene absorption, at least some of which is likely to occur via inhalation. This conclusion
11 is supported by studies in experimental animals, which indicate that benzo[a]pyrene is readily
12 absorbed from carbonaceous particles following inhalation exposure ([Gerde et al., 2001](#); [Hood et
13 al., 2000](#)).

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

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

1 and high-fat diets, as compared with fiber- or protein-rich diets ([O'Neill et al., 1991](#); [Kawamura et](#)
2 [al., 1988](#)). Aqueous vehicles, quercetin, chlorogenic acid, or carbon particles reduced biliary
3 excretion of benzo[a]pyrene, while lipid media such as corn oil increased it ([Stavric and Klassen,](#)
4 [1994](#)). The addition of wheat bran to the benzo[a]pyrene-containing diets increased fecal excretion
5 of benzo[a]pyrene ([Mirvish et al., 1981](#)).

6 Studies of benzo[a]pyrene metabolites or DNA adducts measured in humans exposed
7 dermally to benzo[a]pyrene-containing PAH mixtures demonstrate that benzo[a]pyrene is
8 absorbed dermally. One study of dermal absorption in volunteers found absorption rate constants
9 ranging from 0.036 to 0.135/hour over a 45-minute exposure, suggesting that 20–56% of the dose
10 would be absorbed within 6 hours ([VanRooij et al., 1993](#)). Dermal absorption rates varied 69%
11 between different anatomical sites (forehead, shoulder, volar forearm, palmar side of the hand,
12 groin, and ankle) and only 7% between different individual volunteers ([VanRooij et al., 1993](#)).
13 Metabolism is also an important determinant of permeation, with very low rates observed in
14 nonviable skin ([Kao et al., 1985](#)). The overall absorbed amount of benzo[a]pyrene in explanted
15 viable skin samples from tissue donors (maintained in short-term organ cultures) exposed for
16 24 hours ranged from 0.09 to 2.6% of the dose ([Wester et al., 1990](#); [Kao et al., 1985](#)). Similar
17 amounts of penetration were measured in skin samples from other species including marmosets,
18 rats, and rabbits ([Kao et al., 1985](#)). Skin from mice allowed more of the dose to penetrate (>10%),
19 while that of guinea pig let only a negligible percentage of the dose penetrate ([Kao et al., 1985](#)).

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

29 **D.1.3. Distribution**

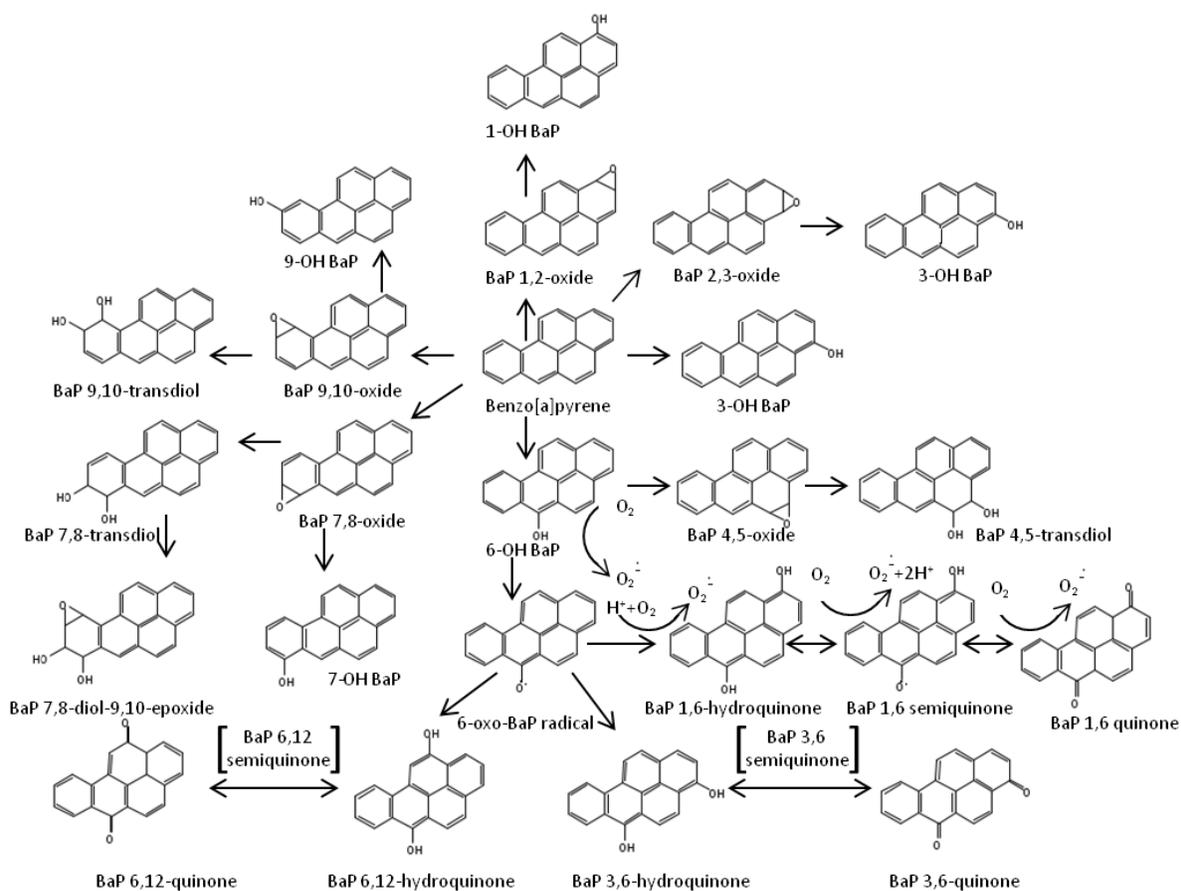
30 No adequate quantitative studies of benzo[a]pyrene tissue distribution in exposed humans
31 were identified. [Obana et al. \(1981\)](#) observed low levels of benzo[a]pyrene in liver and fat tissues
32 from autopsy samples. However, prior exposure histories were not available for the donors.
33 Nevertheless,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

35 Distribution of benzo[a]pyrene has been studied in laboratory animals for multiple routes
36 of exposure, including inhalation, ingestion, dermal, and intravenous (i.v.). Exposure to
37 benzo[a]pyrene in various species (Sprague-Dawley rats, Gunn rats, guinea pigs, and hamsters)

1 results in wide distribution throughout the body and rapid uptake into well-perfused tissues (i.e.,
2 lung, kidney, and liver) ([Weyand and Bevan, 1987, 1986](#)). Benzo[a]pyrene and its metabolites are
3 distributed systemically after administration via many routes of administration including
4 inhalation (or intratracheal instillation), oral, i.v., and dermal exposures ([Saunders et al., 2002](#); [Moir
5 et al., 1998](#); [Neubert and Tapken, 1988](#); [Weyand and Bevan, 1987, 1986](#); [Morse and Carlson, 1985](#)).
6 Intratracheal instillation of radiolabeled benzo[a]pyrene in mice resulted in increased radioactivity
7 in lung-associated lymph nodes, suggesting distribution of benzo[a]pyrene or its metabolites via
8 the lymph ([Schnizlein et al., 1987](#)). Rats with biliary cannulas had high excretion of benzo[a]pyrene
9 and benzo[a]pyrene metabolites in bile. The benzo[a]pyrene thioether and glucuronic acid-
10 conjugated metabolites in intestines indicated enterohepatic recirculation of benzo[a]pyrene and
11 benzo[a]pyrene metabolites ([Weyand and Bevan, 1986](#)). The vehicle for delivery of inhaled
12 benzo[a]pyrene impacts the distribution, with aerosolized benzo[a]pyrene more readily absorbed
13 directly in the respiratory tract than particle-adsorbed benzo[a]pyrene (which is cleared by the
14 mucociliary and then ingested) ([Sun et al., 1982](#)). Exposure of pregnant rats and mice to
15 benzo[a]pyrene via inhalation and ingestion showed a wide tissue distribution of benzo[a]pyrene,
16 consistent with other studies, and demonstrated placental transfer of benzo[a]pyrene and its
17 metabolites ([Withey et al., 1993](#); [Neubert and Tapken, 1988](#); [Shendrikova and Aleksandrov, 1974](#)).
18 The reactive metabolites of benzo[a]pyrene are also transported in the blood and may be
19 distributed to tissues incapable of benzo[a]pyrene metabolism. Serum of benzo[a]pyrene-treated
20 mice incubated with splenocytes or salmon sperm DNA resulted in adduct formation, suggesting
21 that reactive benzo[a]pyrene metabolites were systemically distributed and available for
22 interaction with target tissues ([Ginsberg and Atherholt, 1989](#)).

23 **D.1.4. Metabolism**

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



Source: [Miller and Ramos \(2001\)](#).

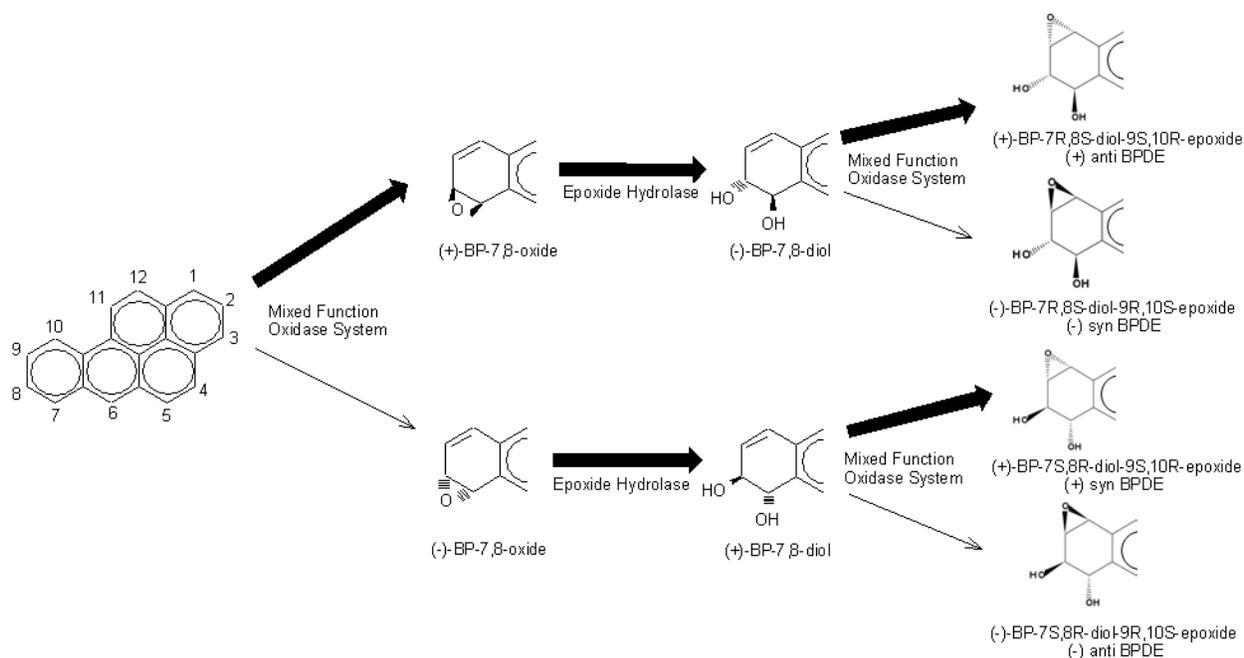
Figure D-1. Metabolic pathways for benzo[a]pyrene.

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

1 **Phase I Metabolism**

2 Phase I reactions of benzo[a]pyrene are catalyzed primarily by cytochrome P450 (CYP450)
3 and produce metabolites including epoxides, dihydrodiols, phenols, and quinones (Figure D-2). The
4 first step of Phase I metabolism is the oxidation of benzo[a]pyrene that forms a series of epoxides,
5 the four major forms of which are the 2,3-, 4,5-, 7,8-, and 9,10-isomers ([Gelboin, 1980](#)). Once
6 formed, these epoxides may undergo three different routes of metabolism: (1) spontaneous
7 rearrangement to phenols; (2) hydration to trans-dihydrodiols catalyzed by microsomal epoxide
8 hydrolase (EH); or (3) the Phase II detoxification of binding with glutathione (either spontaneously
9 or catalyzed by cytosolic glutathione-S-transferases (GSTs) ([IARC, 1983](#))). The metabolism of
10 benzo[a]pyrene to phenols results in five phenol isomers (1-, 3-, 6-, 7, and 9-OH benzo[a]pyrene)
11 ([Pelkonen and Nebert, 1982](#)). Four benzo[a]pyrene epoxides (2,3-, 4,5-, 7,8-, and 9,10-) are
12 hydrated to trans-dihydrodiols. Benzo[a]pyrene-7,8-diol (formed from benzo[a]pyrene-7,8-oxide)
13 has been the focus of much of the study of benzo[a]pyrene metabolism. Benzo[a]pyrene-7,8-diol is
14 the metabolic precursor to the potent DNA-binding metabolite, benzo[a]pyrene-7,8-diol-
15 9,10-epoxide (BPDE). BPDE is formed from trans-benzo[a]pyrene 7,8-diol by multiple mechanisms
16 including catalysis by cytochromes (CYPs) ([Grover, 1986](#); [Deutsch et al., 1979](#)), myeloperoxidase
17 ([Mallet et al., 1991](#)), or prostaglandin h synthase (also known as cyclooxygenase) ([Marnett, 1990](#)),
18 and lipid peroxidation ([Byczkowski and Kulkarni, 1990](#)). The diolepoxides can react further by
19 spontaneously hydrolyzing to tetrols ([Hall and Grover, 1988](#)).

20 The metabolism of benzo[a]pyrene proceeds with a high degree of stereoselectivity. Liver
21 microsomes from rats stereospecifically oxidize the 7,8-bond of benzo[a]pyrene to yield almost
22 exclusively the (+)-benzo[a]pyrene-(7,8)-oxide (see Figure D-2). Each enantiomer of
23 benzo[a]pyrene-7,8-oxide is stereospecifically converted by EH to a different stereoisomeric trans
24 dihydrodiol. The (+)-benzo[a]pyrene-7,8-oxide is preferentially hydrated to the (-)-trans-
25 benzo[a]pyrene-7,8-dihydrodiol enantiomer by rat CYP enzymes and the (-)-trans-
26 benzo[a]pyrene-7,8-dihydrodiol is preferentially oxidized by CYP enzymes to (+)-benzo[a]pyrene-
27 7R,8S-diol-9S,10R-epoxide [(+)-anti-BPDE], which is the most potent carcinogen among the four
28 stereoisomers (Figure D-2). Formation of these stereoisomers does not occur at equimolar ratios,
29 and the ratios differ between biological systems. For example, a study in rabbit livers
30 demonstrated that purified microsomes oxidized the (-)-benzo[a]pyrene-7,8-dihydrodiol to
31 isomeric diol epoxides in a ratio ranging from 1.8:1 to 11:1 in favor of the (+)-anti-BPDE isomer
32 ([Deutsch et al., 1979](#)).



1

2 Source: [Grover \(1986\)](#).

3

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

5 Several studies have attempted to determine which CYP isozyme is predominantly
 6 responsible for the metabolism of benzo[a]pyrene. Dermal administration of tritiated
 7 benzo[a]pyrene to mice that have an aryl hydrocarbon (Ah) receptor (AhR) knock-out (AhR^{-/-})
 8 had significantly decreased formation of (+)-anti-BPDE-DNA adducts compared to wild type (WT)
 9 and 1B1^{-/-} mice ([Kleiner et al., 2004](#)). Gavage administration of benzo[a]pyrene in AhR knock-out
 10 mice found that the AhR^{-/-} mice (with lower levels of CYP1A1) had higher levels of protein
 11 adducts and unmetabolized benzo[a]pyrene than the AhR^{+/+} or ^{+/-} mice ([Sagredo et al., 2006](#)).
 12 Similarly, CYP1A1 (-/-) knock-out mice administered benzo[a]pyrene in feed for 18 days had
 13 higher steady-state blood levels of benzo[a]pyrene and benzo[a]pyrene-DNA adducts ([Uno et al.,](#)
 14 [2006](#)). These findings establish important roles in benzo[a]pyrene metabolism for CYP1A1, but the
 15 relationship is not clear between the CYP enzymes and biological activation or detoxification.

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

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

3 Another pathway of benzo[a]pyrene metabolism is the conversion of benzo[a]pyrene to
4 6-OH benzo[a]pyrene, which can be further oxidized into quinones, primarily the 1,6-, 3,6-, and
5 6,12- isomers. Trans-benzo[a]pyrene-7,8-dihydrodiol can be converted by aldo-keto reductases
6 (AKR) to 7,8-dihydroxybenzo[a]pyrene (benzo[a]pyrene-7,8-catechol), which auto-oxidizes to
7 benzo[a]pyrene-7,8-quinone (BPQ). BPQ can undergo redox cycling in the presence of cellular
8 reducing equivalents. This reaction pathway produces reactive oxygen species (ROS), including
9 peroxide anion radicals, benzo[a]pyrene semiquinone radicals, hydroxyl radicals, and H₂O₂, which
10 in turn can cause extensive DNA fragmentation ([Penning et al., 1999](#); [Flowers et al., 1997](#); [Flowers
11 et al., 1996](#)). 6-Hydroxybenzo[a]pyrene can be oxidized into 6-oxo-benzo[a]pyrene semi-quinone
12 radical and further metabolized into 1,6-, 3,6-, or 6,12-quinones spontaneously, or catalytically by
13 prostaglandin endoperoxide synthetase ([Eling et al., 1986](#)). The CYP and AKR enzymes both can
14 metabolize trans-benzo[a]pyrene-7,8-dihydrodiol to different metabolites, BPDE and BPQ.
15 Reconstituted in vitro systems of human lung cells show that CYP enzymes have faster steady-state
16 reaction rate constants than AKR and basal expression of AKR is higher than CYP in lung cells,
17 suggesting that AKR and CYP enzymes compete for metabolism of trans-benzo[a]pyrene-
18 7,8-dihydrodiol ([Quinn and Penning, 2008](#)).

19 ***Phase II Metabolism***

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

26 The diol epoxides formed from benzo[a]pyrene metabolism by Phase I reactions are not
27 usually found as urinary metabolites. Rather, they are detected as adducts of nucleic acids or
28 proteins or further metabolized by glutathione (GSH) conjugation, glucuronidation, and sulfation.
29 These metabolites make up a significant portion of total metabolites in excreta or tissues. For
30 example, the identified metabolites in bile 6 hours after a 2 µg/kg benzo[a]pyrene dose by
31 intratracheal instillation to male Sprague-Dawley rats were 49% glucuronides (quinol
32 diglucuronides or monglucuronides), 30.4% thioether conjugates, 6.2% sulfate conjugates, and
33 14.4% unconjugated metabolites ([Bevan and Sadler, 1992](#)).

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

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

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

16 Sulfotransferases can catalyze the formation of sulfates of benzo[a]pyrene metabolites. In
17 rat or mouse liver, cytosolic sulfotransferase (in the presence of 3'-phosphoadenosine 5'-phospho-
18 sulfate) catalyzes formation of sulfates of three benzo[a]pyrene metabolites: benzo[a]pyrene-
19 7,8,9,10-tetrahydro-7-ol, benzo[a]pyrene-7,8-dihydrodiol, and benzo[a]pyrene-7,8,9,10-tetrol. The
20 benzo[a]pyrene-7,8,9,10-tetrahydro-7-ol-sulfate is able to form potentially damaging DNA adducts
21 ([Surh and Tannenbaum, 1995](#)). In human lung tissue 3-hydroxybenzo[a]pyrene conjugation to
22 sulfate produces benzo[a]pyrene-3-yl-hydrogen sulfate, a very lipid soluble compound that would
23 not be readily excreted in the urine ([Cohen et al., 1976](#)).

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

35 ***Tissue-Specific Metabolism***

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

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

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

25 Benzo[a]pyrene undergoes extensive metabolism in the GI tract and liver after oral
26 administration. In rats after administration of an oral dose, the majority of benzo[a]pyrene
27 detected in organs is as metabolites ([Ramesh et al., 2004](#); [Ramesh et al., 2001b](#); [Yamazaki and](#)
28 [Kakiuchi, 1989](#)). In rats administered a 100-nmol dose, >90% was recovered in portal blood as
29 metabolites ([Bock et al., 1979](#)). Orally administered benzo[a]pyrene produced strong induction of
30 CYP1A1 in the intestine of mice ([Brooks et al., 1999](#)). DNA post-labeling studies of mice
31 administered benzo[a]pyrene by gavage demonstrated higher benzo[a]pyrene-DNA adduct levels in
32 CYP1A1(-/-) than CYP1A1(+/-) mice in small intestines ([Uno et al., 2004](#)). To compare the
33 relative roles of the liver and intestine in benzo[a]pyrene metabolism and absorption, a
34 multicompartiment perfusion system was developed; it was found that benzo[a]pyrene is
35 extensively metabolized by the intestinal Caco-2 cells and that benzo[a]pyrene and its metabolites
36 are transported to the apical side of the Caco-2 cells away from the liver HepG2 cells ([Choi et al.,](#)
37 [2004](#)).

1 Dermal exposure in humans and animals resulted in benzo[a]pyrene metabolism, and the
2 permeation of benzo[a]pyrene in skin is linked to benzo[a]pyrene metabolism. Human skin
3 samples maintained in short-term organ culture (i.e., human epithelial tissue, samples from human
4 hair follicles, and melanocytes isolated from adult human skin) can metabolize benzo[a]pyrene into
5 dihydrodiols, phenols, quinones, and glucuronide and sulfate conjugates ([Agarwal et al., 1991](#);
6 [Alexandrov et al., 1990](#); [Hall and Grover, 1988](#); [Merk et al., 1987](#)). Nonviable skin is unable to
7 metabolize benzo[a]pyrene (the permeation into nonviable skin is lower than viable skin) as
8 measured in a range of species including humans, rats, mice, rabbits, and marmosets ([Kao et al.,](#)
9 [1985](#)). Viable human skin samples treated with 2 µg/cm² [¹⁴C]-benzo[a]pyrene in acetone and
10 incubated for 24 hours produced the following percentages of benzo[a]pyrene metabolites: 52%
11 water-soluble compounds, 8% polar compounds, 17% diols, 1% phenols, 2.5% quinones, and 18%
12 unmetabolized benzo[a]pyrene ([Kao et al., 1985](#)).

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

17 ***Age-Specific Metabolism***

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

35 **D.1.5. Elimination**

36 Benzo[a]pyrene metabolites have been detected in the urine of exposed humans, but fecal
37 excretion has not been investigated in any detail. Studies of benzo[a]pyrene elimination in animals

1 following exposure via inhalation, ingestion, and dermal routes have shown that benzo[a]pyrene is
2 excreted preferentially in the feces in multiple species of laboratory animals including rat, mice,
3 hamsters, guinea pigs, monkeys, and dogs ([Wang et al., 2003](#); [Likhachev et al., 1992](#); [Wolff et al.,
4 1989](#); [Yang et al., 1989](#); [Petridou-Fischer et al., 1988](#); [Weyand and Bevan, 1987](#); [Sun et al., 1982](#);
5 [Hecht et al., 1979](#)). The metabolites in bile are primarily benzo[a]pyrene conjugates,
6 predominantly thioether conjugates of varying extent in different species ([Weyand and Bevan,
7 1987](#)). Six hours after a single intratracheal instillation of benzo[a]pyrene (2 µg/kg) to male
8 Sprague-Dawley rats, relative metabolite levels were 31.2% diglucuronides, 30.4% thioether
9 conjugates, 17.8% monoglucuronides, 6.2% sulfate conjugates, and 14.4% unconjugated
10 metabolites ([Bevan and Sadler, 1992](#)). Rats administered benzo[a]pyrene via i.v. excrete a larger
11 fraction in urine than via inhalation or oral exposure, suggesting an important role for
12 enterohepatic circulation of benzo[a]pyrene metabolite conjugates ([Moir et al., 1998](#); [Weyand and
13 Bevan, 1986](#); [Hirom et al., 1983](#)). The vehicle impacts the amount of benzo[a]pyrene excreted and
14 may, in part, be due to the elimination rate or to other factors such as the absorption rate. For
15 tritiated benzo[a]pyrene administered to Sprague-Dawley rats in hydrophilic triethylene glycol,
16 70.5% of the dose was excreted into bile within 6 hours. When the lipophilic solvents, ethyl laurate
17 and tricapylin, were used as vehicles, 58.4 and 56.2% of the dose was excreted, respectively
18 ([Bevan and Ulman, 1991](#)). In addition to benzo[a]pyrene and its metabolites, adducts of
19 benzo[a]pyrene with nucleotides have also been identified as a small fraction of the administered
20 dose in feces and urine of animals. The level of BPDE adducts with guanine detected in urine of
21 male Wistar rats was dose-dependent. Forty-eight hours after dosing with 100 µg/kg tritiated
22 benzo[a]pyrene, 0.15% of the administered benzo[a]pyrene dose was excreted in the urine as an
23 adduct with guanine ([Autrup and Seremet, 1986](#)). Overall, the data in humans and laboratory
24 animals are sufficient to describe benzo[a]pyrene elimination qualitatively, but are limited in
25 estimating quantitative rates of elimination.

26 **D.2. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS**

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

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

1 [Roth and Vinegar \(1990\)](#) reviewed the capacity of the lung to impact the disposition of
2 chemicals and used benzo[a]pyrene as a case study. A PBPK model was presented based on data
3 from ([Wiersma and Roth \(1983a\)](#), [1983b](#)) and was evaluated against tissue concentration data
4 from [Schlede et al. \(1970\)](#). The model was structured with compartments for arterial blood, venous
5 blood, lung, liver, fat, and slowly and rapidly perfused tissues and an adequate fit was obtained for
6 some compartments; however, tissue-level data for calibration and validation of this model were
7 limited. Metabolism in liver and lung was estimated using kinetic data from control rats and rats
8 pretreated with 3-methylcholanthrene to induce benzo[a]pyrene metabolism. In microsomal
9 preparations from control and 3-methylcholanthrene induced rat livers and lungs, benzo[a]pyrene
10 hydroxylase activity was 1,000-fold greater in liver. In isolated rat lungs, the clearance of
11 benzo[a]pyrene was about one-sixth of the clearance in isolated rat livers and in
12 3-methylcholanthrene-pretreated rats the clearance in lungs and livers were of similar magnitude.
13 The PBPK simulations model based on these data showed that for a bolus intravascular injection of
14 benzo[a]pyrene in rats, the majority of benzo[a]pyrene metabolism usually occurs in the liver.
15 Except for cases when rats are pretreated with enzyme-inducing agents or where the exposure
16 occurs via inhalation, the metabolic clearance in the lung is minor.

17 [Moir et al. \(1998\)](#) conducted a pharmacokinetic study on benzo[a]pyrene to obtain data for
18 model development. Rats were injected with varying doses of [¹⁴C]-benzo[a]pyrene to 15 mg/kg,
19 and blood, liver, fat, and richly perfused tissue were sampled varying time points after dosing. [Moir](#)
20 [\(1999\)](#) then described a model for lung, liver, fat, richly and slowly perfused tissues, and venous
21 blood, with saturable metabolism occurring in the liver. The fat and richly perfused tissues were
22 modeled as diffusion-limited, while the other tissues were flow-limited. The model predicted the
23 blood benzo[a]pyrene concentrations well, although it overestimated the 6 mg/kg results at longer
24 times (>100 minutes). The model also produced a poor fit to the liver data. The model simulations
25 were also compared to data of [Schlede et al. \(1970\)](#), who injected rats with 0.056 mg/kg body
26 weight of benzo[a]pyrene. The model predicted blood and fat benzo[a]pyrene concentrations well,
27 but still poorly predicted liver benzo[a]pyrene concentrations. The model included only one
28 saturable metabolic pathway, and only parent chemical concentrations were used to establish the
29 model. No metabolites were included in the model. This model was re-calibrated by [Crowell et al.](#)
30 [\(2011\)](#) by optimizing against additional rodent data and altering partition coefficient derivation.
31 However, it still did not incorporate metabolites, and some tissues continued to exhibit poor model
32 fits.

33 An attempt to scale the [Moir et al. \(1998\)](#) rodent PBPK model to humans, relevant to risk
34 assessment of oral exposures to benzo[a]pyrene, was presented by [Zeilmaker et al. \(1999a\)](#) and
35 [Zeilmaker et al. \(1999b\)](#). The PBPK model for benzo[a]pyrene was derived from an earlier model
36 for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats ([Zeilmaker and van Eijkeren, 1997](#)). Most
37 compartments were perfusion-limited, and tissues modeled included blood, adipose (with diffusion
38 limitation), slowly and richly perfused tissues, and liver. However, there was no separate

1 compartment for the lung. The liver compartment featured the AhR-dependent CYP450 induction
2 mechanism and DNA adduct formation as a marker for formation of genotoxic benzo[a]pyrene
3 metabolites. It was assumed that DNA adduct formation and the bulk benzo[a]pyrene metabolism
4 were mediated by two different metabolic pathways. The model was experimentally calibrated in
5 rats with the data for 7-ethoxyresorufin-O-deethylase (EROD) and formation of DNA adducts in the
6 liver after i.v. administration of a single dose and per os administration of a single or repeated doses
7 of benzo[a]pyrene ([Zeilmaker et al., 1999a](#)).

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

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

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

35 PBPK models for benzo[a]pyrene were evaluated to determine the capability to extrapolate
36 from rats to humans, or between oral and inhalation exposure routes. Due to significant
37 uncertainties with respect to the inter-species scaling of the metabolic parameters between rats

1 and humans, these models were not used for cross-species extrapolation. Furthermore, no
2 complete mechanistic PBPK model for the inhalation route was identified, nor was there a model
3 for humans that simulates the typical inhalation exposure to benzo[a]pyrene on poorly soluble
4 carbonaceous particles. This precluded the model's use for cross-route extrapolation to the
5 inhalation pathway.

6 **D.3. HUMAN STUDIES**

7 **D.3.1. Noncancer Endpoints**

8 *Cardiovascular Endpoints*

9 [Burstyn et al. \(2005\)](#) reported the association of death from cardiovascular disease with
10 benzo[a]pyrene exposure in a cohort of 12,367 male European asphalt workers (Table D-1). These
11 workers were first employed in asphalt paving between 1913 and 1999, and worked at least one
12 season. Average duration of follow-up was 17 ± 9 years (mean \pm standard deviation [SD]),
13 encompassing 193,889 person-years of observation. Worker exposure to coal tar was estimated
14 using industrial process and hygiene information and modeling (presented in a previous report),
15 and coal tar exposure was found to be the strongest determinant of exposure to benzo[a]pyrene.
16 Benzo[a]pyrene exposure was assessed quantitatively using measurement-driven mixed effects
17 exposure models, using data collected from other asphalt industry workers, and this model was
18 constructed and validated previously. Due to limited data availability, only information regarding
19 the primary cause of death was collected, and this analysis was limited to diseases of the circulatory
20 system (ICD codes 390–459), specifically ischemic heart disease (IHD: ICD codes 410–414). Diesel
21 exhaust exposure was also assessed in this cohort, but varied little among the asphalt pavers, and
22 was not associated with risk of death from cardiovascular disease. Of the initial cohort, 0.25% was
23 lost to follow-up and 0.38% emigrated during the course of observation. Relative risks (RRs) and
24 associated 95% confidence intervals (CIs) were estimated using Poisson regression, and all models
25 included exposure index for agent of interest (coal tar or benzo[a]pyrene), age, calendar period of
26 exit from cohort, total duration of employment, and country, using the category of lowest exposure
27 as the reference. Confounding by tobacco smoke exposure was considered in relation to the
28 strength of its association with cardiovascular disease and the smoking prevalence in the
29 population. The RR attributed to cigarette smoking in former and current smokers was assumed to
30 be 1.2 and 2, respectively, based upon literature reports. From analysis of smoking incidence in a
31 subcohort, the following smoking distribution was proposed: in the lowest exposure group, 40%
32 never-smokers, 30% former smokers, and 30% current smokers; and among the highest exposed,
33 the proportion shifted to 20/30/50%, respectively.

34 Exposed subjects were stratified into quintiles based upon IHD mortality, with
35 83–86 deaths per exposure category, composing approximately 2/3 of the 660 cardiovascular
36 disease-related deaths. Both cumulative and average exposure indices for benzo[a]pyrene were

1 positively associated with IHD mortality, with a RR of approximately 1.6 in the highest exposure
 2 quintile from both metrics, independent of total employment duration. Similar monotonic trends
 3 were observed for all cardiovascular diseases (combined), although a dose-response relationship
 4 was evident only for IHD and not hypertension or other individual heart disease categories. Similar
 5 trends were also observed for coal tar exposure and IHD. Adjusting the RR to account for possible
 6 confounding by smoking yields a RR of 1.39 under the assumptions mentioned above, and is still
 7 elevated (1.21) if the contribution of smoking to cardiovascular disease etiology was greater than
 8 the original assumptions. Furthermore, the RR for the high versus low exposure quintile is
 9 1.24 even if the distribution of nonsmokers/former smokers/current smokers shifts to 0/30/70%,
 10 using the original assumptions of cigarette smoke casual potency.

11 **Table D-1. Exposure to benzo[a]pyrene and mortality from cardiovascular**
 12 **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	
<i>Diseases of the circulatory system</i>						
Deaths	137	145	118	132	128	0.09
RR	1.00	1.08	1.06	1.24	1.42	
95% CI		0.85–1.38	0.80–1.42	0.89–1.71	0.96–2.09	
<i>IHD</i>						
Deaths	83	83	84	83	85	0.06
RR	1.00	0.99	1.22	1.24	1.58	
95% CI		0.72–1.36	0.86–1.74	0.82–1.85	0.98–2.55	
Effect measured	Average exposure (ng/m ³)					p-value for trend
	0–68 ^a	68–105	106–146	147–272	≥273	
<i>Diseases of the circulatory system</i>						
Deaths	128	142	143	139	108	<0.001
RR	1.00	1.30	1.55	1.45	1.58	
95% CI		1.01–1.67	1.18–2.05	1.09–1.93	1.16–2.15	
<i>IHD</i>						
Deaths	83	83	83	86	83	0.02
RR	1.00	1.13	1.33	1.20	1.64	
95% CI		0.82–1.55	0.94–1.90	0.84–1.71	1.13–2.38	

13
 14 ^aReference category.

15
 16 Source: [Burstyn et al. \(2005\)](#).

17
 18 [Friesen et al. \(2010\)](#) examined the association between benzo[a]pyrene exposure and
 19 deaths from chronic nonmalignant disease in a cohort of 6,423 male and 603 female Canadian
 20 aluminum smelter workers (Table D-2). Inclusion criteria required at least 3 years of continuous

1 employment in either the smelter facility or power-generating station from 1954 to 1997, with
2 worker history collected up through 1999. This cohort was probabilistically linked to the Canadian
3 national mortality database for external comparison to the British Columbia population and
4 calculation of standardized mortality ratios (SMRs), which were adjusted for age, sex, and time
5 period. Ninety-five percent CIs were calculated for the SMRs assuming a Poisson distribution.
6 Internal comparisons were also made during the analysis of IHD mortality in male workers,
7 calculating hazard ratios (HRs) for IHD with or without acute myocardial infarction (AMI) after
8 1969, as AMI could not be differentiated from other IHD on death certificates issued previously.
9 HRs were calculated using Cox regression models, with age as a metamarker of time, also including
10 smoking status, time since first employed and work location status. Smoking information for 77%
11 of this updated cohort was collected by questionnaire, and workers were categorized as 75% ever-
12 smokers and 25% never-smokers. Quantitative exposure to coal tar pitch volatiles were estimated
13 by benzo[a]pyrene measurements, calculated by a job classification and time-based exposure
14 matrix, as described in a previous report; annual arithmetic mean values were calculated for
15 exposures from 1977 to 2000, while pre-1977 levels were backwards-extrapolated from 1977
16 values, incorporating major technological changes in time periods as appropriate.

17 Cumulative exposure metrics were highly skewed. Cumulative benzo[a]pyrene with a
18 5-year lag (past benzo[a]pyrene exposure) and cumulative benzo[a]pyrene in the most recent
19 5 years (recent benzo[a]pyrene exposure) were only slightly positively correlated ($r = 0.10$,
20 $p < 0.001$). Current benzo[a]pyrene exposure was highly correlated with cumulative exposure for
21 the most recent 5 years of exposure ($r = 0.86$, $p < 0.001$), but not with 5-year lagged cumulative
22 exposure ($r = 0.03$, $p < 0.001$). Lagged cumulative exposure metrics (0–10 years) were all highly
23 correlated with each other ($r = 0.96$, all p -values < 0.001); lagged metrics for cumulative exposure
24 were used to distinguish between effects of current versus long-term exposure.

25 When exposed workers were pooled and compared externally to non-exposed referents, the
26 IHD and AMI SMRs were all ≤ 1.00 for males, and the only significant association in females was an
27 SMR of 1.27 for AMI. For internal comparisons, exposed males were stratified into quintiles based
28 upon IHD mortality, with approximately 56 deaths per exposure category. Five-year lagged
29 cumulative benzo[a]pyrene exposure was significantly associated with elevated risk of IHD
30 mortality, HR = 1.62 (95% CI 1.06–2.46) in the highest exposure quintile, while no association was
31 observed between most recent (5 years) exposure and mortality. Restricting IHD events to only
32 AMI (1969 onward) resulted in similar monotonic trends, albeit of lower statistical significance. No
33 association was observed between benzo[a]pyrene exposure and non-AMI IHD. While there was
34 little difference in the exposure-response association among 0-, 2-, and 5-year lagged data, 10-year
35 lagged data resulted in a weaker association. All risk estimates were strengthened by the
36 incorporation of work status and time-since-hire to account for the healthy worker effect, as
37 evidenced by the SMR of 0.87 (95% CI 0.82–0.92) for all chronic nonmalignant diseases combined
38 in male exposed workers versus external referents. Using a continuous variable, the authors

1 calculated the risk of death from IHD as 1.002 (95% CI 1.000–1.005) per $\mu\text{g}/\text{m}^3$ from cumulative
 2 benzo[a]pyrene exposure; however, visual inspection of the categorical relationships indicated that
 3 the association is nonlinear, suggesting that this value may be an underestimate. Restricting the
 4 cohort to only members who died within 30 days of active employment at the worksite, cumulative
 5 benzo[a]pyrene exposure was not significantly associated with IHD or AMI, although the HR for the
 6 highest exposure group was 2.39 (95% CI 0.95–6.05). Exposure-response relationships were
 7 similarly examined in male smelter workers for chronic obstructive pulmonary disease and
 8 cerebrovascular disease, but neither was significantly associated with cumulative benzo[a]pyrene
 9 exposure in either internal or external comparisons.

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

Effect measured	Categorical cumulative exposure with a 5-yr lag ($\mu\text{g}/\text{m}^3\text{-yr}$)					<i>p</i> -value for trend ^a	Continuous ^b
	0	0–7.79	7.79–24.3	24.3–66.7	≥66.7		
<i>All IHD (1957 onward)</i>							
Deaths	56	56	57	56	56	0.053	281
Person-years of follow-up	33,111	37,581	34,838	31,533	13,688		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
<i>AMI (1969 onward)</i>							
	0	0–7.51	7.51–27.7	27.7–67.4	≥67.4		
Deaths	35	37	37	38	37	0.19	184
Person-years of follow-up	25,071	30,454	34,621	24,081	13,261		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

12
 13 ^aTwo-sided test for trend using the person-year-weighted mean value for each category as a linear, continuous
 14 variable.

15 ^bExposure variable was entered as a continuous, linear variable in the model.

16
 17 Source: [Friesen et al. \(2010\)](#).

18 **Reproductive and Developmental Endpoints**

19 [Wu et al. \(2010\)](#) conducted a study of benzo[a]pyrene-DNA adduct levels in relation to risk
 20 of fetal death in Tianjin, China. This case-control study included women who experienced a delayed
 21 miscarriage before 14 weeks gestational age (i.e., a fetal death that remained in utero and therefore
 22 required surgical intervention). Cases were matched by age and gravidity to controls (women
 23 undergoing induced abortion due to an unplanned or unwanted pregnancy). The study excluded
 24 women who smoked, women with chronic disease and pregnancy complications, and women with
 25 occupational exposures to PAHs. Residency within Tianjin for at least 1 year was also an eligibility

1 criterion. The participation rate was high: 81/84 eligible cases participated and 81/89 eligible
2 controls participated. Data pertaining to demographic characteristics, reproductive history, and
3 factors relating to potential PAH exposure were collected using a structured interview, and samples
4 from the aborted tissue were obtained. In two of the four hospitals used in the study, blood
5 samples from the women (n = 51 cases and 51 controls) were also collected. The presence of
6 benzo[a]pyrene-BPDE adducts was assessed in the blood and tissue samples using high-
7 performance liquid chromatography (HPLC). There was no correlation between blood and aborted
8 tissue levels of benzo[a]pyrene adducts ($r = -0.12$ for the 102 blood-tissue pairs, $r = -0.02$ for the
9 51 case pairs, and $r = -0.21$ for the 51 control pairs). (The authors noted that there was little
10 difference between women with and without blood samples in terms of the interview-based
11 measures collected or in terms of the DNA-adduct levels in aborted tissue.) Benzo[a]pyrene-adduct
12 levels were similar but slightly lower in the aborted tissue of cases compared with controls
13 (mean \pm SD 4.8 ± 6.0 in cases and 6.0 ± 7.4 in controls, $p = 0.29$). In the blood samples, however,
14 benzo[a]pyrene-adduct levels were higher in cases (6.0 ± 4.7 and 2.7 ± 2.2 in cases and controls,
15 respectively, $p < 0.001$). In logistic regression analyses using a continuous adduct measure, the
16 odds ratio (OR) was 1.35 (95% CI 1.11–1.64) per adduct/ 10^8 nucleotide. These results were
17 adjusted for education, household income, and gestational age, but were very similar to the
18 unadjusted results. Categorizing exposure at the median value resulted in an adjusted OR of
19 4.27 (95% CI 1.41–12.99) in the high compared with low benzo[a]pyrene-adduct group. There was
20 no relation between benzo[a]pyrene-adduct levels in the aborted tissue and miscarriage in the
21 logistic regression analyses using either the continuous (adjusted OR 0.97, 95% CI 0.93–1.02) or
22 dichotomous exposure measure (adjusted OR 0.76, 95% CI 0.37–1.54). Associations between
23 miscarriage and several interview-based measures of potential PAH exposure were also seen:
24 adjusted ORs of 3.07 (95% CI 1.31–7.16) for traffic congestion near residence, 3.52 (95% CI
25 1.44–8.57) for commuting by walking, 3.78 (95% CI 1.11–12.87) for routinely cooked during
26 pregnancy, and 3.21 (95% CI 0.98–10.48) for industrial site or stack near residence, but there was
27 no association with other types of commuting (e.g., by bike, car, or bus).

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

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

11 [Tang et al. \(2006\)](#) measured BPDE-DNA adducts in maternal and umbilical cord blood
12 obtained at delivery from a cohort of 150 nonsmoking women and their newborns in China.
13 Exposure assessment was related to the seasonal operation of a local, coal-fired power plant;
14 however, airborne PAH concentrations were not measured. Dietary PAH intake was not included as
15 a covariate because it did not significantly contribute to the final models, but ETS, sex, and maternal
16 height and weight were considered as covariates. DNA adduct levels were compared to several
17 birth outcomes and physical development parameters, such as gestational age at birth; infant sex,
18 birth weight, length, head circumference, and malformations; maternal height and pregnancy
19 weight total weight gain; complications of pregnancy and delivery; and medications used during
20 pregnancy.

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

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

37 [Neal et al. \(2008\)](#) examined levels of benzo[a]pyrene and other PAHs in follicular fluid and
38 serum sample from 36 women undergoing in vitro fertilization at a clinic in Toronto, and compared

1 the successful conception rate in relation to benzo[a]pyrene levels. The women were classified by
2 smoking status, with 19 current cigarette smokers, 7 with passive or sidestream smoke exposure
3 (i.e., nonsmoker with a partner who smoked), and 10 nonsmokers exposed. An early follicular
4 phase blood sample and follicular fluid sample from the follicle at the time of ovum retrieval were
5 collected and analyzed for the presence of benzo[a]pyrene, acenaphthelene, phenanthrene, pyrene,
6 and chrysene using gas chromatography/mass spectrometry (MS) (detection limit 5 pg/mL). The
7 frequency of nondetectable levels of serum benzo[a]pyrene was highest in the nonsmoking group
8 (60.0, 14.3, and 21.0% below the detection limit in nonsmoking, sidestream smoke, and active
9 smoking groups, respectively). A similar pattern was seen with follicular fluid benzo[a]pyrene
10 (30.0, 14.3, and 10.5% below the detection limit in nonsmoking, sidestream smoke, and active
11 smoking groups, respectively). In the analyses comparing mean values across groups, an assigned
12 value of 0 was used for nondetectable samples. Follicular fluid benzo[a]pyrene levels were higher
13 in the active smoking group (mean \pm standard error [SE], 1.32 ± 0.68 ng/mL) than in the sidestream
14 (0.05 ± 0.01 ng/mL) or nonsmoking (0.03 ± 0.01 ng/mL) groups ($p = 0.04$). The between-group
15 differences in serum benzo[a]pyrene levels were not statistically significant (0.22 ± 0.15 ,
16 0.98 ± 0.56 , and 0.40 ± 0.13 ng/mL in nonsmoking, sidestream smoke, and active smoking groups,
17 respectively), and there were no differences in relation to smoking status. Among active smokers,
18 the number of cigarettes smoked per day was strongly correlated with follicular fluid
19 benzo[a]pyrene levels ($r = 0.7$, $p < 0.01$). Follicular fluid benzo[a]pyrene levels were significantly
20 higher among the women who did not conceive (1.79 ± 0.86 ng/mL) compared with women who
21 did get pregnant (mean approximately 0.10 ng/mL, as estimated from graph) ($p < 0.001$), but
22 serum levels of benzo[a]pyrene were not associated with successful conception.

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

1 benzo[a]anthracene, and benzo[g,h,i]perylene were found). There was little difference in
2 benzo[a]pyrene levels between cases (mean \pm SE 13.85 \pm 7.06 ppb) and controls (8.83 \pm 5.84 ppb),
3 but elevated levels of fluoranthene (325.91 \pm 45.14 and 208.6 \pm 21.93 ppb in cases and controls,
4 respectively, $p < 0.05$) and benzo[b]fluoranthene (61.91 \pm 12.43 and 23.84 \pm 7.01 ppb in cases and
5 controls, respectively, $p < 0.05$) were seen.

6 **Neurotoxicity**

7 [Niu et al. \(2010\)](#) studied 176 Chinese coke-oven workers with elevated benzo[a]pyrene
8 exposure and compared them against 48 referents (workers in a supply warehouse), matched by
9 socioeconomic status, lifestyle, and health. Blood levels of monoamine, amino acid and chlorine
10 neurotransmitters were measured, and the World Health Organization Neurobehavioral Core Test
11 Battery was administered to assess emotional state, learning, memory, and hand-eye coordination.
12 The authors self-designed a study questionnaire to gather information on worker education,
13 vocational history, smoking and drinking habits, and personal habits, personal and family medical
14 history, as well as any current symptoms and medications used in the previous several weeks.
15 Workers were excluded from the study for any of the following criteria: if they reported feeling
16 depressed at any point during the previous 6 months; if they had taken medicine in the previous
17 2 weeks that could affect nervous system function; or if they reported undertaking vigorous
18 exercise less than 48 hours previously. “Smoking” was defined as ≥ 10 cigarettes/day during the
19 past year. Similarly, “drinking” was defined as wine/beer/spirits consumed ≥ 3 times/week for the
20 past 6 months. Workplace environmental sampling stations were established at each of the
21 physical work locations, including the referent’s warehouse, and dual automatic air sampling
22 pumps collected samples at personal breathing zone height for 6 hours/day, over 3 consecutive
23 days. Benzo[a]pyrene content was determined by HPLC, and relative exposure was compared to
24 post-shift urine levels of a benzo[a]pyrene metabolite, 1-hydroxypyrene (1-OH-Py). Blood was
25 collected in the morning before breakfast; monoamine (norepinephrine and dopamine) and amino
26 acid (glutamate, aspartate, glycine, and gamma-aminobutyric acid [GABA]) neurotransmitter levels
27 were determined by HPLC, acetylcholine levels determined by hydroxyamine chromometry, and
28 acetylcholine esterase (AChE) levels measured in lysed red blood cells (RBCs) using activity kits.

29 Benzo[a]pyrene mean concentrations were 19.56 \pm 13.2, 185.96 \pm 38.6, and
30 1,623.56 \pm 435.8 ng/m³ at the bottom, side, and top of the coke oven, respectively, all of which were
31 higher than the mean at the referents’ warehouse (10.26 \pm 7.6 ng/m³). The authors did not report
32 stratified analysis by different levels of benzo[a]pyrene exposure, and reported only comparisons
33 between the referents and all exposed workers combined (Table D-3), or between workers grouped
34 by urinary benzo[a]pyrene metabolite 1-OH-Py levels (Table D-4). There were no significant
35 differences in age, education, or smoking or alcohol use between the coke oven and warehouse
36 workers. Urinary 1-OH-Py levels were 32% higher in coke oven workers compared to the referent
37 group, corresponding to the higher levels of benzo[a]pyrene detected in all coke oven workstation
38 compared to the supply warehouse. Performance in two neurobehavioral function tests, digit span

1 and forward digit span, were significantly decreased in the exposed oven workers versus the
 2 control group; when stratified by urinary metabolite level, scores significantly decreased with
 3 increasing 1-OH-Py levels. Of the neurotransmitters assessed, norepinephrine, dopamine,
 4 aspartate, and GABA were significantly decreased in exposed versus control workers;
 5 norepinephrine and aspartate were also significantly and inversely related with 1-OH-Py levels.
 6 Dopamine levels appeared to decrease with increased urinary metabolite levels, although the
 7 relationship was not statistically significant. GABA levels were highly variable, and appeared to
 8 increase with increasing 1-OH-Py levels, although this relationship was not statistically significant.
 9 Acetylcholine levels were fourfold higher in coke oven workers compared to referents, and AchE
 10 activity was 30% lower; both acetylcholine and AchE were significantly associated with urinary
 11 benzo[a]pyrene metabolite levels, although acetylcholine increased and AchE activity decreased
 12 with increasing 1-OH-Py. The authors reported the results of correlation analysis, indicating that
 13 digit span scores correlated negatively with acetylcholine and positively with AchE (coefficients of
 14 -0.230 , -0.276 and 0.120 , 0.170 , respectively), although no indication of statistical significance was
 15 given. No other associations were reported.

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

Effect measured	Exposure group		p-value
	Controls (n = 48)	Exposed workers (n = 176)	
<i>Background information (mean ± SD, incidence or percent)</i>			
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
<i>Urine benzo[a]pyrene metabolite (µmol/mol creatinine; mean ± SD)</i>			
1-OH-Py	2.77 ± 1.45	3.66 ± 0.67	0.000
<i>Neurobehavioral function tests (mean ± SD)</i>			
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
<i>Neurotransmitter concentrations (mean ± SD)</i>			
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

18
 19 Source: [Niu et al. \(2010\)](#).

1
2
3
4**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 $\mu\text{mol/mol}$ creatinine	3.09–3.90 $\mu\text{mol/mol}$ creatinine	3.90–5.53 $\mu\text{mol/mol}$ creatinine	
Number of subjects	33	72	36	
<i>Neurobehavioral function tests (mean \pm SD)</i>				
Digit span	18.24 \pm 4.58	16.04 \pm 4.24	15.78 \pm 3.71	0.003
Forward digit span	10.85 \pm 2.12	9.80 \pm 2.86	9.58 \pm 2.33	0.019
Backward digit span	7.20 \pm 3.07	6.38 \pm 2.55	6.20 \pm 2.15	0.089
Right dotting	152.15 \pm 35.43	153.80 \pm 31.55	167.22 \pm 59.21	0.094
<i>Neurotransmitter concentrations (mean \pm SD)</i>				
Norepinephrine (ng/mL)	67.31 \pm 67.45	36.97 \pm 23.58	46.75 \pm 35.88	0.002
Dopamine (ng/mL)	1,614.45 \pm 683.57	1,482.30 \pm 323.66	1,405.06 \pm 332.23	0.134
Aspartate ($\mu\text{g/mL}$)	2.29 \pm 2.13	1.61 \pm 0.71	1.47 \pm 0.58	0.001
Glutamate ($\mu\text{g/mL}$)	11.56 \pm 8.92	9.93 \pm 4.14	9.06 \pm 3.30	0.070
GABA ($\mu\text{g/mL}$)	1.40 \pm 3.59	1.42 \pm 3.44	1.56 \pm 3.24	0.964
Acetylcholine ($\mu\text{g/mL}$)	334.66 \pm 83.75	483.71 \pm 57.87	665.85 \pm 94.34	0.030
AchE activity (U/mg protein)	68.17 \pm 9.28	54.98 \pm 4.23	52.64 \pm 4.60	0.043

5
6Source: [Niu et al. \(2010\)](#).**7 Immunotoxicity**

8 [Zhang et al. \(2012\)](#) studied 129 Chinese coke-oven workers with elevated benzo[a]pyrene
9 exposure and compared them against 37 referents (workers in a supply warehouse), matched by
10 socioeconomic status, lifestyle, and health. Area benzo[a]pyrene levels were quantified in the
11 various work areas, and the primary endpoint was the level of early and late apoptosis in
12 peripheral blood mononuclear cells (PBMCs) isolated from each worker subgroup the morning
13 following an overnight fast. The authors self-designed a study questionnaire to gather information
14 on worker education, vocational history, smoking and drinking habits, personal habits, and
15 personal and family medical history, as well as any current symptoms and medications used in the
16 previous several weeks. “Smoking” was defined as ≥ 10 cigarettes/day during the past year, with
17 “smoking index” defined as cigarettes/day \times years smoking. Similarly, “drinking” was defined as
18 wine/beer/spirits consumed ≥ 3 times/week for the past 6 months, and “drinking index” defined as
19 grams of alcohol consumed/day \times years drinking. Exposed workers were categorized by physical
20 worksite location and expected differences in benzo[a]pyrene exposure: 34 oven bottom workers,
21 48 oven side workers, and 47 oven top workers. Workplace environmental sampling stations were
22 established at each of the physical work locations, including the referent’s warehouse, and dual

1 automatic air sampling pumps collected samples at personal breathing zone height for 6 hours/day,
 2 over 3 consecutive days. Benzo[a]pyrene content was determined by HPLC, and relative exposure
 3 was compared to post-shift urine levels of a benzo[a]pyrene metabolite, 1-OH-Py. Collected and
 4 purified PBMCs were incubated with Annexin-V and PI prior to analysis by flow cytometry; early
 5 apoptotic cells were considered to be Annexin V+/PI-, while late apoptotic cells were considered
 6 Annexin V+/PI+.

7 All apoptosis data were displayed graphically, and in all groupings, early:late apoptotic
 8 PBMCs occurred at an approximate 2:1 frequency. PBMC apoptosis was similar in each of the three
 9 coke oven worker groups, which were all statistically significantly higher than referents
 10 (approximately twofold) for both early and late apoptosis. While self-reported smoking incidence
 11 varied significantly among the worker groups, stratification by smoking years or smoking index did
 12 not reveal any significant association with PBMC apoptosis. Multiple linear stepwise regression
 13 analysis suggested that urine 1-OH-Py levels and years of coke oven operation were positively
 14 associated with increased early and late PBMC apoptosis (Table D-5), and that years of ethanol
 15 consumption was negatively associated with only early apoptosis. These associations were tested
 16 by stratifying workers into three groups by urinary 1-OH-Py levels or coke oven operation years,
 17 and in both cases, the groups with the highest urinary metabolite levels or longest oven operating
 18 experience had statistically significantly higher levels of both early and late apoptotic PBMCs versus
 19 the lowest or shortest duration groups, respectively. Likewise, when sorted into groups based
 20 upon years of ethanol consumption, the highest ethanol “years of consumption” group had
 21 statistically significantly lower early apoptosis rates when compared to the lowest ethanol
 22 consuming group.

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

Effect measured	Exposure group (ng/m ³ ; mean ± SD)				p-value
	10.2 ± 7.6	19.5 ± 13.2	185.9 ± 38.6	1,623.5 ± 435.8	
Number of subjects	37	34	48	47	
<i>Background information (mean ± SD or %)</i>					
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
<i>Urine benzo[a]pyrene metabolite (µmol/mol creatinine; mean ± SD)</i>					
1-OH-Py	2.78 ± 1.04	3.22 ± 0.81*	3.51 ± 0.55*	3.66 ± 0.58*	0.000

25
 26 *p < 0.05 significantly different from control mean.
 27

28 Source: [Zhang et al. \(2012\)](#).

1

2 **D.3.2. Cancer-related Endpoints**3 ***Benzo[a]pyrene-Induced Cytogenetic Damage***

4 Many studies measure cytogenetic damage as biomarkers of early biological effects, which
5 also reflect exposure to genotoxic chemicals. Standard cytogenetic endpoints include chromosomal
6 aberration (CA), sister chromatid exchange (SCE), micronucleus (MN) formation, hypoxanthine
7 guanine phosphoribosyl transferase (hprt) mutation frequency, and glycophorin A mutation
8 frequency ([Gyorffy et al., 2008](#)). These biomarkers are often incorporated in multi-endpoint
9 studies with other biomarkers of exposure. Because they indicate related but different endpoints,
10 there is often a lack of correlation between the different categories of biomarkers.

11 [Merlo et al. \(1997\)](#) evaluated DNA adduct formation (measured by [³²P]-postlabelling) and
12 MN in white blood cells (WBCs) of 94 traffic policemen versus 52 residents from the metropolitan
13 area of Genoa, Italy. All study subjects wore personal air samplers for 5 hours of one work shift,
14 and levels of benzo[a]pyrene and other PAHs were measured. Policemen were exposed to 4.55 ng
15 benzo[a]pyrene/m³ air, compared with urban residents who were exposed to 0.15 ng/m³. DNA
16 adduct levels in policemen were 35% higher than in urban residents ($p = 0.007$), but MN in urban
17 residents were 20% higher than in policemen ($p = 0.02$). Linear regressions of DNA adducts and
18 MN incidence, respectively, versus benzo[a]pyrene exposure levels did not reveal significant
19 correlations.

20 Perera and coworkers assessed DNA damage in Finnish iron foundry workers in two
21 separate studies and using three methodologies. Based on results from personal sampling and
22 stationary monitoring in both studies, three levels of benzo[a]pyrene air concentrations were
23 defined: low (<5 ng/m³ benzo[a]pyrene), medium (5–12 ng/m³), and high (>12 ng/m³) ([Perera et](#)
24 [al., 1994](#); [Perera et al., 1993](#)). In the first study, involving 48 workers, several biomarkers were
25 analyzed for dose-response and interindividual variability ([Perera et al., 1993](#)). PAH-DNA adducts
26 were determined in WBCs using an immunoassay and enzyme-linked immunosorbent assay
27 (ELISA) with fluorescence detection. Mutations at the hprt locus were also measured in WBC DNA.
28 The latter assay is based on the fact that each cell contains only one copy of the hprt gene, which is
29 located on the X-chromosome. While male cells have only one X-chromosome, female cells
30 inactivate one of the two X-chromosomes at random. The gene is highly sensitive to mutations such
31 that in the event of a crucial mutation in the gene, enzyme activity disappears completely from the
32 cell. In addition, mutations at the glycophorin A gene locus were measured in RBCs. The
33 glycophorin A mutation frequency was not correlated with either benzo[a]pyrene exposure or
34 PAH-DNA adduct formation. However, both PAH-DNA adduct levels and hprt mutation frequency
35 increased with increasing benzo[a]pyrene exposure. In addition, there was a highly significant
36 correlation between incidence of hprt mutations and PAH-DNA adduct levels ($p = 0.004$).

1 In a second study, [Perera et al. \(1994\)](#) surveyed 64 iron foundry workers with assessments
2 conducted in 2 successive years; 24 of the workers provided blood samples in both years. Exposure
3 to benzo[a]pyrene, collected by personal and area sampling in the first year of the study, ranged
4 from <5 to 60 ng/m³ and was estimated to have decreased by 40% in the second year. The levels of
5 PAH-DNA adducts were roughly 50% lower in the 2nd year, presumably reflecting decreased
6 exposure. The longer-lived hprt mutations were not as strongly influenced by the decreasing
7 exposure to benzo[a]pyrene. Study subjects who did not have detectable levels of DNA adducts
8 were excluded from the study. As in the previous study, a strong correlation between DNA adduct
9 levels and incidence of hprt mutations was observed ([Perera et al., 1993](#)).

10 [Kalina et al. \(1998\)](#) studied several cytogenetic markers in 64 coke oven workers and
11 34 controls employed at other locations within the same plant. Airborne benzo[a]pyrene and seven
12 other carcinogenic PAHs were collected by personal air samplers, which showed ambient
13 benzo[a]pyrene concentrations ranging widely from 0.002 to 50 µg/m³ in coke oven workers and
14 from 0.002 to 0.063 µg/m³ in controls. CAs, SCEs, high-frequency cells (HFCs), and SCE
15 heterogeneity index were all significantly increased with benzo[a]pyrene exposure. Except for
16 increases in HFCs, no effect of smoking was observed. Consistent with studies of PAH-DNA adduct
17 formation, reduced cytogenetic response at high exposure levels produced a nonlinear dose-
18 response relationship. The authors also evaluated the potential influence of polymorphisms in
19 enzymes involved in the metabolism of benzo[a]pyrene. GSTM1 and N-acetyl transferase-2
20 polymorphisms were studied and no evidence of the two gene polymorphisms having any influence
21 on the incidence of cytogenetic damage was found.

22 [Motykiewicz et al. \(1998\)](#) conducted a similar study of genotoxicity associated with
23 benzo[a]pyrene exposure in 67 female residents of a highly polluted industrial urban area of Upper
24 Silesia, Poland, and compared the results to those obtained from 72 female residents of another
25 urban but less polluted area in the same province of Poland. Urinary mutagenicity and 1-OH-Py
26 levels, PAH-DNA adducts in oral mucosa cells (detected by immunoperoxidase staining), SCEs,
27 HFCs, CAs, bleomycin sensitivity, and GSTM1 and CYP1A1 polymorphisms in blood lymphocytes
28 were investigated. High volume air samplers and gas chromatography were used to quantify
29 ambient benzo[a]pyrene levels, which were 3.7 ng/m³ in the polluted area and 0.6 ng/m³ in the
30 control area during the summer. During winter, levels rose to 43.4 and 7.2 ng/m³ in the two areas,
31 respectively. The cytogenetic biomarkers (CA and SCE/HFC), urinary mutagenicity, and urinary
32 1-OH-Py excretion were significantly increased in females from the polluted area, and differences
33 appeared to be more pronounced during winter time. PAH-DNA adduct levels were significantly
34 increased in the study population, when compared to the controls, only in the winter season. No
35 difference in sensitivity to bleomycin-induced lymphocyte chromatid breaks was seen between the
36 two populations. As with the study by [Kalina et al. \(1998\)](#), genetic polymorphisms assumed to
37 affect the metabolic transformation of benzo[a]pyrene were not associated with any difference in
38 the incidence of DNA damage.

1 In a study of Thai school boys in urban (Bangkok) and rural areas, bulky (including but not
2 limited to BPDE-type) DNA adduct levels were measured in lymphocytes along with DNA single-
3 strand breaks (SSBs), using the comet assay, and DNA repair capacity ([Tuntawiroon et al., 2007](#)).
4 Ambient air and personal breathing zone measurements indicated that Bangkok school children
5 experienced significantly higher exposures to benzo[a]pyrene and total PAHs. A significantly
6 higher level of SSBs (tail length 1.93 ± 0.09 versus 1.28 ± 0.12 μm , +51%; $p < 0.001$) was observed
7 in Bangkok school children when compared with rural children, and this parameter was
8 significantly associated with DNA adduct levels. A significantly reduced DNA repair capacity
9 (0.45 ± 0.01 versus 0.26 ± 0.01 γ -radiation-induced deletions per metaphase, -42%; $p < 0.001$) was
10 also observed in the city school children, again significantly associated with DNA adduct levels. It
11 was not evident why higher environmental PAH exposure would be associated with lowered DNA
12 repair capacity. However, because the personal breathing zone PAH levels and DNA adduct levels
13 were not associated with each other, it is conceivable that the city school children had a priori
14 lower DNA repair capacities that contributed significantly to the high adduct levels. The authors
15 considered genetic differences between the two study populations as a possible reason for this
16 observation.

17 **D.3.3. Epidemiologic Findings in Humans**

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

30 ***Molecular Epidemiology and Case-Control Cancer Studies***

31 Defective DNA repair capacity leading to genomic instability and, ultimately, increased
32 cancer risk is well documented ([Wu et al., 2007](#); [Wu et al., 2005](#)). Moreover, sensitivity to mutagen-
33 induced DNA damage is highly heritable and thus represents an important factor that determines
34 individual cancer susceptibility. Based on studies comparing monozygotic and dizygotic twins, the
35 genetic contribution to BPDE mutagenic sensitivity was estimated to be 48.0% ([Wu et al., 2007](#)).
36 BPDE has been used as an etiologically relevant mutagen in case-control studies to examine the
37 association between elevated lung and bladder cancer risk and individual sensitivity to BPDE-

1 induced DNA damage. Mutagen sensitivity is determined by quantifying chromatid breaks or DNA
2 adducts in phytohemagglutinin-stimulated peripheral blood lymphocytes as an indirect measure of
3 DNA repair capacity.

4 In a hospital-based, case-control study involving 221 lung cancer cases and 229 healthy
5 controls, DNA adducts were measured in stimulated peripheral blood lymphocytes after incubation
6 with BPDE in vitro ([Li et al., 2001](#)). Lung cancer cases showed consistent statistically significant
7 elevations in induced BPDE-DNA adducts in lymphocytes, compared with controls, regardless of
8 subgroup by age, sex, ethnicity, smoking history, weight loss, or family history of cancer. The
9 lymphocyte BPDE-induced DNA adduct levels, when grouped by quartile using the levels in controls
10 as cutoff points, were significantly dose-related with lung cancer risk (ORs 1.11, 1.62, and 3.23;
11 trend test, $p < 0.001$). In a related hospital-based, case-control study involving 155 lung cancer
12 patients and 153 healthy controls, stimulated peripheral blood lymphocytes were exposed to BPDE
13 in vitro ([Wu et al., 2005](#)). DNA damage/repair was evaluated in lymphocytes using the comet assay,
14 and impacts on cell cycle checkpoints were measured using a fluorescence-activated cell-sorting
15 method. The lung cancer cases exhibited significantly higher levels of BPDE-induced DNA damage
16 than the controls ($p < 0.001$), with lung cancer risk positively associated with increasing levels of
17 lymphocyte DNA damage when grouped in quartiles (trend test, $p < 0.001$). In addition, lung cancer
18 patients demonstrated significantly shorter cell cycle delays in response to BPDE exposure to
19 lymphocytes, which correlated with increased DNA damage.

20 Sensitivity to BPDE-induced DNA damage in bladder cancer patients supports the results
21 observed in lung cancer cases. In a hospital-based, case-control study involving 203 bladder cancer
22 patients and 198 healthy controls, BPDE-induced DNA damage was specifically evaluated at the
23 chromosome 9p21 locus in stimulated peripheral blood lymphocytes ([Gu et al., 2008](#)). Deletions of
24 9p21, which includes critical components of cell cycle control pathways, are associated with a
25 variety of cancers. After adjusting for age, sex, ethnicity, and smoking status, individuals with high
26 BPDE-induced damage at 9p21 were significantly associated with increased bladder cancer risk
27 (OR 5.28; 95% CI 3.26–8.59). Categorization of patients into tertiles for BPDE sensitivity relative to
28 controls demonstrated a dose-related association between BPDE-induced 9p21 damage and
29 bladder cancer risk. Collectively, the results of molecular epidemiology studies with lung and
30 bladder cancer patients indicate that individuals with a defective ability to repair BPDE-DNA
31 adducts are at increased risk for cancer and, moreover, that specific genes linked to tumorigenesis
32 pathways may be molecular targets for benzo[a]pyrene and other carcinogens.

33 Due to the importance of the diet as a benzo[a]pyrene exposure source, several population-
34 and hospital-based, case-control studies have investigated the implied association between dietary
35 intake of benzo[a]pyrene and risk for several tumor types. In a study involving 193 pancreatic
36 cancer cases and 674 controls ([Anderson et al., 2005](#)), another involving 626 pancreatic cancer
37 cases and 530 controls ([Li et al., 2007](#)), and a third involving 146 colorectal adenoma cases and
38 228 controls ([Sinha et al., 2005](#)), dietary intake of benzo[a]pyrene was estimated using food

1 frequency questionnaires. In all studies, the primary focus was on estimated intake of
2 benzo[a]pyrene (and other carcinogens) derived from cooked meat. Overall, cases when compared
3 with controls, had higher intakes of benzo[a]pyrene and other food carcinogens, leading to the
4 conclusion that benzo[a]pyrene plays a role in the etiology of these tumors in humans. In a
5 supportive follow-up case-control study of colorectal adenomas, levels of leukocyte PAH-DNA
6 adducts were significantly higher in cases when compared with controls ($p = 0.02$), using a method
7 that recognizes BPDE and several other PAHs bound to DNA ([Gunter et al., 2007](#)).

8 ***Cohort Cancer Studies***

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

20 Cancer incidence in aluminum and electrode production plants

21 Exposure to benzo[a]pyrene and BSM in aluminum smelter workers is strongly associated
22 with bladder cancer and weakly associated with lung cancer ([Boffetta et al., 1997](#); [Tremblay et al.,
23 1995](#); [Armstrong et al., 1994](#); [Gibbs, 1985](#); [Theriault et al., 1984](#)). In an analysis of pooled data from
24 nine cohorts of aluminum production workers, 688 respiratory tract cancer cases were observed
25 versus 674.1 expected (pooled RR 1.03; CI 0.96–1.11) ([Bosetti et al., 2007](#)). A total of 196 bladder
26 cancer cases were observed in eight of the cohorts, compared with 155.7 expected (pooled RR 1.29;
27 CI 1.12–1.49). Based on estimated airborne benzo[a]pyrene exposures from a meta-analysis of
28 eight cohort studies, the predicted lung cancer RR per 100 $\mu\text{g}/\text{m}^3$ -years of cumulative
29 benzo[a]pyrene exposure was 1.16 (95% CI 1.05–1.28) ([Armstrong et al., 2004](#)).

30 [Spinelli et al. \(2006\)](#) reported a 14-year update to a previously published historical cohort
31 study ([Spinelli et al., 1991](#)) of Canadian aluminum reduction plant workers. The results confirmed
32 and extended the findings from the earlier epidemiology study. The study surveyed a total of
33 6,423 workers with ≥ 3 years of employment at an aluminum reduction plant in British Columbia,
34 Canada, between the years 1954 and 1997, and evaluated all types of cancers. The focus was on
35 cumulative exposure to coal tar pitch volatiles, measured as BSM and as benzo[a]pyrene.
36 Benzo[a]pyrene exposure categories were determined from the range of predicted exposures over
37 time from statistical exposure models. There were 662 cancer cases, of which approximately 98%

1 had confirmed diagnoses. The overall cancer mortality rate (SMR 0.97; CI 0.87–1.08) and cancer
2 incidence rate (standardized incidence ratio [SIR] 1.00; CI 0.92–1.08) were not different from that
3 of the British Columbia general population. However, this study identified significantly increased
4 incidence rates for cancers of the bladder (SIR 1.80; CI 1.45–2.21) and stomach (SIR 1.46; CI
5 1.01–2.04). The lung cancer incidence rate was only slightly higher than expected (SIR 1.10; CI
6 0.93–1.30). Significant dose-response associations with cumulative benzo[a]pyrene exposure were
7 seen for bladder cancer ($p < 0.001$), stomach cancer ($p < 0.05$), lung cancer ($p < 0.001$), non-
8 Hodgkin lymphoma ($p < 0.001$), and kidney cancer ($p < 0.01$), although the overall incidence rates
9 for the latter three cancer types were not significantly elevated versus the general population.
10 Similar cancer risk results were obtained using BSM as the exposure measure; the cumulative
11 benzo[a]pyrene and BSM exposures were highly correlated ($r = 0.94$).

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

14 In a study covering 11,103 workers and 272,554 person × years of PAH exposure, cancer
15 incidence was evaluated in six Norwegian aluminum smelters ([Romundstad et al., 2000a](#)) and
16 ([Romundstad et al., 2000b](#)). Reported estimates of PAH exposure concentrations reached a
17 maximum of 3,400 $\mu\text{g}/\text{m}^3$ PAH (680 $\mu\text{g}/\text{m}^3$ benzo[a]pyrene). The overall number of cancers
18 observed in this study did not differ significantly from control values (SIR 1.03; CI 1.0–1.1). The
19 data from this study showed significantly increased incidences for cancer of the bladder (SIR 1.3;
20 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;
21 CI 0.7–2.5), and multiple myeloma (SIR 1.4; CI 0.9–1.9). Incidence rates for bladder, lung, pancreas,
22 and kidney cancer (the latter three with SIRs close to unity) were subjected to a cumulative
23 exposure-response analysis. The incidence rate for bladder cancer showed a trend with increasing
24 cumulative exposure and with increasing lag times (up to 30 years) at the highest exposure level.
25 The incidence of both lung and bladder cancers was greatly increased in smokers. The authors
26 reported that using local county rates rather than national cancer incidence rates as controls
27 increased the SIR for lung cancer (SIR 1.4; CI 1.2–1.6) to a statistically significant level.

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

29 An increased risk of death from lung and bladder cancer is reported in some studies
30 involving coke oven, coal gasification, and iron and steel foundry workers ([Boström et al., 2002](#);
31 [Boffetta et al., 1997](#)). An especially consistent risk of lung cancer across occupations is noted when
32 cumulative exposure is taken into consideration (e.g., RR of 1.16 per 100 unity-years for aluminum
33 smelter workers, 1.17 for coke oven workers, and 1.15 for coal gasification workers). In an analysis
34 of pooled data from 10 cohorts of coke production workers, 762 lung cancer cases were observed
35 versus 512.1 expected (pooled RR 1.58; CI 1.47–1.69) ([Bosetti et al., 2007](#)). Significant variations in
36 risk estimates among the studies were reported, particularly in the large cohorts (RRs of 1.1, 1.2,
37 2.0, and 2.6). There was no evidence for increased bladder cancer risk in the coke production
38 workers. Based on estimated airborne benzo[a]pyrene exposures from a meta-analysis of

1 10 cohort studies, the predicted lung cancer RR per 100 $\mu\text{g}/\text{m}^3$ -years of cumulative benzo[a]pyrene
2 exposure was 1.17 (95% CI 1.12–1.22) ([Armstrong et al., 2004](#)).

3 A meta-analysis of data from five cohorts of gasification workers reported 251 deaths from
4 respiratory tract cancer, compared with 104.7 expected (pooled RR 2.58; 95% CI 2.28–2.92)
5 ([Bosetti et al., 2007](#)). Pooled data from three of the cohorts indicated 18 deaths from urinary tract
6 cancers, versus 6.0 expected (pooled RR 3.27; 95% CI 2.06–5.19). Based on estimated airborne
7 benzo[a]pyrene exposures from a meta-analysis of four gas worker cohort studies, the predicted
8 lung cancer RR per 100 $\mu\text{g}/\text{m}^3$ -years of cumulative benzo[a]pyrene exposure was 1.15 (95% CI
9 1.11–1.20) ([Armstrong et al., 2004](#)).

10 Increased risks were reported in iron and steel foundry workers for cancers of the
11 respiratory tract, bladder, and kidney. In an analysis of pooled data from 10 cohorts,
12 1,004 respiratory tract cancer cases were observed versus 726.0 expected (pooled RR 1.40;
13 CI 1.31–1.49) ([Bosetti et al., 2007](#)). A total of 99 bladder cancer cases were observed in seven of the
14 cohorts, compared with 83.0 expected (pooled RR 1.29; CI 1.06–1.57). For kidney cancer, 40 cases
15 were observed compared with 31.0 expected based on four studies (pooled RR 1.30; 95% CI
16 0.95–1.77).

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

26 There were significant trends for long-term, cumulative benzo[a]pyrene exposure versus
27 lung cancer ($p = 0.004$) or stomach cancer ($p = 0.016$) incidence. For cumulative total
28 benzo[a]pyrene exposures of <0.84 , 0.85–1.96, 1.97–3.2, and ≥ 3.2 $\mu\text{g}/\text{m}^3$ -year, the ORs for lung
29 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
30 cumulative total benzo[a]pyrene exposures of <0.84 , 0.85–1.96, 1.97–3.2, and ≥ 3.2 $\mu\text{g}/\text{m}^3$ -year, the
31 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),
32 respectively. However, the investigators noted that additional workplace air contaminants were
33 measured, which might have influenced the outcome. Of these, asbestos, silica, quartz, and iron
34 oxide-containing dusts may have been confounders. For lung cancers, cumulative exposures to
35 total dust and silica dust both showed significant dose-response trends ($p = 0.001$ and 0.007,
36 respectively), while for stomach cancer, only cumulative total dust exposure showed a marginally
37 significant trend ($p = 0.061$). For cumulative total dust exposures of <69 , 69–279, 280–882, and
38 ≥ 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 1.9 (CI 1.3–2.5), respectively. For cumulative silica dust exposures of <3.7, 3.7–10.39, 10.4–27.71,
2 and ≥27.72 mg/m³, 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),
3 and 1.8 (CI 1.2–2.5), respectively. For cumulative total dust exposures of <69, 69–279, 280–882,
4 and ≥883 mg/m³, ORs for stomach cancer were 1.3 (CI 0.8–2.1), 1.4 (CI 0.9–2.2), 1.2 (CI 0.8–1.9),
5 and 1.6 (CI 1.1–2.5), respectively.

6 Exposure-response data from studies of coke oven workers in the United States have often
7 been used to derive quantitative risk estimates for PAH mixtures, and for benzo[a]pyrene as an
8 indicator substance ([Boström et al., 2002](#)). However, there are numerous studies of coke oven
9 worker cohorts that do not provide estimates of benzo[a]pyrene exposure. An overview of the
10 results of these and other studies can be obtained from the review of [Boffetta et al. \(1997\)](#).

11 Cancer incidence in asphalt workers and roofers

12 These groups encompass different types of work (asphalt paving versus roofing) and also
13 different types of historical exposure that have changed from using PAH-rich coal tar pitch to the
14 use of bitumen or asphalt, both of which are rather low in PAHs due to their source (crude oil
15 refinery) and a special purification process. Increased risks for lung cancer were reported in large
16 cohorts of asphalt workers and roofers; evidence for increased bladder cancer risk is weak
17 ([Burstyn et al., 2007](#); [Partanen and Boffetta, 1994](#); [Chiazze et al., 1991](#); [Hansen, 1991, 1989](#);
18 [Hammond et al., 1976](#)). In an analysis of pooled data from two cohorts of asphalt workers, 822 lung
19 cancer cases were observed versus 730.7 expected (pooled RR 1.14; 95% CI 1.07–1.22) ([Bosetti et](#)
20 [al., 2007](#)). In two cohorts of roofers, analysis of pooled data indicated that 138 lung cancer cases
21 were observed, compared with 91.9 expected (pooled RR 1.51; 95% CI 1.28–1.78) ([Bosetti et al.,](#)
22 [2007](#)).

23 Epidemiology of patients treated with coal tar containing ointments

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

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

Reference and study details	Results								
<i>General population studies</i>									
<p>Mitropoulos and Norman (2005) (United States, Arizona)</p> <p>Case-control study (Southeastern Arizona Health Study-2), population-based; n = 404 squamous cell skin cancer cases, 395 controls, 1992–1996, age ≥30 yrs; controls selected using random digit dialing (frequency matched by 5-yr age group and gender); limited to whites; details regarding participation rates not reported</p> <p>Exposure: Interview, focusing on occupational and other sources of sun exposure, chemical exposures, and coal tar/dandruff shampoo</p> <p>Outcome: Incident squamous cell cancer from regional skin cancer registry</p>	<p>Squamous cell carcinoma (SCC), coal tar/dandruff shampoo use:</p> <table border="1"> <thead> <tr> <th>Cases n (%)</th> <th>Controls n (%)</th> <th>OR^a (95% CI)</th> <th>OR^b (95% CI)</th> </tr> </thead> <tbody> <tr> <td>101 (25)</td> <td>73 (19)</td> <td>1.50 (1.05, 2.14)</td> <td>1.28 (0.85, 1.9)</td> </tr> </tbody> </table> <p>^aAdjusted for age and gender. ^bAdjusted for age, gender, actinic keratosis, current number of arm freckles, and reaction of skin to prolonged sun.</p>	Cases n (%)	Controls n (%)	OR ^a (95% CI)	OR ^b (95% CI)	101 (25)	73 (19)	1.50 (1.05, 2.14)	1.28 (0.85, 1.9)
Cases n (%)	Controls n (%)	OR ^a (95% CI)	OR ^b (95% CI)						
101 (25)	73 (19)	1.50 (1.05, 2.14)	1.28 (0.85, 1.9)						
<i>Studies of patients with skin conditions</i>									
<p>Roelofzen et al. (2010) (Netherlands)</p> <p>Cohort (retrospective); total n = 13,200 (4,315 psoriasis 8,885 eczema patients), identified through hospital records (manual). Diagnosed 1960–1990 (≥3 visits to dermatologist); median age 28 yrs; follow-up through 2003 (median follow-up 21 yrs)</p> <p>Exposure: Coal tar treatment (pix lithantracis and/or liquor carbonis detergens): 8,062 (39%); duration of use obtained from 1,100 users (14%), median = 6 mo</p> <p>Outcome: Skin cancer diagnosis from national cancer registry (operating since 1989) and cause of death registries, with some supplemental questionnaire data from 61% of the cohort</p>	<p>Skin cancer (excluding basal cell carcinoma); includes melanoma and squamous cell [number of cases = 145]</p> <p>HR (95% CI) for use of coal tar; referent category = only used dermatocorticosteroids:</p> <table border="1"> <tbody> <tr> <td>Psoriasis</td> <td>1.08 (0.43, 2.72)</td> </tr> <tr> <td>Eczema</td> <td>1.06 (0.62, 1.83)</td> </tr> <tr> <td>Psoriasis or eczema</td> <td>1.09 (0.69, 1.72)</td> </tr> </tbody> </table> <p>Proportional hazards models, adjusted for age (continuous), gender, severity (>10% of body area affected), interaction term of coal tar and severity, calendar period, psoralen + ultraviolet-A (PUVA) systemic therapy, and smoking (current and ever versus never). Also examined skin type, history of sun exposure, and alcohol consumption. Smoking data imputed for 58% of the cohort.</p>	Psoriasis	1.08 (0.43, 2.72)	Eczema	1.06 (0.62, 1.83)	Psoriasis or eczema	1.09 (0.69, 1.72)		
Psoriasis	1.08 (0.43, 2.72)								
Eczema	1.06 (0.62, 1.83)								
Psoriasis or eczema	1.09 (0.69, 1.72)								
<p>Torinuki and Tagami (1988) (Japan)</p> <p>Cohort (prospective); total n = 151 psoriasis patients including 43 treated with Goeckerman regimen without PUVA treatment, mean age 43 yrs; patients</p>	<p>No skin cancers observed</p>								

Reference and study details	Results															
<p>treated between 1976–1986; follow-up: 5/43 Goeckerman patients followed for >6 yrs</p> <p>Exposure: Goeckerman regimen without PUVA treatment; duration of use not reported</p> <p>Outcome: Skin cancer diagnosis from case records</p>																
<p>Maughan et al. (1980) (United States, Mayo Clinic)</p> <p>Cohort (retrospective); n = 426 atopic dermatitis or neurodermatitis patients, treated with Goeckerman regimen between 1950–1954; follow-up: 305 (72%) followed to approximately 1980 (25 yrs)</p> <p>Exposure: Goeckerman regimen (ultraviolet-B [UVB] + coal tar treatments) at hospital; follow-up questionnaire inquired about other treatment (including coal tar treatment) after hospitalization; coal tar use ranged from none to every day for 26 yrs</p> <p>Outcome: Skin cancer diagnosis by self-report (follow-up questionnaire) with confirmation through histology specimens; 9 of 11 nonmelanoma skin cancers confirmed</p>	<p>Eleven nonmelanoma skin cancer cases (observed) [8 basal cell, 1 squamous cell, 2 unknown]</p> <p>Expected rates from Third National Cancer Survey</p> <table border="1" data-bbox="691 688 1398 856"> <thead> <tr> <th></th> <th>Observed/Expected</th> <th>Expected</th> </tr> </thead> <tbody> <tr> <td>Minneapolis-St Paul</td> <td>6.7</td> <td>1.64</td> </tr> <tr> <td>San Francisco-Oakland</td> <td>9.4</td> <td>1.17</td> </tr> <tr> <td>Iowa</td> <td>5.3</td> <td>2.08</td> </tr> <tr> <td>Dallas-Fort Worth</td> <td>18.8</td> <td>0.59</td> </tr> </tbody> </table> <p>No difference in duration of coal tar use after hospitalization in skin cancer patients compared to those who did not develop skin cancer.</p>		Observed/Expected	Expected	Minneapolis-St Paul	6.7	1.64	San Francisco-Oakland	9.4	1.17	Iowa	5.3	2.08	Dallas-Fort Worth	18.8	0.59
	Observed/Expected	Expected														
Minneapolis-St Paul	6.7	1.64														
San Francisco-Oakland	9.4	1.17														
Iowa	5.3	2.08														
Dallas-Fort Worth	18.8	0.59														
<p>Pittelkow et al. (1981) (United States, Mayo Clinic)</p> <p>Cohort (retrospective); n = 280 psoriasis patients, hospitalized 1950–1954 at Mayo Clinic; 260 (92%) followed to 1978 (25 yrs)</p> <p>Exposure: Goeckerman regimen (UVB + coal tar treatments) at hospital; other treatment (including coal tar treatment) recorded from clinical records. Median duration use approximately 15 d in 1951–1955 and 21 d in 1956–1960</p> <p>Outcome: Skin cancer diagnosis by self-report (follow-up questionnaire) with confirmation through histology specimens; 20 of 22 confirmed</p>	<p>Among patients reporting coal tar therapy use: n = 19 nonmelanoma squamous cell or basal cell (or unknown) skin cancer cases (observed)</p> <p>Expected rates from Third National Cancer Survey</p> <table border="1" data-bbox="691 1442 1398 1610"> <thead> <tr> <th></th> <th>Observed/Expected</th> <th>Expected</th> </tr> </thead> <tbody> <tr> <td>Minneapolis-St Paul</td> <td>18.7</td> <td>1.01</td> </tr> <tr> <td>San Francisco-Oakland</td> <td>23.1</td> <td>0.82</td> </tr> <tr> <td>Iowa</td> <td>15.5</td> <td>1.22</td> </tr> <tr> <td>Dallas-Fort Worth</td> <td>49.2</td> <td>0.39</td> </tr> </tbody> </table>		Observed/Expected	Expected	Minneapolis-St Paul	18.7	1.01	San Francisco-Oakland	23.1	0.82	Iowa	15.5	1.22	Dallas-Fort Worth	49.2	0.39
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Reference and study details	Results								
<i>Coal tar use in studies with combined treatment of PUVA therapy</i>									
<p>(Stern et al. (1998); Stern and Laird (1994)) (United States, 16 centers)</p> <p>Cohort (prospective); total n = 1,380 psoriasis patients, enrolled between 1975 and 1976 in the PUVA cohort study; mean age 44 yrs; follow-up at 12–15-mo intervals through 1996 (approximately 20 years); 1,049 (91%) patients interviewed at final follow-up</p> <p>Exposure: Non-PUVA treatments (including topical coal tar, ultraviolet B, methotrexate, and ionizing radiation) were collected at start of PUVA treatment and during follow-up; coal tar use was noted to be highly correlated with UVB therapy and thus reported as a single parameter; ‘high use’ defined as >45 mo topical tar therapy or >300 UVB treatments</p> <p>Outcome: Skin cancer diagnosis reported at follow-up, confirmed by histopathology</p>	<p>From 1996 follow-up (limited to first occurrence 1986–1996):</p> <table border="0"> <tr> <td>Cancer type</td> <td>OR (95% CI) [n cases]</td> </tr> <tr> <td>Squamous</td> <td>1.4 (1.0, 2.0) [1,047]</td> </tr> <tr> <td>Basal cell</td> <td>1.5 (1.1, 2.0) [821]</td> </tr> </table> <p>OR compares ‘high’ exposure to UVB/tar to ‘low’ exposure to UVB/tar, adjusted for age, sex, geographic area, anatomic site (head and neck, other), PUVA treatments through 1985 (five categories from <100 to >336), PUVA treatments after 1985 (≥50, <50), methotrexate (≥208 weeks, <208 weeks), and Grenz rays or x-rays for therapy (ever/never)</p>	Cancer type	OR (95% CI) [n cases]	Squamous	1.4 (1.0, 2.0) [1,047]	Basal cell	1.5 (1.1, 2.0) [821]		
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<p>Stern et al. (1980) (United States, 16 centers)</p> <p>Nested case-control study based on a study following 1,373 PUVA-treated patients (34 incident cases, 24 prevalent cases; 126 controls); matched by age (within 5 yrs), sex, skin type, geographic area, and ionizing radiation; incident cases also matched for number of PUVA treatments; average follow-up 2.7 yrs</p> <p>Exposure: Exposure to coal tar therapy and/or ultraviolet radiation based on follow-up interview; includes exposures before PUVA trial began; coal tar use quantified as number of months in which crude coal tar preparations was used at least weekly; high coal tar exposure defined as > 90 mo of use; high ultraviolet radiation exposure defined as ≥300 sunlamp treatments. Assumption made that coal tar and ultraviolet radiation have the same quantitative effect on risk of skin cancer</p> <p>Outcome: Skin cancer, prevalent cases occurred before PUVA trial started; incident cases occurred during follow-up period</p>	<p>RR (95% CI) of skin cancer (skin cancer type not specified) among high exposure (≥90 mo of tar use or ≥300 sunlamp treatments)</p> <table border="0"> <tr> <td></td> <td>Matched analysis:</td> </tr> <tr> <td>All cases (n = 58)</td> <td>4.7 (2.2, 10.0)</td> </tr> <tr> <td>Incident cases (n = 34)</td> <td>5.6 (1.9, 16.2)</td> </tr> <tr> <td>Prevalent cases (n = 24)</td> <td>3.8 (1.2, 12.5)</td> </tr> </table>		Matched analysis:	All cases (n = 58)	4.7 (2.2, 10.0)	Incident cases (n = 34)	5.6 (1.9, 16.2)	Prevalent cases (n = 24)	3.8 (1.2, 12.5)
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Reference and study details	Results
<p>Lindelöf and Sigurgeirsson (1993) (Sweden) Nested case-control study based on a study following 4,799 PUVA-treated patients (24 cases, 96 controls); matched by gender, age, diagnosis, PUVA dose, number of treatments, type of psoralen regimen, site of treatment, and skin type; clinic location matching utilized when possible; mean age 52 yrs Exposure: Non-PUVA treatments (including tar, topical corticosteroids, UVB, and anthralin) collected by questionnaire; exposure not quantified and duration not provided Outcome: Skin cancer diagnosis obtained from Swedish cancer registry</p>	<p>SCC with coal tar usage: Cases Controls n (%) n (%) OR (95% CI) 17 (70) 62 (64) 1.3 (0.5, 3.5) (Similar results were seen for UVB exposure [OR 1.3, 95% CI 0.5, 3.5], reflecting the high correlation between these treatments)</p>

1
2 The U.S. Environmental Protection Agency (EPA) noted several limitations with respect to
3 study design and analysis in this literature, precluding the ability to provide a foundation for
4 evaluating the potential association between use of therapeutic coal tar treatment (particularly
5 long-term treatment) and risk of skin cancer. A primary limitation concerns the quality of the
6 exposure assessment. Only one population-based, case-control study was identified ([Mitropoulos
7 and Norman, 2005](#)); this study examined self-reported use of coal tar/dandruff shampoo and
8 incidence of squamous cell cancer in a population in Arizona (adjusted OR 1.28, 95% CI 0.85, 1.9).
9 This exposure measure is likely to be highly susceptible to misclassification bias. EPA considered
10 the likelihood of non-differential misclassification to be high; differential misclassification was also
11 considered to be possible, but of lower likelihood. Non-differential misclassification would arise
12 from lack of awareness of the content of shampoos, inability to recall use of individual shampoos,
13 and the lack of specificity of this particular question. Differential misclassification would arise from
14 differential reporting based on disease status. EPA noted similar concerns regarding exposure
15 quality in the nested case-control study conducted among patients receiving psoralen plus
16 ultraviolet-A (PUVA) treatment (in addition to a variety of other treatments, including coal tar
17 treatments and ultraviolet -B [UVB]) by [Lindelöf and Sigurgeirsson \(1993\)](#). Use of coal tar was
18 collected through a mailed questionnaire, with no information on duration of use and no
19 verification with medical records. A large study of psoriasis and eczema patients
20 (n = 13,200 patients) by [Roelofzen et al. \(2010\)](#) with a 21-year follow-up period obtained data on
21 coal tar treatment through manual chart review; this chart review was conducted in 2003 on
22 medical records going back to 1960. Duration of use (median 6 months) was available for only 14%
23 of the patients who had an indication of use. Thus, considerable non-differential misclassification
24 of exposure (coal tar use) is likely, and the limited exposure data did not allow examination of
25 variation in exposure level. Misclassification of disease was also noted to be a limitation of this
26 study in that [Roelofzen et al. \(2010\)](#) included melanoma, in addition to squamous cell skin cancer,

1 which introduces a lack of specificity of outcome into the analysis as melanoma is not thought to be
2 associated with PAH exposure. Given these issues of exposure and disease misclassification, the
3 RRs from these studies do not provide a sound basis for interpretation as no risk, and would be
4 expected to diminish effect estimates.

5 A common regimen for treatment of psoriasis and other skin conditions combines coal tar
6 treatment with UVB radiation (referred to as the Goeckerman regimen). One study of this regimen
7 was very small (n = 43 patients) with only 5 of the patients followed for more than 6 years
8 ([Torinuki and Tagami, 1988](#)). Two larger Goeckerman treatment studies (280–426 patients) had a
9 longer follow-up period (25 years), but were limited in terms of the choice of referent rates and
10 differences in disease ascertainment between cases and the reference population ([Pittelkow et al.,
11 1981](#); [Maughan et al., 1980](#)). Specifically, dermatology patients were seen at the Mayo Clinic in
12 Rochester, Minnesota, but the reference rates for cancer were obtained from survey data from
13 Minneapolis-St Paul, San Francisco-Oakland, Iowa, and Dallas-Fort Worth. Therefore, it is unclear
14 whether the reference population appropriately represents the case population. In addition, this
15 combination of UVB and coal tar makes it impossible to attribute risk to either individual
16 component. This limitation effects the interpretation of the results of the PUVA trial studies ([Stern
17 et al., 1998](#); [Stern and Laird, 1994](#); [Stern et al., 1980](#)), in which the analysis was conducted using a
18 definition of “high” exposure as >4 months of topical tar therapy or >300 UVB treatments.
19 Similarly, the study by ([Lindelöf and Sigurgeirsson \(1993\)](#)) reported similar prevalence and risk
20 estimates for coal tar use and for UVB, reflecting the high correlation between these treatments.

21 In summary, the available studies examining therapeutic topical coal tar use and risk of skin
22 cancer were limited by low-quality exposure data with high potential of exposure misclassification
23 (e.g., [Roelofzen et al., 2010](#); [Mitropoulos and Norman, 2005](#); [Lindelöf and Sigurgeirsson, 1993](#)),
24 small size and short duration of follow-up (e.g., [Torinuki and Tagami, 1988](#)), and choice of referent
25 rates and differences in disease ascertainment between cases and the reference population (e.g.,
26 [Pittelkow et al., 1981](#); [Maughan et al., 1980](#)). In addition, clinic-based studies focused on the
27 commonly used regimen of coal tar in conjunction with UVB therapy cannot distinguish effects of
28 coal tar from the carcinogenic effects of UVB (e.g., [Stern et al., 1998](#); [Stern and Laird, 1994](#); [Lindelöf
29 and Sigurgeirsson, 1993](#); [Stern et al., 1980](#)). Therefore, the available studies do not provide an
30 adequate basis for examining the potential association between coal tar treated patients and skin
31 cancer.

32 D.4. ANIMAL STUDIES

33 D.4.1. Oral Bioassays

34 *Subchronic Studies*

35 [De Jong et al. \(1999\)](#) treated male Wistar rats (eight/dose group) with benzo[a]pyrene
36 (98.6% purity) dissolved in soybean oil by gavage 5 days/week for 35 days at doses of 0, 3, 10, 30,

1 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
 2 exposure period, rats were necropsied, organ weights were determined, and major organs and
 3 tissues were prepared for histological examination (adrenals, brain, bone marrow, colon, caecum,
 4 jejunum, heart, kidney, liver, lung, lymph nodes, esophagus, pituitary, spleen, stomach, testis, and
 5 thymus). Blood was collected for examination of hematological endpoints, but there was no
 6 indication that serum biochemical parameters were analyzed. Immune parameters included
 7 determinations of serum immunoglobulin (Ig) levels (IgG, IgM, IgE, and IgA), relative spleen cell
 8 distribution, and spontaneous cytotoxicity of spleen cell populations determined in a natural-killer
 9 (NK) cell assay.

10 Body weight gain was decreased beginning at week 2 at the high dose of 90 mg/kg-day;
 11 there was no effect at lower doses ([De Jong et al., 1999](#)). Hematology revealed a dose-related
 12 decrease in RBC count, hemoglobin, and hematocrit at ≥ 10 mg/kg-day (Table D-7). A minimal but
 13 significant increase in mean cell volume and a decrease in mean cell hemoglobin concentration
 14 were noted at 90 mg/kg-day, and may indicate dose-related toxicity for the RBCs and/or RBC
 15 precursors in the bone marrow. A decrease in WBCs, attributed to a decrease in the number of
 16 lymphocytes (approximately 50%) and eosinophils (approximately 90%), was observed at
 17 90 mg/kg-day; however, there was no effect on the number of neutrophils or monocytes. A
 18 decrease in the cell number in the bone marrow observed in the 90 mg/kg-day dose group was
 19 consistent with the observed decrease in the RBC and WBC counts at this dose level. In the
 20 90 mg/kg-day dose group, brain, heart, kidney, and lymph node weights were decreased and liver
 21 weight was increased (Table D-7). Decreases in heart weight at 3 mg/kg-day and in kidney weight
 22 at 3 and 30 mg/kg-day were also observed, but these changes did not show dose-dependent
 23 responses. Dose-related decreases in thymus weight were statistically significant at
 24 ≥ 10 mg/kg-day (Table D-7).

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

Effect	Dose (mg/kg-d)				
	0	3	10	30	90
<i>Hematologic effects</i> (mean \pm SD; n = 7–8)					
WBCs (10^9 /L)	14.96 \pm 1.9	13.84 \pm 3.0	13.69 \pm 1.8	13.58 \pm 2.9	8.53 \pm 1.1*
RBCs (10^9 /L)	8.7 \pm 0.2	8.6 \pm 0.2	8.3 \pm 0.2*	7.8 \pm 0.4*	7.1 \pm 0.4*
Hemoglobin (mmol/L)	10.5 \pm 0.2	10.4 \pm 0.3	9.8 \pm 0.2*	9.5 \pm 0.4*	8.6 \pm 0.6*
Hematocrit (L/L)	0.5 \pm 0.01	0.5 \pm 0.01	0.47 \pm 0.01*	0.46 \pm 0.02*	0.43 \pm 0.02*
<i>Serum Ig levels</i> (mean \pm SD; n = 7–8)					
IgM	100 \pm 13	87 \pm 16	86 \pm 31	67 \pm 16*	81 \pm 26
IgG	100 \pm 40	141 \pm 106	104 \pm 28	106 \pm 19	99 \pm 29
IgA	100 \pm 28	73 \pm 29	78 \pm 67	72 \pm 22	39 \pm 19*

Effect	Dose (mg/kg-d)				
	0	3	10	30	90
IgE	100 ± 65	50 ± 20	228 ± 351	145 ± 176	75 ± 55
<i>Cellularity (mean ± SD; n = 7–8)</i>					
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*
<i>Spleen cell distribution (%)</i>					
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
<i>Body (g) and organ (mg) weights (means; n = 7–8)</i>					
Body weight	305	282*	300	293	250*
Brain	1,858	1,864	1,859	1,784	1,743*
Heart	1,030	934*	1,000	967	863*
Kidney	1,986	1,761*	1,899	1,790*	1,626*
Liver	10,565	9,567	11,250	11,118	12,107*
Thymus	517 ± 47	472 ± 90	438 ± 64*	388 ± 71*	198 ± 65*
Spleen	551	590	538	596	505
Mandibular lymph nodes	152	123	160	141	89*
Mesenteric lymph nodes	165	148	130*	158	107*
Popliteal lymph nodes	19	18	19	17	10*
Thymus cortex surface area (% of total surface area of thymus; mean ± SD; n = 6–8)	77.9 ± 3.8	74.4 ± 2.2	79.2 ± 5.9	75.8 ± 4.0	68.9 ± 5.2*

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

Source: [De Jong et al. \(1999\)](#).

Statistically significant reductions were also observed in the relative cortex surface area of the thymus and thymic medullar weight at 90 mg/kg-day, but there was no difference in cell proliferation between treated and control animals using the proliferating cell nuclear antigen (PCNA) technique. Changes in the following immune parameters were noted: dose-related and statistically significant decrease in the relative number of B cells in the spleen at 10 (13%), 30 (18%), and 90 mg/kg-day (41%); significant decreases in absolute number of cells harvested in the spleen (31%), in the number of B cells in the spleen (61%), and NK cell activity in the spleen (E:T ratio was $40.9 \pm 28.4\%$ 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,

1 suggesting a role of thymus toxicity in the decreased (T-cell dependent) antibody production. In
2 addition to the effects on the thymus and spleen, histopathologic examination revealed treatment-
3 related lesions only in the liver and forestomach at the two highest dose levels, but the incidence
4 data for these lesions were not reported by [De Jong et al. \(1999\)](#). Increased incidence for
5 forestomach basal cell hyperplasia ($p < 0.05$ by Fisher's exact test) was reported at 30 and
6 90 mg/kg-day, and increased incidence for oval cell hyperplasia in the liver was reported at
7 90 mg/kg-day ($p < 0.01$, Fisher's exact test). The results indicate that 3 mg/kg-day was a no-
8 observed-adverse-effect level (NOAEL) for effects on hematological parameters (decreased RBC
9 count, hemoglobin, and hematocrit) and immune parameters (decreased thymus weight and
10 percent of B cells in the spleen) noted in Wistar rats at 10 mg/kg-day (the lowest-observed-
11 adverse-effect level [LOAEL]) and above. Lesions of the liver (oval cell hyperplasia) and
12 forestomach (basal cell hyperplasia) occurred at doses ≥ 30 mg/kg-day.

13 [Knuckles et al. \(2001\)](#) exposed male and female F344 rats (20/sex/dose group) to
14 benzo[a]pyrene (98% purity) at doses of 0, 5, 50, or 100 mg/kg-day in the diet for 90 days. Food
15 consumption and body weight were monitored, and the concentration of benzo[a]pyrene in the
16 food was adjusted every 3–4 days to maintain the target dose. The authors indicated that the actual
17 intake of benzo[a]pyrene by the rats was within 10% of the calculated intake, and the nominal
18 doses were not corrected to actual doses. Hematology and serum chemistry parameters were
19 evaluated. Urinalysis was also performed. Animals were examined for gross pathology, and
20 histopathology was performed on selected organs (stomach, liver, kidney, testes, and ovaries).
21 Statistically significant decreases in RBC counts and hematocrit level (decreases as much as 10 and
22 12%, respectively) were observed in males at doses ≥ 50 mg/kg-day and in females at 100 mg/kg-
23 day. A maximum 12% decrease (statistically significant) in hemoglobin level was noted in both
24 sexes at 100 mg/kg-day. Blood chemistry analysis showed a significant increase in blood urea
25 nitrogen (BUN) only in high-dose (100 mg/kg-day) males. Histopathology examination revealed an
26 apparent increase in the incidence of abnormal tubular casts in the kidney in males at 5 mg/kg-day
27 (40%), 50 mg/kg-day (80%), and 100 mg/kg-day (100%), compared to 10% in the controls. Only
28 10% of the females showed significant kidney tubular changes at the two high-dose levels
29 compared to zero animals in the female control group. The casts were described as molds of distal
30 nephron lumen and were considered by the study authors to be indicative of renal dysfunction.
31 From this study, male F344 rats appeared to be affected more severely by benzo[a]pyrene
32 treatment than the female rats. However, the statistical significance of the kidney lesions is unclear.
33 Several reporting gaps and inconsistencies regarding the reporting of kidney abnormalities in
34 [Knuckles et al. \(2001\)](#) make interpretation of the results difficult. Results of histopathological
35 kidney abnormalities (characterized primarily as kidney casts) were presented graphically and the
36 data were not presented numerically in this report. No indication was given in the graph that any
37 groups were statistically different than controls, although visual examination of the magnitude of
38 response and error bars appears to indicate a fourfold increase in kidney casts in males compared

1 to the control group (40 compared to 10%). The figure legend reported the data as “percentage
2 incidence of abnormal kidney tissues” and reported values as mean ± SD. However, the text under
3 the materials and methods section stated that Fisher’s exact test was used for histopathological
4 data, which would involve the pairwise comparison of incidence and not means. There are
5 additional internal inconsistencies in the data presented. The data appeared to indicate that
6 incidences for males were as follows: control, 10%; 5 mg/kg-day, 40%; 50 mg/kg-day, 80%; and
7 100 mg/kg-day, 100%; however, these incidences are inconsistent with the size of the study
8 groups, which were reported as 6–8 animals per group. The study authors were contacted, but did
9 not respond to EPA’s request for clarification of study design and/or results. Due to issues of data
10 reporting, a LOAEL could not be established for the increased incidence of kidney lesions. Based on
11 the statistically significant hematological effects including decreases in RBC counts, hematocrit, and
12 BUN, the NOAEL in males was 5 mg/kg-day and the LOAEL was 50 mg/kg-day, based on in F344
13 rats. No exposure-related histological lesions were identified in the stomach, liver, testes, or
14 ovaries in this study.

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

26 A significant, but not dose-dependent, increase in food consumption in males at
27 ≥1.5 mg/kg-day and a decrease in food consumption in females at ≥5 mg/kg-day was observed
28 ([Kroese et al., 2001](#)). Water consumption was statistically significantly altered in males only: a
29 decrease at 1.5, 5, and 15 mg/kg-day and an increase at 50 mg/kg-day. Organ weights of lung,
30 spleen, kidneys, adrenals, and ovaries were not affected by treatment. There was a dose-related,
31 statistically significant decrease in thymus weight in males at 15 and 50 mg/kg-day (decreased by
32 28 and 33%, respectively) and a significant decrease in thymus weight in females at 50 mg/kg-day
33 (decreased by 17%) (Table D-8). In both sexes, liver weight was statistically significantly increased
34 only at 50 mg/kg-day by about 18% (Table D-8).

1 **Table D-8. Exposure-related effects in Wistar rats exposed to benzo[a]pyrene**
 2 **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*

3
 4 *Significantly ($p < 0.05$) different from control mean; $n = 10/\text{sex}/\text{group}$.

5
 6 Source: [Kroese et al. \(2001\)](#).

7
 8 Hematological evaluation revealed only statistically nonsignificant, small, dose-related
 9 decreases in hemoglobin in both sexes and RBC counts in males. Clinical chemistry analysis
 10 showed a small, but statistically significant, increase in creatinine levels in males only at
 11 1.5 mg/kg-day, but this effect was not dose-dependent. A dose-dependent induction of liver
 12 microsomal EROD activity was observed, with a 5-fold induction at 1.5 mg/kg-day compared to
 13 controls, reaching 36-fold in males at 50 mg/kg-day; the fold induction in females at the top dose
 14 was less than in males. At necropsy, significant, dose-dependent macroscopic findings were not
 15 observed.

16 Histopathology examination revealed a statistically significant increase in basal cell
 17 hyperplasia in the forestomach of females at doses ≥ 15 mg/kg-day ([Kroese et al., 2001](#)). The
 18 induction of liver microsomal EROD was not accompanied by any adverse histopathologic findings
 19 in the liver at the highest dose, 50 mg/kg-day, so the livers from intermediate-dose groups were,
 20 therefore, not examined. An increased incidence of brown pigmentation of red pulp (hemosiderin)
 21 in the thymus was observed in treated animals of both sexes. However, this tissue was not
 22 examined in intermediate-dose groups. This range-finding, 5-week study identified a NOAEL of
 23 5 mg/kg-day and a LOAEL of 15 mg/kg-day, based on decreased thymus weight and forestomach
 24 hyperplasia in Wistar rats.

25 [Kroese et al. \(2001\)](#) exposed Wistar (Riv:TOX) rats (10/sex/dose group) to benzo[a]pyrene
 26 (98.6% purity, dissolved in soybean oil) by gavage at 0, 3, 10, or 30 mg/kg body weight-day,
 27 5 days/week for 90 days. The rats were examined daily for behavior and clinical symptoms and by
 28 palpation. Food and water consumption, body weights, morbidity, and mortality were monitored.

1 At the end of the exposure period, rats were subjected to macroscopic examination and organ
2 weights were recorded. Blood was collected for hematology and serum chemistry evaluation, and
3 urine was collected for urinalysis. All gross abnormalities, particularly masses and lesions
4 suspected of being tumors, were evaluated. The liver, stomach, esophagus, thymus, lung, spleen,
5 and mesenteric lymph node were examined histopathologically. In addition, cell proliferation in
6 forestomach epithelium was measured as the prevalence of S-phase epithelial cells displaying
7 bromodeoxyuridine (BrdU) incorporation.

8 There were no obvious effects on behavior of the animals, and no difference was observed
9 in survival or food consumption between exposed animals and controls ([Kroese et al., 2001](#)).
10 Higher water consumption and slightly lower body weights than the controls were observed in
11 males, but not females, at the high dose of 30 mg/kg-day. Hematological investigations showed
12 only nonsignificant, small dose-related decreases in RBC count and hemoglobin level in both sexes.
13 Clinical chemistry evaluation did not show any treatment-related group differences or dose-
14 response relationships for alanine aminotransferase, serum aspartate transaminase (AST), lactate
15 dehydrogenase (LDH), or creatinine, but a small dose-related decrease in γ -glutamyl transferase
16 activity was observed in males only. Urinalysis revealed an increase in urine volume in males at
17 30 mg/kg-day, which was not dose related. At the highest dose, both sexes showed increased levels
18 of urinary creatinine and a dose-related increase in urinary protein. However, no further
19 investigation was conducted to determine the underlying mechanisms for these changes. At
20 necropsy, reddish to brown/gray discoloration of the mandibular lymph nodes was consistently
21 noted in most rats; occasional discoloration was also observed in other regional lymph nodes
22 (axillary). Statistically significant increases in liver weight were observed at 10 and 30 mg/kg-day
23 in males (15 and 29%) and at 30 mg/kg-day in females (17%). A decrease in thymus weight was
24 seen in both sexes at 30 mg/kg-day (17 and 33% decrease in females and males, respectively,
25 compared with controls) (Table D-9). At 10 mg/kg-day, thymus weight in males was decreased by
26 15%, but the decrease did not reach statistical significance.

1 **Table D-9. Means \pm SD^a for liver and thymus weights in Wistar rats exposed to**
 2 **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 \pm 0.97	8.00 \pm 0.85	8.62 \pm 1.30*	9.67 \pm 1.17*
Females	5.54 \pm 0.70	5.42 \pm 0.76	5.76 \pm 0.71	6.48 \pm 0.78*
Thymus weight (mg)				
Males	380 \pm 60	380 \pm 110	330 \pm 60	270 \pm 40*
Females	320 \pm 60	310 \pm 50	300 \pm 40	230 \pm 30*

3
 4 *Significantly ($p < 0.05$) different from control mean; student t-test (unpaired, two-tailed); n = 10/sex/group.

5 ^aReported as SE, but judged to be SD (and confirmed by study authors).

6
 7 Source: [Kroese et al. \(2001\)](#).

8
 9 Histopathologic examination revealed what was characterized by [Kroese et al. \(2001\)](#) as
 10 basal cell disturbance in the epithelium of the forestomach in males ($p < 0.05$) and females
 11 ($p < 0.01$) at 30 mg/kg-day. The basal cell disturbance was characterized by increased number of
 12 basal cells, mitotic figures, and remnants of necrotic cells; occasional early nodule development;
 13 infiltration by inflammatory cells (mainly histiocytes); and capillary hyperemia, often in
 14 combination with the previous changes ([Kroese et al., 2001](#)). Incidences for these lesions (also
 15 described as “slight basal cell hyperplasia”) in the 0, 3, 10, and 30-mg/kg-day groups were 0/10,
 16 2/10, 3/10, and 7/10, respectively, in female rats and 2/10, 0/10, 6/10, and 7/10, respectively, in
 17 male rats. Nodular hyperplasia was noted in one animal of each sex at 30 mg/kg-day. A significant
 18 ($p < 0.05$) increase in proliferation of forestomach epithelial cells was detected at doses
 19 ≥ 10 mg/kg-day by morphometric analysis of nuclei with BrdU incorporation. The mean numbers
 20 of BrdU-staining nuclei per unit surface area of the underlying lamina muscularis mucosa were
 21 increased by about two- and three–fourfold at 10 and 30 mg/kg-day, respectively, compared with
 22 controls. A reduction of thymus weight and increase in the incidence of thymus atrophy (the report
 23 described the atrophy as slight, but did not specify the full severity scale used in the pathology
 24 examination) was observed in males only at 30 mg/kg-day ($p < 0.01$ compared with controls).
 25 Respective incidences for thymus atrophy for the control through high-dose groups were 0/10,
 26 0/10, 0/10, and 3/10 for females and 0/10, 2/10, 1/10, and 6/10 for males. No significant
 27 differences were observed in the lungs of control and treated animals. In the esophagus,
 28 degeneration and regeneration of muscle fibers and focal inflammation of the muscular wall were
 29 judged to be a result of the gavage dosing rather than of benzo[a]pyrene treatment.

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

1 **Chronic Studies and Cancer Bioassays**

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

18 At 104 weeks, survival in the control group was 65% (males) and 50% (females), whereas
19 mortality in the 30 mg/kg-day dose group was 100% after about week 70. At 80 weeks, survival
20 percentages were about 90, 85, and 75% in female rats in the 0, 3, and 10 mg/kg-day groups,
21 respectively; in males, respective survival percentages were ~95, 90, and 85% at 80 weeks.
22 Survival of 50% of animals occurred at 104, 104, ~90, and 60 weeks for control through high-dose
23 females; for males, the respective times associated with 65% survival were 104, 104, 104, and
24 ~60 weeks. The high mortality rate in high-dose rats was attributed to liver or forestomach tumor
25 development, not to noncancer systemic effects. After 20 weeks, body weight was decreased
26 (compared with controls by >10%) in 30-mg/kg-day males, but not in females. This decrease was
27 accompanied by a decrease in food consumption. Body weights and food consumption were not
28 adversely affected in the other dose groups compared to controls. In males, there was a dose-
29 dependent increase in water consumption starting at week 13, but benzo[a]pyrene treatment had
30 no significant effects on water consumption in females.

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

1 (without diffuse hyperplasia) in the forestomach of rats in the preliminary range-finding and
 2 90-day exposure studies. At the highest dose level, 59% of rats with forestomach tumors had
 3 malignant carcinomas (60/102; Table D-10). Other tissue sites with significantly elevated
 4 incidences of tumors in the 30 mg/kg-day dose group included the oral cavity (papilloma and
 5 squamous cell carcinoma [SCC]) in both sexes, and the jejunum (adenocarcinoma), kidney (cortical
 6 adenoma), and skin (basal cell adenoma and carcinoma) in male rats (Table D-10). In addition,
 7 auditory canal tumors (carcinoma or squamous cell papilloma originating from pilo-sebaceous
 8 units including the Zymbal’s gland) were also detected in both sexes at 30 mg/kg-day, but auditory
 9 canal tissue was not histologically examined in the lower dose groups and the controls
 10 (Table D-10). Gross examination revealed auditory canal tumors only in the high-dose group.

11 **Table D-10. Incidences of exposure-related neoplasms in Wistar rats treated**
 12 **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*

Supplemental Information—Benzo[a]pyrene

	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.

^aThis group had significantly decreased survival.

^bIncidences 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

1 occurrence. However, incidences were reported for nonneoplastic lesions in tissues or organs in
2 which tumors were detected (i.e., oral cavity, esophagus, forestomach, jejunum, liver, kidney, skin,
3 mammary, and auditory canal). The reported nonneoplastic lesions associated with exposure were
4 the forestomach basal cell hyperplasia and clear cell foci of cellular alteration in the liver.
5 Incidences for forestomach basal cell hyperplasia in the control through high-dose groups were
6 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
7 hepatic clear cell foci of cellular alteration were 22/52, 33/52, 4/52, and 2/52 for females and
8 8/52, 22/52, 1/52, and 1/52 for males. These results indicate that the lowest dose group,
9 3 mg/kg-day, was a LOAEL for increased incidence of forestomach hyperplasia and hepatic
10 histological changes in male and female Wistar rats exposed by gavage to benzo[a]pyrene for up to
11 104 weeks (see Table D-10). The lack of an increase in incidence of these nonneoplastic lesions in
12 the forestomach and liver at the intermediate and high doses (compared with controls) was
13 associated with increased incidences of forestomach and liver tumors at these dose levels. The
14 authors of this study noted that nonneoplastic effects were not quantified in organs with tumors.

15 As an adjunct study to the 2-year gavage study with Wistar rats, [Kroese et al. \(2001\)](#)
16 sacrificed additional rats (6/sex/group) after 4 and 5 months of exposure (0, 1, 3, 10, or
17 30 mg/kg-day) for analysis of DNA adduct formation in WBCs and major organs and tissues.
18 Additional rats (6/sex/time period) were exposed to 0.1 mg/kg-day benzo[a]pyrene for 4 and
19 5 months for analysis of DNA adduct formation. Using the [³²P]-postlabeling technique, five
20 benzo[a]pyrene-DNA adducts were identified in all of the examined tissues at 4 months (WBCs,
21 liver, kidney, heart, lung, skin, forestomach, glandular stomach, brain). Only one of these adducts
22 (adduct 2) was identified based on co-chromatography with a standard. This adduct, identified as
23 10β-(deoxyguanosin-N2-yl)-7β,8α,9α-trihydroxy-7,8,9,10 tetrahydro-benzo[a]pyrene, was the
24 predominant adduct in all organs of female rats exposed to 10 mg/kg-day, except the liver and
25 kidney, in which another adduct (unidentified adduct 4) was predominant. Levels of total adducts
26 (number of benzo[a]pyrene-DNA adducts per 10¹⁰ nucleotides) in examined tissues (from the
27 single 10 mg/kg-day female rat) showed the following order: liver > heart > kidney > lung > skin >
28 forestomach ≈ WBCs > brain. Mean values for female levels of total benzo[a]pyrene-DNA adducts
29 (number per 10¹⁰ nucleotides) in four organs showed the same order, regardless of exposure
30 group: liver > lung > forestomach ≈ WBCs; comparable data for males were not reported. Mean
31 total benzo[a]pyrene-DNA adduct levels in livers increased in both sexes from about 100 adducts
32 per 10¹⁰ nucleotides at 0.1 mg/kg-day to about 70,000 adducts per 10¹⁰ nucleotides at 30 mg/kg-
33 day. In summary, these results suggest that total benzo[a]pyrene-DNA adduct levels in tissues at 4
34 months were not independently associated with the carcinogenic responses noted after 2 years of
35 exposure to benzo[a]pyrene. The liver showed the highest total DNA adduct levels and a
36 carcinogenic response, but total DNA adduct levels in heart, kidney, and lung (in which no
37 carcinogenic responses were detected) were higher than levels in forestomach and skin (in which
38 carcinogenic responses were detected).

1 Groups of Sprague-Dawley rats (32/sex/dose) were fed diets delivering a daily dose of
2 0.15 mg benzo[a]pyrene/kg body weight every ninth day or 5 times/week ([Brune et al., 1981](#)).
3 Other groups (32/sex/dose) were given gavage doses of 0.15 mg benzo[a]pyrene (in aqueous 1.5%
4 caffeine solution)/kg every ninth day, every third day, or 5 times/week. The study included an
5 untreated control group (to compare with the dietary exposed groups) and a gavage vehicle control
6 group (each with 32 rats/sex). Rats were treated until moribundity or death occurred, with
7 average annual doses reported in Table D-11 [mg/kg-year, calculated by [Brune et al. \(1981\)](#)]. The
8 following tissues were prepared for histopathological examination: tongue, larynx, lung, heart,
9 trachea, esophagus, stomach, small intestine, colon, rectum, spleen, liver, urinary bladder, kidney,
10 adrenal gland, and any tissues showing tumors or other gross changes. Survival was similar among
11 the groups, with the exception that the highest gavage-exposure group showed a decreased median
12 time of survival (Table D-11). Significantly increased incidences of portal-of-entry tumors
13 (forestomach, esophagus, and larynx) were observed in all of the gavage-exposed groups and in the
14 highest dietary exposure group (Table D-11). Following dietary administration, all observed
15 tumors were papillomas. Following gavage administration, two malignant forestomach tumors
16 were found (one each in the mid- and high-dose groups) and the remaining tumors were benign.
17 The data in Table D-11 show that the carcinogenic response to benzo[a]pyrene was stronger with
18 the gavage protocol compared with dietary exposure, and that no distinct difference in response
19 was apparent between the sexes. Tumors at distant sites (mammary gland, kidney, pancreas, lung,
20 urinary bladder, testes, hematopoietic, and soft tissue) were not considered treatment-related as
21 they were also observed at similar rates in the control group (data not provided). The study report
22 did not address noncancer systemic effects.

23

1 **Table D-11. Incidences of alimentary tract tumors in Sprague-Dawley rats**
 2 **chronically exposed to benzo[a]pyrene in the diet or by gavage in caffeine**
 3 **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)
<i>Benzo[a]pyrene by gavage in 1.5% caffeine solution</i>				
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
<i>Benzo[a]pyrene in diet</i>				
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

4
5 *Significantly ($p < 0.1$) different from control using a modified χ^2 test that accounted for group differences in
6 survival time.

7 **Significantly ($p < 0.05$) different from control using a modified χ^2 test that accounted for group differences in
8 survival time.

9 ^aAverage annual dose divided by 365 days.

10 ^bNo sex-specific forestomach tumor incidence data were reported by [Brune et al. \(1981\)](#).

11 ^cSex-specific incidences for total alimentary tract tumors were reported as follows:

12 Gavage (control, high dose): Male: 6/32, 7/32, 15/32, 8/32

13 Female: 0/32, 6/32, 11/32, 6/32

14 Diet (control, high dose): Male: 3/32, 3/32, 8/32

15 Female: 0/32, 0/32, 2/32

16
17 Source: [Brune et al. \(1981\)](#).

18
19 In the other modern cancer bioassay with benzo[a]pyrene, female B6C3F₁ mice (48/dose
20 group) were administered benzo[a]pyrene (98.5% purity) at concentrations of 0 (acetone vehicle),
21 5, 25, or 100 ppm in the diet for 2 years ([Beland and Culp, 1998](#); [Culp et al., 1998](#)). This study was
22 designed to compare the carcinogenicity of coal tar mixtures with that of benzo[a]pyrene and it
23 included groups of mice fed diets containing one of several concentrations of two coal tar mixtures.
24 Benzo[a]pyrene was dissolved in acetone before mixing with the feed. Control mice received only
25 acetone-treated feed. Female mice were chosen because they have a lower background incidence of
26 lung tumors than male B6C3F₁ mice. [Culp et al. \(1998\)](#) reported that the average daily intakes of
27 benzo[a]pyrene in the 25- and 100-ppm groups were 104 and 430 $\mu\text{g}/\text{day}$, but did not report the
28 intake for the 5-ppm group. Based on the assumption that daily benzo[a]pyrene intake at 5 ppm
29 was one-fifth of the 25-ppm intake (about 21 $\mu\text{g}/\text{day}$), average daily doses for the three

1 benzo[a]pyrene groups are estimated as 0.7, 3.3, and 16.5 mg/kg-day. Estimated doses were
2 calculated using time-weighted average (TWA) body weights of 0.032 kg for the control, 5- and
3 25-ppm groups and 0.026 kg for the 100-ppm group (estimated from graphically presented data).
4 Food consumption, body weights, morbidity, and mortality were monitored at intervals, and lung,
5 kidneys, and liver were weighed at sacrifice. Necropsy was performed on all mice that died during
6 the experiment or survived to the end of the study period. Limited histopathologic examinations
7 (liver, lung, small intestine, stomach, tongue, esophagus) were performed on all control and high-
8 dose mice and on all mice that died during the experimental period, regardless of treatment group.
9 In addition, all gross lesions found in mice of the low- and mid-dose groups were examined
10 histopathologically.

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

23 Papillomas and/or carcinomas of the forestomach, esophagus, tongue, and larynx at
24 elevated incidences occurred in groups of mice exposed to 25 or 100 ppm, but no exposure-related
25 tumors occurred in the liver or lung ([Beland and Culp, 1998](#); [Culp et al., 1998](#)). The forestomach
26 was the most sensitive tissue, demonstrated the highest tumor incidence among the examined
27 tissues, and was the only tissue with an elevated incidence of tumors at 25 ppm (Table D-12). In
28 addition, most of the forestomach tumors in the exposed groups were carcinomas, as 1, 31, and
29 45 mice had forestomach carcinomas in the 5-, 25-, and 100-ppm groups, respectively.
30 Nonneoplastic lesions were also found in the forestomach at significantly ($p < 0.05$) elevated
31 incidences: hyperplasia at ≥ 25 ppm and hyperkeratosis at ≥ 25 ppm (Table D-12). The esophagus
32 was the only other examined tissue showing elevated incidence of a nonneoplastic lesion (basal cell
33 hyperplasia, see Table D-12). Tumors (papillomas and carcinomas) were also significantly elevated
34 in the esophagus and tongue at 100 ppm (Table D-12). Esophageal carcinomas were detected in
35 1 mouse at 25 ppm and 11 mice at 100 ppm. Tongue carcinomas were detected in seven 100-ppm
36 mice; the remaining tongue tumors were papillomas. Although incidences of tumors of the larynx
37 were not significantly elevated in any of the exposed groups, a significant dose-related trend was
38 apparent (Table D-12).

1 **Table D-12. Incidence of nonneoplastic and neoplastic lesions in female**
 2 **B6C3F₁ 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)

3
 4 *Significantly different from control incidence ($p < 0.05$); using a modified Bonferonni procedure for multiple
 5 comparisons to the same control.

6 ^aSignificant ($p < 0.05$) dose-related trend calculated for incidences of these lesions.

7
 8 Sources: [Beland and Culp \(1998\)](#); [Culp et al. \(1998\)](#).

9
 10 [Neal and Rigdon \(1967\)](#) fed benzo[a]pyrene (purity not reported) at concentrations of 0, 1,
 11 10, 20, 30, 40, 45, 50, 100, and 250 ppm to male and female CFW-Swiss mice in the diet.
 12 Corresponding doses (in mg/kg-day) were calculated¹ as 0, 0.2, 1.8, 3.6, 5.3, 7.1, 8, 8.9, 17.8, and
 13 44.4 mg/kg-day. The age of the mice ranged from 17 to 180 days old and the treatment time was
 14 from 1 to 197 days; the size of the treated groups ranged from 9 to 73. There were 289 mice
 15 (number of mice/sex not stated) in the control group. No forestomach tumors were reported at 0,
 16 0.2, or 1.8 mg/kg-day. The incidences of forestomach tumors at 20, 30, 40, 45, 50, 100, and
 17 250 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,
 18 23/34, 19/23, and 66/73, respectively.

¹Calculation: 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.

1 **Other Oral Exposure Cancer Bioassays in Mice**

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

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

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

Supplemental Information—Benzo[a]pyrene

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

Supplemental Information—Benzo[a]pyrene

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.	<table border="0"> <thead> <tr> <th>ppm</th> <th>Exposure (d)</th> <th>Fore-stomach tumor incidence</th> </tr> </thead> <tbody> <tr><td>1</td><td>110</td><td>0/25</td></tr> <tr><td>10</td><td>110</td><td>0/24</td></tr> <tr><td>20</td><td>110</td><td>1/23</td></tr> <tr><td>30</td><td>110</td><td>0/37</td></tr> <tr><td>40</td><td>110</td><td>1/40</td></tr> <tr><td>45</td><td>110</td><td>4/40</td></tr> <tr><td>50</td><td>152</td><td>24/34</td></tr> <tr><td>100</td><td>110</td><td>19/23</td></tr> <tr><td>250</td><td>118</td><td>66/73</td></tr> </tbody> </table>	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)
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																																
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: Carcinoma papilloma Dose (µg) 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)																														

Supplemental Information—Benzo[a]pyrene

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)

1 **D.4.2. Inhalation Studies**

2 **Short-Term and Subchronic Studies**

3 [Wolff et al. \(1989\)](#) exposed groups of 40 male and 40 female F344/Crl rats, via nose only, to
4 7.5 mg benzo[a]pyrene/m³ for 2 hours/day, 5 days/week for 4 weeks (corresponding to a TWA of
5 0.45 mg/m³). Rats were 10–11 weeks old at the beginning of the experiment. Benzo[a]pyrene
6 (>98% pure) aerosols were formed by heating and then condensing the vaporized benzo[a]pyrene.
7 The particle mass median aerodynamic diameter (MMAD) was 0.21 µm. Subgroups of these
8 animals (six/sex/dose) were exposed for 4 days or 6 months after the end of the 4-week exposure
9 to radiolabeled aluminosilicate particles. Lung injury was assessed by analyzing clearance of
10 radiolabeled aluminosilicate particles and via histopathologic evaluations. Body and lung weights,
11 measured in subgroups from 1 day to 12 months after the exposure did not differ between controls
12 and treated animals. Radiolabeled particle clearance did not differ between the control and treated
13 groups, and there were no significant lung lesions. This study identified a NOAEL for lung effects of
14 0.45 mg/m³ for a short-term exposure.

15 **Chronic Studies and Cancer Bioassays**

16 [Thyssen et al. \(1981\)](#) conducted an inhalation study in which male Syrian golden hamsters
17 were exposed to benzo[a]pyrene for their natural lifetime. Groups of 24 animals (8 weeks old)
18 were exposed by nose-only inhalation to NaCl aerosols (controls; 240 µg NaCl/m³) or
19 benzo[a]pyrene condensed onto NaCl aerosols at three target concentrations of 2, 10, or 50 mg
20 benzo[a]pyrene/m³ for 3–4.5 hours/day, 5 days/week for 1–41 weeks, followed by 3 hours/day,
21 7 days/week for the remainder of study (until hamsters died or became moribund). [Thyssen et al.](#)
22 [\(1981\)](#) reported average measured benzo[a]pyrene concentrations to be 0, 2.2, 9.5, or 46.5 mg/m³.
23 More than 99% of the particles were between 0.2 and 0.5 µm in diameter, and over 80% had
24 diameters between 0.2 and 0.3 µm. The particle analysis of the aerosols was not reported to
25 modern standards (MMAD and geometric SD were not reported). Final overall group sizes were
26 larger as animals dying during the first 12 months of the study were replaced.

27 Review of the individual animal data (including individual animal pathology reports, time-
28 to-death data, and exposure chamber monitoring data) provided by Thyssen et al. to EPA ([U.S. EPA,](#)
29 [1990](#)) revealed several discrepancies in the reported exposure protocol. The actual exposure

1 protocol was as follows: 4.5 hours/day, 5 days/week on weeks 1–12; 3 hours/day, 5 days/week on
2 weeks 13–29; 3.7 hours/day, 5 days/week on week 30; 3 hours/day, 5 days/week on weeks 31–41;
3 and 3 hours/day, 7 days/week for the remainder of the experiment.

4 Analytical chamber monitoring data were generally recorded about once or twice per week,
5 with some exceptions ranging from no measurements for a 3-week period to as many as five
6 measurements in 1 week. Individual measurements (in mg/m³) were 0.2–4.52, 1.16–19.2, and
7 0.96–118.6 in the 2, 10, and 50 mg/m³ target concentration groups, respectively. Overall, weekly
8 average exposure concentrations varied two- to fivefold from the overall average for each group
9 over the course of the study, with no particular trends over time (data not shown). The 95%
10 confidence limits for the average exposure level over time in each group varied within 4–7% of the
11 averages. Because some animals were started at different times and the exposure protocol changed
12 over time, each individual animal had an exposure history somewhat different than others in the
13 same exposure group. In order to address this variability, [U.S. EPA \(1990\)](#) used the individual
14 animal data and the chamber monitoring data to calculate a lifetime average continuous exposure
15 for each individual hamster. Group averages of these individual TWA concentrations were 0, 0.25,
16 1.01, and 4.29 mg/m³ for the control through high-exposure groups.

17 Statistical analysis of outcomes was not reported by [Thyssen et al. \(1981\)](#). Survival was
18 similar in the control, low-, and mid-exposure groups, but was decreased about 40% in the high-
19 exposure group. Average survival times in the control, low-, mid-, and high-exposure groups were
20 96.4 ± 27.6 , 95.2 ± 29.1 , 96.4 ± 27.8 , and 59.5 ± 15.2 weeks, respectively. After the 60th week, body
21 weights decreased and mortality increased steeply in the highest exposure group. Histologic
22 examination of organs² revealed an exposure-related increase in the mid- and high-exposure
23 groups of benign and malignant tumors of the upper respiratory tract, including the nasal cavity,
24 larynx, and trachea, and of the upper digestive tract, including the pharynx, esophagus, and
25 forestomach (Table D-13). No lung tumors were observed. Tumors were detected in other sites,
26 but none of these appeared to be related to exposure.

27

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

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

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

4
 5 ^aHistopathology incidence data from the raw data obtained from the Thyssen study ([Clement Associates, 1990](#)),
 6 adjusted to show animals only on study long enough to be at risk of tumor development: at least 1 year (0 2, or
 7 10 mg/m³ groups) or until the first tumor occurrence (week 40 in the 50 mg/m³ group). See Table E-17 for a list of
 8 all animals with histopathology results.

9 ^bSee text.

10 ^cExcludes animals with unexamined tissues, unless a tumor was diagnosed in the tissues that were examined.

11 ^dFractions represent the number of animals diagnosed with at least one of the specified tumors, among the
 12 animals examined for each tissue.

13 ^eStatistically significant trends by Cochran-Armitage trend test, conducted by EPA: all tumors: $p < 0.0001$,
 14 malignant tumors only: $p < 0.0001$.

15 ^fIncludes one animal with an in situ carcinoma in the larynx.

16
 17 The tumor types observed in the upper respiratory and upper digestive tract were very
 18 similar, characterized as polyps, papillomas, papillary polyps, and squamous carcinomas, with the
 19 exceptions of one in situ carcinoma and one adenocarcinoma (both in the mid-exposure group),
 20 reflecting similar cell types. Consequently, evaluation of the overall cancer hazard included
 21 consideration of the joint incidence of these tumor types. The pharynx and larynx (including the
 22 epiglottis), clearly the main cancer targets, can be difficult to distinguish given their close proximity.
 23 There were a few instances of nasal cavity or trachea tumors among animals without larynx or
 24 pharynx tumors. Tumors of the upper digestive tract may have been a consequence of mucociliary
 25 particle clearance ([Thyssen et al., 1981](#)), but the tumors in the esophagus and forestomach
 26 observed in the mid- and high-exposure groups all occurred in animals that also had pharynx or
 27 respiratory tract tumors. Overall, there were increasing trends in tumor incidence with increasing
 28 exposure, both for the combined incidence of benign or malignant tumors, or for only malignant
 29 tumors (Table D-14), and earlier occurrence of tumors with increasing exposure levels. Several
 30 studies have investigated the carcinogenicity of benzo[a]pyrene in hamsters exposed by
 31 intratracheal instillation. Single-dose studies verified that benzo[a]pyrene is tumorigenic, but do

1 not provide data useful for characterizing dose-response relationships because of their design
2 ([Kobayashi, 1975](#); [Renzik-Schüller and Mohr, 1974](#); [Henry et al., 1973](#); [Mohr, 1971](#); [Saffiotti et al.,](#)
3 [1968](#); [Gross et al., 1965](#); [Herrold and Dunham, 1962](#)). One multiple-dose study, which utilized very
4 low doses (0.005, 0.02, and 0.04 mg once every 2 weeks), failed to find any tumorigenic response
5 ([Kunstler, 1983](#)). Tumorigenic responses (mostly in the respiratory tract) were found at higher
6 dosage levels (0.25–2 mg benzo[a]pyrene once per week for 30–52 weeks) in four multiple-dose
7 studies ([Feron and Kruysse, 1978](#); [Ketkar et al., 1978](#); [Feron et al., 1973](#); [Saffiotti et al., 1972](#)).
8 These studies identify the respiratory tract as a cancer target with exposure to benzo[a]pyrene by
9 intratracheal instillation and provide supporting evidence for the carcinogenicity of
10 benzo[a]pyrene at portal-of-entry sites.

11 **D.4.3. Dermal studies**

12 ***Skin-Tumor Initiation-Promotion Assays***

13 Results from numerous studies indicate that acute dermal exposure to benzo[a]pyrene
14 induces skin tumors in mice when followed by repeated exposure to a potent tumor promoter
15 ([Weyand et al., 1992](#); [Cavalieri et al., 1991](#); [Rice et al., 1985](#); [El-Bayoumy et al., 1982](#); [Lavoie et al.,](#)
16 [1982](#); [Raveh et al., 1982](#); [Cavalieri et al., 1981](#); [Slaga et al., 1980](#); [Wood et al., 1980](#); [Slaga et al.,](#)
17 [1978](#); [Hoffmann et al., 1972](#)). The typical exposure protocol in these studies involved the
18 application of a single dose of benzo[a]pyrene (typically ≥ 20 nmol per mouse) to dorsal skin of mice
19 followed by repeated exposure to a potent tumor promoter, such as 12-O-tetradecanoylphorbol-
20 13-acetate (TPA).

21 ***Carcinogenicity Bioassays***

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

32 This section discusses complete carcinogenicity bioassays in mice that provide the best
33 available dose-response data for skin tumors caused by repeated dermal exposure to
34 benzo[a]pyrene ([Sivak et al., 1997](#); [Higginbotham et al., 1993](#); [Albert et al., 1991](#); [Grimmer et al.,](#)
35 [1984](#); [Habs et al., 1984](#); [Grimmer et al., 1983](#); [Habs et al., 1980](#); [Schmähl et al., 1977](#); [Schmidt et al.,](#)
36 [1973](#); [Roe et al., 1970](#); [Poel, 1963, 1959](#)). Early studies of benzo[a]pyrene complete carcinogenicity

1 in mouse skin ([Wynder and Hoffmann, 1959](#); [Wynder et al., 1957](#)) are not further described herein,
2 because the investigators applied solutions of benzo[a]pyrene at varying concentrations on the
3 skin, but did not report volumes applied. As such, applied doses in these studies cannot be
4 determined. Other complete carcinogenicity mouse skin tumor bioassays with benzo[a]pyrene are
5 available, but these are not described further in this review, because: (1) they only included one
6 benzo[a]pyrene dose level (e.g., [Emmett et al., 1981](#)) or only dose levels inducing 90–100%
7 incidence of mice with tumors (e.g., [Wilson and Holland, 1988](#); [Warshawsky and Barkley, 1987](#)) and
8 thus provide no information about the shape of the dose-response relationship; (2) they used a
9 1-time/week (e.g., [Nesnow et al., 1983](#)) or 1-time every 2 weeks (e.g., [Levin et al., 1977](#)) exposure
10 protocol, which is less useful for extrapolating to daily human exposure; or (3) they used a vehicle
11 demonstrated to interact with or enhance benzo[a]pyrene carcinogenicity ([Bingham and Falk,
12 1969](#)).

13 [Poel \(1959\)](#) applied benzo[a]pyrene in toluene to shaved interscapular skin of groups of
14 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
15 for up to 103 weeks or until the appearance of a tumor by gross examination (3 times weekly).
16 Some organs (not further specified) and interscapular skin in sacrificed mice were examined
17 histologically. With increasing dose level, the incidence of mice with skin tumors increased and the
18 time of tumor appearance decreased (see Table D-15). Doses >3.8 µg were associated with 100%
19 mortality after increasingly shorter exposure periods, none greater than 44 weeks. [Poel \(1959\)](#) did
20 not mention the appearance of exposure-related tumors in tissues other than interscapular skin.

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

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

3
 4 ^aIndicated doses were applied to interscapular skin 3 times/week for up to 103 weeks or until time of appearance
 5 of a grossly detected skin tumor.

6 ^bCarcinomas were histologically confirmed.

7 ^cRanges reflect differing information in Tables 4 and 6 of [Poel \(1959\)](#).

8
 9 Source: [Poel \(1959\)](#).

10
 11 [Poel \(1963\)](#) applied benzo[a]pyrene in a toluene vehicle to shaved interscapular skin of
 12 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,
 13 19.0, 94.0, or 470 μg benzo[a]pyrene per application, until mice died or a skin tumor was observed.
 14 Time ranges for tumor observations were provided, but not times of death for mice without tumors,
 15 so it was not possible to evaluate differential mortality among all dose groups or the length of
 16 exposure for mice without tumors. With increasing dose level, the incidence of mice with skin
 17 tumors increased and the time of tumor appearance decreased (Table D-16). The lowest dose level
 18 did not induce an increased incidence of mice with skin tumors in any strain, but strain differences
 19 in susceptibility were evident at higher dose levels. SWR and C3HeB mice showed skin tumors at
 20 doses ≥ 0.38 μg benzo[a]pyrene, whereas AH/e mice showed tumors at doses ≥ 19 μg
 21 benzo[a]pyrene (Table D-16). Except for metastases of the skin tumors to lymph nodes and lung,
 22 [Poel \(1963\)](#) did not mention the appearance of exposure-related tumors in tissues other than
 23 interscapular skin.

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

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

4
 5 ^aIndicated doses were applied 3 times/week for life or until a skin tumor was detected. Mice were 10–14 weeks
 6 old at initial exposure.

7 ^bIncidence of mice exposed ≥ 10 weeks with a skin tumor.

8
 9 Source: [Poel \(1963\)](#).

10
 11 [Roe et al. \(1970\)](#) treated groups of 50 female Swiss mice with 0 (acetone vehicle), 0.1, 0.3, 1,
 12 3, or 9 μg benzo[a]pyrene applied to the shaved dorsal skin 3 times/week for up to 93 weeks; all
 13 surviving mice were killed and examined for tumors during the following 3 weeks. The dorsal skin
 14 of an additional control group was shaved periodically but was not treated with the vehicle. Mice
 15 were examined every 2 weeks for the development of skin tumors at the site of application.
 16 Histologic examinations included: (1) all skin tumors thought to be possibly malignant; (2) lesions
 17 of other tissues thought to be neoplastic; and (3) limited nonneoplastic lesions in other tissues. As
 18 shown in Table D-17, markedly elevated incidences of mice with skin tumors were only found in
 19 the two highest dose groups (3 and 9 μg), compared with no skin tumors in the control groups.
 20 Malignant skin tumors (defined as tumors with invasion or penetration of the panniculus carnosus
 21 muscle) were detected in 4/41 and 31/40 mice in the 3- and 9- μg groups, respectively, surviving to
 22 at least 300 days. Malignant lymphomas were detected in all groups, but the numbers of cases were
 23 not elevated compared with expected numbers after adjustment for survival differences. Lung
 24 tumors were likewise detected in control and exposed groups at incidences that were not
 25 statistically different.

1 **Table D-17. Tumor incidence in female Swiss mice dermally exposed to**
 2 **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%)

3
 4 ^aDoses were applied 3 times/week for up to 93 weeks to shaved dorsal skin.

5 ^bNumerator: number of mice detected with a skin tumor. Denominator: number of mice surviving to 300 days for
 6 all groups except the highest dose group. For the highest dose group (in which skin tumors were first detected
 7 between 200 and 300 days), the number of mice surviving to 200 days was used as the denominator.

8 ^cNumerator: number of mice detected with specified tumor. Denominator: number of mice surviving to 300 days
 9 unless a tumor was detected earlier, in which case, the number dying before 300 days without a tumor was
 10 subtracted from the number of animals reported to have been examined.

11
 12 Source: [Roe et al. \(1970\)](#).

13
 14 [Schmidt et al. \(1973\)](#) dermally administered benzo[a]pyrene in acetone to female NMRI
 15 mice (100/group) and female Swiss mice. Benzo[a]pyrene was applied to the shaved dorsal skin
 16 twice weekly at doses of 0, 0.05, 0.2, 0.8, or 2 μg until spontaneous death occurred or until an
 17 advanced carcinoma was observed. Skin carcinomas were identified by the presence of crater-
 18 shaped ulcerations, infiltrative growth, and the beginning of physical wasting (i.e., cachexia).
 19 Necropsy was performed for all animals, and histopathological examination of the dermal site of
 20 application and any other tissues with gross abnormalities was conducted. Skin tumors were
 21 observed at the two highest doses in both strains of female mice (see Table D-18), with induction
 22 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
 23 Swiss mice, respectively. The authors indicated that the latency period for tumor formation was
 24 highly variable, and significant differences among exposure groups could not be identified, but no
 25 further timing information was available, including overall survival. Carcinoma was the primary
 26 tumor type seen after lifetime application of benzo[a]pyrene to mouse skin.

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

Dose (μg) ^a	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma
<i>Female NMRI mice</i>			
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%)
<i>Female Swiss mice</i>			
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%)

3
 4 ^aMice were exposed until natural death or until they developed a carcinoma at the site of application; indicated
 5 doses were applied 2 times/week to shaved skin of the back.

6
 7 Source: [Schmidt et al. \(1973\)](#).

8
 9 [Schmähl et al. \(1977\)](#) applied benzo[a]pyrene 2 times/week to the shaved dorsal skin of
 10 female NMRI mice (100/group) at doses of 0, 1, 1.7, or 3 μg in 20 μL acetone. The authors reported
 11 that animals were observed until natural death or until they developed a carcinoma at the site of
 12 application. The effective numbers of animals at risk was about 80% of the nominal group sizes,
 13 which the authors attributed to autolysis; no information was provided concerning when tumors
 14 appeared in the relevant groups, how long treatment lasted in each group, or any times of death.
 15 Necropsy was performed on all mice and the skin of the back, as well as any organs that exhibited
 16 macroscopic changes, were examined histopathologically. The incidence of all types of skin tumors
 17 was increased in a dose-related manner compared to controls (see Table D-19). Carcinoma was the
 18 primary tumor type observed following chronic dermal exposure to benzo[a]pyrene, and skin
 19 papillomas occurred infrequently. Dermal sarcoma was not observed.

1 **Table D-19. Skin tumor incidence in female NMRI mice dermally exposed to**
 2 **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%)

3
 4 ^aMice were exposed until natural death or until they developed a carcinoma at the site of application; indicated
 5 doses were applied 2 times/week to shaved skin of the back.

6 ^bSarcoma.

7
 8 Source: [Schmähl et al. \(1977\)](#).

9
 10 [Habs et al. \(1980\)](#) applied benzo[a]pyrene to the shaved interscapular skin of female NMRI
 11 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
 12 age until natural death or gross observation of infiltrative tumor growth. Latency of tumors, either
 13 as time of first appearance or as average time of appearance of tumors, was not reported. Necropsy
 14 was performed on all animals, and the dorsal skin, as well as any organs showing gross alterations
 15 at autopsy, was prepared for histopathological examination. Age-standardized mortality rates,
 16 using the total population of the experiment as the standard population, were used to adjust tumor
 17 incidence findings in the study. Benzo[a]pyrene application was associated with a statistically
 18 significant increase in the incidence of skin tumors at each dose level (see Table D-20).

19 **Table D-20. Skin tumor incidence in female NMRI mice dermally exposed to**
 20 **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%

21
 22 ^aMice were exposed until natural death or until they developed a carcinoma at the site of application; indicated
 23 doses were applied 2 times/week to shaved skin of the back.

24 ^bMortality data of the total study population were used to derive the age-standardized tumor incidence.

25
 26 Source: [Habs et al. \(1980\)](#).

27
 28 [Grimmer et al. \(1984\)](#) and [Grimmer et al. \(1983\)](#) applied benzo[a]pyrene (in 0.1 mL of a
 29 1:3 solution of acetone:dimethyl sulfoxide [DMSO]) to the interscapular skin of female CFLP mice

1 (65–80/group) 2 times/week for 104 weeks. Doses were 0, 3.9, 7.7, and 15.4 µg in the 1983
 2 experiment, and 0, 3.4, 6.7, and 13.5 µg in the 1984 experiment. Mice were observed until
 3 spontaneous death, unless an advanced tumor was observed or if animals were found moribund.
 4 Survival information was not provided; incidences reflect the number of animals placed on study.
 5 Necropsy was performed on all mice. Histopathological examination of the skin and any other
 6 organ showing gross abnormalities was performed. Chronic dermal exposure to benzo[a]pyrene
 7 produced a dose-related increase in skin tumor incidence and a decrease in tumor latency (see
 8 Table D-21). Carcinoma was the primary tumor type observed and a dose-response relationship
 9 was evident for carcinoma formation and incidence of all types of skin tumors.

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

Dose (µg) ^a	Skin tumor incidence (all types)	Incidence of papilloma	Incidence of carcinoma	Tumor appearance (Wks)
<u>Grimmer et al. (1983)</u>				
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
<u>Grimmer et al. (1984)</u>				
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)

12
 13 ^aIndicated doses were applied twice/week to shaved skin of the back.
 14 ^bMean ± SD.
 15 ^cMedian with 95% CI.

16
 17 Sources: [Grimmer et al. \(1984\)](#) and [Grimmer et al. \(1983\)](#).

18
 19 [Habs et al. \(1984\)](#) applied benzo[a]pyrene (in 0.01 mL acetone) to the shaved interscapular
 20 skin of female NMRI mice at doses of 0, 2, or 4 µg, 2 times/week for life. Animals were observed
 21 twice daily until spontaneous death, unless an invasive tumor was observed. All animals were
 22 necropsied and histopathological examination was performed on the dorsal skin and any other
 23 organ with gross abnormalities. Chronic dermal exposure to benzo[a]pyrene did not affect body
 24 weight gain, but appeared to reduce survival at the highest dose with mean survival times of 691,
 25 648, and 528 days for the 0, 2, and 4 µg/day groups, respectively. The total length of exposure for

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

7 **Table D-22. Skin tumor incidence in female NMRI mice dermally exposed to**
 8 **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)

9
 10 ^aMice were exposed until natural death or until they developed an invasive tumor at the site of application;
 11 indicated doses were applied 2 times/week to shaved interscapular skin.

12
 13 Source: [Habs et al. \(1984\)](#).

14
 15 Groups of 23–27 female Ah-receptor-responsive Swiss mice were treated on a shaved area
 16 of dorsal skin with 0, 1, 4, or 8 nmol (0, 0.25, 1, or 2 μg /treatment) benzo[a]pyrene (>99% pure) in
 17 acetone 2 times weekly for 40 weeks ([Higginbotham et al., 1993](#)). Surviving animals were
 18 sacrificed 8 weeks later. Complete necropsies were performed, and tissues from the treated area,
 19 lung, liver, kidney, spleen, urinary bladder, ovary, and uterus were harvested for histopathologic
 20 examination. Histopathologic examination was performed on tissues from the treated area, lungs,
 21 liver, kidneys, spleen, urinary bladder, uterus, and ovaries, as well as any other grossly abnormal
 22 tissue. Lung adenomas occurred in each group (1/27, 2/24, 1/23, 1/23), and other tumors were
 23 noted in isolated mice (i.e., malignant lymphoma [spleen] in one low-dose and one mid-dose mouse;
 24 malignant lymphoma with middle organ involvement in one high-dose mouse; and hemangioma
 25 [liver] in one mid-dose mouse) and were not considered dose related. In addition, benzo[a]pyrene
 26 showed no skin tumors under the conditions of this bioassay.

27 [Sivak et al. \(1997\)](#) designed a study to compare the carcinogenicity of condensed asphalt
 28 fumes (including benzo[a]pyrene and other PAHs) with several doses of benzo[a]pyrene alone. For
 29 the purposes of this assessment, the exposure groups exposed to PAH mixtures are not discussed.
 30 Groups of 30 male C3H/HeJ mice were treated dermally twice/week to 0, 0.0001, 0.001, or 0.01%
 31 (0, 0.05, 0.5, or 5 μg) benzo[a]pyrene in a 50 μL volume of cyclohexanone/acetone (1:1) for
 32 104 weeks beginning at 8 weeks of age. Mice dying during the exposure period or sacrificed at the
 33 24-month termination were necropsied; mice with skin tumors that persisted for 4 consecutive

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

12 **Table D-23. Skin tumor incidence in male C3H/HeJ mice dermally exposed to**
 13 **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

14
 15 ^aIndicated doses were applied twice/week to shaved dorsal skin.

16 ^bNumber of skin tumor-bearing mice. In the high-dose group, 1 papilloma and 28 carcinomas were detected; in
 17 the 0.5 μg group, 2 papillomas and 3 carcinomas were detected.

18
 19 Source: [Sivak et al. \(1997\)](#).

20
 21 To examine dose-response relationships and the time course of benzo[a]pyrene-induced
 22 skin damage, DNA adduct formation, and tumor formation, groups of 43–85 female Harlan mice
 23 were treated dermally with 0, 16, 32, or 64 μg of benzo[a]pyrene in 50 μL of acetone once per week
 24 for 29 weeks ([Albert et al., 1991](#)). Interscapular skin of each mouse was clipped 3 days before the
 25 first application and every 2 weeks thereafter. Additional groups of mice were treated for 9 weeks
 26 with 0, 8, 16, 32, or 64 μg radiolabeled benzo[a]pyrene to determine BPDE-DNA adduct formation
 27 in the epidermis at several time points (1, 2, 4, and 9 weeks). Tumor formation was monitored only
 28 in the skin.

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

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

18 **D.4.4. Reproductive and Developmental Toxicity Studies**

19 ***Oral***

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

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

7 [Chung et al. \(2011\)](#) evaluated the effects of low-dose benzo[a]pyrene exposure on
8 spermatogenesis and the role of altered steroidogenesis on the sperm effects. Groups of
9 20–25 male Sprague-Dawley rats (8 weeks old) were given daily gavage doses of 0, 0.001, 0.01, or
10 0.1 mg/kg-day benzo[a]pyrene in DMSO for 90 consecutive days. At the end of exposure, the
11 animals were sacrificed for removal of the pituitary, testes, and epididymides, and collection of
12 serum and testicular interstitial fluid. Subgroups of each exposure group were used for various
13 analyses. Serum levels of testosterone and luteinizing hormone (LH) were measured, as was
14 testosterone concentration in the interstitial fluid (ELISA). Body and testes weights were recorded.
15 Sections of the testis were analyzed for apoptotic germ cells using the terminal deoxynucleotidyl
16 transferase dUTP nick end labeling (TUNEL) assay. Evaluation of the epididymis included
17 histopathology as well as measurement of caput and caudal epididymal tubule diameters. In
18 addition, sperm were isolated from the cauda epididymis for analysis of sperm number and
19 motility, acrosomal integrity, and immunocytochemistry for ADAM3 (a disintegrin and
20 metalloproteinase domain 3; a sperm surface protein associated with fertilization).

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

30 At termination of exposure, body weights of treated animals were similar to controls, as
31 were absolute testes weights ([Chung et al., 2011](#)). Testosterone concentrations in both serum and
32 testicular interstitial fluid were significantly reduced at the high dose of benzo[a]pyrene
33 (0.1 mg/kg-day); based on visual inspection of the data, the mean serum concentration in this
34 group was ~20% of the control and the mean interstitial fluid concentration was ~60% of the
35 control (n = 9 animals/dose for these evaluations). In addition, baseline production of testosterone
36 by cultured Leydig cells was significantly decreased (~50% based on data shown graphically) at
37 0.1 mg/kg-day. Both hCG- and dbcAMP-stimulated testosterone production measurements were
38 lower (~60% lower than controls) in Leydig cells from rats exposed to either 0.01 or 0.1

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

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

19 Statistically significant effects observed at the lowest dose (0.001 mg/kg-day) of
20 benzo[a]pyrene in this study included decreased caput and cauda epididymal tubule diameters
21 (~10–15% lower than controls) and increased numbers of apoptotic germ cells (~twofold higher
22 than controls) by TUNEL assay ([Chung et al., 2011](#)). The authors reported that “sperm motility was
23 significantly reduced in the benzo[a]pyrene-exposed groups in comparison to that of the control”
24 but provided quantitative data only for the middle dose group, which exhibited a ~30% decrease in
25 percent motile sperm. No statistically significant decrease in sperm count was reported at any
26 dose. The middle dose (0.01 mg/kg-day) is considered to be a LOAEL based on reduced sperm
27 motility.

28 [Gao et al. \(2011\)](#) examined effects of benzo[a]pyrene exposure via on cervical cell
29 morphology. Female ICR mice (18–22 g) were exposed to doses of 0, 2.5, 5, or 10 mg/kg twice per
30 week for 14 weeks, either by gavage or by intraperitoneal (i.p.) injection (for this review, only oral
31 results are reported). After adjustment for equivalent continuous dosing (2/7 days/week), the
32 equivalent daily doses are estimated to be 0.7, 1.4, and 2.9 mg/kg-day. Both vehicle (sesame oil)
33 and untreated control groups were maintained. Body weights were determined weekly. Groups of
34 26 mice per dose per exposure route were sacrificed at the end of exposure for evaluation of
35 cervical weight and histopathology. Additional groups of 10 mice were exposed for 14 weeks and
36 used for determination of lipid peroxidation (malondialdehyde and glutathione-S-transferase
37 levels) and CYP1A1 activity (EROD) in both liver and cervix, as well as creatine kinase activity, AST
38 activity, and IL-6 levels in cervix and serum.

1 Mortality was observed in all exposure groups with the exception of the low-dose oral
 2 exposure group; the authors did not indicate the timing or causes of death ([Gao et al., 2011](#)). There
 3 were no control deaths. Mortality incidences in the oral exposure groups (low to high dose) were
 4 0/26 (untreated control), 0/26 (vehicle control), 0/26, 1/36, and 2/26. Benzo[a]pyrene treatment
 5 resulted in dose-dependent decreases in body weight gain. In the high-dose group of both
 6 treatments, body weight began to decline after ~7 weeks of exposure. Based on visual examination
 7 of data presented graphically, mean terminal body weights in the low-, mid-, and high-dose oral
 8 exposure groups were ~10, 15, and 30% lower (respectively) than the vehicle control mean. The
 9 untreated control mean body weight for the oral exposure group was similar to the vehicle control
 10 mean body weight. Cervical weight as a function of body weight was not affected by oral
 11 benzo[a]pyrene exposure. Microscopic examination of the cervix revealed increased incidences of
 12 epithelial hyperplasia and inflammatory cells in the cervix of all groups of exposed mice, and
 13 atypical hyperplasia of the cervix in mice exposed to 1.4 or 2.9 mg/kg -day benzo[a]pyrene.
 14 Statistical analysis of the findings was conducted, but was poorly reported in the publication.
 15 Table D-24 shows the incidences in the oral exposure groups, along with the results of Fisher's
 16 exact tests performed for this review.

17 **Table D-24. Mortality and cervical histopathology incidences in female ICR**
 18 **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*

19 *Significantly different from vehicle control by Fisher's exact test performed for this review (one-sided $p < 0.05$).
 20

21 Source: [Gao et al. \(2011\)](#).
 22

23
 24 Levels of malondialdehyde in both the cervix and liver were significantly higher than
 25 controls in all dose groups of animals treated by either oral (1.5–2-fold higher in the cervix and
 26 ~3–7-fold higher in the liver after oral exposure, $p < 0.05$) or i.p. exposure. Concomitant decreases
 27 in GST activity (~15–50% lower than controls in the cervix and ~30–60% lower in the liver after
 28 oral exposure, $p < 0.05$) were also observed at all doses and in both organs and both treatments.
 29 EROD activity was increased in the cervix (~4–~12-fold) and liver (~12–~35-fold) of all exposure
 30 groups. Measurement of creatine kinase and AST activity in the cervix and serum also showed
 31 significant increases at all doses and after both exposures (~1.5–2-fold in the cervix, and ~20–50%

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

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

8 [Mohamed et al. \(2010\)](#) investigated multi-generational effects in male mice following
9 exposure of 6-week-old C57BL/6 mice (10/group) to 0 (corn oil), 1, or 10 mg/kg-day
10 benzo[a]pyrene for 6 weeks by gavage. Following final treatment, male mice were allowed to
11 stabilize for 1 week prior to being mated with two untreated female mice to produce an
12 F1 generation. Male mice were sacrificed 1 week after mating. F1 males were also mated with
13 untreated female mice, as were F2 males. The mice of the F1, F2, and F3 generations were not
14 exposed to benzo[a]pyrene. The F0, F1, F2, and F3 mice were all sacrificed at the same age
15 (14 weeks) and endpoints including testis histology, sperm count, sperm motility, and in vitro
16 sperm penetration (of hamster oocytes) were evaluated. These endpoints were analyzed
17 statistically using ANOVA and Tukey's honest significance test and results were reported
18 graphically as means \pm SD.

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

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

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

12 [Xu et al. \(2010\)](#) treated female Sprague-Dawley rats (6/group) to 0 (corn oil only), 5, or
13 10 mg/kg-day benzo[a]pyrene by gavage every other day for a duration of 60 days. This resulted in
14 TWA doses of 0, 2.5, and 5 mg/kg-day over the study period of 60 days. Endpoints examined
15 included ovary weight, estrous cycle, 17 β -estradiol blood level, and ovarian follicle populations
16 (including primordial, primary, secondary, atretic, and corpora lutea). Animals were observed daily
17 for any clinical signs of toxicity and following sacrifice, gross pathological examinations were made
18 and any findings were recorded. All animals survived to necropsy. A difference in clinical signs was
19 not observed for the treated groups and body weights were not statistically different in treated
20 animals (although they appear to be depressed 6% at the high dose). Absolute ovary weight was
21 statistically significantly reduced in both the low- and high-dose groups (11 and 15%, respectively)
22 (see Table D-25). Animals treated with the high dose were noted to have a statistically significantly
23 prolonged duration of the estrous cycle and nonestrus phase compared to controls. Animals in the
24 high-dose group also had statistically significantly depressed levels of estradiol (by approximately
25 25%) and decreased numbers of primordial follicles (by approximately 20%). This study also
26 indicated a strong apoptotic response of ovarian granulosa cells as visualized through TUNEL
27 labeling; however, the strongest response was seen at the low dose; decreased apoptosis was also
28 observed at the high dose. Based on decreased ovary weight following a 60-day oral exposure to
29 benzo[a]pyrene, a LOAEL of 2.5 mg/kg-day was established from this study (no NOAEL was
30 identified).

1 **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

2
3 *Statistically different from controls ($p < 0.05$) using one-way ANOVA.

4 ^aTWA doses over the 60-day study period.

5
6 Source: [Xu et al. \(2010\)](#).

7
8 [Zheng et al. \(2010\)](#) treated male Sprague-Dawley rats to 0 (corn oil only), 1, or 5 mg/kg-day
9 benzo[a]pyrene by daily gavage for a duration of 30 (8/group) or 90 days (8/group). At necropsy,
10 the left testis of each animal was collected and weighed. Testes testosterone concentrations were
11 determined by radioimmunoassay and results were expressed as ng/g testis and reported
12 graphically. Testicular testosterone was statistically significantly decreased in the high-dose group
13 approximately 15% following 90 days of exposure. The low-dose group also appeared to have a
14 similar average depression of testosterone levels; however, the change did not reach statistical
15 significance. Testosterone levels measured in animals sacrificed following 30 days of
16 benzo[a]pyrene exposure were not statistically different than controls. Based on decreased
17 testicular testosterone levels following a 90-day oral exposure to benzo[a]pyrene, a LOAEL of
18 5 mg/kg-day and a NOAEL of 1 mg/kg-day were identified.

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

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

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

6 ***Reproductive Effects of In Utero Exposure Via Oral Route***

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

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

1 **Table D-26. Reproductive effects in male and female CD-1 F1 mice exposed in**
 2 **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*

3
4 *Significantly ($p < 0.05$) different from control by unspecified tests.

5 ^aPregnant F0 mice were administered daily doses of benzo[a]pyrene in corn oil on GDs 7–16.

6 ^bBeginning at 7 weeks of age, each F1 male mouse (20–45/group) was exposed to 10 untreated females over a
7 period of 25 days. Index = (females pregnant/females exposed to males) × 100.

8 ^cBeginning at 6 weeks of age, each F1 female mouse (20–55/group) was cohabitated with an untreated male for a
9 period of 6 months.

10
11 SEM = standard error of the mean.

12
13 Source: [Mackenzie and Angevine \(1981\)](#).

14
15 Exposure to benzo[a]pyrene caused a marked dose-related decrease in the size of the
16 gonads. In F1 males, testes weights were statistically significantly reduced. Testes from animals
17 exposed in utero to 10 and 40 mg/kg-day weighed approximately 42 and 82%, respectively, of the
18 weight of testes from the control animals (no F2 offspring were produced in the high-dose group).
19 This was confirmed by histopathologic observation of atrophic seminiferous tubules in the
20 40 mg/kg-day group that were smaller than those of controls and were empty except for a basal
21 layer of cells. The number of interstitial cells in the testes was also increased in this group. Males
22 from the 10 mg/kg-day group showed limited testicular damage; although all exhibited evidence of
23 tubular injury, each animal had some seminiferous tubules that displayed active spermatogenesis.
24 Ovarian tissue was absent or reduced in F1 females such that organ weights were not possible to
25 obtain. Examination of available tissue in these females revealed hypoplastic ovaries with few
26 follicles and corpora lutea (10 mg/kg-day) or with no evidence of folliculogenesis (40 mg/kg-day).
27 Ovarian tissue was not examined in highest-dose females.

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

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

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

19 **Table D-27. Effect of prenatal exposure to benzo[a]pyrene on indices of**
 20 **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)

21
 22 *Significantly ($p < 0.05$) different from control group by Wilcoxon rank sum test or Kruskal-Wallis two-tailed test.
 23 ^aGroups of nine female NMRI F0 mice were administered 0 or 10 mg benzo[a]pyrene/kg-day by gavage in corn oil
 24 on GDs 7–16. One F1 female from each litter was continuously bred with an untreated male for 6 months.

25
 26 Source: [Kristensen et al. \(1995\)](#).

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

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

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

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

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

9 For all measurements of neonatal sensory and motor development, results from both sexes
10 were analyzed together since benzo[a]pyrene was reported to have no significant interaction with
11 sex by 3-way ANOVA. No significant differences were observed in either the cliff aversion or
12 forelimb grip strength tests. In the surface righting reflex test, latency was increased in the
13 0.2 mg/kg-day group at PND 12, in the 0.02 and 2 mg/kg-day groups at PND 14, and in only the
14 high-dose group at PND 16; latency was not significantly different in any group at PND 18. At
15 PND 12, there was a dose-related increase in negative geotaxis latency associated with 0.02, 2, and
16 2 mg/kg-day benzo[a]pyrene, which was also present in the 2 mg/kg-day group at PND 14, but
17 returned to control levels at PND 16 and 18. In the open field test, there were no significant
18 differences in either locomotion or rearing activity at PND 18 or 20. At PND 34, the 2 mg/kg-day
19 group exhibited significantly increased movement, but increases in rearing were not significant. At
20 PND 69, increased locomotion was observed in both the 0.2 and 2 mg/kg-day groups, while rearing
21 was significantly increased in only the 2 mg/kg-day treatment group.

22 The elevated plus maze performance was only evaluated in adolescent and adult rats.
23 Unlike the previous tests, 3-way ANOVA revealed a statistically significant interaction between
24 neonatal benzo[a]pyrene treatment and sex, so male and female performance was analyzed
25 independently. No significant differences in PND 35 males were observed, and the only significant
26 observation in PND 35 females was increased time spent in the open maze arms by the
27 2 mg/kg-day treatment group. Significantly decreased latency time to first open arm entry was
28 observed in PND 70 males and females in both 0.2 and 2 mg/kg-day treatment groups; these groups
29 also spent significantly more time in open maze arms, along with the 0.02 mg/kg-day female group.
30 At PND 70, the 2 mg/kg-day males, along with the 0.2 and 2 mg/kg-day females, entered more
31 frequently into open arms and less frequently into closed arms than the vehicle controls. In the
32 Morris water maze, escape latency (time to reach the platform during each of the four testing days)
33 was consistently increased in the 2 mg/kg-day treatment group of both sexes, in both adolescent
34 and adult animals. These increases were statistically significant in both males and females treated
35 with 2 mg/kg-day benzo[a]pyrene at both PNDs 39 and 74, and were also significantly elevated in
36 0.2 mg/kg-day animals of both sexes at PND 74. Likewise, performance during the 5th test day, in
37 the absence of the escape platform, was significantly adversely affected by both metrics (decreased
38 time spent in the target quadrant and decreased number of attempts to cross the platform location)

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

6 [Jules et al. \(2012\)](#) treated pregnant Long-Evans Hooded rats with benzo[a]pyrene
7 (unspecified purity) dissolved in 0.875 mL peanut oil by gavage daily on GDs 14–17, at doses of
8 150, 300, 600, and 1,200 µg benzo[a]pyrene/kg body weight, with animals weighed daily. Cage-
9 side observations were performed until pup weaning, and litter size was evaluated for each
10 treatment group. Pups from four to five individual litters were analyzed for each endpoint, which
11 was independently repeated for a total of three replicates. Delivery was designated PND 0, and
12 pups were harvested on PNDs 0–15 for benzo[a]pyrene metabolite identification, or for other
13 endpoints as young adults at PND 53. Systolic/diastolic blood pressure and heart rate was
14 recorded by a volume pressure recording sensor and occlusion tail-cuff applied to conscious, non-
15 anesthetized animals. Animals were preconditioned to the restraint device and tail-cuff by daily
16 acclimatization sessions during PNDs 46–50, to minimize stress effects during data collection.
17 Cardiac function values were averaged from 15 readings each collected over a 1-minute interval
18 every other minute for 30 minutes on PND 53.

19 No significant differences in litter size or pup weight gain from PND 0 to 15 were reported
20 in any treatment group, and no convulsions, tremors, or abnormal movements were reproducibly
21 observed. Most analytical data were reported graphically, as mean ± standard error of the mean
22 (SEM) of three replicates of 3–5 offspring measured/group. Plasma and heart tissue total
23 benzo[a]pyrene metabolite levels were maximal at PND 0 (the first time point sampled) and
24 progressively decreased from PNDs 0 to 13. Compared to the low-dose group (150 µg/kg), plasma
25 metabolite levels were significantly elevated in the 600 and 1,200 µg/kg-day benzo[a]pyrene
26 groups through PND 13, while heart metabolite levels were significantly increased through PND 11.
27 Metabolites in mid-dose group, 300 µg/kg-day, trended between the 150 and 600 µg/kg-day group
28 levels from PND 0 to 7, while not achieving statistically significant differences in pair-wise
29 comparisons. Three principal groups of benzo[a]pyrene metabolites were identified. More than
30 70% of the total heart metabolite burden was composed of diol metabolites through PND 13, while
31 the more reactive hydroxyl metabolites increased in relative composition from PND 9 to 13, and the
32 dione population remained constant at ≤5%.

33 Cardiovascular function was evaluated in pups exposed in utero to 600 or 1,200 µg/kg-day
34 benzo[a]pyrene versus controls (see Table D-28). A dose-related and statistically significant
35 increase in both systolic (20, 50%) and diastolic pressure (30, 80%) was observed in mid- and
36 high-dose pups, respectively. Heart rate was also significantly altered; a 10% increased heart rate
37 was reported in the 600 µg/kg-day benzo[a]pyrene group, while the average heart rate of the
38 1,200 µg/kg-day benzo[a]pyrene groups decreased 8%.

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

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

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

Source: [Jules et al. \(2012\)](#).

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

Pup neuromotor maturation and behavior was assessed during pre-weaning by four standard methods (administered between 10 am and 1 pm on testing days, and in temporal order as indicated): (1) *righting reflex test*, maximum duration of 120 seconds, administered on PNDs 3, 5, 7, and 9; (2) *negative geotaxis test*, maximum duration of 120 seconds, administered on PNDs 5, 7, 9, and 11; (3) *forelimb grip test*, duration until failure, administered on PNDs 9 and 11; and (4) *open field test*, 6-minute evaluation of locomotor activity and rearing following a 1-minute habituation period, administered on PND 15. Adolescent function was evaluated by three methods: *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

1 PND 40 for 5 minutes, in which the percentage of spontaneous alternation was calculated by: [(the
2 number of successful overlapping triplets)/(total number of arm entries – 2) × 100%].

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

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

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

18
19 * $p < 0.001$ significantly different from control mean.

20 ** $p < 0.01$.

21
22 Source: [Bouayed et al. \(2009a\)](#).

23
24 Behavioral test data was reported graphically, as mean ± SEM of n = 20/group. For the pre-
25 weaning neuromotor developmental tests, benzo[a]pyrene treatment was found to not depend on
26 sex; therefore, data from male and female pups were combined. Pups nursing from mothers
27 administered 2 or 20 mg/kg-day benzo[a]pyrene had significantly elevated righting reflex times at
28 PNDs 3–5, which decreased to control times at PNDs 7–9. Only pups from the 20 mg/kg-day

1 treatment group demonstrated significantly increased negative geotaxis latency, which was twofold
2 greater than controls at PNDs 5, 7, and 9, but returned to control levels at PND 11. Interestingly,
3 mice in the 20 mg/kg-day group had increased forelimb grip strength, which was significantly
4 greater than control mice at PNDs 9 and 11, corresponding to increased body weight in the
5 benzo[a]pyrene-treated mice versus controls. Mice in the 2 mg/kg-day group also performed
6 better than controls at PND 9, but were equivalent at PND 11. No treatment or sex-related effects
7 were reported on locomotion or rearing activity during the open field test. Sex-dependency on test
8 performance became evident during the analysis of the WESPOC test data: female pups were not
9 significantly affected using any metric, while males in the 20 mg/kg-day group demonstrated a
10 statistically significantly longer pole-grasping latency (threefold), and took 13 times longer to
11 escape the pole and board the safety platform versus vehicle controls. While performance of male
12 pups from the 2 mg/kg-day group was not statistically significantly worse than vehicle controls by
13 pair-wise comparison, latency for both pole-grasping and escape in this treatment group
14 contributed to a significant trend for treatment dose and these effects. In the evaluation of the
15 elevated plus maze, treatment effects did not appear to depend upon sex, so both male and female
16 performance was analyzed together. Mice in both benzo[a]pyrene treatment groups demonstrated
17 decreased latency time to first entering an open arm (30–50%), as well as entered open arms
18 2 times more frequently and spent twice as much time there versus vehicle controls. While mice in
19 the 2 mg/kg-day treatment group entered into closed arms 20% less frequently than controls, mice
20 in the 20 mg/kg-day group were not significantly different. Likewise, mice nursing from mothers
21 treated with 2 mg/kg-day benzo[a]pyrene performed 15% more spontaneous alternations in the
22 Y-maze spontaneous alternation test compared to controls, while mice in the high-dose group were
23 not significantly different. The brains of pups nursing from the 20 mg/kg-day group expressed
24 approximately 50% lower levels of 5-hydroxytryptamine (serotonin) 1A (5HT1A), and mu 1-opioid
25 (MOR1) mRNA, and a trend was observed in the low-dose group as well. No significant changes in
26 alpha-1D adrenergic or GABA-A mRNA levels were detected.

27 ***Reproductive Effects in Adults and Repeated Oral Exposure***

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

1 provided. The only treatment-related effect was a lack of weight gain related to feed unpalatability.
2 While this study suggests that pre-mating exposure of male or female mice to doses up to
3 122 mg/kg-day for 20 days may not affect fertility, the sample sizes were too small and the study
4 designs were too inconsistent to provide reliable NOAELs and LOAELs for reproductive/
5 developmental toxicity.

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

16 **D.4.5. Inhalation**

17 ***Reproductive Toxicity and In Utero Exposure via Inhalation***

18 [Archibong et al. \(2002\)](#) evaluated the effect of exposure to inhaled benzo[a]pyrene on fetal
19 survival and luteal maintenance in timed-pregnant F344 rats. Prior to exposure on GD 8,
20 laparotomy was performed to determine the number of implantation sites, and confirmed pregnant
21 rats were divided into three groups, consisting of rats that had four to six, seven to nine, or more
22 than nine conceptuses in utero. Rats in these groups were then assigned randomly to the treatment
23 groups or control groups to ensure a similar distribution of litter sizes. Animals (10/group) were
24 exposed to benzo[a]pyrene:carbon black aerosols at concentrations of 25, 75, or 100 µg/m³ via
25 nose-only inhalation, 4 hours/day on GDs 11–20. Control animals were either sham-exposed to
26 carbon black or remained entirely unexposed. Results of particle size analysis of generated
27 aerosols were reported by several other reports from this laboratory ([Inyang et al., 2003](#); [Ramesh
28 et al., 2001a](#); [Hood et al., 2000](#)). Aerosols showed a trimodal distribution (average of cumulative
29 mass, diameter) <95%, 15.85 µm; 89%, <10 µm; 55%, <2.5 µm; and 38%, <1 µm ([Inyang et al.,
30 2003](#)). [Ramesh et al. \(2001a\)](#) reported that the MMAD (± geometric SD) for the 55% mass fraction
31 with diameters <2.5 µm was 1.7 ± 0.085. Progesterone, estradiol-17β, and prolactin concentrations
32 were determined in plasma collected on GDs 15 and 17. Fetal survival was calculated as the total
33 number of pups divided by the number of all implantation sites determined on GD 8. Individual
34 pup weights and crown-rump length per litter per treatment were determined on PND 4
35 (PND 0 = day of parturition).

36 [Archibong et al. \(2001\)](#) reported that exposure of rats to benzo[a]pyrene caused
37 biologically and statistically significant ($p \leq 0.05$) reductions in fetal survival compared with the

1 two control groups; fetal survival rates were 78.3, 38.0, and 33.8% per litter at 25, 75, and
 2 100 µg/m³, respectively, and 96.7% with carbon black or 98.8% per litter in untreated controls (see
 3 Table D-30). Consequently, the number of pups per litter was also decreased in a concentration-
 4 dependent manner. The decrease was ~50% at 75 µg/m³ and ~65% at 100 µg/m³, compared with
 5 sham-exposed and unexposed control groups. No effects on hormone levels were observed on
 6 GDs 15 or 17 at the low dose. Biologically significant decreases in mean pup weights (expressed as
 7 g per litter) of >5% were observed at doses ≥75 µg/m³ (14 and 16% decreases at 75 and
 8 100 µg/m³, respectively, *p* < 0.05). Exposure to benzo[a]pyrene did not affect crown-rump length
 9 (see Table D-30).

10 **Table D-30. Pregnancy outcomes in female F344 rats treated with**
 11 **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

12
 13 *Significantly different from controls at *p* < 0.05 by one-tailed post-hoc t-testing following ANOVA.

14 ^aValues presented as means ± SEM.

15
 16 Source: [Archibong et al. \(2002\)](#).

17
 18 Benzo[a]pyrene exposure at 75 µg/m³ caused a statistically significant decrease in plasma
 19 progesterone, estradiol, and prolactin on GD 17; these levels were not determined in the rats
 20 exposed to 100 µg/m³ ([Archibong et al., 2002](#)). Plasma prolactin is an indirect measure of the
 21 activity of decidual luteotropin, a prolactin-like hormone whose activity is necessary for luteal
 22 maintenance during pregnancy in rats. Control levels of prolactin increased from GD 15 to 17, but
 23 this increase did not occur in the rats exposed to 75 µg/m³. Although the progesterone
 24 concentration at 75 µg/m³ was significantly lower than in controls on GD 17, the authors thought
 25 that the circulating levels should have been sufficient to maintain pregnancy; thus, the increased
 26 loss of fetuses was thought to be caused by the lower prolactin levels rather than progesterone
 27 deficiency. The reduced circulating levels of progesterone and estradiol-17β among
 28 benzo[a]pyrene-treated rats were thought to be a result of limited decidual luteotropic support for
 29 the corpora lutea. The authors proposed the following mechanism for the effects of benzo[a]pyrene

1 on fertility: benzo[a]pyrene or its metabolites decreased prolactin and decidual luteotropin levels,
2 compromising the luteotropic support for the corpora lutea and thereby decreasing the plasma
3 levels of progesterone and estradiol-17 β . The low estradiol-17 β may decrease uterine levels of
4 progesterone receptors, thereby resulting in fetal mortality. Based on biologically and statistically
5 significant decreases in pups/litter and percent fetal survival/per litter, the LOAEL was 25 $\mu\text{g}/\text{m}^3$;
6 no NOAEL was identified.

7 ***Neurotoxicity and In Utero Exposure via Inhalation***

8 To evaluate the effects of benzo[a]pyrene on the developing central nervous system,
9 [Wormley et al. \(2004\)](#) exposed timed-pregnant F344 rats (10/group) to benzo[a]pyrene:carbon
10 black aerosols by nose-only inhalation on GDs 11–21 for 4 hours/day at a concentration of
11 100 $\mu\text{g}/\text{m}^3$. Results of particle size analysis of generated aerosols were reported by other reports
12 from this laboratory ([Ramesh et al., 2001a](#); [Hood et al., 2000](#)). Particle size analysis of a 100- $\mu\text{g}/\text{m}^3$
13 aerosol showed a trimodal distribution (average of cumulative mass, diameter): <95%, 15.85 μm ;
14 90%, <10 μm ; 67.5%, <2.5 μm ; and 66.2%, <1 μm ; the MMAD \pm geometric SD for the latter fraction
15 was 0.4 \pm 0.02 μm ([Hood et al., 2000](#)). Dams were maintained to term and pups were weaned on
16 PND 30. Benzo[a]pyrene reduced the number of live pups to one-third of control values without
17 affecting the number of implantation sites. During PNDs 60–70, electrical stimulation and evoked
18 field potential responses were recorded via electrodes implanted into the brains of the animals.
19 Direct stimulation of perforant paths in the entorhinal region revealed a diminution in long-term
20 potentiation of population spikes across the perforant path-granular cell synapses in the dentate
21 gyrus of the hippocampus of F1 generation benzo[a]pyrene-exposed animals; responses in exposed
22 offspring were about 25% weaker than in control offspring. Additionally, NMDA receptor subunit 1
23 protein (important for synaptic functioning) was down-regulated in the hippocampus of
24 benzo[a]pyrene-exposed F1 pups. The authors interpreted their results as suggesting that
25 gestational exposure to benzo[a]pyrene inhalation attenuates the capacity for long-term
26 potentiation (a cellular correlate of learning and memory) in the F1 generation.

27 In another study by this same group of investigators, [Wu et al. \(2003a\)](#) evaluated the
28 generation of benzo[a]pyrene metabolites in F1 generation pups, as well as the developmental
29 profile for AhR and mRNA. In this study, confirmed-pregnant F344 rats were exposed to
30 benzo[a]pyrene:carbon black aerosols at 25, 75, or 100 $\mu\text{g}/\text{m}^3$ via nose-only inhalation,
31 4 hours/day, for 10 days (GDs 11–21). Control animals either were exposed to carbon black
32 (sham) to control for inert carrier effects or remained untreated. Each benzo[a]pyrene
33 concentration had its own set of controls (carbon black and untreated). Two randomly selected
34 pups were sacrificed on each of PNDs 0, 3, 5, 10, 15, 20, and 30. Body, brain, and liver weights were
35 recorded. Benzo[a]pyrene metabolites were analyzed in the cerebral cortex, hippocampus, liver,
36 and plasma. A dose-related increase in plasma and cortex benzo[a]pyrene metabolite
37 concentrations in pups was observed. Dihydrodiols (4,5-; 7,8-; 9,10-) dominated the metabolite
38 distribution profile up to PND 15 and the hydroxy (3-OH-benzo[a]pyrene; 9-OH-benzo[a]pyrene)

1 metabolites after PND 15 at 100 $\mu\text{g}/\text{m}^3$ (the only exposure concentration reported). Results
2 indicated a dose-related decrease in the ratio of the total number of pups born per litter to the total
3 number of implantation sites per litter. The number of resorptions at 75 and 100 $\mu\text{g}/\text{m}^3$, but not at
4 25 $\mu\text{g}/\text{m}^3$, was statistically significantly increased compared with controls.

5 ***Adult Male Reproductive Effects and Repeated Inhalation Exposure***

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

19 Regardless of the exposure concentration, inhaled benzo[a]pyrene did not affect testis
20 weight or the density of stored sperm compared with controls. However, inhaled benzo[a]pyrene
21 caused a concentration-dependent reduction in the progressive motility of stored sperm.
22 Progressive motility was similar at 75 and 100 $\mu\text{g}/\text{m}^3$, but these values were significantly lower
23 ($p < 0.05$) than in any other group. The reduction in sperm motility postcessation of exposure was
24 thought to be the result of benzo[a]pyrene limiting epididymal function. Benzo[a]pyrene exposure
25 to 75 $\mu\text{g}/\text{m}^3$ caused a decrease in circulating concentrations of testosterone compared with controls
26 from the time of cessation of exposure (time 0) to 48 hours posttermination of exposure ($p < 0.05$).
27 However, the decrease was followed by a compensatory increase in testosterone concentration at
28 72 hours postcessation of exposure. Exposure to 75 $\mu\text{g}/\text{m}^3$ caused a nonsignificant increase in
29 plasma LH concentrations at the end of exposure compared with controls, which increased further
30 and turned significant ($p < 0.05$) for the remaining time of the study period. The decreased plasma
31 concentration of testosterone, accompanied by an increased plasma LH level, was thought to
32 indicate that benzo[a]pyrene did not have a direct effect on LH. The authors also noted that the
33 decreased circulating testosterone may have been secondary to induction of liver CYP450 enzymes
34 by benzo[a]pyrene. The authors concluded that subacute exposure to benzo[a]pyrene contributed
35 to impaired testicular endocrine function that ultimately impaired epididymal function. For this
36 study, the NOAEL was 25 $\mu\text{g}/\text{m}^3$ and the LOAEL was 75 $\mu\text{g}/\text{m}^3$, based on a statistically significant
37 reduction in the progressive motility of stored sperm and impairment of testicular function with
38 10 days of exposure at 75 $\mu\text{g}/\text{m}^3$.

1 In a follow-up study with longer exposure duration, adult male F344 rats (10 per group)
 2 were exposed to benzo[a]pyrene:carbon black aerosols at 75 µg/m³ via nose-only inhalation,
 3 4 hours/day for 60 days ([Archibong et al., 2008](#); [Ramesh et al., 2008](#)). Rats in the control group
 4 were subjected to the nose-only restraint, but were not exposed to the carbon black carrier. Blood
 5 samples were collected at 0, 24, 48, and 72 hours after exposure terminated, and the animals were
 6 sacrificed for tissue analyses following the last blood sampling. Data were analyzed statistically for
 7 benzo[a]pyrene effects on weekly body weights, total plasma testosterone and LH concentrations,
 8 testis weights, density of stored spermatozoa, sperm morphological forms and motility,
 9 benzo[a]pyrene metabolite concentrations and aryl hydrocarbon hydroxylase (AHH) activity, and
 10 morphometric assessments of testicular histologies. Relative to controls, the results indicated 34%
 11 reduced testis weight ($p < 0.025$), reduced daily sperm production ($p < 0.025$), and reduced
 12 intratesticular testosterone concentrations ($p < 0.025$). Plasma testosterone concentrations were
 13 reduced to about one-third of the level in controls on the last day of exposure (day 60) and at 24,
 14 48, and 72 hours later ($p < 0.05$). However, plasma LH concentrations in benzo[a]pyrene-exposed
 15 rats were elevated throughout the blood sampling time periods compared with controls ($p < 0.05$).
 16 In testis, lung, and liver, AHH activity and benzo[a]pyrene-7,8-dihydrodiol (precursor to the
 17 DNA-reactive BPDE) and benzo[a]pyrene-3,6-dione metabolites were significantly ($p < 0.05$)
 18 elevated relative to controls. Progressive motility and mean density of stored spermatozoa were
 19 significantly reduced ($p < 0.05$). Weekly body weight gains were not affected by benzo[a]pyrene
 20 exposure. These results indicate that a 60-day exposure of adult male rats to benzo[a]pyrene:
 21 carbon black aerosols at 75 µg/m³ produced decreased testis weight; decreased intratesticular and
 22 plasma testosterone concentrations; and decreased sperm production, motility, and density.

23 D.5. OTHER PERTINENT TOXICITY INFORMATION

24 D.5.1. Genotoxicity Information

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

27 **Table D-31. In vitro genotoxicity studies of benzo[a]pyrene in non-**
 28 **mammalian cells**

	Result		Reference
	+S9	-S9	
Endpoint/test system: <i>prokaryotic cells</i>			
Forward mutation			
<i>Salmonella typhimurium</i> TM677	+	-	Rastetter et al. (1982)
<i>S. typhimurium</i> TM677	+	ND	Babson et al. (1986)
Reverse mutation			

Supplemental Information—Benzo[a]pyrene

	Result		Reference
	+S9	-S9	
<i>S. typhimurium</i> TA98, TA1538	+	ND	Ames et al. (1975)
<i>S. typhimurium</i> TA98, TA100, TA1538	+	ND	McCann et al. (1975)
<i>S. typhimurium</i> TA1538, TA98	+	-	Wood et al. (1976)
<i>S. typhimurium</i> TA98, TA100, TA1537	+	-	Epler et al. (1977)
<i>S. typhimurium</i> TA98, TA100	+	-	Obermeier and Froberg (1977)
<i>S. typhimurium</i> TA98	+	-	Pitts et al. (1978)
<i>S. typhimurium</i> TA98, TA100	+	ND	Lavoie et al. (1979)
<i>S. typhimurium</i> TA98, TA100	+	-	Simmon (1979a)
<i>S. typhimurium</i> TA98	+	ND	Hermann (1981)
<i>S. typhimurium</i> TA98, TA100	+	ND	Alfheim and Ramdahl (1984)
<i>S. typhimurium</i> TA98, TA100, TA1538	ND	-	Glatt et al. (1985)
<i>S. typhimurium</i> TA97, TA98, TA100	+	-	Sakai et al. (1985)
<i>S. typhimurium</i> TA97, TA98, TA100, TA1537	+	-	Glatt et al. (1987)
<i>S. typhimurium</i> TA97, TA98, TA100	+	ND	Marino (1987)
<i>S. typhimurium</i> TA98	+	-	Alzieu et al. (1987)
<i>S. typhimurium</i> TA98, TA100	+	-	Prasanna et al. (1987)
<i>S. typhimurium</i> TA98	+	ND	Ampy et al. (1988)
<i>S. typhimurium</i> TA98, TA100	+	ND	Bos et al. (1988)
<i>S. typhimurium</i> TA98	+	ND	Lee and Lin (1988)
<i>S. typhimurium</i> TA98	+	ND	Antignac et al. (1990)
<i>S. typhimurium</i> TA98	-	ND	Gao et al. (1991)
<i>S. typhimurium</i> TA98	+	ND	Balansky et al. (1994)
<i>S. typhimurium</i> TA100	+	ND	Norpoth et al. (1984)
<i>S. typhimurium</i> TA100	+	-	Carver et al. (1986)
<i>S. typhimurium</i> TA100	+	ND	Pahlman and Pelkonen (1987)
<i>S. typhimurium</i> TA100	+	ND	Tang and Friedman (1977)
<i>S. typhimurium</i> TA100	+	ND	Bruce and Heddele (1979)
<i>S. typhimurium</i> TA100	+	ND	Phillipson and Ioannides (1989)
<i>S. typhimurium</i> TA100	-	ND	Balansky et al. (1994)
<i>S. typhimurium</i> TA1537, TA1538	+	-	Ames et al. (1973)
<i>S. typhimurium</i> TA1537, TA1538	+	-	Glatt et al. (1975)
<i>S. typhimurium</i> TA1537	+	ND	Oesch et al. (1976)

	Result		Reference
	+S9	-S9	
<i>S. typhimurium</i> TA1538	+	ND	Egert and Greim (1976)
<i>S. typhimurium</i> TA1538	+	-	Rosenkranz and Poirier (1979)
<i>S. typhimurium</i> TA1535	-	-	Ames et al. (1973)
<i>S. typhimurium</i> TA 1535	-	-	Glatt et al. (1975)
<i>S. typhimurium</i> TA 1535	-	ND	Mccann et al. (1975)
<i>S. typhimurium</i> TA1535	-	-	Epler et al. (1977)
DNA damage			
<i>Escherichia coli/pol A</i>	+	-	Rosenkranz and Poirier (1979)
<i>E. coli/differential killing test</i>	+	-	Tweats (1981)
<i>E. coli</i> WP2-WP100/rec-assay	+	ND	Mamber et al. (1983)
<i>E. coli/SOS chromotest Pq37</i>	+	-	Mersch-Sundermann et al. (1992)
Endpoint/test system: nonmammalian eukaryotes			
Mitotic recombination			
<i>Saccharomyces cerevisiae</i> D4-RDII	ND	-	Siebert et al. (1981)
<i>S. cerevisiae</i> D3	-	-	Simmon (1979b)

1
2 + = positive; - = negative; ND = not determined.
3

4 **Table D-32. In vitro genotoxicity studies of benzo[a]pyrene in mammalian**
5 **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 (<i>hprt</i>)	ND	+	Crespi et al. (1985)
Human lymphoblastoid (AHH-1) cell line	ND	+	Chen et al. (1996)
Human fibroblast (MRC5CV1) cell line (<i>hprt</i>)	-	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)

Supplemental Information—Benzo[a]pyrene

Assay/test system	Result		Reference
	+S9	-S9	
Mouse <i>lacZ</i> transgenic Muta TM 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 (<i>aprt</i>)	+	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)
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			
<i>DNA adducts</i>			
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)
<i>Unscheduled DNA synthesis</i>			
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)

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Supplemental Information—Benzo[a]pyrene

Assay/test system	Result		Reference
	+S9	-S9	
Rat hepatocytes	ND	+	Michalopoulos et al. (1978)
Rat tracheal cells	ND	-	Roggeband et al. (1994)
<i>DNA repair</i>			
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			
<i>CAs</i>			
Human blood cells	ND	+	Salama et al. (2001)
Human WI38 fibroblasts	+	-	Weinstein et al. (1977)
Chinese hamster lung cells	+	-	Matsuoka et al. (1979)
Chinese hamster V79-4 lung epithelial cells	-	-	Popescu et al. (1977)
Mouse lymphoma (L5178Y/TK+/-) cells	+	ND	Arce et al. (1987)
Rat Liver RL1 cells	+	ND	Dean (1981)
<i>MN</i>			
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)
<i>DNA strand breaks</i>			
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)
<i>SCEs</i>			

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Assay/test system	Result		Reference
	+S9	-S9	
Human C-HC-4 and C-HC-20 hepatoma cells	ND	+	(Abe et al. (1983a), 1983b)
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), 1983b)
Chinese hamster V79 lung epithelial cells	+	-	Popescu et al. (1977)
Chinese hamster V79 lung epithelial cells	+	ND	Mane et al. (1990)
Chinese hamster V79 lung epithelial cells	+	ND	Wojciechowski et al. (1981)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Chinese hamster V79 lung epithelial cells	ND	+	Kulka et al. (1993a)
CHO cells	+	-	de Raat (1979)
CHO cells	+	-	Husgafvel-Pursiainen et al. (1986)
CHO cells	ND	+	Wolff and Takehisa (1977)
CHO cells	ND	+	Pal et al. (1978)
Chinese hamster lung cells	ND	+	Shimizu et al. (1984)
Rabbit peripheral blood lymphocytes	ND	+	Takehisa and Wolff (1978)
Rat ascites hepatoma AH66-B	ND	+	(Abe et al. (1983a), 1983b)
Rat esophageal tumor R1	ND	+	(Abe et al. (1983a), 1983b)
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)
<i>Aneuploidy</i>			
Chinese hamster V79-MZ cells	ND	+	Matsuoka et al. (1998)
<i>Cell transformation</i>			
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)

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Assay/test system	Result		Reference
	+S9	-S9	
Golden hamster embryo cells	+	ND	Mager et al. (1977)
Syrian hamster embryo (SHE) cells	ND	+	(Dipaolo et al. (1971); Dipaolo et al. (1969))
SHE cells	ND	+	Dunkel et al. (1981)
SHE cells	ND	+	Leboeuf et al. (1990)
SHE cells/focus assay	ND	+	Casto et al. (1977)
Fetal Syrian hamster lung cells	ND	+	(Emura et al. (1987); Emura et al. (1980))
Virus infected rat embryo RLV/RE and RAT cells; mouse embryo AKR/Me cells; Syrian hamster embryo cells	ND	+	Heidelberger et al. (1983)
Virus transformed SHE and mouse C3H10T1/2 cells	ND	+	Arce et al. (1987)
Mouse C3H/10T1/2 embryo fibroblasts	ND	+	(Nesnow et al. (2002); Nesnow et al. (1997))
Mouse embryo fibroblast (C3H/10T1/2 CL 8) cells	ND	+	Peterson et al. (1981)
Mouse embryo fibroblast (C3H/10T1/2 CL 8) cells	ND	+	Lubet et al. (1983)
Mouse SHE cells; BALB/c-3t3 cells; C3H/10T1/2 cells; prostate cells	ND	+	Heidelberger et al. (1983)
Mouse BALB/c-3T3 cells	ND	+	Dunkel et al. (1981)
Mouse BALB/c-3T3 cells	ND	+	Matthews (1993)
Mouse BALB/c-3T3 clone A31-1-1	ND	+	Little and Vetrovs (1988)
Rat/Fischer, embryo cells (leukemia virus transformed)	ND	+	Dunkel et al. (1981)
Rat/Fischer, embryo cells/Oua ^R	ND	+	Mishra et al. (1978)

- 1
2 + = positive; - = negative; CHO = Chinese hamster ovary; ND = not determined; SHE = Syrian hamster embryo;
3 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 nonsmokers); hprt locus mutation assay	T-cells of lung cancer patients (smokers and nonsmokers from lung cancer patients and population controls with known smoking status) analyzed for hprt locus mutations.	+	Smokers and nonsmokers	Splicing mutations, base-pair substitutions, frameshift, and deletion mutations observed. Smokers and nonsmokers had GC→TA transversions (13 and 6%, respectively) and GC→AT transitions (24 and 35%, respectively) in hprt gene consistent with in vitro mutagenicity of benzo[a]pyrene.	Hackman et al. (2000)
Mutation, germline	Mouse, T-stock, (SEC × C57BL)F1, (C3H × 101)F1, or (C3H × C57BL)F1 for females; (101 × C3H)F1 or (C3H × 101)F1 for males; dominant-lethal mutation assay	12-wk-old males dosed with benzo[a]pyrene i.p. and mated 3.5–6.5 d posttreatment with 12-wk-old females from different stocks; sacrificed on d 12–15 after vaginal plug was observed; females kept in a 5-hr dark phase to synchronize ovulation 5 wks before the start of the experiment; fertilized eggs collected 9–11 hrs after mating and first-cleavage metaphase chromosomes prepared 20 hrs after mating.	+	500 mg/kg	The percent of dominant lethal mutations were in the order of T-stock = (C3H × 101)F1 > (SEC × C57BL)F1 > (C3H × C57BL)F1.	Generoso et al. (1979)
Mutation, germline	Mouse, male stocks: (101 × C3H)F1; female stocks (A): (101 × C3H)F1, (B): (C3H × 101)F1, (C): (C3H × C57BL)F1, (D):(SEC × C57BL)F1, (E):T-stock females; dominant lethal mutations	In dominant lethal assay, 12-wk-old males dosed i.p. with benzo[a]pyrene and mated with 10–12-wk-old (#1) stock A females; or (#2) stock B females on the day of dosing; or with (#3a) with stocks B, C, and D females 3.5–7.5 d postdosing, or with (#3b) with stocks B, C, D, and E females 3.5–6.5 d postdosing. Control group mated at time corresponding to 1.5–4.5 d posttreatment in the test groups.	+	500 mg/kg	Dominant lethal effects were observed in early to middle (4.5–5.5 and 6.5–7.5 d posttreatment, respectively) spermatozoa and in preleptotene spermatocytes (32.5–33.5 and 34.5–35.5 d posttreatment).	Generoso et al. (1982)

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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; 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, <i>cII</i> 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 <i>cII</i> 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 <i>Xpc</i> ^{-/-} with pUR288 <i>lacZ</i> reporter gene	Benzo[a]pyrene given via gavage in sunflower oil 3 times/wk for 1, 4, or 6 wks (<i>Xpc</i> ^{-/-}) or 6 wks (WT). Spleen, testis, and sperm cells analyzed for <i>lacZ</i> mutation frequency, and DNA adducts analyzed in testis by [³² P]-postlabeling.	+	13 mg/kg	Statistically significant increases in <i>lacZ</i> mutation frequencies in <i>Xpc</i> ^{-/-} 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 <i>lacZ</i> 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 <i>lacZ</i> reporter gene in <i>E. coli</i> 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, <i>lacZ</i> 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 <i>lacZ</i> mutation frequency.	+	25, 50, and 75 mg/kg-d	Highest <i>lacZ</i> mutation frequency observed in small intestine, followed by bone marrow, glandular stomach, and liver.	Lemieux et al. (2011)
Mutation	Mouse, <i>lacZ</i> 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 <i>lacZ</i> mutation frequency.	+	125 mg/kg-d	Highest mutation frequency observed in colon followed by ileum > forestomach > bone marrow = spleen > glandular stomach > liver = lung > kidney = heart.	Hakura et al. (1998)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
Mutation	Mouse, C57BL/6J <i>Dlb-1</i> congenic; <i>Dlb-1</i> 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 (<i>lacZ</i> negative and <i>XPA</i> ^{+/+} and <i>XPA</i> ^{-/-}); 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: <i>XPA</i> ^{-/-} > <i>XPA</i> ^{+/+} .	Bol et al. (1998)
Mutation	Mouse, Cockayne syndrome-deficient (<i>Csb</i> ^{-/-}); heterozygous (<i>Csb</i> ^{+/-}) and WT controls (<i>Csb</i> ^{+/+}); hprt mutation frequency assay	<i>Csb</i> ^{-/-} / <i>lacZ</i> ^{+/-} and <i>Csb</i> ^{+/-} / <i>lacZ</i> ^{+/-} 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 <i>lacZ</i> mutation frequency analysis, mice were sacrificed 3 d after last treatment and liver, lung, and spleen were collected.	+	13 mg/kg	<i>lacZ</i> mutation frequency detected in all tissues but no differences between WT and <i>Csb</i> ^{-/-} mice; hprt mutations significantly higher in <i>Csb</i> ^{-/-} mice than control mice. BPDE-dGuo adducts in hprt gene are preferentially removed in WT mice than <i>Csb</i> ^{-/-} mice.	Wijnhoven et al. (2000)
Mutation	Mouse, B6C3F ₁ , forestomach <i>H-ras</i> , <i>K-ras</i> , and <i>p53</i> mutations	Benzo[a]pyrene given in feed in a 2-yr chronic feeding study.	+	5, 25, or 100 ppm	68% <i>K-ras</i> (codons 12, 13), 10% <i>H-ras</i> (codon 13), 10% <i>p53</i> mutations; all G→T transversions.	Culp et al. (2000)
Mutation	Mouse, <i>lacZ/galE</i> (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 polymerase chain reaction and direct sequencing.	+	600 nmol/mouse	About 90% of papillomas contained Ha-ras mutations, all of them being transversions at codons 12 (20% GGA→GTA), 13 (50% GGC→GTC), and 61 (20% CAA→CTA).	Colapietro et al. (1993)
Mutation	Rat, Wistar	Single dose by gavage; urine and feces collected 0–24, 24–48, and 48–72 hrs posttreatment; urine and extracts of feces tested in <i>S. typhimurium</i> 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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, WBCs	96 people occupationally or medically exposed to PAH mixtures (psoriatic patients, coke oven workers, chimney sweeps, and aluminum anode plant workers); adducts measured by HPLC/fluorescence analysis.	+		Percentages of subjects with adduct levels greater than the 95 th 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)
BPDE-DNA adducts	Human, WBCs	67 highly exposed coke oven workers were tested for genetic factors that can modulate individual responses to carcinogenic PAHs; adducts measured by HPLC/fluorescence analysis.	+		Levels of BPDE-DNA adducts were significantly associated with workplace PAH exposure (as correlated with urinary excretion of 1-pyrenol), lack of GSTM1 activity, and low nucleotide excision repair capacity.	Pavanello et al. (2005)
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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Human, maternal and umbilical cord blood	Maternal and umbilical cord blood obtained following normal delivery from 329 nonsmoking pregnant women exposed to emissions from fires during the 4 wks following the collapse of the WTC building in New York City on 09/11/2001.	+		BPDE-DNA 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)
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/*2a) > CYP1A1*1/*1; occupational > environmental exposure; smokers > nonsmokers; adducts increased with dose and duration of smoking.	Rojas et al. (2000)
BPDE-DNA adducts	Human, WBCs	Coke oven workers were exposed to PAHs and benzo[a]pyrene-WBC DNA analyzed by HPLC-fluorescence detection for BPDE-DNA adducts.	±	0.14 µg/m ³	Median detectable BPDE-DNA adducts in workers versus controls not significant due to low number of subjects (9 workers, 26 controls); 4/9 workers had adducts substantially higher than all controls. No significant difference between smokers and nonsmokers; no correlation with air benzo[a]pyrene levels and adduct levels.	Mensing et al. (2005)
BPDE-DNA adducts	Mouse, <i>lacZ</i> 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)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
BPDE-DNA adducts	Mouse, (<i>Ahr</i> ^{+/+} , <i>Ahr</i> ^{+/-} , <i>Ahr</i> ^{-/-})	Gavage; sacrificed 24 hrs posttreatment.	+	100 mg/kg	No induction of CYP in <i>Ahr</i> ^{-/-} , but all alleles positive for adduct formation.	Sagredo et al. (2006)
BPDE-DNA adducts	Mouse, C57BL/6J <i>Cyp1a1</i> (+/-) and <i>Cyp1a1</i> (-/-)	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 <i>Cyp1a1</i> (-/-) mice than <i>Cyp1a1</i> (+/-) mice.	Uno et al. (2001)
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 d after last treatment; DNA analyzed by [³² P]-postlabeling assay.	+	4 × 1.2 µmol/animal	Five adducts spots detected.	Reddy et al. (1984)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
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)
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 tricapylin; 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 benzo[a]pyrene-induced CAs and AHH inducibility. No differences in bone marrow mitotic index of males of different strains between control and treatment groups.	Adler et al. (1989)
CAs	Mouse, 1C3F1 hybrid (101/E1 × C31 × E1)F1; CAs in bone marrow	Single dose by gavage; sacrificed 30 hrs postdosing; bone marrow from femur isolated and analyzed for CAs.	+	63 mg/kg	Significant increase in CAs in benzo[a]pyrene-treated animals compared to controls.	Adler and Ingwersen (1989)
CAs	Rat, Wistar; peripheral blood lymphocytes	Single dose by gavage; sacrificed 6, 24, and 48 hrs posttreatment; blood from abdominal aorta collected, whole blood cultures set up, CAs scored in 100 first-division peripheral blood lymphocytes per animal.	-	0, 10, 100, or 200 mg/kg	No difference between control and treatment groups at any dose or at any sampling time observed.	Willems et al. (1991)
CAs	Hamster; bone marrow	Single, i.p. injection of benzo[a]pyrene dissolved in tricapyline; 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, <i>lacZ</i> 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 tricapylin; animals sacrificed 30 hrs post-exposure.	-	100, 300, or 500 mg/kg		Bayer (1978)
MN	Fish (carp, rainbow trout, clams); blood and hemolymph		+	0.05, 0.25, 0.5, or 1 ppm		Kim and Hyun (2006)

Endpoint	Test system	Test conditions	Results	Dose	Comment	Reference
DNA strand breaks	Rat, Sprague-Dawley; comet assay	Instilled intratracheally with: (1) single dose of benzo[a]pyrene in aqueous suspension; sacrificed at 3, 24, and 48 hrs posttreatment; alveolar macrophages, lung cells, lymphocytes, and hepatocytes collected or (2) dose-response study and sacrificed at 24 hrs posttreatment; lungs collected; controls received normal saline instillation; all cells analyzed by comet assay.	+	Experiment #1: 3 mg of benzo[a]pyrene; Experiment #2: dose-response study with 0.75, 1.5, or 3 mg benzo[a]pyrene	All time points showed significant increase in SSBs (Experiment #1); a dose-response in SSBs was observed (Experiment #2).	(Garry et al. (2003a) , 2003b)
DNA strand breaks	Aquatic organisms: carp (<i>Cyprinus carpio</i>), rainbow trout (<i>Oncorhynchus mykiss</i>), and clams (<i>Spisula sachalinensis</i>); 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 (s.c.) with BrdU tablet; 2 hrs later given phorone (125 or 250 mg/kg) i.p.; another 2 hrs later dosed i.p. with benzo[a]pyrene; 24 hrs post-BrdU dosing, animals injected with colchicine 10 mg/kg body weight, sacrificed 2 hrs later; bone marrow from femur prepared for SCE assay.	+	50 or 100 mg/kg	SCEs 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)
SCEs	Hamster; bone marrow	Not available	+	2.5, 25, 40, 50, 75, or 100 mg/kg	Frequency of SCEs increased ≥40 mg/kg body weight.	Bayer (1978)

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

^aFM6 = 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.

1 D.5.2. Tumor Promotion and Progression

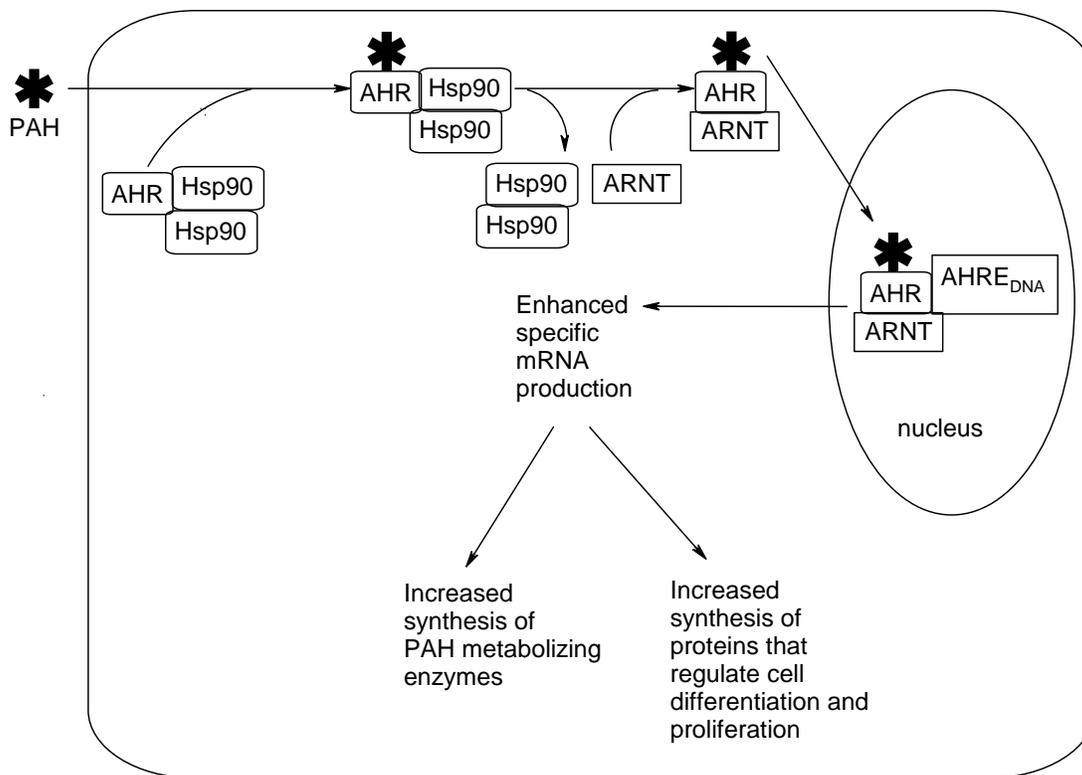
2 *Cytotoxicity and Inflammatory Response*

3 The cytotoxicity of benzo[a]pyrene metabolites may contribute to tumor promotion via
4 inflammatory responses leading to cell proliferation ([Burdick et al., 2003](#)). Benzo[a]pyrene is
5 metabolized to *o*-quinones, which are cytotoxic, and can generate ROS ([Bolton et al., 2000](#); [Penning
6 et al., 1999](#)). Benzo[a]pyrene *o*-quinones reduce the viability and survival of rat and human
7 hepatoma cells ([Flowers-Geary et al., 1996](#); [Flowers-Geary et al., 1993](#)). Cytotoxicity was also
8 induced by benzo[a]pyrene and BPDE in a human prostate carcinoma cell line ([Nwagbara et al.,
9 2007](#)). Inflammatory responses to cytotoxicity may contribute to the tumor promotion process.
10 For example, benzo[a]pyrene quinones (1,6-, 3,6-, and 6,12-benzo[a]pyrene-quinone) generated
11 ROS and increased cell proliferation by enhancing the epidermal growth factor receptor pathway in
12 cultured breast epithelial cells ([Burdick et al., 2003](#)).

13 Several studies have demonstrated that exposure to benzo[a]pyrene increases the
14 production of inflammatory cytokines, which may contribute to cancer progression. [Garçon et al.
15 \(2001a\)](#) and [Garçon et al. \(2001b\)](#) exposed Sprague-Dawley rats by inhalation to benzo[a]pyrene
16 with or without ferrous oxide (Fe₂O₃) particles. They found that benzo[a]pyrene alone or in
17 combination with Fe₂O₃ particles elicited mRNA and protein synthesis of the inflammatory
18 cytokine, IL-1. [Tamaki et al. \(2004\)](#) also demonstrated a benzo[a]pyrene-induced increase in IL-1
19 expression in a human fibroblast-like synoviocyte cell line (MH7A). Benzo[a]pyrene increases the
20 expression of the mRNA for CCL1, an inflammatory chemokine, in human macrophages ([N'Diaye et
21 al., 2006](#)). The benzo[a]pyrene-induced increase in CCL1 mRNA was inhibited by the potent AhR
22 antagonist, 3'-methoxy-4'-nitroflavone.

23 *AhR-Mediated Effects*

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



1

2 AHRE_{DNA} = Ah-responsive elements of DNA; ARNT = Ah receptor nuclear translocator.

3

4 Source: [Okey et al. \(1994\)](#).5 **Figure D-3. Interaction of PAHs with the AhR.**

6

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

1 in AhR knock-out mice compared with WT C57Bl6/J mice following a single dermal application of
2 33 mg/kg benzo[a]pyrene for 24 hours. [Ma and Lu \(2007\)](#) further noted that Ah-nonresponsive
3 mice were at greater risk of toxicity and tumorigenicity in remote organs, distant from the site of
4 exposure (i.e., bone marrow). As an example, [Uno et al. \(2006\)](#) showed that benzo[a]pyrene
5 (125 mg/kg-day, orally for 18 days) caused marked wasting, immunosuppression, and bone
6 marrow hypocellularity in CYP1A1 knock-out mice, but not in WT mice.

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

25 [Uno et al. \(2006\)](#) also demonstrated a paradoxical increase in liver DNA adducts in AhR
26 knock-out mice following oral exposure to benzo[a]pyrene. WT C57BL/6 mice and several knock-
27 out mouse strains (CYP1A2^{-/-} and CYP1B1^{-/-} single knock-out, CYP1A1/1B1^{-/-} and
28 CYP1A2/1B1^{-/-} double knock-out) were studied. Benzo[a]pyrene was administered in the feed at
29 1.25, 12.5, or 125 mg/kg for 18 days (this dose is well-tolerated by WT C57BL/6 mice for 1 year,
30 but lethal within 30 days to the CYP1A1^{-/-} mice). Steady-state blood levels of benzo[a]pyrene,
31 reached within 5 days of treatment, were ~25 times higher in CYP1A1^{-/-} and ~75 times higher in
32 CYP1A1/1B1^{-/-} than in WT mice, while clearance was similar to WT mice in the other knock-out
33 mouse strains. DNA adduct levels, measured by [³²P]-postlabeling in liver, spleen, and bone
34 marrow, were highest in the CYP1A1^{-/-} mice at the two higher doses, and in the CYP1A1/1B1^{-/-}
35 mice at the mid dose only. Adduct patterns, as revealed by 2-dimensional chromatography, differed
36 substantially between organs in the various knock-out types.

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

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

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

26 Inhibition of gap junctional intercellular communication (GJIC)

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

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

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

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

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

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

18 The Gene Expression Omnibus and Array Express microarray repositories were searched
 19 for studies that used benzo[a]pyrene as a test chemical and raw data were available. The search
 20 terms used and the number of studies retrieved are listed in Table D-34. Many of the search terms
 21 included terms for specific PAH mixtures, as benzo[a]pyrene is commonly used as a reference
 22 chemical in PAH mixture studies, to ensure the available and usable benzo[a]pyrene microarray
 23 data were identified.

24 **Table D-34. Search terms and the number of studies retrieved from the gene**
 25 **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

1
2 Forty responsive gene expression datasets were identified, representing 26 peer-reviewed
3 publications. These datasets were further culled for analysis by focusing on publicly available
4 results and species and organs represented by more than one available dataset on the same
5 microarray platform. Crossing microarray platforms and species boundaries adds significant
6 uncertainty to the interpretation with respect to comparisons of the probes being measured, how
7 those different probes align to the genome and are mapped to specific genes, and creates an open
8 question regarding the discovery and mapping of orthologous genes across species. Thus, the
9 analysis included two studies that focused on mouse in vivo transcriptomic studies of the liver
10 (Gene Expression Omnibus accessions: GSE24907 and GSE18789).

11 The first study ([Malik et al., 2012](#)), GSE24907, exposed five male Muta mice (a LacZ
12 transgenic mouse line) per group to 25, 50, or 75 mg/kg benzo[a]pyrene or olive oil vehicle for
13 28 days by gavage. The second study ([Yauk et al., 2011](#)), GSE18789, exposed 27–30-day-old male
14 B6C3F₁ mice to 150 mg/kg benzo[a]pyrene by gavage for 3 days and sacrificed 4 or 24 hours after
15 the final dose. Both studies were subjected to study quality evaluation by the Systematic Omics
16 Analysis Review (SOAR) tool.

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

24 **Data Analysis Overview**

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

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

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

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

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

19 Data were analyzed using limma and an empirical Bayes moderated t-test ([Smyth, 2004](#)).
20 Following analysis, active genes were identified. A gene was considered active if it exhibited a
21 1.5-fold-change and a *p*-value <0.1 in at least one condition or group (e.g., time-point or dose).

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

32 The pathway analysis is a powerful method for comparing study results and identifying
33 consistency than a direct comparison of the active gene list. For instance, differentially expressed
34 gene lists reported in the peer-reviewed literature are not reproducible across similar studies ([Shi
35 et al., 2008](#); [Chuang et al., 2007](#); [Ein-Dor et al., 2005](#); [Lossos et al., 2004](#); [Fortunel et al., 2003](#)). In
36 one example, three different studies aimed at identifying genes that confer “stemness” (i.e., genes
37 which are responsible for conferring stem-cell like capabilities) each yielded 230, 283, and
38 385 active genes, yet the overlap between them was only one gene ([Fortunel et al., 2003](#)). This

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

4 Three candidate pathways were identified. These are:

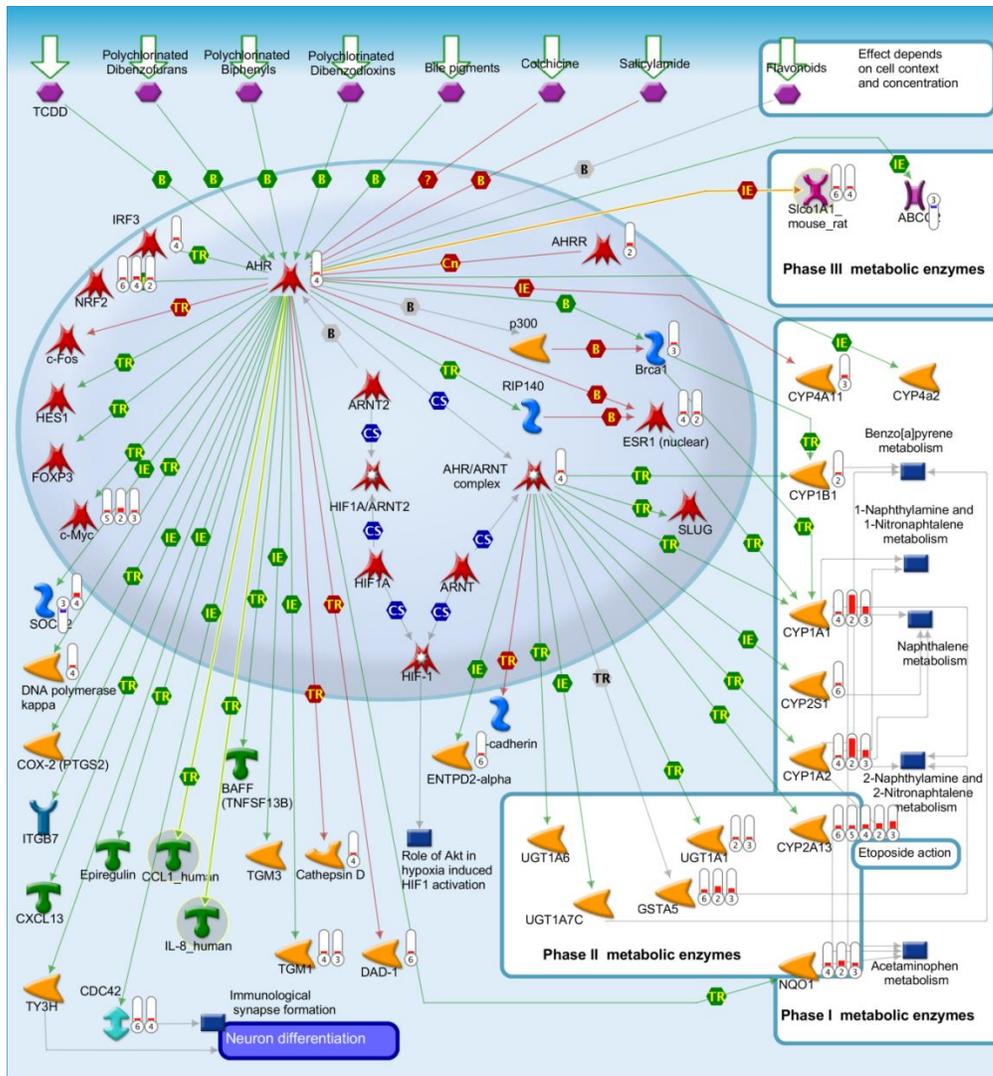
- 5 • AhR signaling
- 6 • DNA damage regulation of the G1/S phase transition
- 7 • Nrf2 regulation of oxidative stress

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

14 **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 hrs after final dose)	Yauk et al. (2011)
3	150 mg/kg	3-d exposure (sacrificed 24 hrs after final dose)	Yauk et al. (2011)
4	75 mg/kg	28-d exposure	Malik et al. (2012)
5	50 mg/kg	28-d exposure	Malik et al. (2012)
6	25 mg/kg	28-d exposure	Malik et al. (2012)

15



1

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5

Figure D-4. AhR 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-34). For instance, NRF2 is upregulated in the 25 mg/kg.

6

AhR Signaling

7

8

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10

11

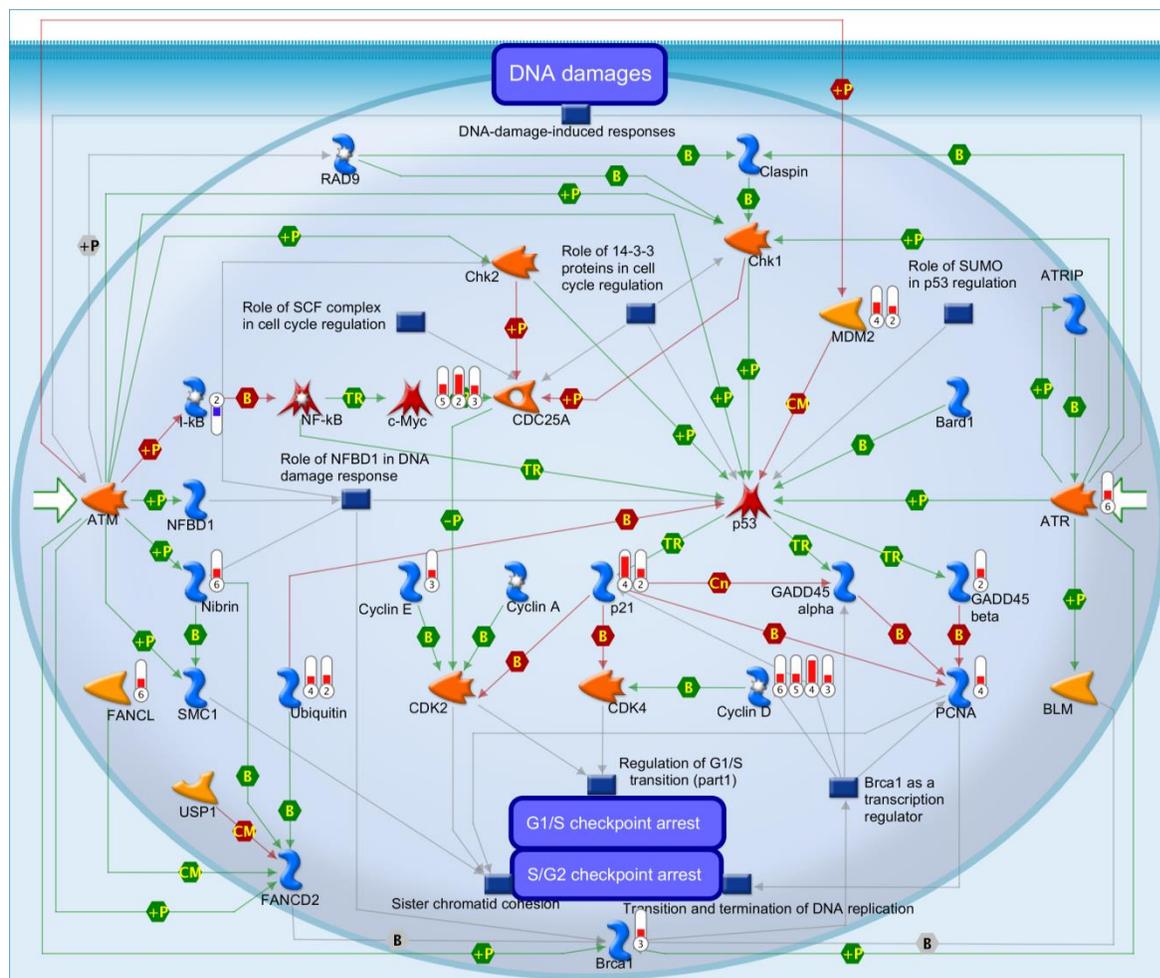
12

13

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

1 [et al., 2000](#)). The AhR has been demonstrated to bind and regulate the Nrf2 promoter ([Dere et al.](#)
2 [2011](#); [Lo et al., 2011](#); [Nair et al., 2008](#)).

3



4

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

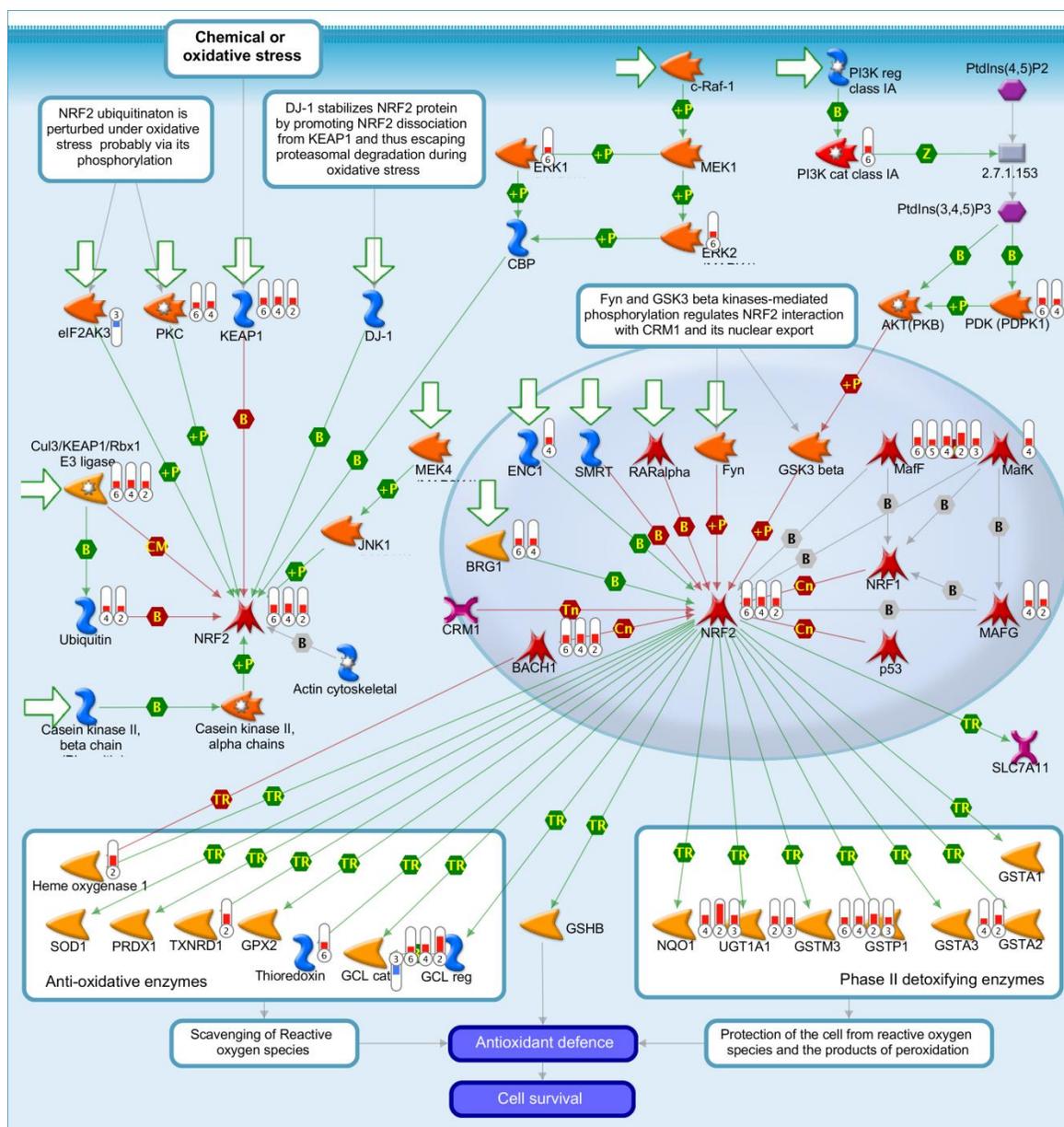
8 **DNA Damage Signaling**

9 The strong upregulation of *p21* and *MDM2* at 4 hours and 75 mg/kg suggests that *p53* is
10 activated following exposure to benzo[a]pyrene, suggesting that benzo[a]pyrene induces DNA
11 damage as early as the 4-hour time-point, and at 75 mg/kg in mice (Figure D-5). *MDM2* is a target
12 gene of *p53*, and also negatively feedback inhibits *p53* signaling through ubiquitination. Ubiquitin is
13 also upregulated at 4 hours and 75 mg/kg, further suggesting that that *p53* may initially be
14 upregulated at times prior to 4 hours and prior to sacrifice in the 75 mg/kg groups, and that at the
15 time of sacrifice, the *p53* signal may be degraded due to *MDM2*-mediated ubiquitination. Coupled
16 with the upregulation of *Cyclin D* and *PCNA* at 75 mg/kg (among other conditions), this suggests a

1 pro-mitotic shift may be occurring which could lead to cellular proliferation in the liver in the mice
2 exposed to 75 mg/kg per day.

3 ***Nrf2 Signaling***

4 Nrf2 transcription may be upregulated by benzo[a]pyrene through activation of the AhR
5 (Figure D-4). The Nrf2 protein heterodimerizes with the MafF protein ([Surh et al., 2008](#); [Marini et](#)
6 [al., 2002](#); [Kim et al., 2000](#)) to regulate the transcription of Phase II metabolism and anti-oxidative
7 enzymes (Figure D-6). Activated *p53* competes with Nrf2 anti-oxidant signaling, perhaps to ensure
8 a large oxidative stress response is present in the cell to promote the induction of apoptosis
9 ([Faraonio et al., 2006](#)). Upregulation of Cul3 at 4 hours and the 75 mg/kg dose in concert with the
10 upregulation of ubiquitin at the same time and dose suggests that repression of Nrf2 activity may
11 occur. This would support the *p53*-mediated pro-oxidant hypothesis, which is further
12 substantiated by the lack of upregulation of anti-oxidant genes at 75 mg/kg, with the exception of
13 GCL cat.
14



1

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

5 **Pathway Analysis Summary**

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

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

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

26
27

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

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

E.1. NONCANCER ENDPOINTS

E.1.1. 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), then the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p -value < 0.10), then the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using

1 either constant variance or modeled variance, models for the mean response were tested for
 2 adequacy of fit using a likelihood ratio test (BMD5 Test 4, with χ^2 *p*-value <0.10 indicating
 3 inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual
 4 fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

5 Model selection

6 For each endpoint selected for modeling (see Table E-1), the BMDL estimate (95% lower
 7 confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and
 8 Akaike’s Information Criterion (AIC) value were used to select a best-fit model from among the
 9 models exhibiting adequate fit. If the BMDL estimates were “sufficiently close,” that is, differed by
 10 at most threefold, then the model selected was the one that yielded the lowest AIC value. If the
 11 BMDL estimates were not sufficiently close, then the lowest BMDL was selected as the POD.

12 **Table E-1. Noncancer endpoints selected for dose-response modeling for**
 13 **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. (2011)	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

14
 15 *Significantly (*p* < 0.05) different from control mean; student t-test (unpaired, two-tailed); n = 10/sex/group.
 16 **Statistically different (*p* < 0.05) from controls using one-way analysis of variance (ANOVA).

17 ^aReported as standard error (SE), but judged to be standard deviation (SD) (and confirmed by study authors).

18 ^bTime-weighted average (TWA) doses over the 60-day study period.

19 ^cDoses converted to mg/kg-day after adjustment for equivalent continuous dosing (2/7 days/week).

1 Modeling results

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

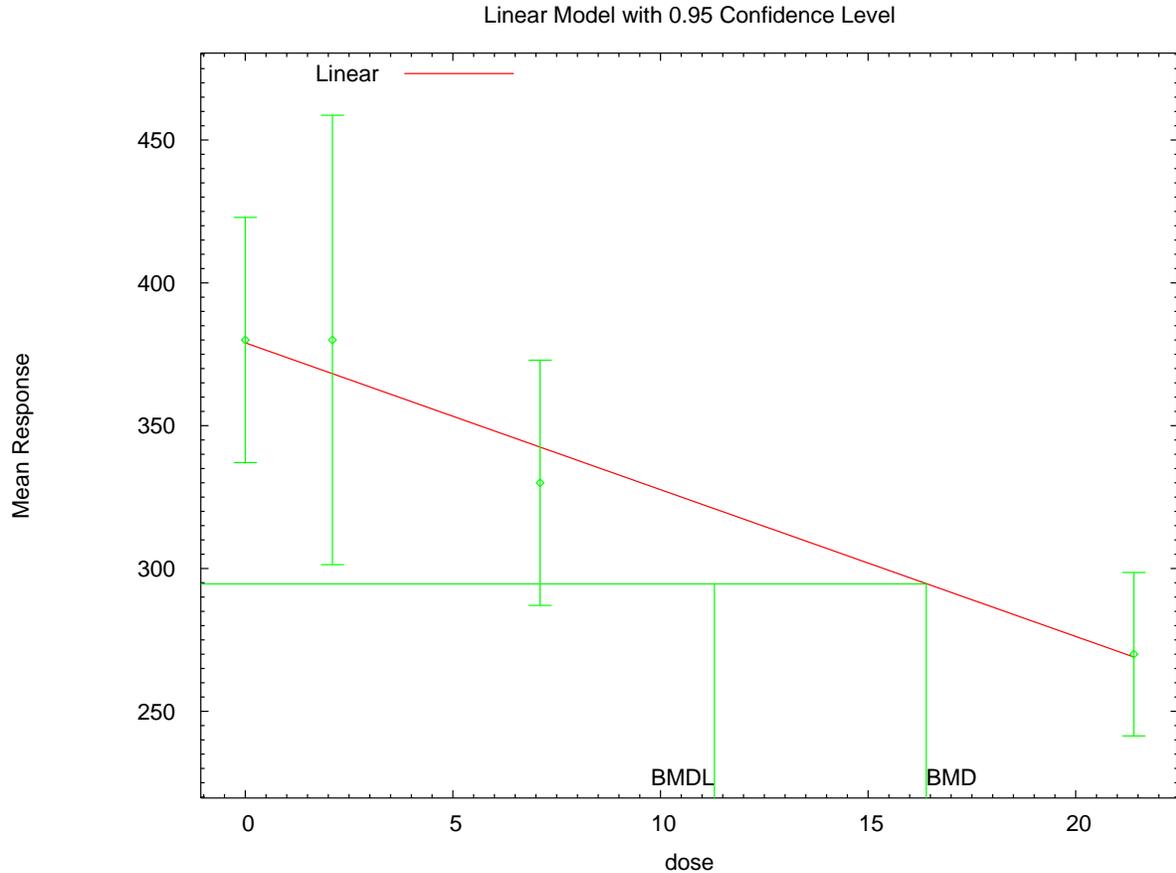
4 **Table E-2. Summary of BMD modeling results for decreased thymus weight in**
 5 **male Wistar rats exposed to benzo[a]pyrene by gavage for 90 days ([Kroese et](#)**
 6 **[al., 2001](#)); BMR = 1 SD change from the control mean**

Model	Variance <i>p</i> -value ^a	Goodness of fit		BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)
		<i>p</i> -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

7
 8 ^aValues <0.10 fail to meet conventional goodness-of-fit criteria.

9 ^bPower restricted to ≥1.

10



1 15:33 10/15 2009

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

3 **Figure E-1. Fit of linear model (nonconstant variance) to data on decreased**
 4 **thymus weight in male Wistar rats—90 days (Kroese et al., 2001).**

```

5 =====
6     Polynomial Model. (Version: 2.13; Date: 04/08/2008)
7     Input Data File:
8     C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2Linkrolin.(
9     d)
10    Gnuplot Plotting File:
11    C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2Linkrolin.p
12    lt
13    =====
14
15    BMDS Model Run
16    ~~~~~
17
18    The form of the response function is:
19
20    Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
21
22
23    Dependent variable = mean
24    Independent variable = dose
25    The polynomial coefficients are restricted to be negative
26    The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
27
28    Total number of dose groups = 4
    
```

Supplemental Information—Benzo[a]pyrene

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\text{lalpha} + \text{rho} \cdot \ln(\mu(i)))$
 Model A3 uses any fixed variance parameters that were specified by the user

Model R: $Y_i = \mu + e(i)$
 $\text{Var}\{e(i)\} = \sigma^2$

Likelihoods of Interest

Supplemental Information—Benzo[a]pyrene

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

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1 **Table E-3. Summary of BMD modeling results for decreased thymus weight in**
 2 **female Wistar rats exposed to benzo[a]pyrene by gavage for 90 days ([Kroese](#)**
 3 **[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 <i>p</i> -value ^a	Mean <i>p</i> -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				

4
5 ^aValues <0.10 fail to meet conventional goodness-of-fit criteria.

6 ^bPower restricted to ≥1.

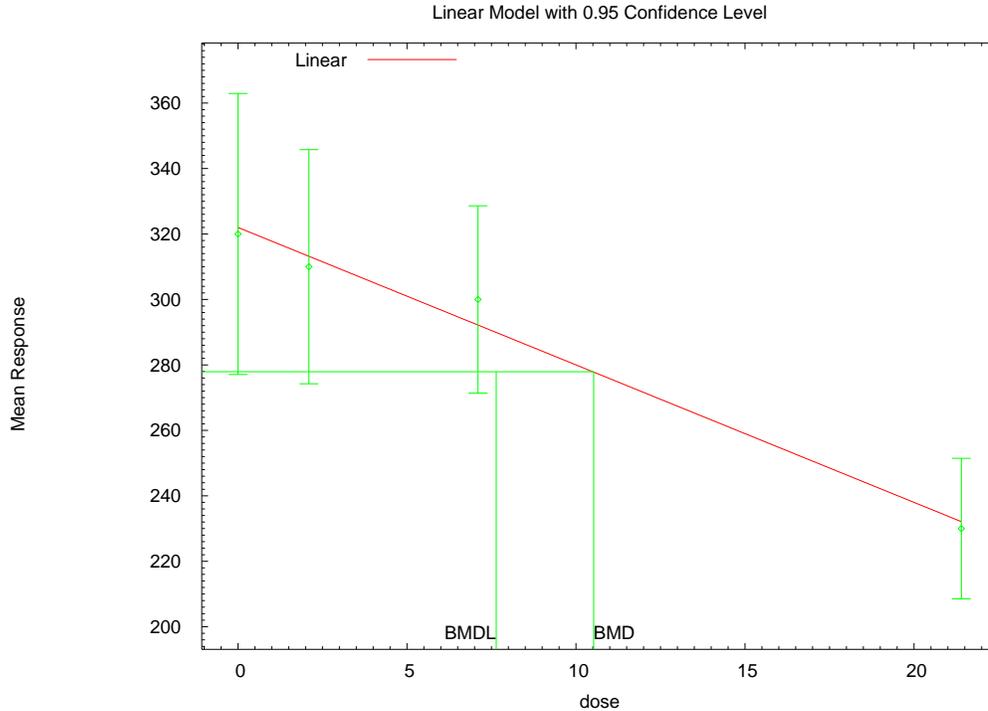
7 ^cCoefficients restricted to be negative.

8 ^dLowest degree polynomial with an adequate fit is reported.

9
10 BMD/BMC = maximum likelihood estimate (MLE) of the dose/concentration associated with the selected BMR;

11 NA = not applicable; model failed to generate.

12



1 16:27 10/15 2009

2 BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.
3

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

```

6 =====
7 Polynomial Model. (Version: 2.13; Date: 04/08/2008)
8 Input Data File:
9 C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\female\durationadjusted\2Linkrolin
10 .(d)
11 Gnuplot Plotting File:
12 C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\female\durationadjusted\2Linkrolin
13 .plt
14 Thu Oct 15 16:27:44 2009
15 =====
16
17 BMDS Model Run
18 ~~~~~
19
20 The form of the response function is:
21
22 Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
23
24
25 Dependent variable = mean
26 Independent variable = dose
27 rho is set to 0
28 The polynomial coefficients are restricted to be negative
29 A constant variance model is fit
30
31 Total number of dose groups = 4
32 Total number of records with missing values = 0
33 Maximum number of iterations = 250
34 Relative Function Convergence has been set to: 1e-008
35 Parameter Convergence has been set to: 1e-008

```

Supplemental Information—Benzo[a]pyrene

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$
 Model A3 uses any fixed variance parameters that were specified by the user

Model R: $Y_i = \mu + e(i)$
 $\text{Var}\{e(i)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-171.357252	5	352.714504
A2	-168.857234	8	353.714467

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Supplemental Information—Benzo[a]pyrene

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.

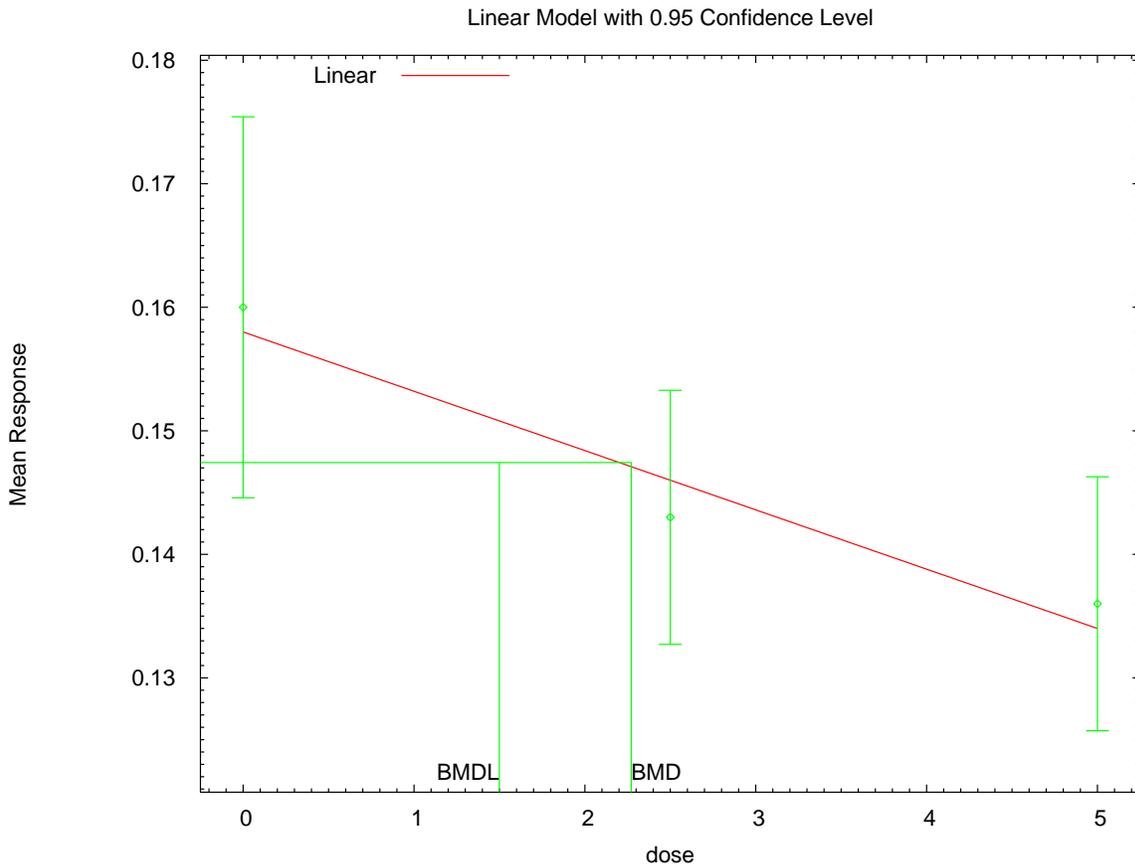
Benchmark Dose Computation

Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 10.5228
BMDL = 7.64037

1 **Table E-4. Summary of BMD modeling results for decreased ovary weight in**
 2 **female Sprague-Dawley rats exposed to benzo[a]pyrene by gavage for 60 days**
 3 **([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

4
 5 ^aNA = not applicable; model failed to generate.



6 16:03 12/14 2010

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

```

9 =====
10 Polynomial Model. (Version: 2.16; Date: 05/26/2010)
11 Input Data File:
12 C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1SD. (d)
13 Gnuplot Plotting File:
14 C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1SD.plt
15 Tue Dec 14 13:51:32 2010
16 =====
17 ~~~~~
    
```

The form of the response function is:

$$Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 \cdot \text{dose} + \text{beta}_2 \cdot \text{dose}^2 + \dots$$

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$

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$$\text{Var}\{e(ij)\} = \text{Sigma}(i)^2$$

Model A3: $Y_{ij} = \text{Mu}(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \text{Sigma}^2$
 Model A3 uses any fixed variance parameters that were specified by the user

Model R: $Y_i = \text{Mu} + e(i)$
 $\text{Var}\{e(i)\} = \text{Sigma}^2$

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

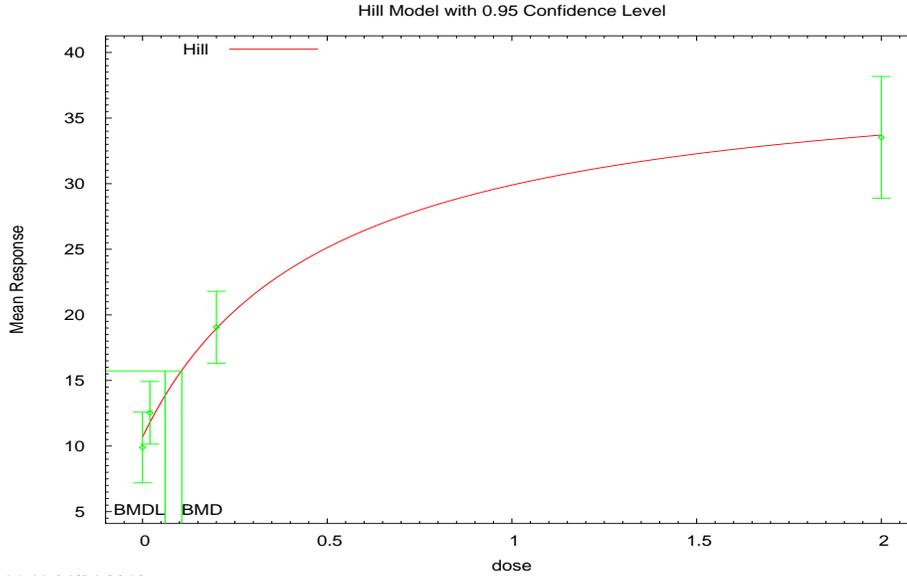
1 **Table E-5. Summary of BMD modeling results for Morris water maze: escape**
 2 **latency in male and female Sprague-Dawley rats exposed to benzo[a]pyrene**
 3 **by gavage for 90 days ([Chen et al., 2012](#)); BMR = 1 SD change from the control**
 4 **mean**

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

5
 6 ^aIncludes modeling of heterogeneous variances (BMDS Test 3, $p = 0.313$).

7 ^bPower parameter n was estimated to be 1 (boundary of parameter space).

8
 9 Data from Morris water maze was presented graphically in [Chen et al. \(2012\)](#), but dose
 10 group means and standard deviations (SDs) were provided upon request by the study authors,
 11 which enabled modeling of this endpoint. In addition, the data for male and female rats were
 12 combined for dose-response analysis because there was no substantive difference between males
 13 and females for each dose group (supported by statistical testing using two-way analysis of
 14 variance [ANOVA], and allowing for interactions), and because there was no rationale or
 15 information available suggesting there would be sex-mediated differences for these
 16 neurobehavioral tests.
 17



14:41 04/24 2012

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:

$$Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$$

Dependent variable = Mean

Independent variable = Dose

Power parameter restricted to be greater than 1

The variance is to be modeled as $\text{Var}(i) = \exp(\text{lalpha} + \text{rho} * \ln(\text{mean}(i)))$

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

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Supplemental Information—Benzo[a]pyrene

(*** 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: $Y_{ij} = \mu(i) + e(ij)$
 $Var\{e(ij)\} = \sigma^2$
- Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $Var\{e(ij)\} = \sigma(i)^2$
- Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $Var\{e(ij)\} = \exp(lalpha + rho * \ln(\mu(i)))$
Model A3 uses any fixed variance parameters that were specified by the user
- Model R: $Y_i = \mu + e(i)$
 $Var\{e(i)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-192.799518	5	395.599036
A2	-186.795503	8	389.591006

Supplemental Information—Benzo[a]pyrene

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.

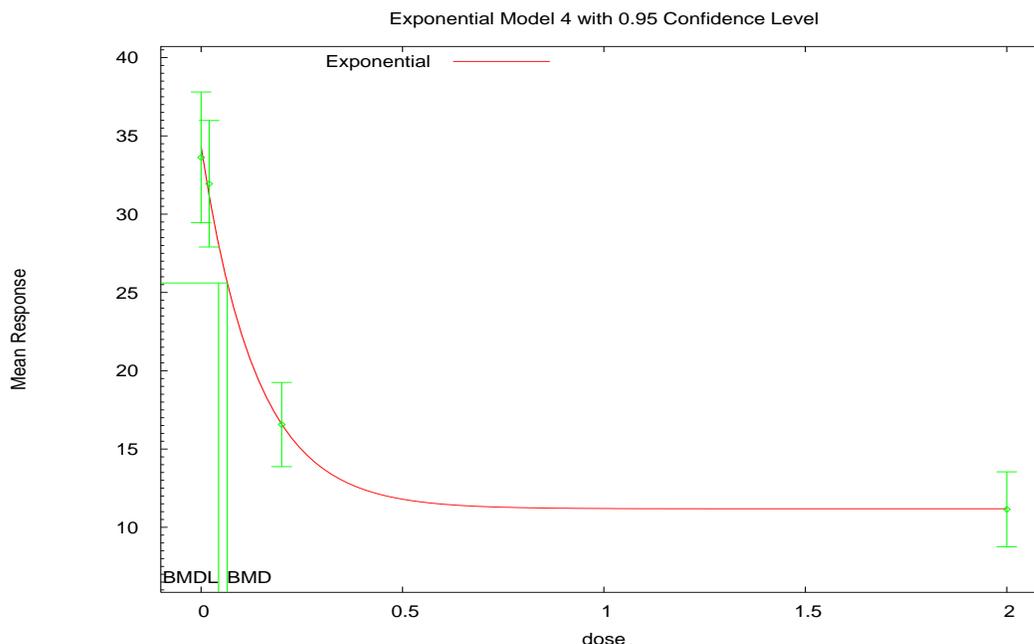
Benchmark Dose Computation

Specified effect =	1
Risk Type =	Estimated standard deviations from the control mean
Confidence level =	0.95
BMD =	0.106284
BMDL =	0.0609511

1 **Table E-6. Summary of BMD modeling results for Morris water maze: time**
 2 **spent in quadrant for in male and female Sprague-Dawley rats exposed to**
 3 **benzo[a]pyrene by gavage for 90 days (Chen et al., 2012); BMR = 1 SD change**
 4 **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

5
 6 ^aIncludes modeling of heterogenous variances (BMDS Test 3, $p = 0.919$).
 7 ^bNA: insufficient degrees of freedom to evaluate χ^2 .



8 14:35 04/24 2012

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

```

11 =====
12 Exponential Model. (Version: 1.7; Date: 12/10/2009)
13 Input Data File: C:\Documents and Settings\...\exp_Chen.FM.target_Exp-ModelVariance-
14 BMR1Std-Down. (d)
15 =====
16
17 BMDS Model Run
18 ~~~~~
19
20 The form of the response function by Model:
21 Model 2: Y[dose] = a * exp$$$sign * b * dose}
22 Model 3: Y[dose] = a * exp$$$sign * (b * dose)^d}
    
```

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

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: $\exp(\ln\alpha + \rho * \ln(Y[\text{dose}]))$
 The variance is to be modeled as $\text{Var}(i) = \exp(\ln\alpha + \log(\text{mean}(i)) * \rho)$

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: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\alpha + \log(\text{mean}(i)) * \rho)$

Model R: $Y_{ij} = \mu + e(i)$
 $\text{Var}\{e(ij)\} = \sigma^2$

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

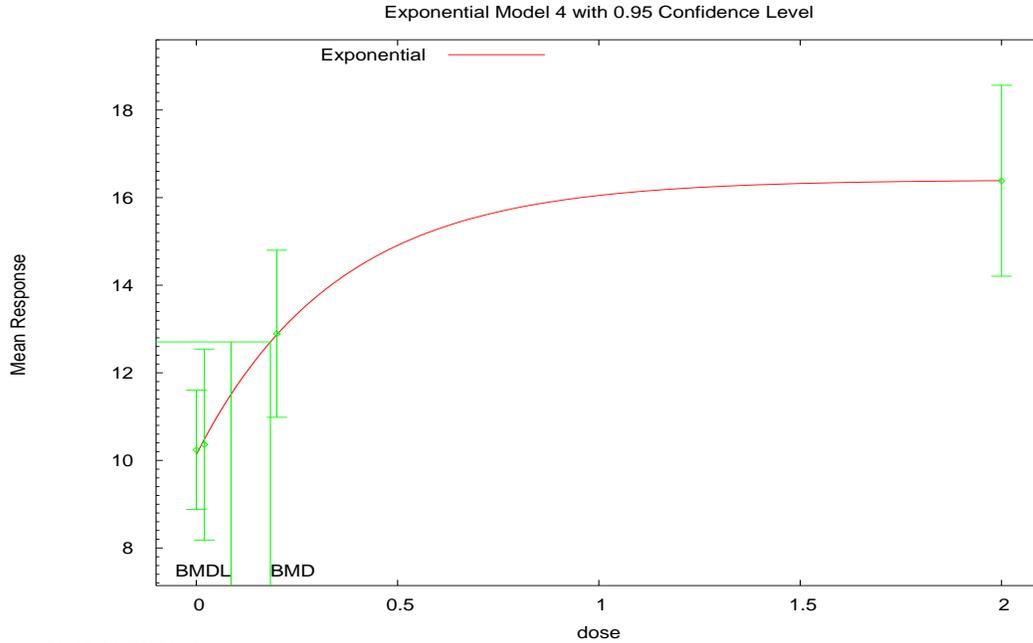
^aConstant variance models are presented (BMDS Test 2 p-value = 0.46), with the selected model in bold. Scaled residuals for selected model for doses 0, 0.02, 0.2, and 2 mg/kg-d were 0.13, -0.15, 0.03, and -0.003, respectively.

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.

^bExponential M5 and Hill model required four parameters and there are four dose groups, leaving no degrees of freedom for the goodness-of-fit test. Therefore, these were not considered for model selection

For the elevated plus maze data, although effects of exposure were observed across multiple ages and both sexes, the results from female rats at PND 70 were chosen for dose-response analyses as effects in females and older animals were of greater severity. While it is preferred that elevated plus maze results be presented as percent of open arm entries or percent of time in the open arms (as a function of total arm entries or time) in order to rule out potential differences in motor activity or general exploration (Hogg, 1996), the data provided in the study were sufficient to rule out that total arm entries were affected by treatment.



1 12:35 08/02 2012

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

```

4 =====
5 Exponential Model. (Version: 1.7; Date: 12/10/2009)
6 Input Data File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\_CAST
7 plus\BaP\BMDs\exp_ChenFO70_Exp-ConstantVariance-BMR1Std-Up.(d)
8 Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\_CAST
9 plus\BaP\BMDs\exp_ChenFO70_Exp-ConstantVariance-BMR1Std-Up.plt
10 Thu Aug 02 12:35:33 2012
11 =====
12
13 BMDs Model Run
14 ~~~~~
15
16 The form of the response function by Model:
17 Model 2: Y[dose] = a * exp{sign * b * dose}
18 Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
19 Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
20 Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
21
22 Note: Y[dose] is the median response for exposure = dose;
23 sign = +1 for increasing trend in data;
24 sign = -1 for decreasing trend.
25
26 Model 2 is nested within Models 3 and 4.
27 Model 3 is nested within Model 5.
28 Model 4 is nested within Model 5.
29
30
31 Dependent variable = Mean
32 Independent variable = Dose
33 Data are assumed to be distributed: normally
34 Variance Model: exp(lnalpha +rho *ln(Y[dose]))
35 rho is set to 0.
36 A constant variance model is fit.
37
38 Total number of dose groups = 4
39 Total number of records with missing values = 0
40 Maximum number of iterations = 250
    
```

Supplemental Information—Benzo[a]pyrene

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: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$
- Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$
- Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\ln\alpha + \log(\text{mean}(i)) * \rho)$
- Model R: $Y_{ij} = \mu + e(i)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest

Supplemental Information—Benzo[a]pyrene

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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

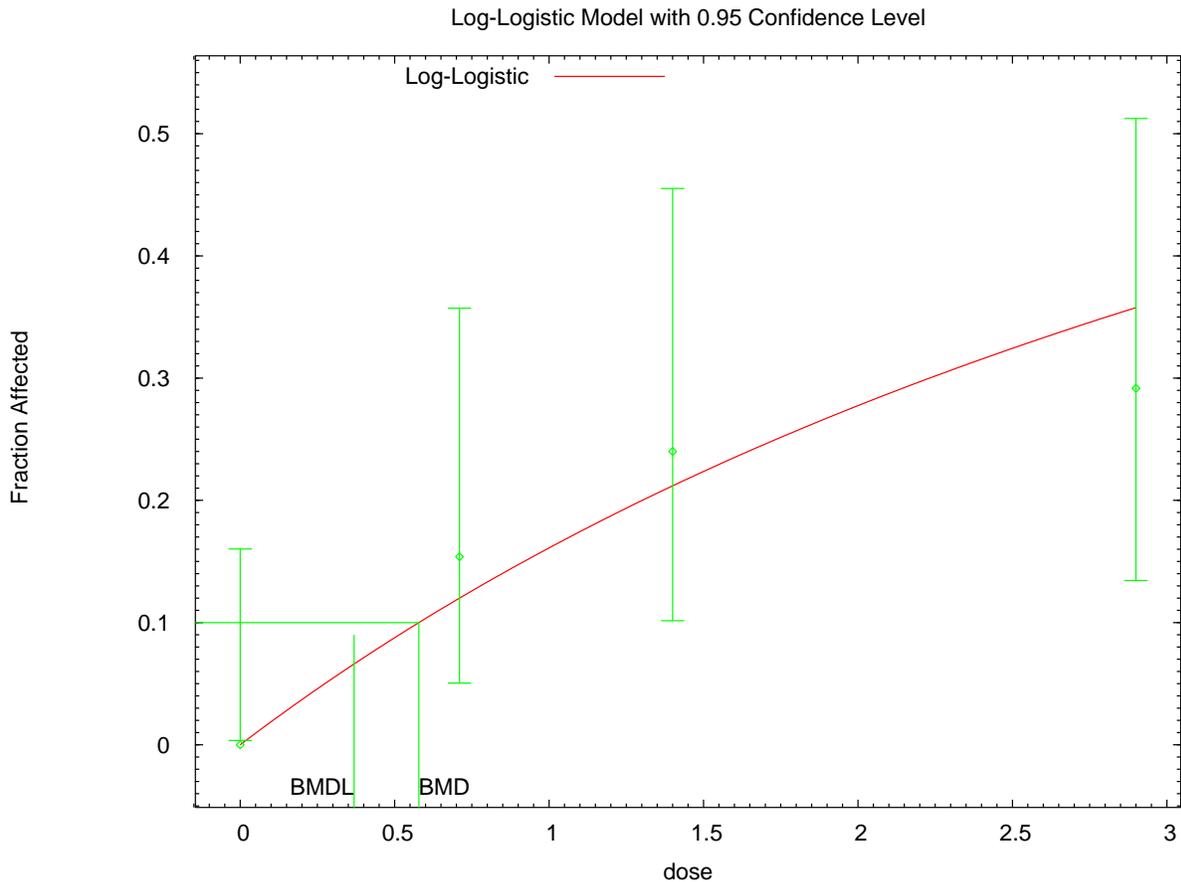
Confidence Level = 0.950000

BMD = 0.184087

BMDL = 0.0864691

1 **Table E-8. Summary of BMD modeling results for incidence of cervical**
 2 **epithelial hyperplasia in female ICR mice exposed to benzo[a]pyrene by oral**
 3 **exposure for 98 days ([Gao et al., 2011](#)); BMR = 1 SD change from the control**
 4 **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



5 19:01 08/26 2011

6 **Figure E-7. Fit of log-logistic model to data on cervical epithelial hyperplasia**
 7 **([Gao et al., 2011](#))**

Supplemental Information—Benzo[a]pyrene

```

1      =====
2      Logistic Model. (Version: 2.13; Date: 10/28/2009)
3      Input Data File: C:\Users\hclynch\Documents\Active Projects\_FA498 IRIS\xBaP\IASC Aug
4      2011\bmd modeling\lnl_gao 2011 inflamm cells_Opt.(d)
5      Gnuplot Plotting File: C:\Users\hclynch\Documents\Active Projects\_FA498
6      IRIS\xBaP\IASC Aug 2011\bmd modeling\lnl_gao 2011 inflamm cells_Opt.plt
7      =====

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9      BMDS_Model_Run
10     ~~~~~

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12     The form of the probability function is:
14     P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

```

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17     Dependent variable = Col3
18     Independent variable = Col1
19     Slope parameter is restricted as slope >= 1
21     Total number of observations = 4
22     Total number of records with missing values = 0
23     Maximum number of iterations = 250
24     Relative Function Convergence has been set to: 1e-008
25     Parameter Convergence has been set to: 1e-008

```

```

29     User has chosen the log transformed model

```

```

32     Default Initial Parameter Values
33     background = 0
34     intercept = -1.60901
35     slope = 1

```

```

38     Asymptotic Correlation Matrix of Parameter Estimates
39
40     ( *** The model parameter(s) -background -slope
41     have been estimated at a boundary point, or have been specified by the user,
42     and do not appear in the correlation matrix )

```

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44     intercept
45
46 intercept      1

```

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50     Parameter Estimates
51
52     95.0% Wald Confidence Interval
53     Variable      Estimate      Std. Err.      Lower Conf. Limit      Upper Conf. Limit
54     background      0              *              *              *
55     intercept     -1.6502        *              *              *
56     slope          1              *              *              *

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58     * - Indicates that this value is not calculated.

```

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62     Analysis of Deviance Table
63
64     Model      Log(likelihood)  # Param's  Deviance  Test d.f.  P-value
65     Full model      -39.4267         4
66     Fitted model    -39.8502         1      0.847034   3          0.8382
67     Reduced model   -45.7739         1      12.6945   3          0.005346
68
69     AIC:           81.7004

```

Supplemental Information—Benzo[a]pyrene

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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

Chi² = 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

1 **E.1.2. Reference Concentration (RfC)**

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

3 Dosimetry Modeling for Estimation of Human Equivalent Concentrations

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

10

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

11 where:

12 BW = body weight (kg)

13 V_E = ventilation rate (L/minute)

14 F_{TOT} = total fractional deposition

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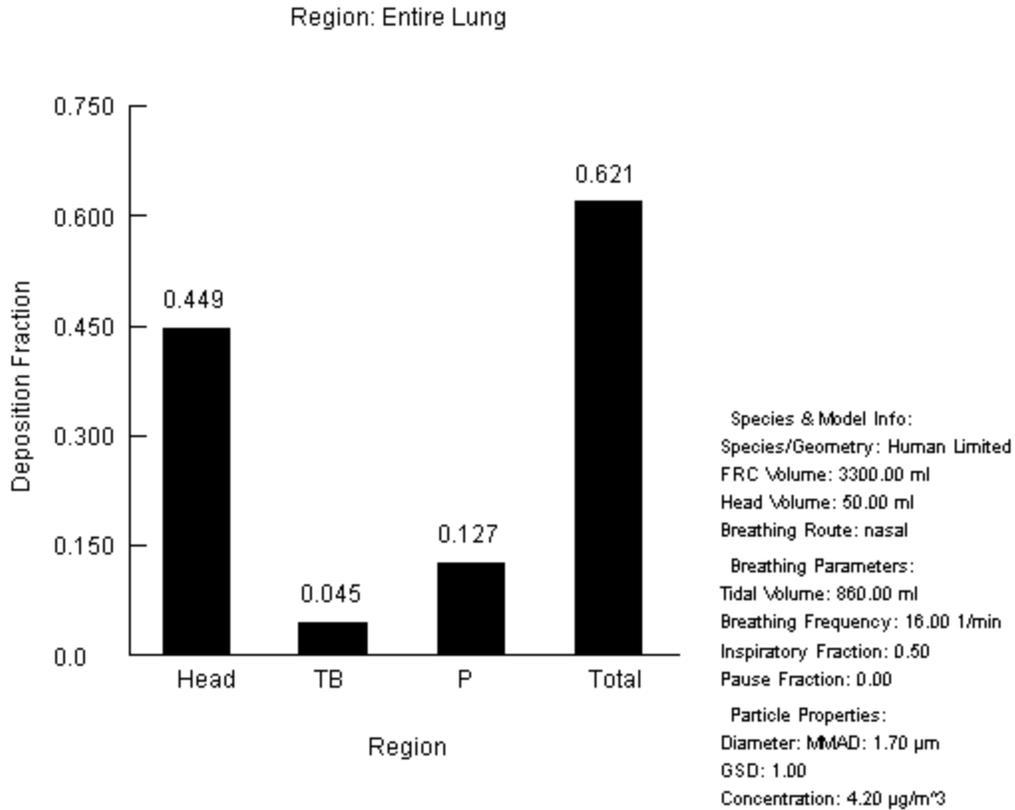
19

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21

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

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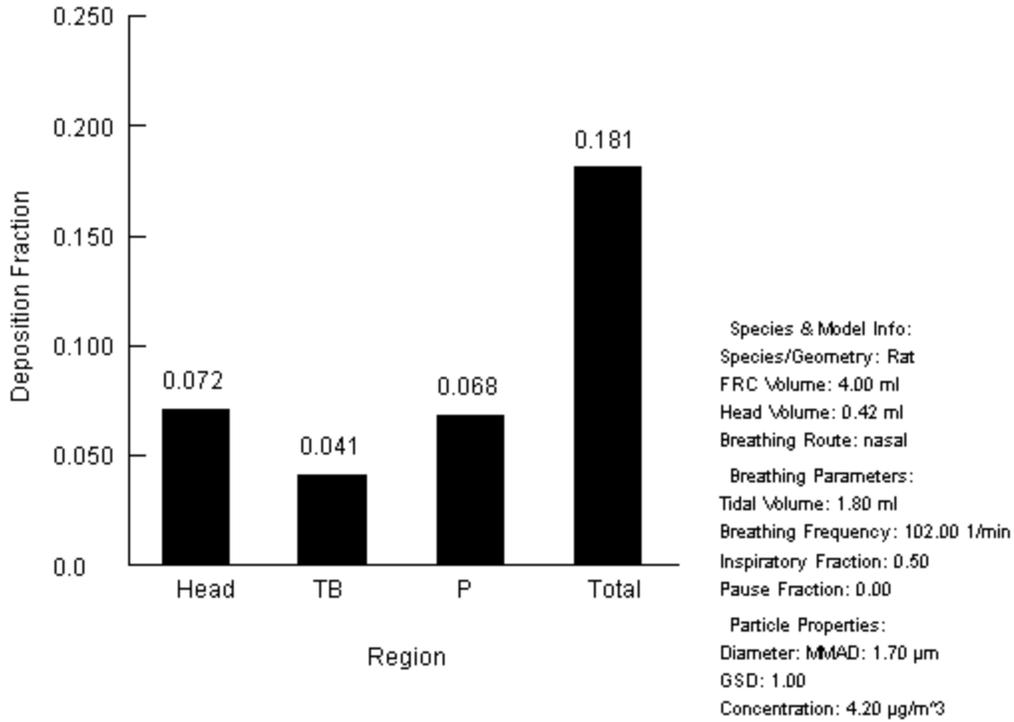
Figure E-8. Human fractional deposition.

3 Species = humanlimited
 4 FRC = 3300.0
 5 Head volume = 50.0
 6 Density = 1.0
 7 Number of particles calculated = single
 8 Diameter = 1.7000000000000002 µm MMAD
 9 Inhalability = yes
 10 GSD = 1.0
 11 Breathing interval: One single breath
 12 Concentration = 4.2
 13 Breathing Frequency = 16.0
 14 Tidal Volume = 860.0
 15 Inspiratory Fraction = 0.5
 16 Pause Fraction = 0.0
 17 Breathing Route = nasal

18
 19 Region: Entire Lung
 20 Region: Entire Lung
 21 Region Deposition Fraction
 22 -- --
 23 **Head 0.449**
 24 **TB 0.045**
 25 **P 0.127**
 26 **Total 0.621**

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Region: Entire Lung



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Figure E-9. Rat fractional deposition.

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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
    
```

1 E.2. Cancer Endpoints

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

3 *Dose-Response Models*

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

$$10 \quad P(d, t) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \dots + q_kd^k) \times (t \pm t_0)^c],$$

11 where $P(d, t)$ represents the lifetime risk (probability) of cancer at dose d (i.e., human equivalent
12 exposure in this case) and age t (in bioassay weeks); parameters $q_i \geq 0$, for $i = 0, 1, \dots, k$; t is the time
13 at which the tumor was observed; and c is a parameter which characterizes the change in response
14 with age. The parameter t_0 represents the time between when a potentially fatal tumor becomes
15 observable and when it causes death, and is generally set to 0 either when all tumors are
16 considered incidental or because of a lack of data to estimate the time reliably. The dose-response
17 analyses were conducted using the computer software program MultiStage-Weibull ([U.S. EPA,
18 2010](#)), which is based on Weibull models drawn from [Krewski et al. \(1983\)](#). Parameters were
19 estimated using the method of maximum likelihood. From specific model fits using stages up to
20 $n - 1$, where n is the number of dose groups, the model fit with the lowest AIC was selected.

21 *Data Adjustments Prior to Modeling*

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

29 Additionally, the multistage-Weibull model can address relative severity of tumor types to
30 some extent by distinguishing between tumors as being either fatal or incidental to the death of an
31 animal in order to adjust partially for competing risks. In contrast to fatal tumors, incidental
32 tumors are those tumors thought not to have caused the death of an animal. Cause-of-death
33 information for most early animal deaths was provided by the investigators of both bioassays. In

1 the rat study of [Kroese et al. \(2001\)](#), tumors of the forestomach or liver were the principal cause of
2 death for most animals dying or sacrificed (due to moribundity) before the end of the study, while
3 tumors of the forestomach were the most common cause of early deaths in the mouse study of
4 [Beland and Culp \(1998\)](#). The incidence data modeled are listed in Tables E-9 (male rats), E-10
5 (female rats), and E-11 (female mice).

6 Human-equivalent dose (HED) estimates used for dose-response modeling were based on
7 scaling by body weight^{3/4}, as there were no pharmacokinetic models or data to inform another
8 approach. The dose estimates are provided in Tables E-12 ([Kroese et al., 2001](#)) and E-13 ([Beland
9 and Culp, 1998](#)).

10 ***Evaluation of Model Fit and Model Selection***

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

15 PODs for estimating low-dose risk were identified at doses at the lower end of the observed
16 data, generally corresponding to 10% extra risk, where extra risk is defined as $[P(d) - P(0)] /$
17 $[1 - P(0)]$. The lifetime oral cancer slope factor for humans is defined as the slope of the line from
18 the lower 95% bound on the exposure at the POD to the control response (slope factor =
19 $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit (UCL), represents a plausible upper bound
20 on the true risk.

21 ***Overall Risk***

22 Although the time-to-tumor modeling helps account for competing risks associated with
23 decreased survival times and other tumors, considering the tumor sites individually still does not
24 convey the total amount of risk potentially arising from the sensitivity of multiple sites (i.e., the risk
25 of developing any combination of the increased tumor types, not just the risk of developing all
26 simultaneously). One approach suggested in the *Guidelines for Carcinogen Risk Assessment* ([U.S.
27 EPA, 2005](#)) would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used
28 this approach until the National Research Council (NRC) document *Science and Judgment in Risk
29 Assessment* ([NRC, 1994](#)) made a case that this approach would tend to underestimate overall risk
30 when tumor types occur in a statistically independent manner. In addition, application of one
31 model to a composite data set does not accommodate biologically relevant information that may
32 vary across sites or may only be available for a subset of sites. For instance, the time courses of the
33 multiple tumor types evaluated varied, as is suggested by the variation in estimates of c , from
34 1.5 (e.g., male rat skin or mammary gland basal cell tumors), indicating relatively little effect of age
35 on tumor incidence, to 3.7 (e.g., male mouse alimentary tract tumors), indicating a more rapidly
36 increasing response with increasing age (in addition to exposure level). The result of fitting a
37 model with parameters that can reflect underlying mechanisms, such as z in the multistage-Weibull

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

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

- 11 1) It was assumed that the tumor groupings modeled above were statistically independent
12 (i.e., that the occurrence of a liver tumor was not dependent upon whether there was a
13 forestomach tumor). This assumption cannot currently be verified, and if not correct, could
14 lead to an overestimate of risk from summing across tumor sites. However, [NRC \(1994\)](#)
15 argued that a general assumption of statistical independence of tumor-type occurrences
16 within animals was not likely to introduce substantial error in assessing carcinogenic
17 potency from rodent bioassay data.
- 18 2) The models previously fitted to estimate the BMDs and BMDLs were used to extrapolate to a
19 lower level of risk (R), in order to reach the region of each estimated dose-response
20 function where the slope was reasonably constant and upper bound estimation was still
21 numerically stable. For these data, a 10^{-3} risk was generally the lowest risk necessary. The
22 oral slope factor for each site was then estimated by $R/BMDL_R$, as for the estimates for each
23 tumor site above.
- 24 3) The maximum likelihood estimates (MLE) of unit potency (i.e., risk per unit of exposure)
25 estimated by R/BMD_R , were summed across the alimentary tract, liver, and jejunum/
26 duodenum in female rats.
- 27 4) An estimate of the 95% (one-sided) upper bound on the summed oral slope factor was
28 calculated by assuming a normal distribution for the individual risk estimates, and deriving
29 the variance of the risk estimate for each tumor site from its 95% UCL according to the
30 formula:

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

31 rearranged to:

$$32 \text{SD} = (\text{UCL} - \text{MLE}) / 1.645,$$

33
34 where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval (CI) and
35 >120 degrees of freedom, and the SD is the square root of the variance of the MLE. The variances
36 (variance = SD^2) for each site-specific estimate were summed across tumor sites to obtain the
37 variance of the sum of the MLEs. The 95% UCL on the sum of MLEs was calculated from the
38 expression above for the UCL, using the variance of the sum of the MLE to obtain the relevant SD
39 ($\text{SD} = \text{variance}^{1/2}$).
40

1 **Table E-9. Tumor incidence data, with time to death with tumor for male**
 2 **Wistar rats exposed by gavage to benzo[a]pyrene for 104 weeks ([Kroese et al.](#)**
 3 **[2001](#))**

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

Supplemental Information—Benzo[a]pyrene

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

Supplemental Information—Benzo[a]pyrene

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:							
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors	Skin or mammary gland		Kidney urothelial carcinoma
			Incidental ^a	Fatal ^a	Incidental	Fatal		Basal cell tumors	Squamous cell tumors	
							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

1
2 ^a“Incidental” denotes presence of tumors not known to have caused death of particular animals. “Fatal” denotes
3 incidence of tumors reported by the study investigators to have caused death of particular animals.

1 **Table E-10. Tumor incidence data, with time to death with tumor for female**
 2 **Wistar rats exposed by gavage to benzo[a]pyrene for 104 weeks ([Kroese et al.](#)**
 3 **[2001](#))**

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

Supplemental Information—Benzo[a]pyrene

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

Supplemental Information—Benzo[a]pyrene

Dose (mg/kg-d)	Wk of death	Total examined	Numbers of animals with:				
			Oral cavity or forestomach tumors		Liver tumors		Duodenum or jejunum tumors
			Incidental ^a	Fatal ^a	Incidental	Fatal	Incidental
30	26	1	0	0	0	0	0
	44	4	4	0	3	1	0
	47	3	3	0	2	1	0
	48	1	1	0	0	1	0
	54	1	0	0	1	0	0
	55	3	3	0	1	2	0
	56	2	2	0	0	2	0
	57	2	2	0	2	0	0
	58	4	3	1	0	4	0
	59	2	1	1	0	2	0
	60	1	0	0	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	3	1	3	1
71	4	3	3	1	3	0	
72	2	2	1	1	0	0	

1
2 ^a“Incidental” denotes presence of tumors not known to have caused death of particular animals. “Fatal” denotes
3 incidence of tumors indicated by the study investigators to have caused death of particular animals.
4

1 **Table E-11. Tumor incidence, with time to death with tumor; B6C3F₁ female**
 2 **mice exposed to benzo[a]pyrene via diet for 2 years ([Beland and Culp, 1998](#))**

Dose group (ppm in diet)	Wk of death	Total examined	Number of animals with alimentary tract squamous cell tumors	
			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
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

Supplemental Information—Benzo[a]pyrene

Dose group (ppm in diet)	Wk of death	Total examined	Number of animals with alimentary tract squamous cell tumors	
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	

Supplemental Information—Benzo[a]pyrene

Dose group (ppm in diet)	Wk of death	Total examined	Number of animals with alimentary tract squamous cell tumors	
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	1	0

- 1
- 2 ^a“Incidental” denotes presence of tumors not known to have caused death of particular animals. “Fatal” denotes
- 3 incidence of tumors indicated by the study investigators to have caused death of particular animals.

1 **Table E-12. Derivation of HEDs to use for BMD modeling of Wistar rat tumor**
 2 **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)
<i>Male</i>			
3	0.349	0.27	0.54
10	0.349	0.27	1.81
30	0.288	0.25	5.17
<i>Female</i>			
3	0.222	0.24	0.49
10	0.222	0.24	1.62
30	0.222	0.24	4.85

3
 4 ^aScaling factors were calculated using [U.S. EPA \(1988\)](#) reference body weights for humans (70 kg), and the TWA
 5 body weights for each dose group: rat-to-human = (TWA body weight/70)^{0.25} = scaling factor.

6 ^bHED = administered dose × scaling factor.

7 **Table E-13. Derivation of HEDs for dose-response modeling of B6C3F₁ female**
 8 **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

9
 10 ^aAdministered doses in mg/kg-day were calculated from dietary concentrations of benzo[a]pyrene using the TWA
 11 body weight and reported food intakes for mice.

12 ^bScaling factors were calculated using [U.S. EPA \(1988\)](#) reference body weights for humans (70 kg), and the TWA
 13 body weights for each dose group: mouse-to-human = (TWA body weight/70)^{0.25} = scaling factor.

14 ^cHED = administered dose × scaling factor.

1 **Dose-Response Modeling Results**

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

6 **Table E-14. Summary of BMD modeling results for best-fitting multistage-**
 7 **Weibull models, using time-to-tumor data for Wistar rats exposed to**
 8 **benzo[a]pyrene via gavage for 104 weeks ([Kroese et al., 2001](#)); BMR = 10%**
 9 **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	577.8	0.104	0.281–0.612	Lowest AIC, best fit to low dose data
		2	407.6	0.678		
		3	229.0	0.453		
	Hepatocellular tumors	1	367.3	0.181	0.449–0.772	Lowest AIC, best fit to low dose data
		2	301.5	0.472		
		3	289.1	0.651		
Duodenum and jejunum tumors	1	69.6	2.64	2.38–3.87	Best fit to data	
	2	65.9	3.04			
	3	66.9	3.03			
Kidney: urothelial carcinoma	1	31.9	9.16	2.50–9.01	Best fit to data	
	2	31.7	5.71			
	3	32.8	4.65			
Skin and mammary gland: basal cell tumors	1	110.6	1.88	2.35–3.62	Lowest AIC, best fit to low dose data	
	2	105.1	2.58			
	3	104.7	2.86			
Skin and mammary gland: squamous cell tumors	1	63.5	3.36	1.77–4.42	Best fit to low dose data	
	2	64.3	2.75			
	3	65.3	2.64			
Female rats	Oral cavity and forestomach: squamous cell tumors	1	277.1	0.245	0.328–0.717	Lowest AIC, best fit to low dose data
		2	211.6	0.428		
		3	201.0	0.539		
Hepatocellular tumors	1	595.5	0.146	0.507–0.630	Lowest AIC, best fit to low dose data	
	2	774.9	0.370			
	3	468.3	0.575			
Duodenum and jejunum tumors	1	37.9	6.00	1.95–5.70	Best fit to low dose data	
	2	37.0	4.33			
	3	37.8	3.43			

10

1 **Male Rat ([Kroese et al., 2001](#)): Squamous Cell Papilloma or Carcinoma in Oral Cavity or**
 2 **Forestomach**

3 =====
 4 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 5 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 6 Input Data File: OralForstKroeseM3.(d)
 7 =====

8 The form of the probability function is:
 9 $P[\text{response}] = 1 - \exp\left[-(t - t_0)^c \cdot (\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3)\right]$
 10

11 The parameter betas are restricted to be positive

12
 13 Dependent variable = CONTEXT
 14 Independent variables = DOSE, TIME

15
 16 Total number of observations = 208
 17 Total number of records with missing values = 0
 18 Total number of parameters in model = 6
 19 Total number of specified parameters = 0
 20 Degree of polynomial = 3

21
 22 Maximum number of iterations = 64
 23 Relative Function Convergence has been set to: 2.22045e-016
 24 Parameter Convergence has been set to: 1.49012e-008

25
 26
 27
 28 Default Initial Parameter Values
 29 c = 3.6
 30 t_0 = 39.1111
 31 beta_0 = 0
 32 beta_1 = 8.8911e-009
 33 beta_2 = 1.60475e-031
 34 beta_3 = 1.95818e-008
 35

36
 37 Asymptotic Correlation Matrix of Parameter Estimates
 38 (*** The model parameter(s) -beta_0 -beta_2
 39 have been estimated at a boundary point, or have been specified by the user,
 40 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

41
 42
 43
 44
 45
 46
 47
 48
 49
 50
 51
 52
 53 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

54
 55
 56
 57
 58
 59
 60
 61
 62
 63 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
 64 and thus has no standard error.

65
 66
 67 Log(likelihood) # Param AIC
 68 Fitted Model -108.512 6 229.024

69
 70
 71 Data Summary
 72 CONTEXT

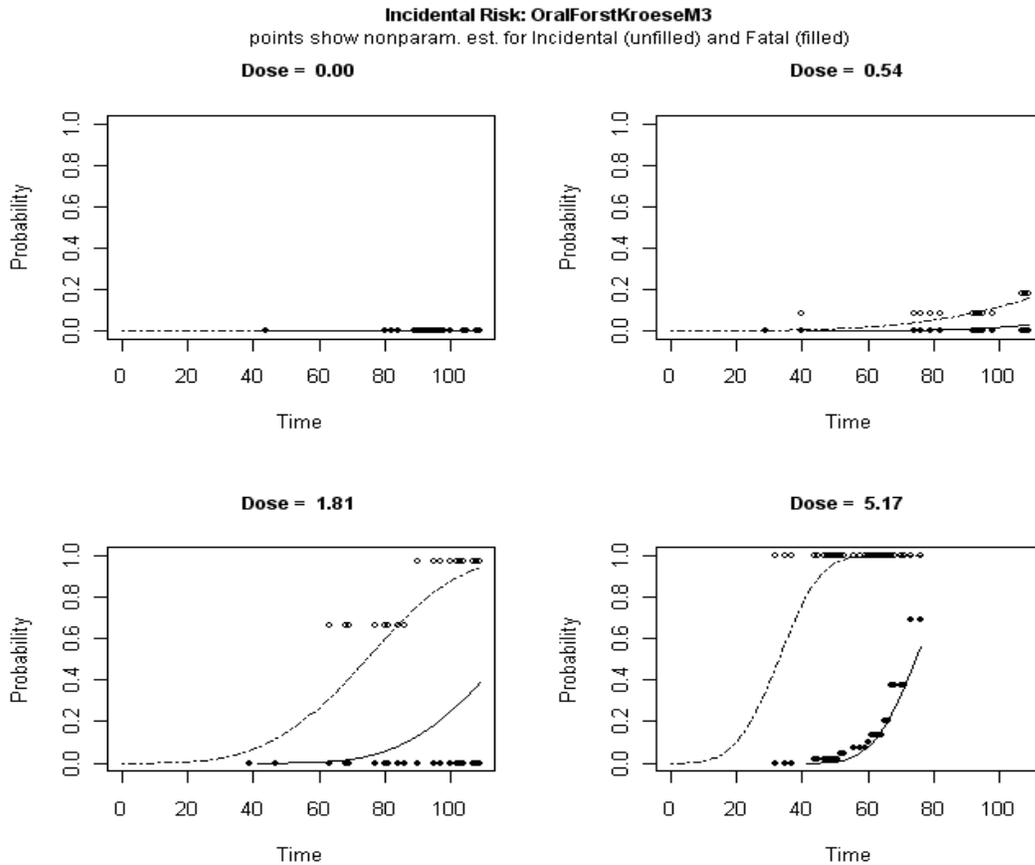
1
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16

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

Minimum observation time for F tumor context = 44

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

Specified effect = 0 . 1 0.01 0.001
 BMD = 0 . 4 5 3 4 7 1 0.0633681 0.00636659
 BMDL = 0 . 2 8 1 0 4 4 0.0286649 0.00285563
 BMDU = 0 . 6 1 2 4 6 2 0.248377 > 0.0509326



17
18
19
20
21

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).

1 **Male Rat ([Kroese et al., 2001](#)): Hepatocellular Adenoma or Carcinoma**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: LiverKroeseM3.(d)
 6 =====

7
 8 The form of the probability function is:
 9 $P[\text{response}] = 1 - \exp\left\{ -\left(t - t_0 \right)^c \cdot \left(\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3 \right) \right\}$
 10

11 The parameter betas are restricted to be positive

12
 13 Dependent variable = CONTEXT
 14 Independent variables = DOSE, TIME

15
 16
 17 Total number of observations = 208
 18 Total number of records with missing values = 0
 19 Total number of parameters in model = 6
 20 Total number of specified parameters = 0
 21 Degree of polynomial = 3

22
 23
 24 Maximum number of iterations = 64
 25 Relative Function Convergence has been set to: 2.22045e-016
 26 Parameter Convergence has been set to: 1.49012e-008

27
 28
 29 Default Initial Parameter Values
 30 c = 3.6
 31 t_0 = 34.6667
 32 beta_0 = 0
 33 beta_1 = 2.73535e-009
 34 beta_2 = 8.116e-028
 35 beta_3 = 1.43532e-008
 36

37
 38 Asymptotic Correlation Matrix of Parameter Estimates
 39 (*** The model parameter(s) -beta_0 -beta_2
 40 have been estimated at a boundary point, or have been specified by the user,
 41 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

52
 53
 54 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

55
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 57
 58
 59
 60
 61
 62
 63
 64 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
 65 and thus has no standard error.

66
 67
 68
 69 Fitted Model Log(likelihood) # Param AIC
 70 -138.544 6 289.088

71
 72 Data Summary
 73 CONTEXT
 74 C F I U Total Expected Response

1
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14
15

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

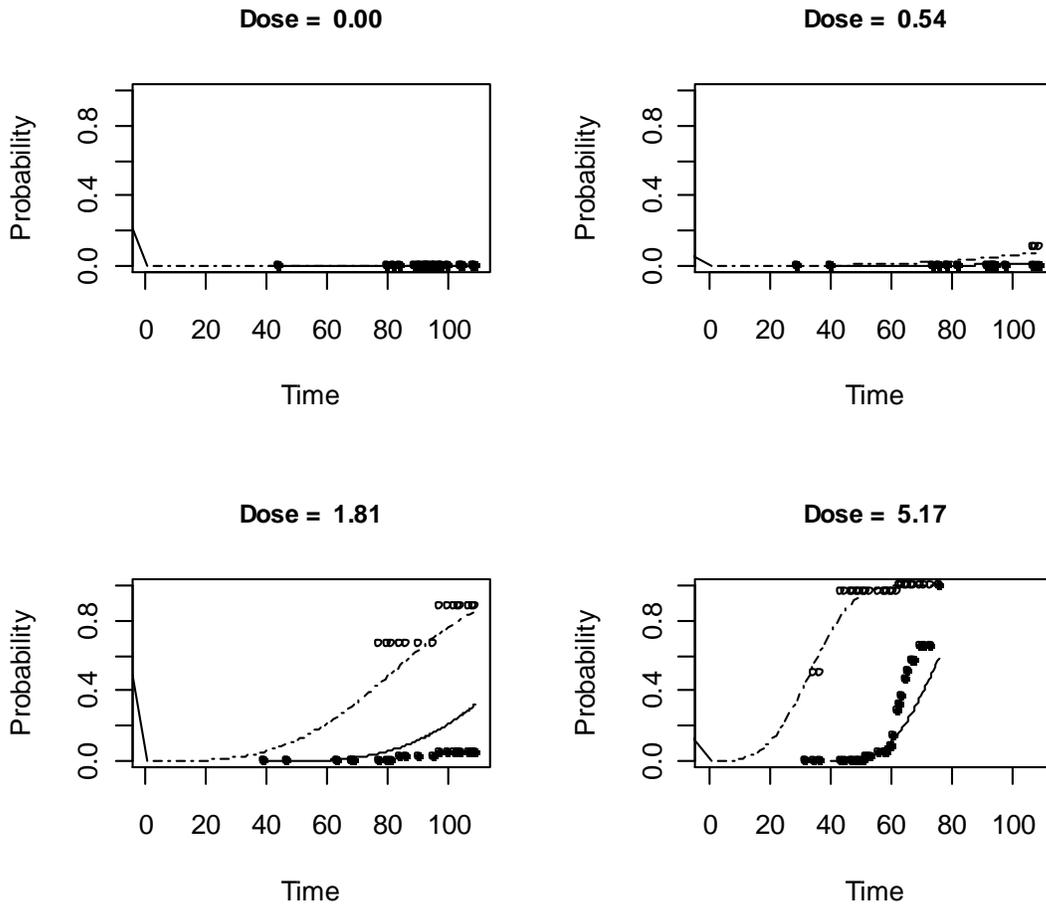
Minimum observation time for F tumor context = 52

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	.	6	5	0	7	0.173556	0.0199908		
BMDL =	0	.	4	4	8	6	8	0.0530469	0.00530386	
BMDU =	0	.	7	7	2	4	6	7	0.352684	> 0.159927

Incidental Risk: Hepatocellular_Kroese_M3

points show nonparam. est. for Incidental (unfilled) and Fatal (filled)



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19
20

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).

1 **Male Rat ([Kroese et al., 2001](#)): Duodenum or Jejunum Adenocarcinoma**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: DuoJeyJKroeseM3.(d)
 6 =====

7
 8 The form of the probability function is:
 9 $P[\text{response}] = 1 - \exp\left[-(t - t_0)^c \cdot (\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3)\right]$
 10

11 The parameter betas are restricted to be positive

12
 13 Dependent variable = CONTEXT
 14 Independent variables = DOSE, TIME

15
 16 Total number of observations = 208
 17 Total number of records with missing values = 0
 18 Total number of parameters in model = 6
 19 Total number of specified parameters = 1
 20 Degree of polynomial = 3
 21

22
 23
 24
 25 User specifies the following parameters:
 26 $t_0 = 0$
 27

28 Maximum number of iterations = 64
 29 Relative Function Convergence has been set to: 2.22045e-016
 30 Parameter Convergence has been set to: 1.49012e-008
 31

32
 33 Default Initial Parameter Values
 34 $c = 1.63636$
 35 $t_0 = 0$ Specified
 36 $\beta_0 = 4.31119e-027$
 37 $\beta_1 = 2.96347e-025$
 38 $\beta_2 = 0$
 39 $\beta_3 = 1.76198e-006$
 40

41
 42 Asymptotic Correlation Matrix of Parameter Estimates
 43 (*** The model parameter(s) $-t_0$ $-\beta_0$ $-\beta_1$ $-\beta_2$
 44 have been estimated at a boundary point, or have been specified by the user,
 45 and do not appear in the correlation matrix)
 46

	c	beta_3
c	1	-1
beta_3	-1	1

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

63 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
 64 and thus has no standard error.
 65

66
 67
 68 Fitted Model Log(likelihood) # Param AIC
 69 -28.4387 5 66.8773
 70

Data Summary						
CONTEXT						
	C	F	I	U	Total	Expected Response
DOSE						

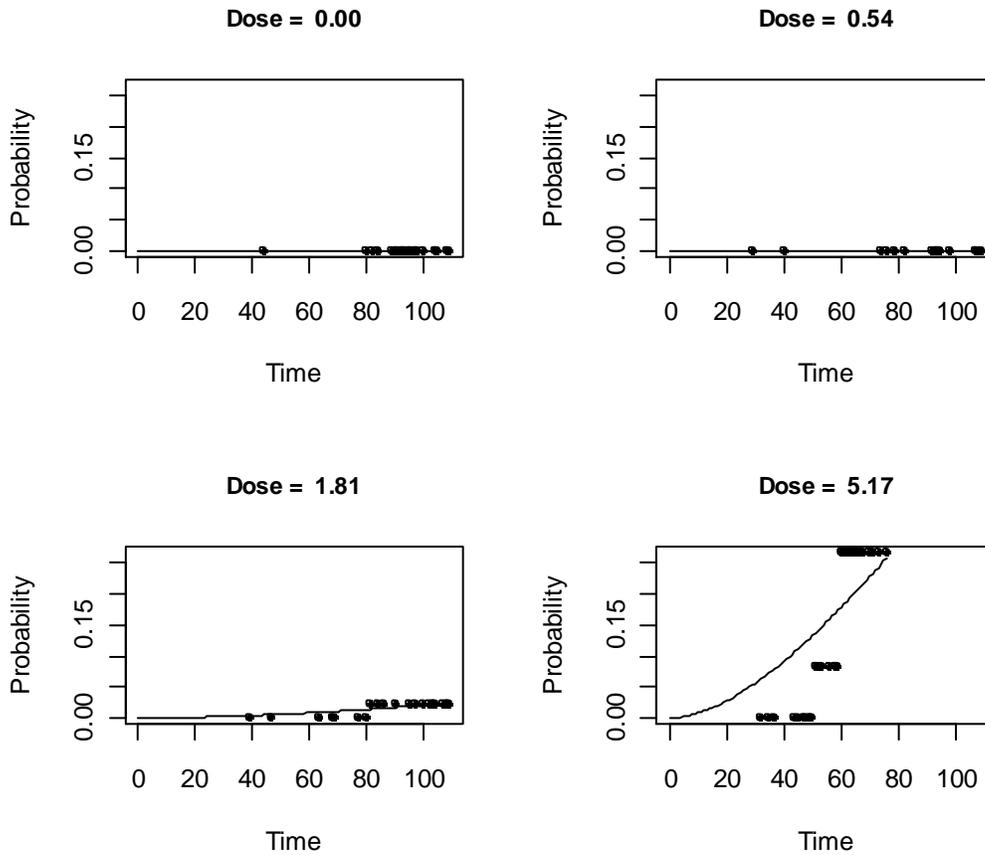
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14

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	.	0	3	2	9	1	1.38578	0.642252
BMDL =	2	.	3	7	7	8	2	0.418285	0.0420835
BMDU =	3	.	8	7	1	8	3	1.76166	0.811476

Incidental Risk: DuoJej_Kroese_M3



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18
19

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).

1 **Male Rat ([Kroese et al., 2001](#)): Skin or Mammary Gland Basal Cell Tumors**

```

2 =====
3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
5 Input Data File: SKinMamBasalKroeseM3.(d)
6 =====

```

```

7
8 The form of the probability function is:
9 P[response] = 1-EXP$$-(t - t_0)^c *
10 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)
11

```

```

12 The parameter betas are restricted to be positive
13

```

```

14 Dependent variable = CONTEXT
15 Independent variables = DOSE, TIME
16

```

```

17 Total number of observations = 208
18 Total number of records with missing values = 0
19 Total number of parameters in model = 6
20 Total number of specified parameters = 1
21 Degree of polynomial = 3
22

```

```

23
24
25 User specifies the following parameters:
26 t_0 = 0
27

```

```

28 Maximum number of iterations = 64
29 Relative Function Convergence has been set to: 2.22045e-016
30 Parameter Convergence has been set to: 1.49012e-008
31

```

```

32
33 Default Initial Parameter Values
34 c = 1.38462
35 t_0 = 0 Specified
36 beta_0 = 3.84298e-005
37 beta_1 = 1.06194e-028
38 beta_2 = 0
39 beta_3 = 6.84718e-006
40

```

```

41
42 Asymptotic Correlation Matrix of Parameter Estimates
43 ( *** The model parameter(s) -t_0 -beta_1 -beta_2
44 have been estimated at a boundary point, or have been specified by the user,
45 and do not appear in the correlation matrix )
46

```

	c	beta_0	beta_3
c	1	-1	-1
beta_0	-1	1	0.99
beta_3	-1	0.99	1

47 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

```

64 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
65 and thus has no standard error.
66

```

```

67
68
69 Log(likelihood) # Param AIC
70 Fitted Model -47.3623 5 104.725
71

```

```

72
73 Data Summary
74 CONTEXT

```

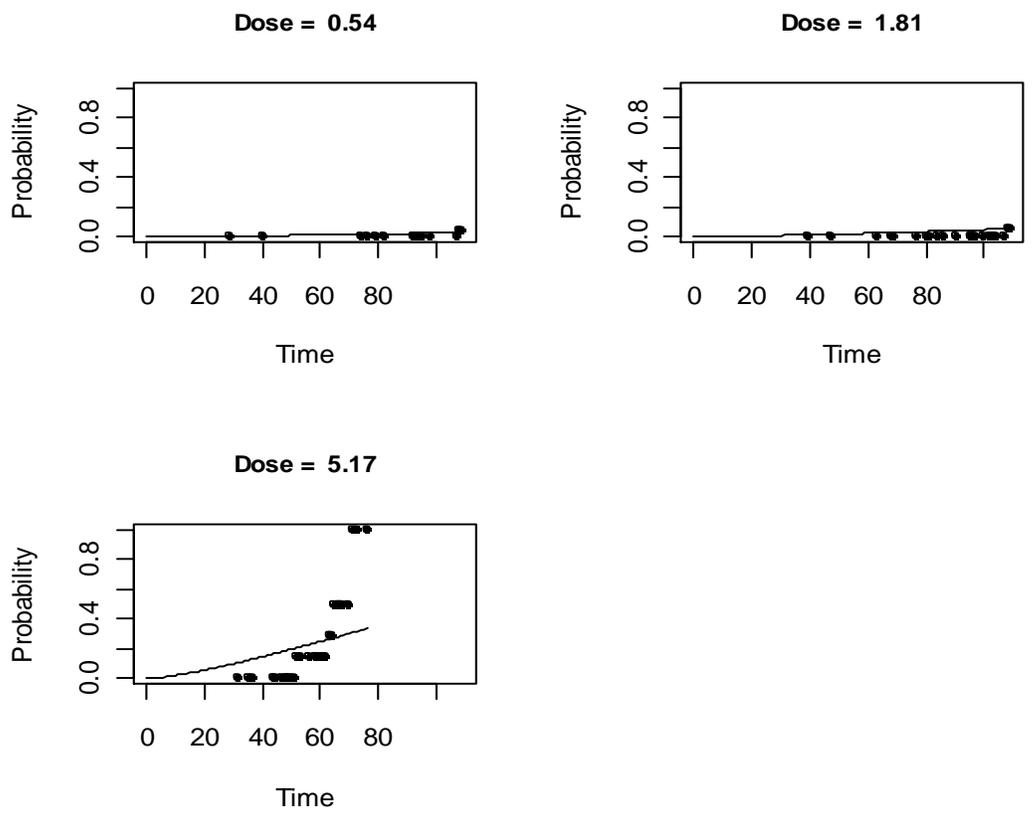
1
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4
5
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8
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12
13
14

	C	F	I	U	Total	Expected Response
DOSE						
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 . 8 6 2 7 6 1.30804 0.606222
 BMDL = 2 . 3 5 1 1 8 0.415897 0.0424277
 BMDU = 3 . 6 2 2 5 8 1.69571 0.761447

Incidental Risk: Skin_Mam_Basal_Kroese_M3



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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).

1 **Male Rat ([Kroese et al., 2001](#)): Skin or Mammary Gland Squamous Cell Tumors**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: SKinMamSCCKroeseM3.(d)
 6 =====

7
 8 The form of the probability function is:
 9 $P[\text{response}] = 1 - \exp\left[-(t - t_0)^c \cdot (\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3)\right]$
 10

11 The parameter betas are restricted to be positive

12
 13 Dependent variable = CONTEXT
 14 Independent variables = DOSE, TIME

15
 16
 17 Total number of observations = 208
 18 Total number of records with missing values = 0
 19 Total number of parameters in model = 6
 20 Total number of specified parameters = 1
 21 Degree of polynomial = 3

22
 23
 24
 25 User specifies the following parameters:
 26 $t_0 = 0$
 27

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

31
 32
 33 Default Initial Parameter Values
 34 $c = 3$
 35 $t_0 = 0$ Specified
 36 $\beta_0 = 0$
 37 $\beta_1 = 1.25256e-008$
 38 $\beta_2 = 1.25627e-030$
 39 $\beta_3 = 3.34696e-009$
 40

41
 42 Asymptotic Correlation Matrix of Parameter Estimates
 43 (*** The model parameter(s) $-t_0$ $-\beta_0$ $-\beta_2$
 44 have been estimated at a boundary point, or have been specified by the user,
 45 and do not appear in the correlation matrix)
 46

	c	beta_1	beta_3
c	1	-0.99	-1
beta_1	-0.99	1	0.99
beta_3	-1	0.99	1

47
 48
 49
 50
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 52
 53
 54
 55

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

56
 57
 58
 59
 60
 61
 62
 63
 64 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
 65 and thus has no standard error.

66
 67
 68
 69 Fitted Model Log(likelihood) # Param AIC
 70 -27.652 5 65.304

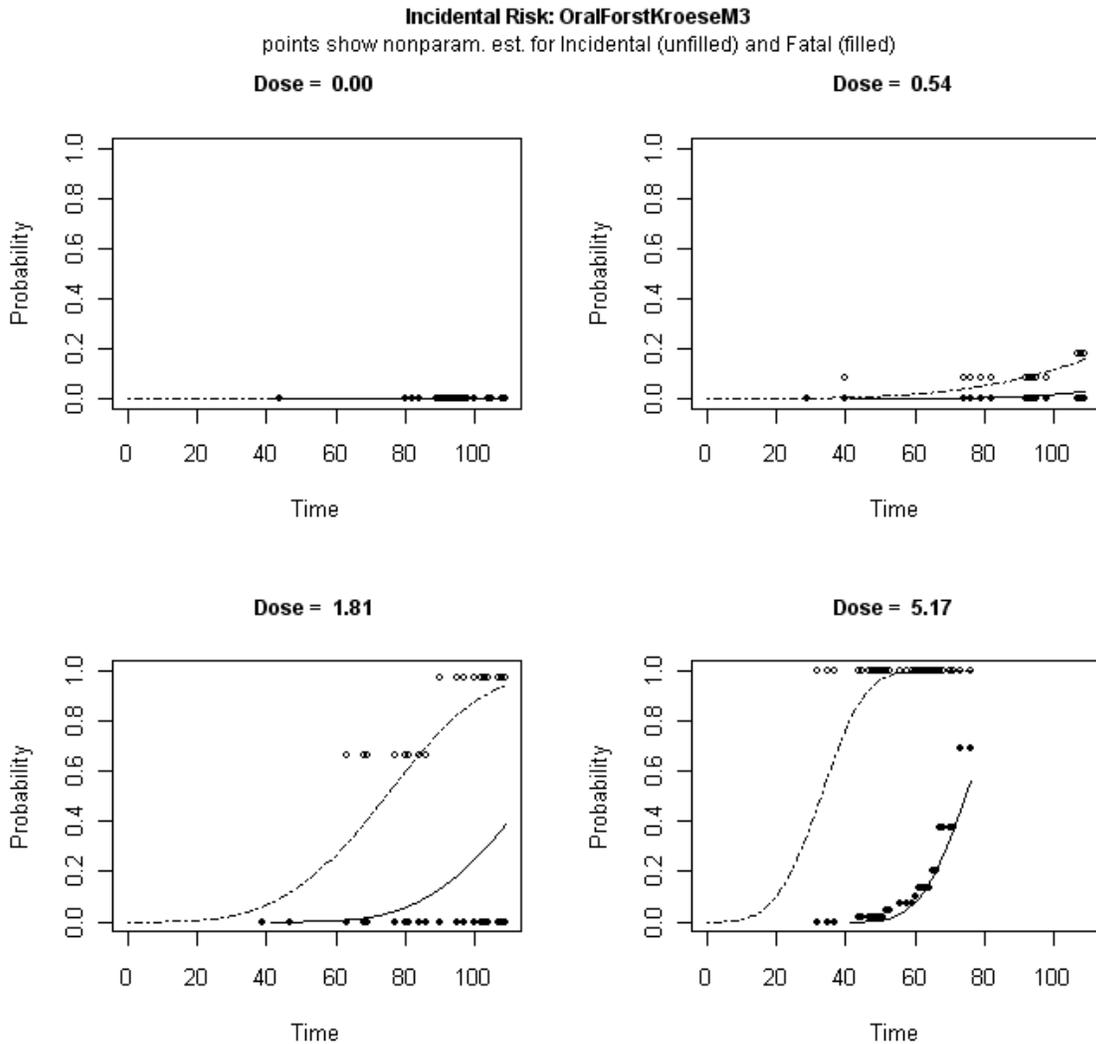
71
 72 Data Summary
 73 CONTEXT
 74 C F I U Total Expected Response

1
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13

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 . 6 4 1 4 0.64109 0.070558
 BMDL = 1 . 7 6 9 3 1 0.211043 0.0210552
 BMDU = 4 . 4 2 1 4 5 2.03605 > 0.564463



14
15
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18

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).

1 **Male Rat ([Kroese et al., 2001](#)): Kidney Urothelial Carcinomas**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: KidneyUrothelialCarKroeseM3.(d)
 6 =====

7
 8 The form of the probability function is:
 9 $P[\text{response}] = 1 - \exp\left[-(t - t_0)^c \cdot (\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3)\right]$
 10

11 The parameter betas are restricted to be positive

12
 13 Dependent variable = CONTEXT
 14 Independent variables = DOSE, TIME

15
 16
 17 Total number of observations = 208
 18 Total number of records with missing values = 0
 19 Total number of parameters in model = 6
 20 Total number of specified parameters = 1
 21 Degree of polynomial = 3

22
 23
 24
 25 User specifies the following parameters:
 26 $t_0 = 0$
 27

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

31
 32
 33 Default Initial Parameter Values
 34 $c = 1.63636$
 35 $t_0 = 0$ Specified
 36 $\beta_0 = 3.78734e-027$
 37 $\beta_1 = 1.59278e-027$
 38 $\beta_2 = 2.718e-024$
 39 $\beta_3 = 4.96063e-007$
 40

41
 42 Asymptotic Correlation Matrix of Parameter Estimates
 43 (*** The model parameter(s) $-t_0$ $-\beta_0$ $-\beta_1$ $-\beta_2$
 44 have been estimated at a boundary point, or have been specified by the user,
 45 and do not appear in the correlation matrix)
 46

	c	beta_3
c	1	-1
beta_3	-1	1

47
 48
 49
 50
 51
 52
 53
 54
 55

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

56
 57
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 61
 62
 63 NA - Indicates that this parameter has hit a
 64 bound implied by some inequality constraint
 65 and thus has no standard error.
 66

67
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 74

Fitted Model	Log(likelihood)	# Param	AIC
	-11.3978	5	32.7956

Data Summary
 CONTEXT
 C F I U Total Expected Response

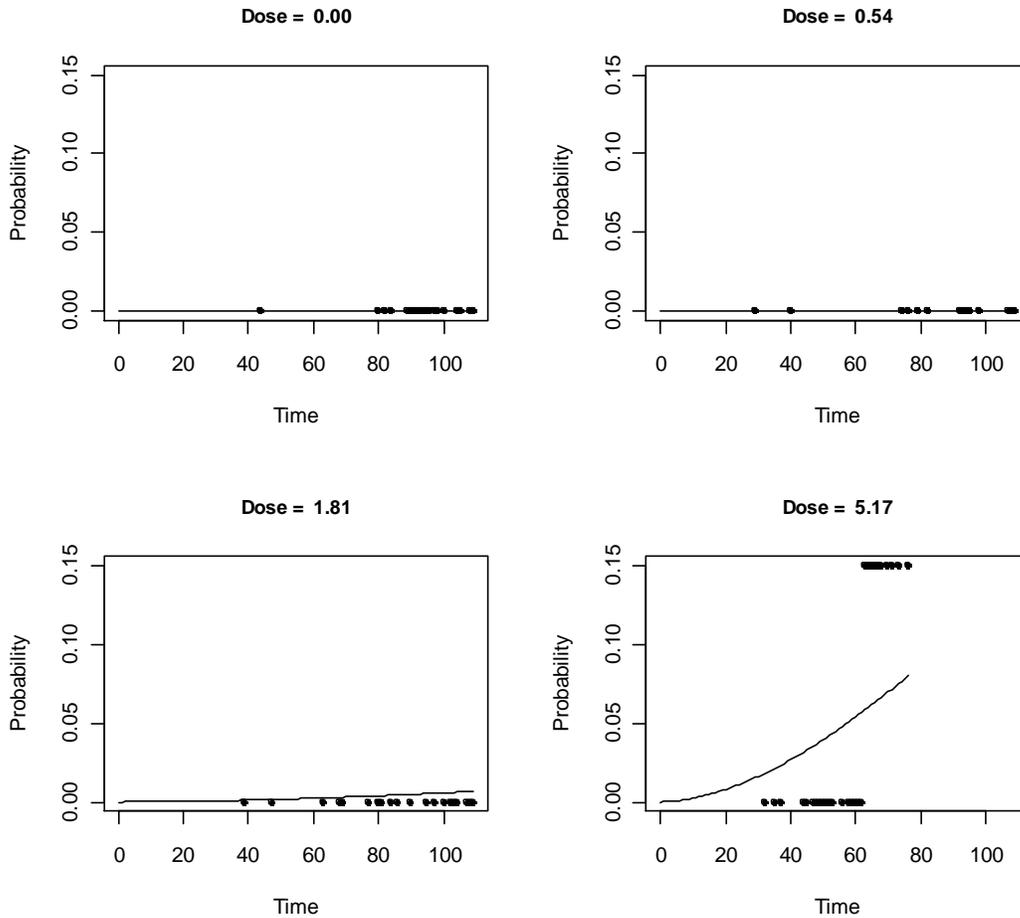
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12
13
14

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 . 6 4 8 8 6			2.12413	0.984449
BMDL =	2 . 4 9 9 7 2			0.734665	0.0748097
BMDU =	9 . 0 1 0 2 3			3.49311	1.61892

Incidental Risk: Kidney_Kroese_M3



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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).

1 **Female Rat ([Kroese et al., 2001](#)): Oral Cavity or Forestomach, Squamous Cell Papilloma or**
 2 **Carcinoma**

3 =====
 4 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 5 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 6 Input Data File: OralForstKroeseF3.(d)
 7 =====

8
 9 The form of the probability function is:

$$10 \text{ P[response]} = 1 - \exp\left\{ -\left(t - t_0 \right)^c \cdot \right. \\
 11 \left. \left(\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3 \right) \right\}$$

12
 13 The parameter betas are restricted to be positive

14
 15 Dependent variable = CONTEXT

16 Independent variables = DOSE, TIME

17
 18 Total number of observations = 208

19 Total number of records with missing values = 0

20 Total number of parameters in model = 6

21 Total number of specified parameters = 0

22 Degree of polynomial = 3

23
 24 Maximum number of iterations = 64

25 Relative Function Convergence has been set to: 2.22045e-016

26 Parameter Convergence has been set to: 1.49012e-008

27
 28
 29 Default Initial Parameter Values

30 c = 3.6
 31 t_0 = 45.1111
 32 beta_0 = 1.11645e-009
 33 beta_1 = 4.85388e-009
 34 beta_2 = 0
 35 beta_3 = 1.95655e-008
 36

37 Asymptotic Correlation Matrix of Parameter Estimates

38 (*** The model parameter(s) -beta_2
 39 have been estimated at a boundary point, or have been specified by the user,
 40 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

41
 42
 43
 44
 45
 46
 47
 48
 49
 50
 51
 52
 53
 54
 55 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

56
 57
 58
 59
 60
 61
 62
 63
 64
 65 NA - Indicates that this parameter has hit a bound implied by some inequality constraint
 66 and thus has no standard error.

67
 68
 69
 70
 71
 72
 73

	Log(likelihood)	# Param	AIC
Fitted Model	-94.5119	6	201.024

Data Summary

1
2
3
4
5
6
7
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11
12
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15
16

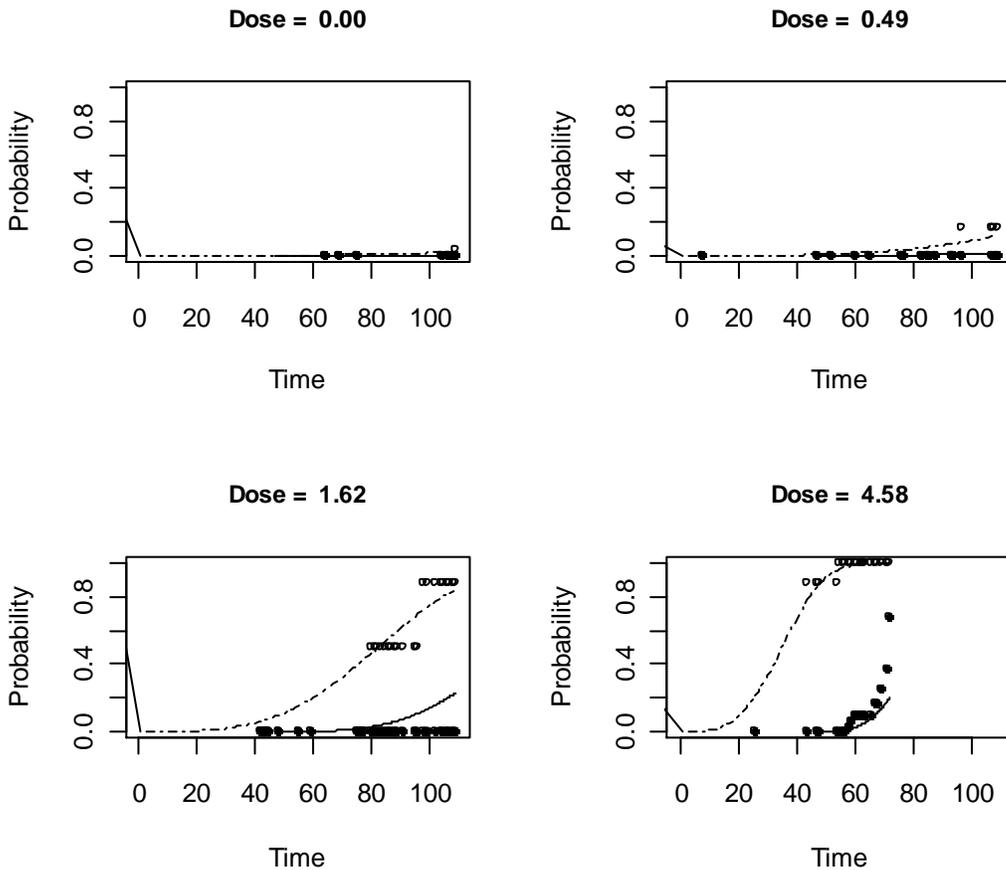
DOSE	CONTEXT				Total	Expected Response
	C	F	I	U		
0	51	0	1	0	52	1.14
0.49	46	0	6	0	52	4.90
1.6	22	0	30	0	52	31.81
4.6	2	7	43	0	52	49.43

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 . 5 3 8 8 0 1 0.0981283 0.0100797
 BMDL = 0 . 3 2 8 1 3 5 0.0345104 0.00344714
 BMDU = 0 . 7 1 7 1 2 7 0.325909 > 0.0806373

Incidental Risk: OralForstKroeseF3
 points show nonparam. est. for Incidental (unfilled) and Fatal (filled)



17
18
19
20
21

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).

1 **Female Rat ([Kroese et al., 2001](#)): Hepatocellular Adenoma or Carcinoma**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: LiverKroeseF3.(d)
 6 Fri Apr 16 09:08:03 2010
 7 =====

8
 9 Timer to Tumor Model, Liver Hepatocellular Tumors, Kroese et al, Female

10 ~~~~~
 11
 12 The form of the probability function is:
 13 $P[\text{response}] = 1 - \text{EXP}\$\$-(t - t_0)^c * (\beta_0 + \beta_1 * \text{dose} + \beta_2 * \text{dose}^2 + \beta_3 * \text{dose}^3)$
 14
 15

16 The parameter betas are restricted to be positive

17
 18
 19 Dependent variable = CONTEXT
 20 Independent variables = DOSE, TIME

21
 22 Total number of observations = 208
 23 Total number of records with missing values = 0
 24 Total number of parameters in model = 6
 25 Total number of specified parameters = 0
 26 Degree of polynomial = 3

27
 28
 29 Maximum number of iterations = 64
 30 Relative Function Convergence has been set to: 2.22045e-016
 31 Parameter Convergence has been set to: 1.49012e-008

32
 33
 34 Default Initial Parameter Values
 35 c = 3.6
 36 t_0 = 31.7778
 37 beta_0 = 0
 38 beta_1 = 4.9104e-031
 39 beta_2 = 5.45766e-030
 40 beta_3 = 3.44704e-008
 41

42
 43 Asymptotic Correlation Matrix of Parameter Estimates
 44 (*** The model parameter(s) -beta_0 -beta_1 -beta_2
 45 have been estimated at a boundary point, or have been specified by the user,
 46 and do not appear in the correlation matrix)
 47

	c	t_0	beta_3
c	1	-0.9	-1
t_0	-0.9	1	0.92
beta_3	-1	0.92	1

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Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

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

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	Log(likelihood)	# Param	AIC
Fitted Model	-228.17	6	468.34

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Data Summary
CONTEXT

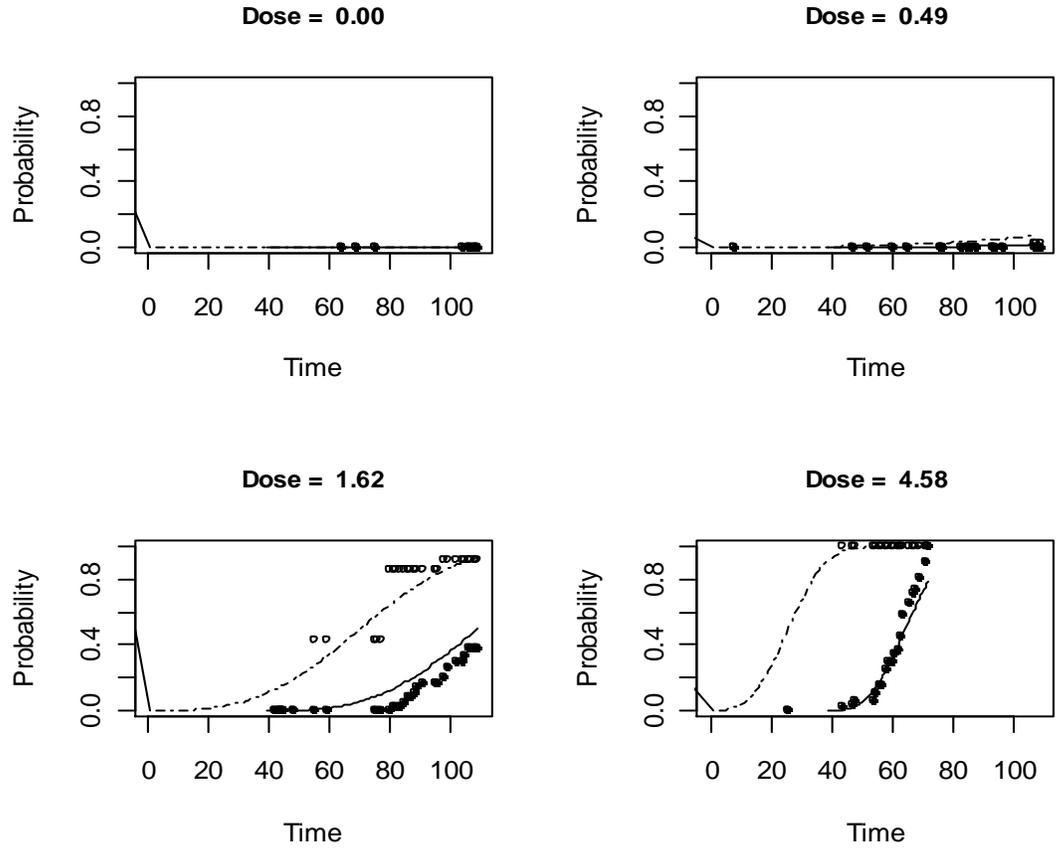
DOSE	C	F	I	U	Total	Expected Response
0	52	0	0	0	52	0.00
0.49	51	0	1	0	52	3.02
1.6	13	12	27	0	52	38.36
4.6	1	38	13	0	52	51.36

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	. 5 7 5 1 2 7		0.262783	0.12179
BMDL =	0	. 5 0 6 6 3 3		0.134213	0.0152934
BMDU =	0	. 6 2 9 8 0 6		0.287232	0.133064

Incidental Risk: Hepatocellular_Kroese_F3
 points show nonparam. est. for Incidental (unfilled) and Fatal (filled)



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20
21
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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).

1 **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: DuoJeyJkroeseF3.(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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

```

DOSE	Data Summary				Total	Expected Response
	C	F	I	U		
0	52	0	0	0	52	0.00
0.49	52	0	0	0	52	0.01

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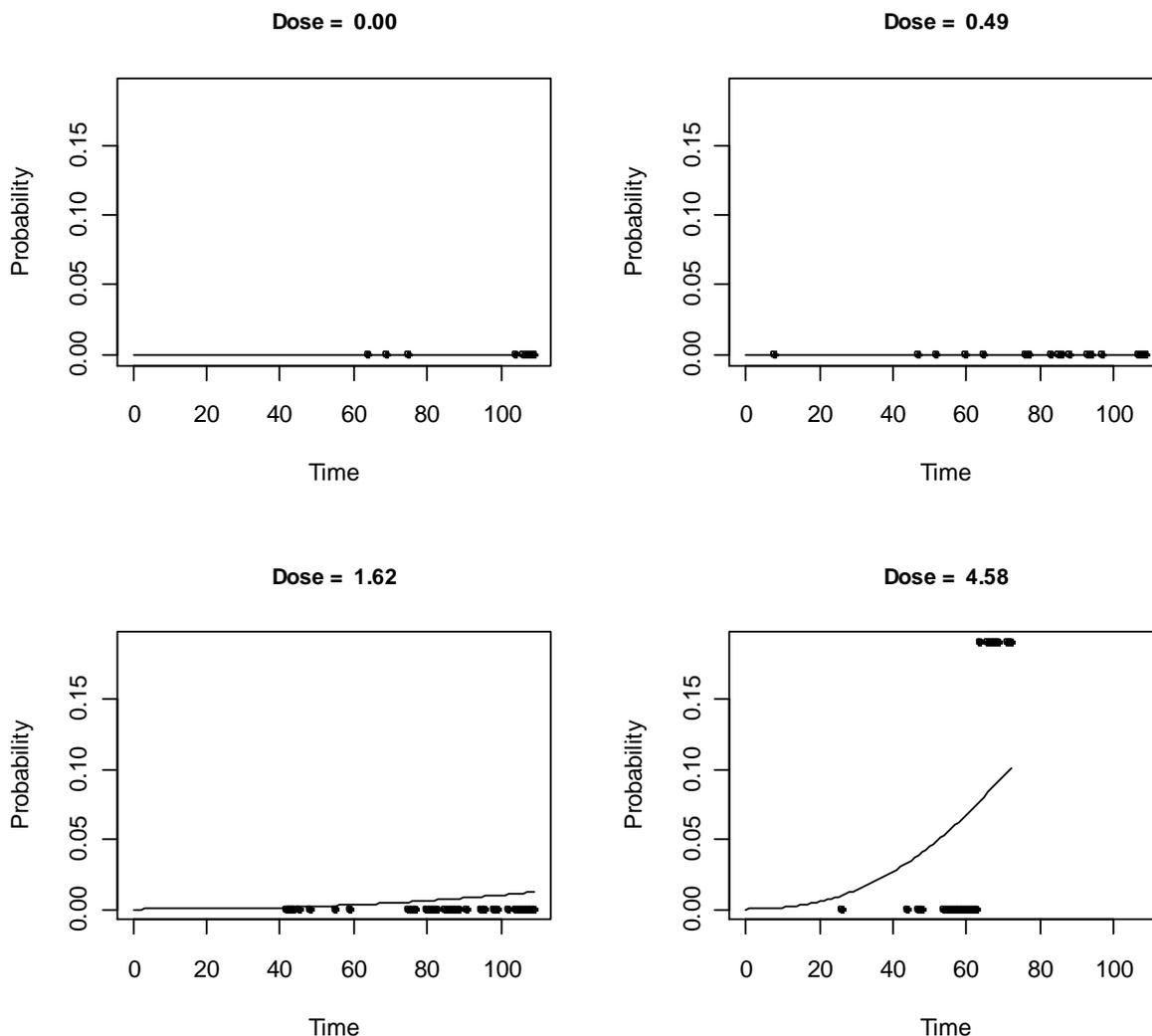
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10

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	.	4	3	1	2	9	1.56781	0.726615
BMDL =	1	.	9	4	7	4	5	0.560867	0.0584891
BMDU =	5	.	7	0	1	0	8	2.61447	1.21046

Incidental Risk: DuoJej_Kroese_F3



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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).

1 **Table E-15. Summary of human equivalent overall oral slope factors, based on**
 2 **tumor incidence in male and female Wistar rats exposed to benzo[a]pyrene by**
 3 **gavage for 104 weeks (Kroese et al., 2001)**

Data set	Tumor site	BMD ₀₀₁	BMDL ₀₀₁	Risk value ^a at		SD	SD ²	Proportion of total variance
				BMD ₀₀₁	BMDL ₀₀₁			
Males	Oral cavity/forestomach	6.37×10^{-3}	2.86×10^{-3}	1.57×10^{-1}	3.50×10^{-1}	1.17×10^{-1}	1.38×10^{-2}	0.64
	Liver	2.00×10^{-2}	5.30×10^{-3}	5.00×10^{-2}	1.89×10^{-1}	8.42×10^{-2}	7.09×10^{-3}	0.33
	Duodenum/jejunum	6.42×10^{-1}	4.21×10^{-2}	1.56×10^{-3}	2.38×10^{-2}	1.35×10^{-2}	1.82×10^{-4}	0.01
	Skin/mammary gland: basal cell	6.06×10^{-1}	4.24×10^{-2}	1.65×10^{-3}	2.36×10^{-2}	1.33×10^{-2}	1.78×10^{-4}	0.01
	Skin/mammary gland: squamous cell	7.06×10^{-2}	2.11×10^{-2}	1.42×10^{-2}	4.75×10^{-2}	2.03×10^{-2}	4.10×10^{-4}	0.02
	Kidney	9.84×10^{-1}	7.48×10^{-2}	1.02×10^{-3}	1.34×10^{-2}	7.51×10^{-3}	5.64×10^{-5}	0.00
	Sum, risk values at BMD ₀₀₁ :			2.25×10^{-1}	Sum, SD ² :		2.17×10^{-2}	
	Overall SD ^b :						1.47×10^{-1}	
Upper bound on sum of risk estimates ^c :					4.68×10^{-1}			
Females	Oral cavity/forestomach	3.45×10^{-3}	1.01×10^{-2}	2.90×10^{-1}	9.92×10^{-2}	1.16×10^{-1}	1.35×10^{-2}	0.91
	Liver	1.53×10^{-2}	1.22×10^{-1}	6.54×10^{-2}	8.21×10^{-3}	3.48×10^{-2}	1.21×10^{-3}	0.08
	Duodenum/jejunum	5.85×10^{-2}	7.27×10^{-1}	1.71×10^{-2}	1.38×10^{-3}	9.56×10^{-3}	9.13×10^{-5}	0.01
	Sum, risk values at BMD ₀₀₁ :			1.09×10^{-1}	Sum, SD ² :		1.48×10^{-2}	
	Overall SD:						1.22×10^{-1}	
Upper bound on sum of risk estimates ^c :					3.09×10^{-1}			

4
 5 ^aRisk value = 0.001/BMDL₀₀₁.

6 ^bOverall SD = (sum, SD²)^{0.5}.

7 ^cUpper bound on the overall risk estimate = sum of BMD₀₀₁ risk values + 1.645 × overall SD.

8 **Table E-16. Summary of BMD model selection among multistage-Weibull**
 9 **models fit to alimentary tract tumor data for female B6C3F₁ mice exposed to**
 10 **benzo[a]pyrene for 2 years (Beland and Culp, 1998)**

Model stages	AIC	BMD ₁₀ ^a	BMDL ₁₀ –BMDU ₁₀ ^a	Basis for model selection
1	688.5	0.104		Lowest AIC, best fit to low dose data
2	629.2	0.102		
3	624.5	0.127	0.071–0.179	

11
 12 ^aEvaluated at 104 weeks

13

1 **Female Mice ([Beland and Culp, 1998](#)): Alimentary Tract Squamous Cell Tumors**

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4 =====
5 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
6 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
7 Input Data File: C:\msw10-09\benzo[a]pyrene_FemaleSquamF3i.(d)
8 =====

9 The form of the probability function is:
10 $P[\text{response}] = 1 - \exp\left\{-(t - t_0)^c \cdot \right.$
11 $\left. (\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2 + \beta_3 \cdot \text{dose}^3)\right\}$
12

13 The parameter betas are restricted to be positive

14
15 Dependent variable = Class
16 Independent variables = Dose, time

17
18 Total number of observations = 191
19 Total number of records with missing values = 0
20 Total number of parameters in model = 6
21 Total number of specified parameters = 0
22 Degree of polynomial = 3

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25 Maximum number of iterations = 64
26 Relative Function Convergence has been set to: 2.22045e-016
27 Parameter Convergence has been set to: 1.49012e-008
28
29
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31 User Inputs Initial Parameter Values

32 c = 2
33 t_0 = 15
34 beta_0 = 1.6e-014
35 beta_1 = 0
36 beta_2 = 5.5e-012
37 beta_3 = 4.4e-012
38

39
40 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

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Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Fitted Model Log(likelihood) # Param AIC
-306.265 6 624.53

Data Summary

Class
C F I U Total Expected Response

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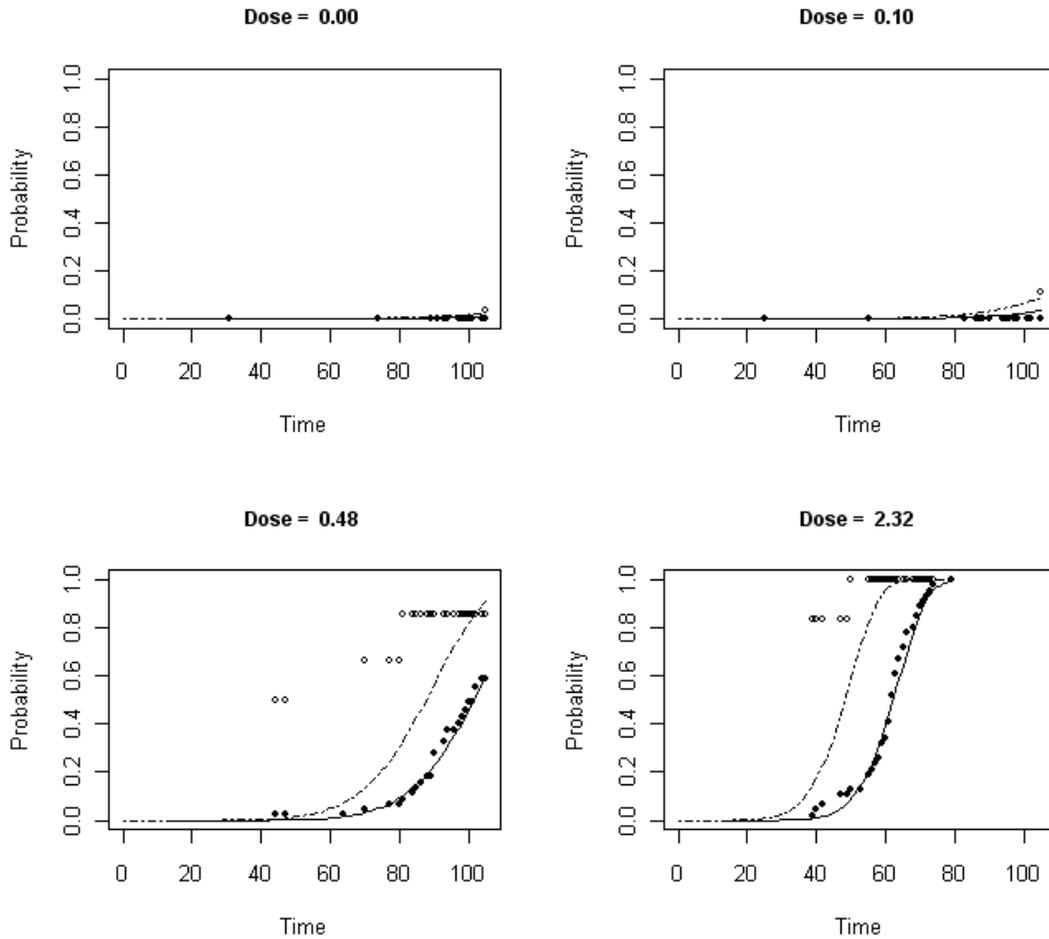
Dose						
0	47	0	1	0	48	0.93
0.1	45	0	3	0	48	3.21
0.48	8	23	15	1	47	30.82
2.3	1	46	0	1	48	41.91

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

Incidental Risk: BaP_FemaleSquamF3i

points show nonparam. est. for Incidental (unfilled) and Fatal (filled)



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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).

1 E.2.2. Dose-Response Modeling for the Inhalation Unit Risk

2 *Modeling Methods*

3 As with the tumor data used for the oral slope factor (see Section E.2.1, *Dose Response-*
4 *modeling for the Oral Slope Factor*), there was earlier occurrence of tumors with increasing
5 exposure, and early termination of the high-dose group ([Thyssen et al., 1981](#); see [Appendix D for](#)
6 [study details](#)). The computer software program Multistage Weibull ([U.S. EPA, 2010](#)) was used as
7 described in the analysis of the oral carcinogenicity data. See Section E.2.1 for details of the
8 modeling methods. A previous time-to-tumor analysis ([U.S. EPA, 1990](#)) was not used because of
9 several discrepancies between the summarized dose-response data and the individual pathology
10 reports, because the use of age at necropsy rather than the time since first exposure, and because
11 multistage Weibull provides a corrected estimate of the confidence bounds on the BMD.

12 *Data Adjustments Prior to Modeling*

13 As with the oral slope factor (see Section E.2.1, *Dose Response-modeling for the Oral Slope*
14 *Factor*), etiologically similar tumor types (i.e., benign and malignant tumors of the same cell type)
15 were combined for dose-response modeling. Here the benign tumors (papillomas, polyps, and
16 papillary polyps) were judged to be of the same cell type as the squamous cell carcinomas (SCCs).
17 As described in Section 2.4.2, the overall incidences of benign or malignant tumors in the
18 respiratory tract (larynx, trachea, and nasal cavity) and pharynx were used for dose-response
19 modeling.

20 [Thyssen et al. \(1981\)](#) did not determine cause of death for any of the animals. Since the
21 investigators for the oral bioassays considered the same tumors to be fatal at least some of the time,
22 bounding estimates for the [Thyssen et al. \(1981\)](#) data were developed by treating the tumors
23 alternately as either all incidental or all fatal. In either case, therefore, an estimate of t_0 (the time
24 between a tumor first becoming observable and causing death) could not be estimated and was set
25 to 0. The data analyzed are summarized in Table E-17. Animals without confirmation of one or
26 more of the pharynx or respiratory tract tissues being examined were not included in the
27 incidences, unless a tumor was diagnosed in those that were examined. Group average TWA
28 continuous exposures, based on chamber air monitoring data and individual hamsters' time on
29 study, of 0, 0.25, 1.01, and 4.29 mg/m³ corresponded to the 0, 2, 10, and 50 mg/m³ nominal study
30 concentrations, respectively ([U.S. EPA, 1990](#)).

31

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Table E-17. Individual pathology and tumor incidence data for male Syrian golden hamsters exposed to benzo[a]pyrene via inhalation for lifetime—[Thyssen et al. \(1981\)](#)^a

Exposure concentration: target (lifetime average continuous exposure) ^b , mg/m ³	Time of tumor observed (d)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors	
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach		
0 (0)	112	0	— ^c	0	0	0	0	—	
	270	0	0	0	0	0	0	0	
	314	0	0	0	0	0	0	0	
	553	0	0	0	0	0	0	0	
	577	0	0	0	—	0	0	—	
	594	0	—	0	0	0	0	—	
	596	0	0	0	0	0	0	0	
	611	0	0	0	0	0	0	0	
	611	0	0	0	0	0	0	0	
	616	0	0	0	0	0	0	0	
	619	0	0	0	0	0	0	0	
	623	0	0	0	0	0	0	0	
	704	0	0	0	0	0	0	0	
	710	0	0	0	0	0	0	0	
	721	0	0	0	0	0	0	0	
	739	0	0	0	0	0	0	0	
	751	0	0	0	0	0	0	0	
	762	0	0	0	0	0	0	0	
	779	0	0	0	0	0	0	0	
	800	0	0	0	0	0	0	0	
808	0	—	0	0	0	0	0	—	
847	0	0	0	0	0	0	0	0	
857	0	0	0	0	0	0	0	0	
867	—	0	0	0	0	0	0	—	
868	0	0	0	0	0	0	0	0	
885	0	—	0	0	0	0	0	—	
917	0	0	0	0	0	0	0	0	
2 (0.25)	93	—	—	—	0	0	0	—	
	247	0	0	0	0	0	0	0	
	370	0	0	0	0	0	0	0	
	407	0	0	0	0	0	0	0	
	489	0	0	0	0	0	0	0	
	539	0	0	0	0	0	0	0	
	554	0	0	0	0	0	0	0	
	591	0	0	0	0	0	0	0	
	612	0	0	0	0	0	0	0	
	650	0	0	0	0	0	0	0	
	682	0	—	0	0	0	0	0	—
	690	0	0	0	0	0	0	0	0
	717	0	0	0	0	0	0	0	0

Exposure concentration: target (lifetime average continuous exposure) ^b , mg/m ³	Time of tumor observed (d)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach	
	717	0	0	0	0	0	0	0
	755	0	0	0	0	0	0	0
	788	0	0	0	0	0	0	0
	795	0	0	0	0	0	0	0
	802	0	0	0	0	0	0	0
	808	0	0	0	0	0	0	0
	836	0	0	0	0	0	0	0
	848	—	—	0	0	0	0	—
	925	0	0	0	0	0	0	0
10 (1.01)	212	0	0	0	0	0	0	0
	227	0	0	0	0	0	0	0
	357	0	0	0	0	0	0	0
	465	0	0	0	0	0	0	0
	509	0	0	0	0	0	0	0
	530	0	1 (1)	0	0	0	0	1
	531	0	1 (1)	0	0	0	0	1
	557	1 (1) ^d	0	0	0	0	0	0
	597	0	0	0	0	0	0	0
	653	1 (1)	0	0	0	0	0	1
	695	0	0	0	0	0	0	0
	712	0	1 (0)	0	0	0	0	1
	732	1 (1)	1 (1)	0	0	0	0	1
	773	0	1 (1)	0	0	0	0	1
	788	0	1 (0)	0	0	0	0	1
	796	1 (1)	1 (1)	0	0	0	0	1
	803	1 (1)	—	1 (0)	1 (0)	0	0	1
	808	0	—	1 (0)	1 (1) ^e	0	0	1
	812	1 (0)	—	0	0	0	0	1
	822	1 (1)	1 (1)	0	0	0	0	1
826	1 (0)	0	0	0	0	0	1	
826	0	—	0	0	0	0	—	
826	1 (1)	0	0	1 (0)	0	1 (1)	1	
848	1 (0)	0	0	0	0	0	1	
867	1 (1)	1 (1)	0	0	0	0	1	
868	0	0	0	1 (0)	0	0	1	
50 (4.29)	144	—	—	—	0	0	0	—
	151	—	—	—	0	0	0	—
	178	—	—	—	0	0	0	—
	210	—	—	—	0	0	0	—
	211	0	—	0	0	0	0	—
	213	—	—	—	0	0	0	—
	242	—	—	—	0	0	0	—
	253	0	0	0	0	0	0	0
	255	—	—	—	0	0	0	—
	263	—	—	—	0	0	0	0

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Exposure concentration: target (lifetime average continuous exposure) ^b , mg/m ³	Time of tumor observed (d)	Incidence of papillomas, polyps, papillary polyps, or carcinomas (total malignant tumors)						Incidence of respiratory tract or pharynx tumors
		Larynx	Pharynx	Trachea	Nasal cavity	Esophagus	Fore-stomach	
	281	0 ^f	1 (1)	1 (0)	0	0	0	1
	284	0	0	0	0	0	0	0
	284	—	—	—	0	0	0	—
	294	0	0	0	0	0	0	0
	296	0	0	0	0	0	0	0
	324	1 (1)	1 (1)	0	0	0	0	1
	329	0	1 (1)	0	0	0	0	1
	371	0	—	0	0	0	0	—
	388	1 (1)	1 (1)	0	0	0	0	1
	395	0	1 (1)	0	0	0	0	1
	421	0	1 (1)	0	0	0	0	1
	436	0	0	0	0	0	0	0
	442	0	1 (0)	0	0	0	1 (0)	1
	462	1 (1)	1 (1)	0	0	—	—	1
	471	0	1 (1)	0	0	0	0	1
	486	1 (0)	1 (1)	0	0	1 (0)	0	1
	494	1 (1)	1 (1)	1 (1)	0	0	0	1
	498	1 (0)	1 (1)	0	0	0	1 (0)	1
	504	1 (1)	1 (1)	0	0	0	0	1
	506	1 (1)	1 (1)	0	0	0	0	1
	572	0	1 (1)	0	0	0	0	1
	575	1 (1)	1 (1)	0	0	1 (0)	0	1
	578	1 (0)	1 (1)	0	0	0	0	1
	717 ^g	1 (1)	1 (1)	1 (0)	1 (0)	0	0	1

1

2 ^aHistopathology incidence from ([Clement Associates \(1990\)](#); [U.S. EPA \(1990\)](#)).3 ^bSee Section D.4.2.4 ^cTissue was not examined.5 ^dIn situ carcinoma; not included in overall tumor incidence.6 ^eAdenocarcinoma; not included in overall tumor incidence.7 ^fMetastasis from pharynx not shown.8 ^g Necropsy occurred 24 weeks after 79 weeks of exposure.

9

1 **Dose-Response Modeling Results**

2 Table E-18 summarizes the modeling results supporting the derivation of an inhalation unit
 3 risk value for benzo[a]pyrene. The model outputs and graphs (Figures E-20 and E-21) following
 4 Table E-18 provide more details for the best-fitting models under the conditions of taking all
 5 tumors to be incidental to the cause of death, or to be the cause of death, respectively.

6 **Table E-18. Summary of BMD model selection among multistage-Weibull**
 7 **models fit to tumor data for male Syrian golden hamsters exposed to**
 8 **benzo[a]pyrene via inhalation for lifetime ([Thyssen et al., 1981](#))**

Tumor context	Model stages	AIC	BMD ₁₀ ^a	BMDL ₁₀ ^a	Basis for model selection
All tumors considered incidental to cause of death	1	50.5	0.076	0.052	Lowest AIC, best fit to data (BMDU ₁₀ = 0.324)
	2	40.4	0.254	0.163	
All tumors considered to be cause of death	1	315.0	0.135	0.104	Lowest AIC; best fit to data (BMDU ₁₀ = 0.544)
	2	302.9	0.468	0.256	

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10 **Output for Squamous Cell Neoplasia Following Inhalation Exposure to Benzo[a]pyrene: All**
 11 **Tumors Considered Incidental to Cause of Death**

12 =====
 13 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 14 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 15 Input Data File: ThyssenInc2sL104noUB70.(d)
 16 Tue Mar 11 12:58:28 2014
 17 =====

18
 19 The form of the probability function is:
 20 $P[\text{response}] = 1 - \text{EXP}\{-(t - t_0)^c * (\text{beta}_0 + \text{beta}_1 * \text{dose}^1 + \text{beta}_2 * \text{dose}^2)\}$
 21

22
 23 The parameter betas are restricted to be positive

24
 25 Dependent variable = CONTEXT
 26 Independent variables = DOSE, TIME

27
 28 Total number of observations = 88
 29 Total number of records with missing values = 0
 30 Total number of parameters in model = 5
 31 Total number of specified parameters = 1
 32 Degree of polynomial = 2

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 35
 36 User specifies the following parameters:
 37 $t_0 = 0$
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39
 40 Maximum number of iterations = 64
 41 Relative Function Convergence has been set to: 2.22045e-016
 42 Parameter Convergence has been set to: 1.49012e-008
 43

44
 45 Default Initial Parameter Values
 46 $c = 4.5$
 47 $t_0 = 0$ Specified
 48 $\text{beta}_0 = 1.32176e-037$
 49 $\text{beta}_1 = 3.02455e-036$
 50 $\text{beta}_2 = 2.03765e-013$

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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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	4.71714	0.957627	2.84023	6.59406
beta_0	0	NA		
beta_1	0	NA		
beta_2	5.16891e-014	3.13384e-013	-5.62533e-013	6.65911e-013

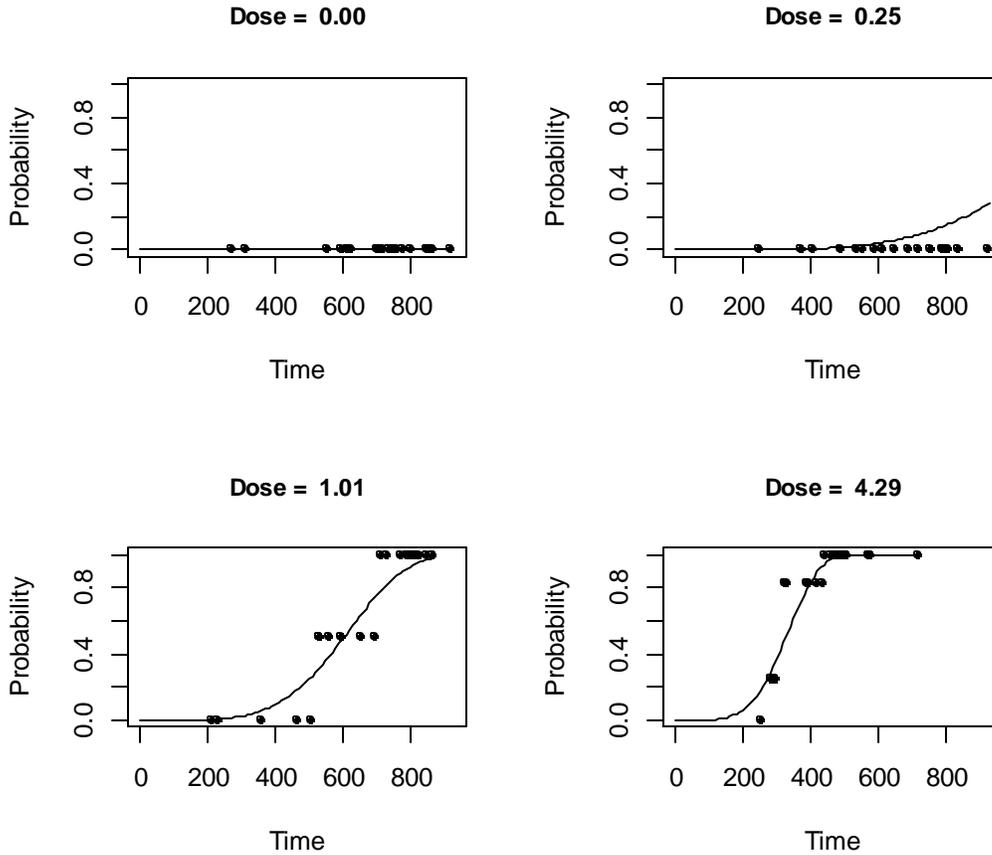
NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-16.2088	4	40.4176

DOSE	Data Summary				U	Total	Expected Response
	C	F	I	U			
0	21	0	0	0	21	0.00	
0.25	19	0	0	0	19	1.63	
1	8	0	17	0	25	16.27	
4.3	5	0	18	0	23	17.75	

Benchmark Dose Computation
 Risk Response = Incidental
 Risk Type = Extra
 Specified effect = 0.1
 Confidence level = 0.9
 Time = 728
 BMD = 0.253569
 BMDL = 0.163052
 BMDU = 0.323781

Incidental Risk: ThyssenInc2sL104noU



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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

Time of tumor observation was converted to weeks in order to run this form of the multistage-Weibull model.

```

=====
Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
Solutions are obtained using donlp2-intv, (c) by P. Spellucci
Input Data File: ThyssenF2sL104noU.(d)
Thu Mar 13 14:30:45 2014
=====

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

The parameter betas are restricted to be positive

Dependent variable = CONTEXT
Independent variables = DOSE, TIME

Total number of observations = 88
Total number of records with missing values = 0
    
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Total number of parameters in model = 5
Total number of specified parameters = 1
Degree of polynomial = 2

User specifies the following parameters:
t_0 = 0

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

Default Initial Parameter Values
c = 6
t_0 = 0 Specified
beta_0 = 2.0496e-036
beta_1 = 4.12988e-014
beta_2 = 3.37033e-013

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

	c	beta_2
c	1	-1
beta_2	-1	1

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	6.61992	0.915036	4.82649	8.41336
beta_0	0	NA		
beta_1	0	NA		
beta_2	2.13816e-014	8.96466e-014	-1.54323e-013	1.97086e-013

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

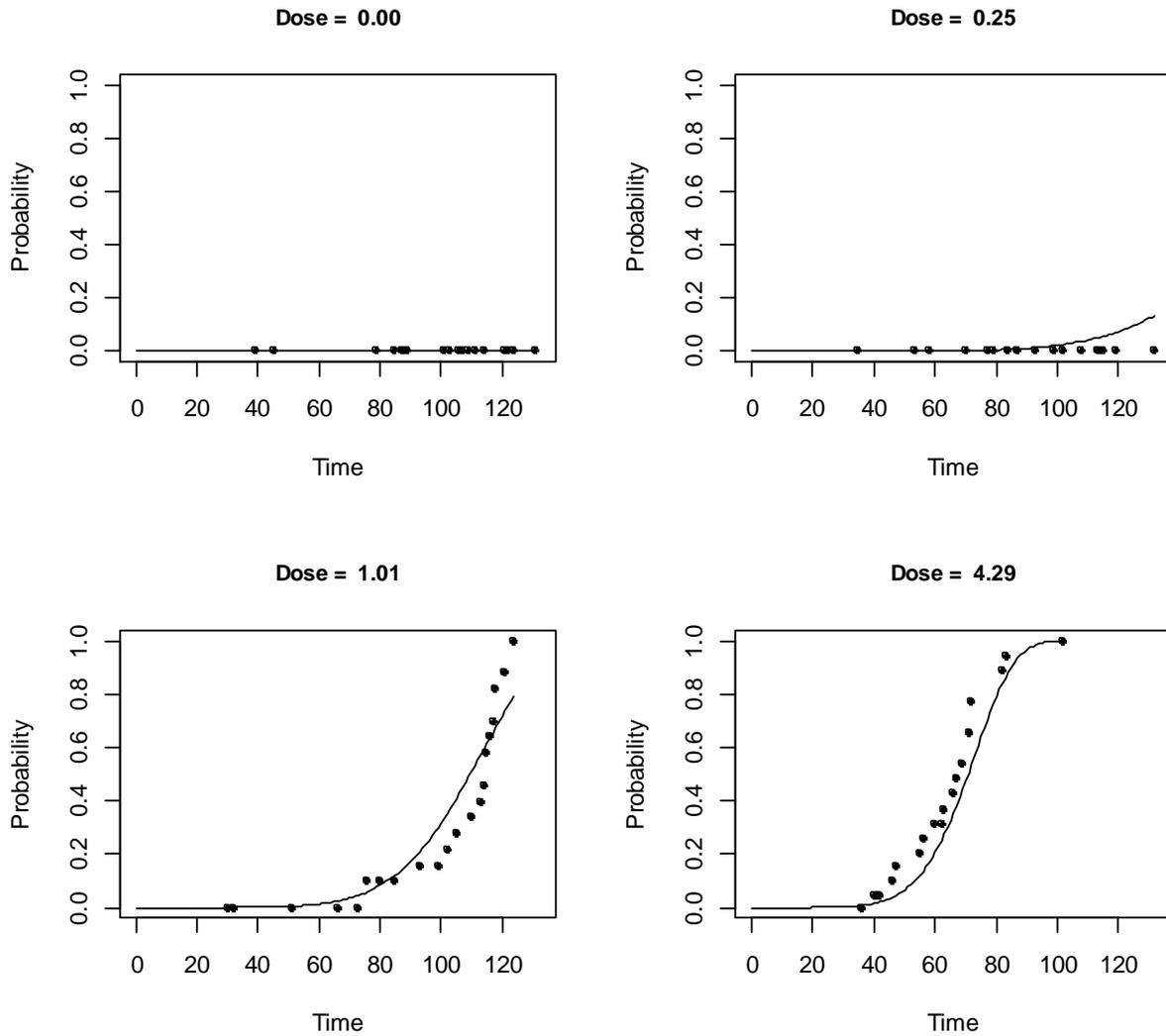
Fitted Model	Log(likelihood)	# Param	AIC
	-147.66	4	303.319

DOSE	Data Summary				Total
	C	F	I	U	
0	21	0	0	0	21
0.25	19	0	0	0	19
1	8	17	0	0	25
4.3	5	18	0	0	23

Minimum observation time for F tumor context = 40

Benchmark Dose Computation
Risk Response = Fatal
Risk Type = Extra
Specified effect = 0.1
Confidence level = 0.9
Time = 104
BMD = 0.467752
BMDL = 0.256206
BMDU = 0.543965

Fatal Risk:



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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.

1 E.2.3. Dose-Response Modeling for the Dermal Slope Factor

2 *Modeling Methods*

3 As with the tumor data used for the oral slope factor (see Section E.2.1, *Dose Response-*
4 *modeling for the Oral Slope Factor*) and the inhalation unit risk (see Section E.2.2, *Dose Response-*
5 *modeling for the Inhalation Unit Risk*), there was earlier occurrence of tumors with increasing
6 exposure, as shown in the individual animal data in the technical report for the National Institute
7 for Occupational Safety and Health (NIOSH) study ([Sivak et al., 1997](#); [Arthur D Little, 1989](#)). The
8 computer software program Multistage Weibull ([U.S. EPA, 2010](#)) was used for the analysis of the
9 dermal carcinogenicity data. See Section E.2.1 for details of the modeling approach, including
10 evaluation of model fit and model selection. Tumors were classified as incidental because the
11 appearance of tumors generally preceded death by weeks in all cases, and time of first appearance
12 of tumors or time on study without tumors were used for the time input.

13 For the other supporting studies identified in Section 2.5.1, multistage models [BMDS; ([U.S.](#)
14 [EPA, 2012a](#)); v 2.1] were used. See Section E.1.1 for details of fitting the multistage model. The
15 BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood
16 method) and AIC value were used to select a best-fit model from among the models exhibiting
17 adequate fit. The data modeled are summarized in Tables E-19 through E-23.

18 *Data Adjustments Prior to Modeling*

19 For time-to-tumor modeling, no adjustment other than an estimate of daily exposure was
20 used. The data modeled are provided in Table E-19.

21 For the remaining studies, two types of adjustments were considered: (1) for study groups
22 that were reported to end before 104 weeks, but well after 1 year of exposure, it was judged
23 reasonable to assume that the tumor incidence observed at the time of early termination could have
24 been realized in a full lifetime study using a lower dose; and (2) reductions of the group sizes when
25 there was mortality prior to the first appearance of tumors, in order to estimate the effective
26 number at risk. Equivalent lifetime doses were estimated by multiplying the relevant average daily
27 doses by $(L_e/104)^3$, where L_e is the length of exposure, based on observations that tumor incidence
28 tends to increase with age ([Doll, 1971](#)). Note that exposure periods <52 weeks would lead to a
29 relatively large adjustment [i.e., $(52/104)^3 = 0.125$, or an eightfold lower dose than administered],
30 reflecting considerable uncertainty in lifetime equivalent dose estimates generated from relatively
31 short studies. This adjustment was applied to all dose groups in [Poel \(1959\)](#) and [Roe et al. \(1970\)](#),
32 and the highest dose group in [Habs et al. \(1980\)](#) and the grouped data reported by [Sivak et al.](#)
33 [\(1997\)](#). The following discussion summarizes how each adjustment was carried out when relevant.

34 [Roe et al. \(1970\)](#) applied benzo[a]pyrene dermally for 93 weeks or until natural death; with
35 the exception of the highest dose group, each group had approximately 20 animals (or ~40%
36 survival) at 600 days (see Table D-17). The tumors were first observed in the lowest and highest
37 dose groups during the interval of days 200–300. Mice dying before day 200 were likely not at risk
38 long enough for tumor development. However, because tumor incidence and mortality were
39 reported in 100-day intervals, mice that had not been on study long enough to develop tumors were

1 not easily identifiable. Incidence denominators reflect the number of animals alive at day 200, and
2 may thus lead to underestimates of tumor risk if the number of animals at risk has been
3 overestimated. Table E-20 summarizes the dose adjustments to estimate equivalent 104-week
4 exposures, as well as incidence data adjusted for mortality prior to skin tumor appearance.

5 [Schmidt et al. \(1973\)](#) did not report survival information; instead, the authors provided
6 incidences based on the numbers of mice initially included in each dose group at the start of the
7 study. Overall latency was reported for the two high-dose groups in each series, but these data only
8 describe the survival of mice with tumors (animals were removed from study when a tumor
9 appeared). It is not clear how long exposures lasted overall in each dose group, or whether some
10 mice may have died on study from other causes before tumors appeared. While it is possible that
11 no mice died during the study, all of the other studies considered here demonstrate mortality.
12 However, the data were modeled as reported, with no adjustments, recognizing the possibility of
13 underestimating risk associated with incidences reported and lack of duration of exposure (see
14 Table E-20).

15 [Schmähl et al. \(1977\)](#) reported that reduced numbers of animals at risk (77–88 mice per
16 dose group compared with the initial group sizes of 100) resulted from varying rates of autolysis.
17 No other survival or latency information was provided, so all exposures were assumed to have
18 lasted for 104 weeks and were modeled as reported. Given the results of the other studies, it seems
19 possible that the numbers at risk in each group may be overestimated, which could lead to an
20 underestimate of lifetime risk (see Table E-20).

21 [Habs et al. \(1980\)](#) reported age-standardized skin tumor incidence rates, indicating earlier
22 mortality in the two highest dose groups (2.8 and 4.6 µg/application). These rates were used to
23 estimate the number at risk for dose-response modeling, by dividing the number of mice with
24 tumors by the age-standardized rates. Exposure lasted longer than 104 weeks in the two lower
25 exposure groups, at about 120 and 112 weeks, and until about 88 weeks in the highest exposure
26 group. Incidence in the two lower exposure groups may be higher than if the exposure had lasted
27 just 104 weeks. There was mortality in the first 52 weeks of exposure, about 10–15% in the three
28 exposure groups, but because there was no information concerning when tumors first appeared, it
29 is not possible to determine how much the early mortality may have impacted the number of mice
30 at risk in each group (see Table E-20).

31 [Habs et al. \(1984\)](#) reported mean survival times (with 95% CIs) for each dose group. The
32 CIs supported the judgment that the control and lower dose groups were treated for 104 weeks.
33 The higher dose group (4 µg/application) was probably treated for <104 weeks, because the upper
34 95% confidence limit for the mean survival was approximately 79 weeks. However, since it was
35 not possible to estimate a more realistic duration for this group, an estimate of 104 weeks was used
36 (see Table E-20).

37 The [Poel \(1959\)](#) study was conducted in male mice and used toluene as the vehicle. In
38 addition to a control group, there were nine dose groups. All C57L mice in dose groups with
39 >3.8 µg/application died by week 44 of the study. Therefore, these five dose groups were omitted
40 prior to dose-response modeling because of the relatively large uncertainty in extrapolating cancer
41 risk as a result of lifetime exposure. Four dose groups in addition to control remained. Among

1 these groups, mice survived and were exposed until weeks 83–103. According to the lifespan
2 ranges provided, at least one mouse in each dose group died before the first appearance of tumor,
3 but insufficient information was available to determine how many; consequently, the incidence
4 denominators were not adjusted. Although mice in the lowest exposure group were treated up to
5 103 weeks, the control group demonstrated a median survival time of 60 weeks and concluded at
6 week 92, suggesting a lifetime <104 weeks is more relevant for this mouse strain. Doses were
7 adjusted to approximate exposures resulting in the same responses at 104 weeks of exposure for
8 comparability among these lifetime studies. This adjustment had little impact on the lowest
9 exposure level, which had an observed response of 9%, close to the BMR of 10% extra risk; in this
10 case, the adjustment is not expected to affect the BMD₁₀ significantly. The dose-response data are
11 summarized in Table E-21.

12 [Grimmer et al. \(1984\)](#) and [Grimmer et al. \(1983\)](#) studied female CFLP mice, using
13 acetone:dimethyl sulfoxide (DMSO) (1:3) as the vehicle. Mean or median latency times were
14 reported (as well as measures of variability), but no information concerning overall survival was
15 included in the results. The total of tumor-bearing mice and the reported percentages of mice with
16 any skin tumors was reported, and varied at most one animal from the number of animals initially
17 placed on study. The decreasing latency and variability and increasing tumor incidence with
18 increasing benzo[a]pyrene exposure suggests that exposure probably did not last for the full
19 scheduled 104 weeks in at least the high-dose group. The data reported were modeled under the
20 assumption that at least some animals in each group were treated and survived until week 104 (see
21 Table E-22).

22

1 **Table E-19. Tumor incidence, with time to observation of tumor or death;**
 2 **CeH/HeJ male mice exposed dermally to benzo[a]pyrene^a (Sivak et al., 1997;**
 3 **[Arthur D Little, 1989](#))**

Average daily dose (µg/d)	Time of first appearance of tumor, or time on study (d) ^b	Total examined	Number of animals with papillomas or carcinomas	Average daily dose (µg/d)	Time of first appearance of tumor, or time on study (d) ^b	Total examined	Number of animals with papillomas or carcinomas	
0	104	1	0	0.14	354	1	0	
	237	1	0		453	1	1	
	239	1	0		460	1	0	
	444	1	0		509	1	0	
	483	1	0		525	1	0	
	504	1	0		537	1	0	
	509	1	0		560	1	1	
	537	1	0		573	1	1	
	545	1	0		601	1	0	
	590	1	0		663	1	1 ^b	
	609	1	0		669	1	0	
	613	1	0		677	1	0	
	615	2	0		685	1	0	
	629	1	0		701	1	0	
	630	1	0		727	1	1	
	658	1	0		731	1	0	
	699	1	0		732	1	0	
	719	1	0		734	13	0	
	748	11	0					
	Totals		30		0	Totals		30
0.014	235	1	0	1.4	233	1	0	
	238	1	0		234	1	0	
	430	1	0		235	1	0	
	446	1	0		307	1	1	
	456	1	0		348	2	2	
	463	1	0		355	2	2	
	470	1	0		362	2	2	
	503	1	0		369	3	3	
	511	1	0		376	1	1	
	590	1	0		383	1	1	
	604	1	0		390	1	1	
	663	1	0		397	3	3	
	680	1	0		404	1	1	
	684	1	0		411	5	5	
	727	1	0		418	1	1	
	747	15	0		440	2	2	
					446	1	1	
			474	1	1			
Totals		30	0	Totals		30	27	

4
 5 ^aDoses were applied twice/weekly to shaved dorsal skin. Vehicle for all groups was 1:1 cyclohexanone/acetone.

6 ^bFor animals with skin tumors, the time of the tumor; if no skin tumor was observed, the time is the time of death
 7 or sacrifice.

8 ^cThe tumor diagnosis was keratoacanthoma.

1 **Table E-20. Skin tumor incidence, benign or malignant in female Swiss or**
 2 **NMRI mice dermally exposed to benzo[a]pyrene; data from [Roe et al. \(1970\)](#),**
 3 **[Schmidt et al. \(1973\)](#), [Schmähl et al. \(1977\)](#), [Habs et al. \(1980\)](#), [Habs et al.](#)**
 4 **[\(1984\)](#)**

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	–	<i>104^d</i>	0	0/100 (0%)
		0.05	0.01	–	<i>104</i>	0.01	0/100 (0%)
		0.2	0.06	–	<i>104</i>	0.06	0/100 (0%)
		0.8	0.23	53 ^e	<i>104</i>	0.23	2/100 (2%)
		2	0.57	76 ^e	<i>104</i>	0.57	30/100 (30%)
	Swiss	0 (acetone)	0	–	<i>104</i>	0	0/80 (0%)
		0.05	0.01	–	<i>104</i>	0.01	0/80 (0%)
		0.2	0.06	–	<i>104</i>	0.06	0/80 (0%)
		0.8	0.23	58 ^e	<i>104</i>	0.23	5/80 (6%)
		2	0.57	61 ^e	<i>104</i>	0.57	45/80 (56%)
Schmähl et al. (1977) ^c	NMRI	0 (acetone)	0	–	<i>104</i>	0	1/81 (1%)
		1	0.29	NR	<i>104</i>	0.29	11/77 (14%)
		1.7	0.49	NR	<i>104</i>	0.49	25/88 (28%)
		3	0.86	NR	<i>104</i>	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%)

5
6 ^aDoses were applied 3 times/week for up to 93 weeks to shaved dorsal skin.

7 ^bNumerator: number of mice detected with a skin tumor. Tumors were thought to be malignant based on
8 invasion or penetration of the panniculus carnosus muscle. Denominator: number of mice surviving to 29 weeks
9 (200 days).

10 ^cDoses were applied 2 times/week to shaved skin of the back. Mice were exposed until natural death or until they
11 developed a carcinoma at the site of application.

12 ^dExposure periods not reported were assumed to be 104 weeks; indicated in italics.

13 ^eCentral tendency estimates; range or other variability measure not reported.

14 ^fThe percentages were reported by the authors as age-standardized incidences of animals with local tumors,
15 derived using mortality data from the entire study population. The incidences reflect reported counts of tumor-
16 bearing animals and denominators estimated from the reported age-standardized rates. The authors did not
17 report the percentages of local tumors which were carcinomas or papillomas.

18
19 NR = not reported.

1 **Table E-21. Skin tumor incidence, benign or malignant, in C57L male mice**
 2 **dermally exposed to benzo[a]pyrene; data from [Poel \(1959\)](#)**

Study	Mouse strain	Dose (μg) ^a	Average daily dose ($\mu\text{g}/\text{d}$)	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%)

3
 4 ^aDoses were applied to interscapular skin 3 times/week for up to 103 weeks or until time of appearance of a
 5 grossly detected skin tumor. See Table E-15 for data of five highest dose groups (19–752 μg) in which all mice
 6 died by week 44. These groups were not considered for dose-response modeling.

7 ^bSee text in this section for discussion of extrapolation to lifetime average daily doses.

8 ^cTumors were histologically confirmed as epidermoid carcinomas.

9 **Table E-22. Skin tumor incidence, benign or malignant, in female CFLP mice**
 10 **dermally exposed to benzo[a]pyrene; data from [Grimmer et al. \(1983\)](#),**
 11 **[Grimmer et al. \(1984\)](#)**

Study	Dose (μg) ^a	Average daily dose ($\mu\text{g}/\text{d}$)	Mean or median time of tumor appearance (wks)	Length of exposure (wks) ^b	Lifetime average daily dose ($\mu\text{g}/\text{d}$)	Skin tumor incidence (all types) ^c
Grimmer et al. (1983)	0 (1:3 acetone:DMSO)	0	–	<i>104</i>	0	0/80 (0%)
	3.9	1.1	74.6 \pm 16.8 ^d	<i>104</i>	1.1	22/65 (34%)
	7.7	2.2	60.9 \pm 13.9	<i>104</i>	2.2	39/64 (61%)
	15.4	4.4	44.1 \pm 7.7	<i>104</i>	4.4	56/64 (88%)
Grimmer et al. (1984)	0 (1:3 acetone:DMSO)	0	–	<i>104</i>	0	0/80 (0%)
	3.4	0.97	61 (53–65) ^e	<i>104</i>	0.97	43/64 (67%)
	6.7	1.9	47 (43–50)	<i>104</i>	1.9	53/65 (82%)
	13.5	3.9	35 (32–36)	<i>104</i>	3.9	57/65 (88%)

12 ^aIndicated doses were applied twice/week to shaved skin of the back for up to 104 weeks.

13 ^bAssumed exposure period is indicated in italics.

14 ^cIncidence denominators were calculated from reported tumor-bearing animals and reported percentages.

15 ^dMean \pm SD.

16 ^eMedian and 95% confidence limit.

18 ***Dose-Response Modeling Results***

19 The modeling results are summarized in Tables E-23 (time-to-tumor modeling of individual
 20 data) and E-24 (multistage modeling of group incidence data). The modeling details are provided
 21 with Figures E-22 through E-33.

22 Adequate model fits for the supporting studies were found using the multistage model for
 23 all but one of the mouse skin tumor incidence data sets (Table E-24). The data from [Grimmer et al.](#)

1 [\(1984\)](#) could not be adequately fit by the multistage model initially, and the other dichotomous
 2 models available in BMDS were used. Due to the supralinear shape of the dose-response data, only
 3 the log-logistic and dichotomous Hill models provided adequate fits. Also due to the supralinear
 4 dose-response shape, the POD for slope factor derivation was identified near the lowest response of
 5 ~70%, because of the lack of data to inform the dose-response relationship at lower doses. Overall,
 6 model fits demonstrated typical statistical variability at the PODs, with BMDLs generally less than
 7 twofold lower than corresponding BMDs.

8 A comparison of model types was feasible for the NIOSH study data ([Arthur D Little, 1989](#)),
 9 which included the keratoacanthoma in the mid-dose group. The multistage model fit of the
 10 grouped data reported by ([Sivak et al., 1997](#)) yielded BMD₁₀ and BMDL₁₀ values of 0.109 and
 11 0.058 µg/day, respectively, while the multistage-Weibull fit of the corresponding individual data
 12 yielded BMD₁₀ and BMDL₁₀ values of 0.0890 and 0.0514 µg/day, respectively. Use of the
 13 multistage-Weibull model was associated with a 10–20% lower POD for these data.

14 **Table E-23. Summary of BMD modeling results for best-fitting multistage-**
 15 **Weibull models, using time-to-tumor data for male CeH/HeJ mice exposed**
 16 **dermally to benzo[a]pyrene ([Sivak et al., 1997](#); [Arthur D Little, 1989](#))**

Endpoints	Model stages	AIC	BMD ₁₀ (µg/d)	BMDL ₁₀ –BMDU ₁₀ (µg/d)	Basis for model selection
Skin papilloma or carcinoma	1	51.9	0.0410	0.0600–0.1466	Lowest AIC, best fit to low dose data
	2	42.9	0.0985		
	3	44.3	0.1047		
Skin papilloma, keratoacanthoma or carcinoma	1	53.7	0.0391	0.0514–0.1303	Lowest AIC, best fit to low dose data
	2	46.2	0.0890		
	3	47.9	0.0919		

17

1 **Male Mice (Sivak et al., 1997; Arthur D Little, 1989): Skin Papilloma or Carcinoma**

2 =====
 3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
 4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
 5 Input Data File: SivakCarPapM2.(d)
 6 =====

7 The form of the probability function is:
 8 $P[\text{response}] = 1 - \text{EXP}\{-(t - t_0)^c * (\text{beta}_0 + \text{beta}_1 * \text{dose}^1 + \text{beta}_2 * \text{dose}^2)\}$
 9

10 The parameter betas are restricted to be positive

11 Dependent variable = CONTEXT
 12 Independent variables = DOSE, TIME

13 Total number of observations = 120
 14 Total number of records with missing values = 0
 15 Total number of parameters in model = 5
 16 Total number of specified parameters = 1
 17 Degree of polynomial = 2

18 User specifies the following parameters:
 19 $t_0 = 0$

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

23 Default Initial Parameter Values
 24 c = 2.57143
 25 $t_0 = 0$ Specified
 26 $\text{beta}_0 = 0$
 27 $\text{beta}_1 = 5.45626e-028$
 28 $\text{beta}_2 = 3.97261e-007$

29 Asymptotic Correlation Matrix of Parameter Estimates
 30 (*** The model parameter(s) $-t_0$ $-\text{beta}_0$ $-\text{beta}_1$
 31 have been estimated at a boundary point, or have been specified by the user,
 32 and do not appear in the correlation matrix)

33 c beta_2
 34 c 1 -1
 35 beta_2 -1 1

36 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	2.75512	0.94014	0.912478	4.59776
beta_0	0	NA		
beta_1	0	NA		
beta_2	1.31225e-007	7.47141e-007	-1.33315e-006	1.5956e-006

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

40 Fitted Model Log(likelihood) # Param AIC
 41 -17.4278 4 42.8557

42 Data Summary

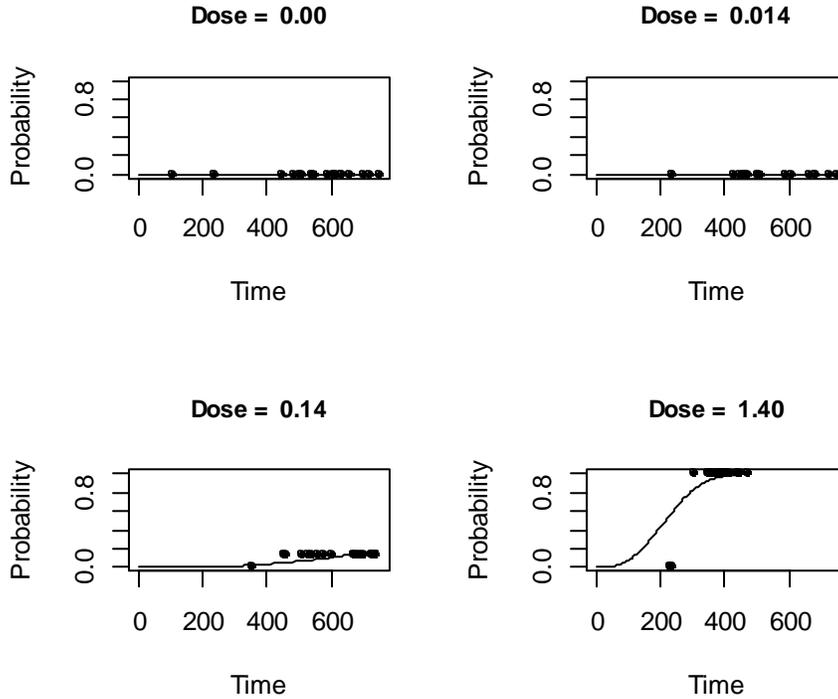
DOSE	CONTEXT				U	Total	Expected Response
	C	F	I				
0	30	0	0	0	30	0.00	
0.014	30	0	0	0	30	0.05	
0.14	26	0	4	0	30	4.38	
1.4	3	0	27	0	30	27.72	

43 Benchmark Dose Computation
 44 Risk Response = Incidental
 45 Risk Type = Extra
 46 Confidence level = 0.9
 47 Time = 748

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Specified effect = 0.1	0.01	0.001
BMD = 0.0984828	0.0304167	0.00959688
BMDL = 0.0599711	0.00728535	0.000745583
BMDU = 0.14663	0.0434989	0.0137323

Incidental Risk: SivakCarPapM2



8
9

10 **Figure E-22. Fit of multistage Weibull model to skin carcinomas or papilloma**
 11 **for male CeH/HeJ mice exposed dermally to benzo[a]pyrene ([Sivak et al.](#)**
 12 **[1997](#)); BMR = 10% extra risk.**

13

1 **Male Mice ([Sivak et al., 1997](#)): Skin papilloma, Keratoacanthoma, or Carcinoma**

```

2 =====
3 Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
4 Solutions are obtained using donlp2-intv, (c) by P. Spellucci
5 Input Data File: SivakCarPapKerM2.(d)
6 =====

```

```

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

```

10 The parameter betas are restricted to be positive

```

11 Dependent variable = CONTEXT
12 Independent variables = DOSE, TIME

```

```

13 Total number of observations = 120
14 Total number of records with missing values = 0
15 Total number of parameters in model = 5
16 Total number of specified parameters = 1
17 Degree of polynomial = 2

```

```

18 User specifies the following parameters:
19 t_0 = 0

```

```

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

```

```

23 Default Initial Parameter Values
24 c = 3
25 t_0 = 0 Specified
26 beta_0 = 4.46003e-033
27 beta_1 = 0
28 beta_2 = 3.21731e-008

```

```

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

```

```

33 c          c          beta_2
34 beta_2     1          -1
35          -1          1

```

```

36 Parameter Estimates
37 Variable          Estimate          Std. Err.          95.0% Wald Confidence Interval
38 c                  2.97877          0.91338          Lower Conf. Limit  Upper Conf. Limit
39 beta_0              0              NA              1.18857          4.76896
40 beta_1              0              NA
41 beta_2              3.6606e-008      2.0366e-007      -3.62561e-007    4.35773e-007

```

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

```

55 Log(likelihood) # Param          AIC
56 Fitted Model    -19.076          4          46.1521

```

```

57 Data Summary
58 CONTEXT
59 C      F      I      U      Total  Expected Response
60 DOSE
61 0      30     0      0      30     0.00
62 0.014  30     0      0      30     0.05
63 0.14   25     0      5      30     5.12
64 1.4    3      0      27     30     27.79

```

```

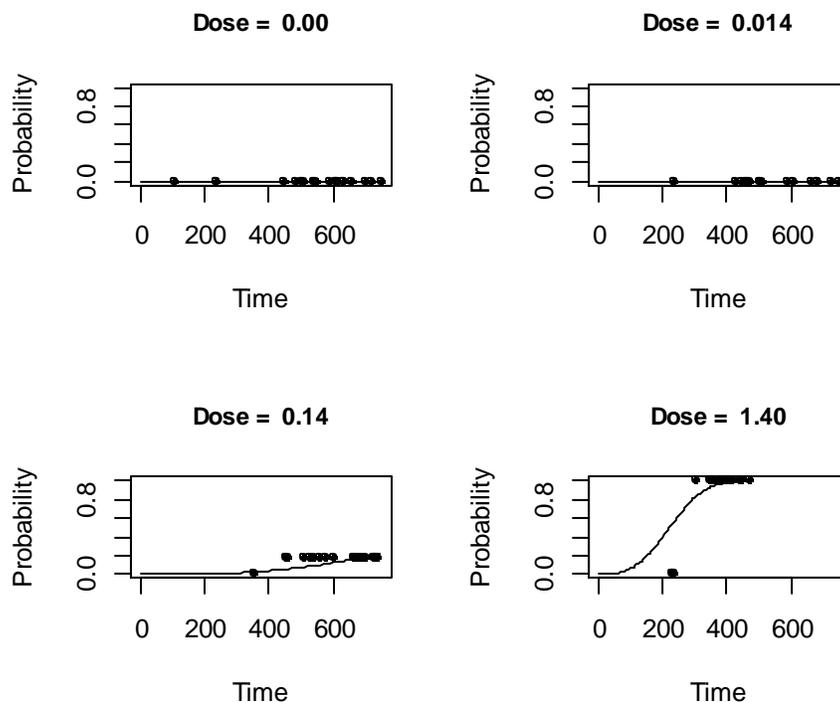
65 Benchmark Dose Computation
66 Risk Response = Incidental
67 Risk Type = Extra
68 Confidence level = 0.9
69 Time = 748

```

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Specified effect = 0.1	0.01	0.001
BMD = 0.0889653	0.0274772	0.00866942
BMDL = 0.0514113	0.00585974	0.000598209
BMDU = 0.130325	0.0401556	0.012538

Incidental Risk: SivakCarPapKerM2



6
7

8 **Figure E-23. Fit of multistage Weibull model to skin carcinomas,**
 9 **keratoacanthoma or papilloma for male CeH/HeJ mice exposed dermally to**
 10 **benzo[a]pyrene ([Sivak et al., 1997](#)); BMR = 10% extra risk.**

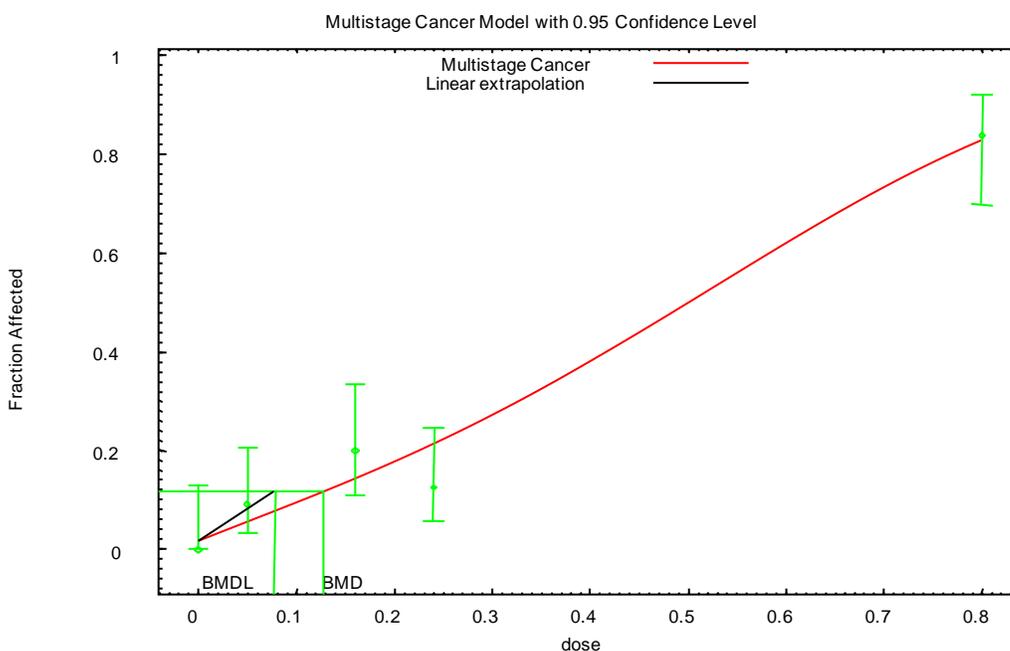
11

1
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3**Table E-24. Summary of BMD model selection and modeling results using multistage models, for multiple data sets of skin tumors in mice following lifetime 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	No significant improvement in model fit with higher stage	E-24
	Multistage 2°	0.027	188.6	0.134	0.078		
	Multistage 3°	0.053	186.9	0.127	0.078		
	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	No significant improvement in model fit with higher stages	E-25
	Multistage 2°	0.485	123.6	0.748	0.480		
	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	No significant improvement in model fit with higher stages	E-26
	Multistage 2°	0.609	147.4	0.329	0.287		
	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	No significant improvement in model fit with higher stage	E-27
	Multistage 2°	0.514	153.3	0.216	0.192		
	Multistage 3°	0.983	151.3	0.282	0.223		
	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	No significant improvement in model fit with higher stage	E-28
	Multistage 2°	0.939	296.3	0.233	0.149		
	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	Only model with adequate fit	E-29
	Multistage 2°	0.009	84.4	0.198	0.143		
	Multistage 3°	0.207	76.7	0.294	0.215		
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-30
	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-31
	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) Lowest AIC among adequately fitting models. (Same as Multistage 1°)	E-32 E-33
	LogLogistic	0.919	195.8	1.07	0.479		
	Dichotomous-Hill	1.000	197.7	0.902	0.533		
	LogProbit	0.047	200.2	1.33	1.11		
	Gamma, Weibull	0.003	205.3	0.132	0.113		
	Logistic	0.0	250.5	2.03	1.76		
	Probit	0.0	255.4	2.29	2.03		
Multistage 1°, high dose dropped	0.499	—	1.21	1.01	E-34		

Data set	Model	Goodness-of-fit		BMD ₁₀ (µg/d)	BMDL ₁₀ (µg/d)	Basis for model selection ^a	Figure number
		p-value	AIC				
Sivak et al. (1997)	Multistage 1°	0.059	57.8	0.036	0.026	No significant improvement in model fit with higher stage	E-35
Male CeH/HeJ	Multistage 2°	0.998	48.6	0.109	0.058		
	Multistage 3°	0.998	48.6	0.109	0.052		

1
2 ^aAdequate fit: goodness-of-fit $p > 0.05$, scaled residuals < 2.0 , good fit near BMR, lack of extreme curvature not
3 reflected in the observed data.
4 ^bThe POD for [Grimmer et al. \(1984\)](#), using a BMR of 70% (near response at the lowest dose), was based on the
5 LogLogistic model. For comparison purposes, the multistage model was fit to the [Grimmer et al. \(1984\)](#) data
6 with the highest dose dropped (AIC not provided because it is not comparable to fits of the full dataset).



7
8 **Figure E-24. Fit of multistage model to skin tumors in C57L mice exposed**
9 **dermally to benzo[a]pyrene ([Poel, 1959](#)).**

```

10 =====
11 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
12 Input Data File: C:\Usepa\BMDs21\Data\msc_benzo[a]pyrene_Poel_1959_MultiCanc3_0.1.(d)
13 Gnuplot Plotting File:
14 C:\Usepa\BMDs21\Data\msc_benzo[a]pyrene_Poel_1959_MultiCanc3_0.1.plt
15 =====
16
17 The form of the probability function is:
18
19 P[response] = background + (1-background)*[1-EXP(
20 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]
21
22 The parameter betas are restricted to be positive
23
24 Dependent variable = NumAff
25 Independent variable = LADD
26
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28

```

1
2 Total number of observations = 5
3 Total number of records with missing values = 0
4 Total number of parameters in model = 4
5 Total number of specified parameters = 0
6 Degree of polynomial = 3

7
8
9 Maximum number of iterations = 250
10 Relative Function Convergence has been set to: 1e-008
11 Parameter Convergence has been set to: 1e-008
12

13
14
15 Default Initial Parameter Values
16 Background = 0.0449589
17 Beta(1) = 0.490451
18 Beta(2) = 0
19 Beta(3) = 2.68146
20

21
22 Asymptotic Correlation Matrix of Parameter Estimates

23
24 (*** The model parameter(s) -Beta(2)
25 have been estimated at a boundary point, or have been specified by the user,
26 and do not appear in the correlation matrix)
27

	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

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38 Parameter Estimates

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

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47 * - Indicates that this value is not calculated.
48
49

50
51 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				

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62 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

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71 Chi^2 = 5.88 d.f. = 2 P-value = 0.0528
72
73

74 Benchmark Dose Computation
75

Supplemental Information—Benzo[a]pyrene

1 Specified effect = 0.1
2
3 Risk Type = Extra risk
4
5 Confidence level = 0.95
6
7 BMD = 0.126567
8
9 BMDL = 0.0777875
10
11 BMDU = 0.272961
12
13 Taken together, (0.0777875, 0.272961) is a 90 % two-sided confidence
14 interval for the BMD
15
16 Multistage Cancer Slope Factor = 1.28555
17

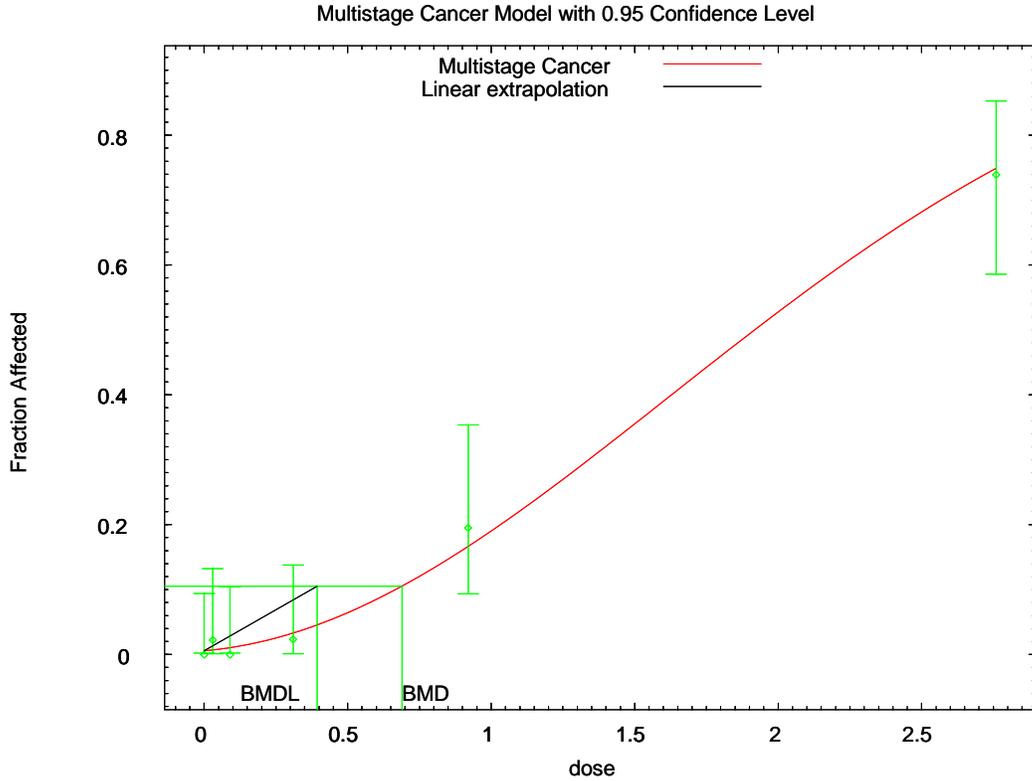


Figure E-25. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Roe et al., 1970).

```

=====
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
=====
BMSD 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
    
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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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

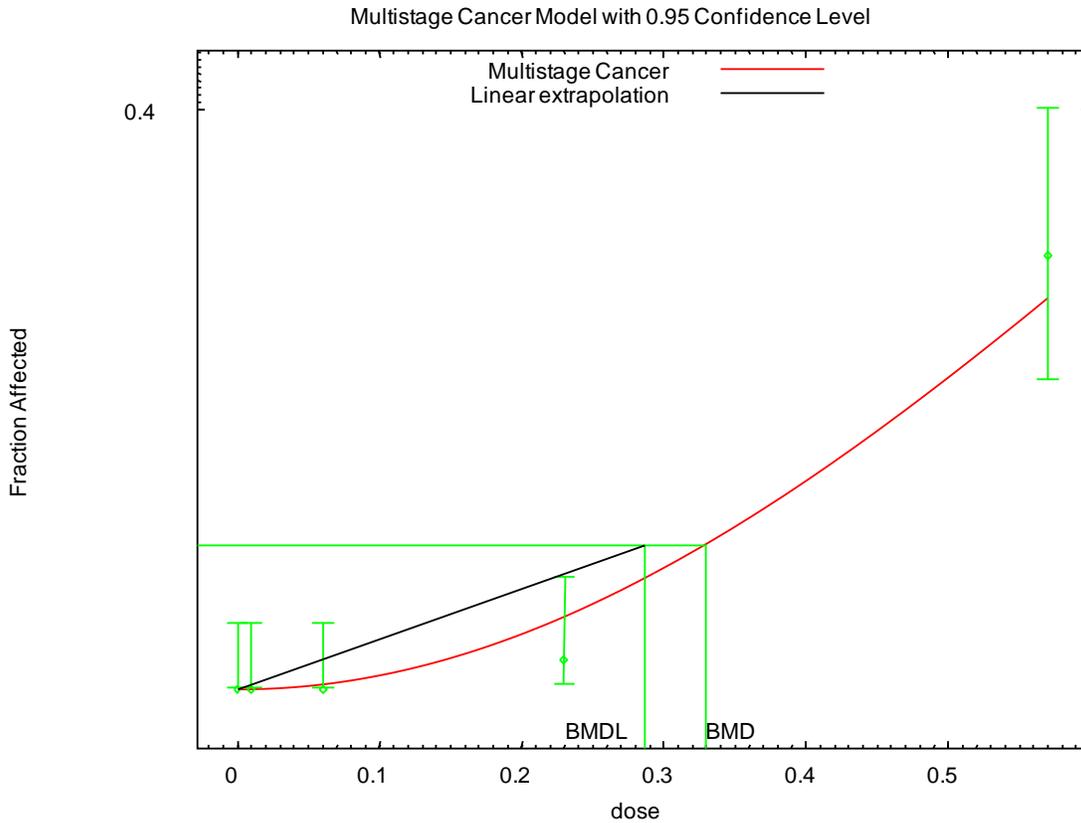
Risk Type = Extra risk

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



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Figure E-26. 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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

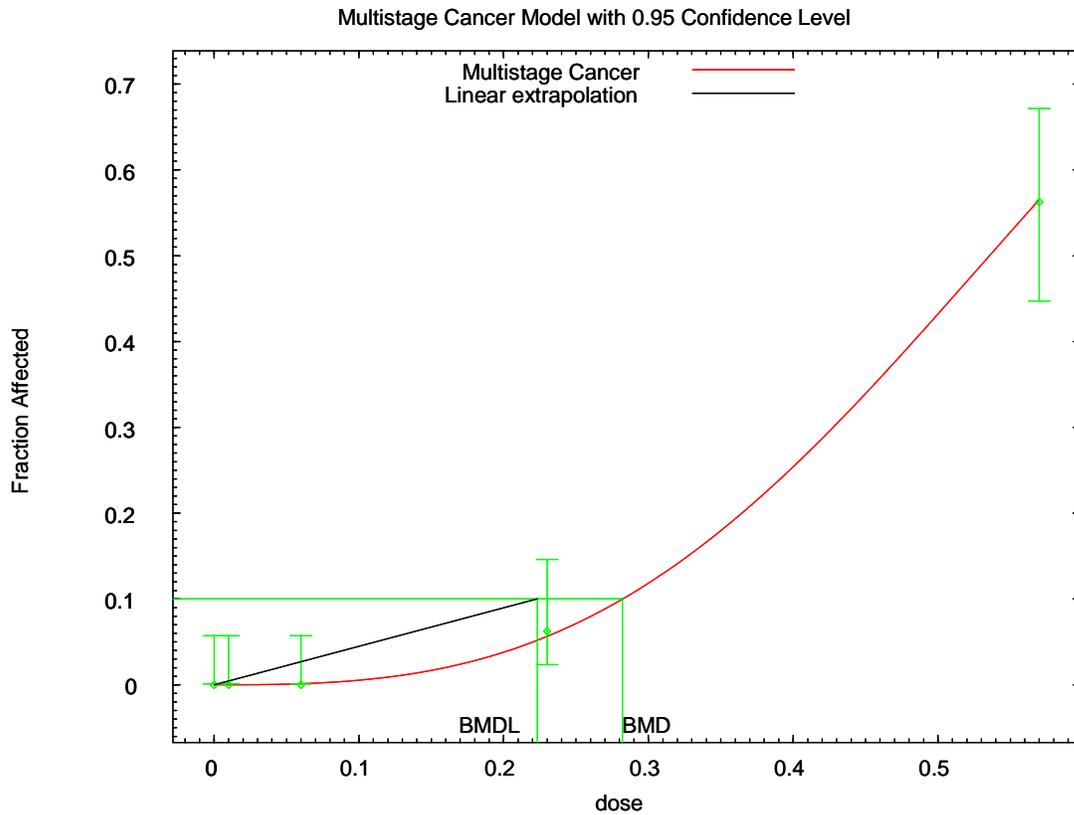


Figure E-27. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Schmidt et al., 1973).

```

=====
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
=====
BMD5 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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

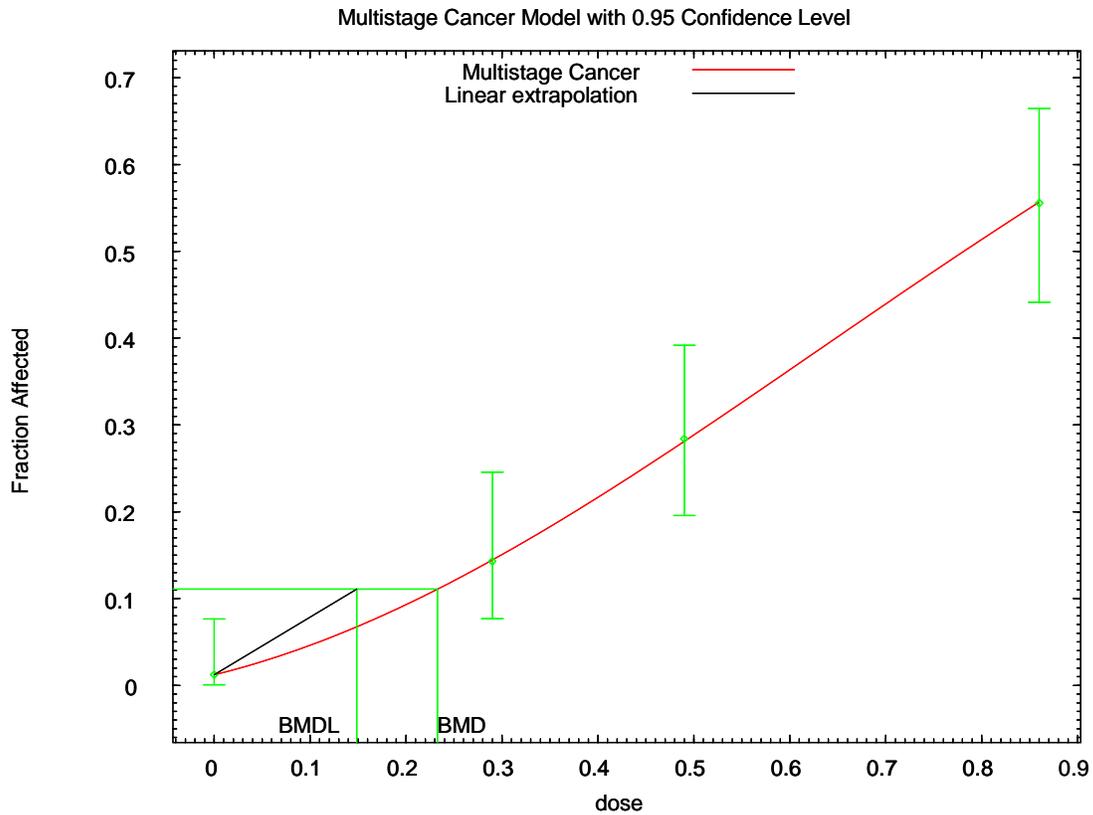
Chi^2 = 0.16 d.f. = 3 P-value = 0.9833

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



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Figure E-28. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Schmähl et al. 1977).

```

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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 ****

```

Supplemental Information—Benzo[a]pyrene

**** 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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

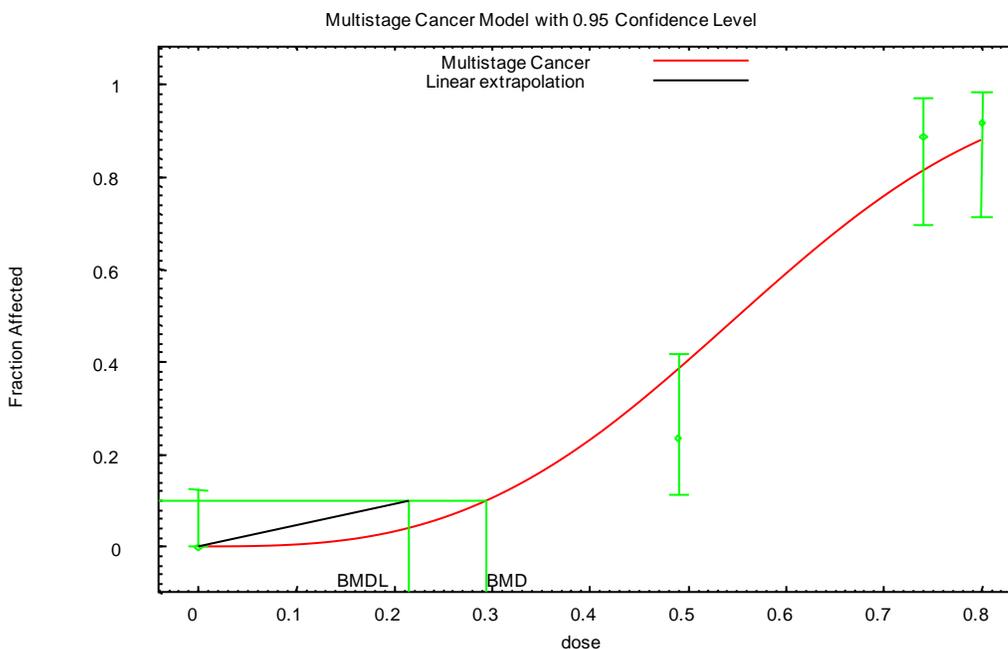
Chi^2 = 0.01 d.f. = 1 P-value = 0.9393

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



1
2 **Figure E-29. Fit of multistage model to skin tumors in female NMRI mice**
3 **exposed dermally to benzo[a]pyrene (Habs et al., 1980).**

4 =====
5 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
6 Input Data File: M:_BMDS\msc_BAP_HABS1980_MultiCanc3_0.1.(d)
7 Gnuplot Plotting File: M:_BMDS\msc_BAP_HABS1980_MultiCanc3_0.1.plt
8 =====

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10
11 BMDS Model Run
12 ~~~~~

13
14 The form of the probability function is:

15
16
$$P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{beta1} * \text{dose}^1 - \text{beta2} * \text{dose}^2 - \text{beta3} * \text{dose}^3)]$$

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19 The parameter betas are restricted to be positive

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22 Dependent variable = NumAff
23 Independent variable = LADD

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25 Total number of observations = 4
26 Total number of records with missing values = 0
27 Total number of parameters in model = 4
28 Total number of specified parameters = 0
29 Degree of polynomial = 3

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

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38 Default Initial Parameter Values
39 Background = 0
40 Beta(1) = 0
41 Beta(2) = 4.23649
42 Beta(3) = 0
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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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

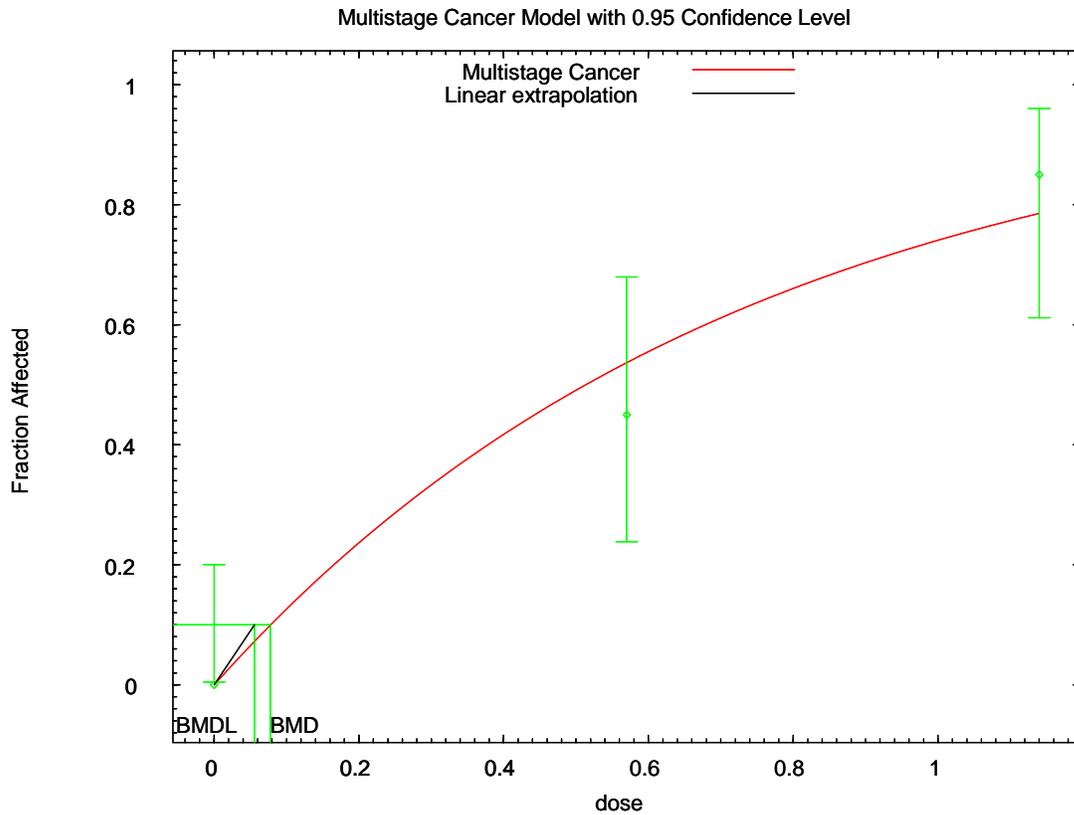
Chi^2 = 4.56 d.f. = 3 P-value = 0.2067

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



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Figure E-30. 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
=====

BMSD Model Run
~~~~~

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
               -beta*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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

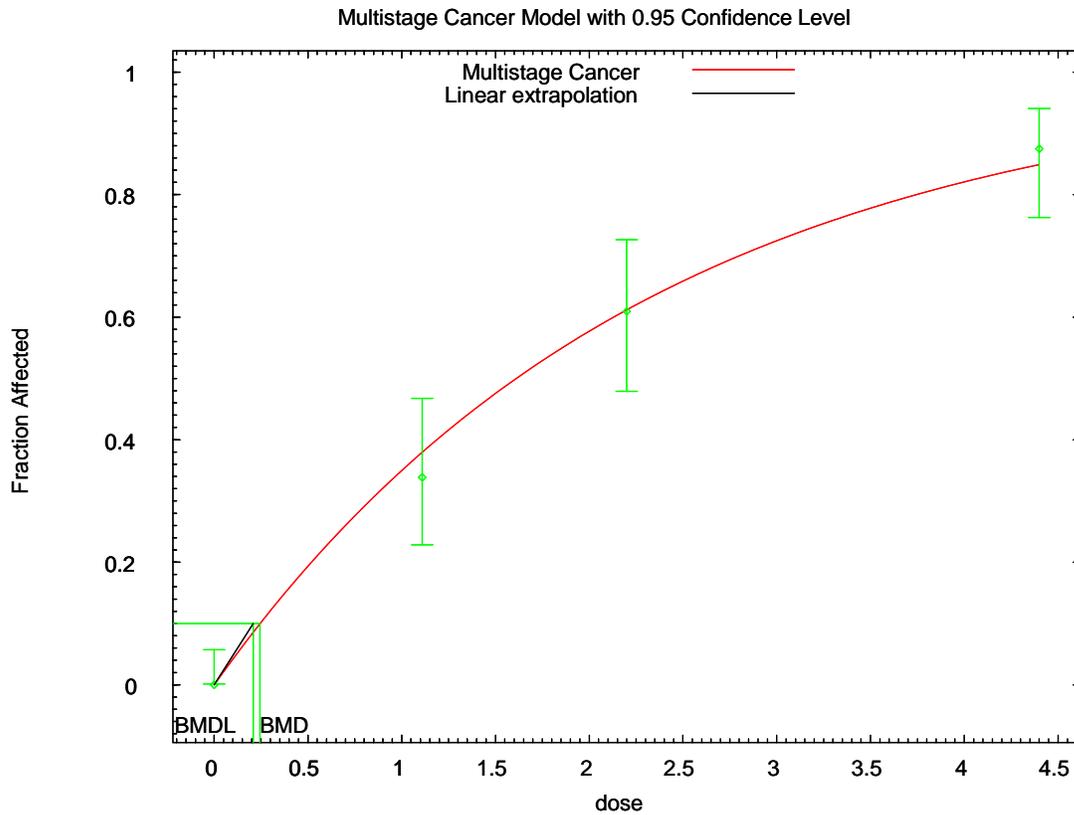


Figure E-31. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1983).

```

=====
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
=====

BMSD 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 ****

```

Supplemental Information—Benzo[a]pyrene

**** 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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

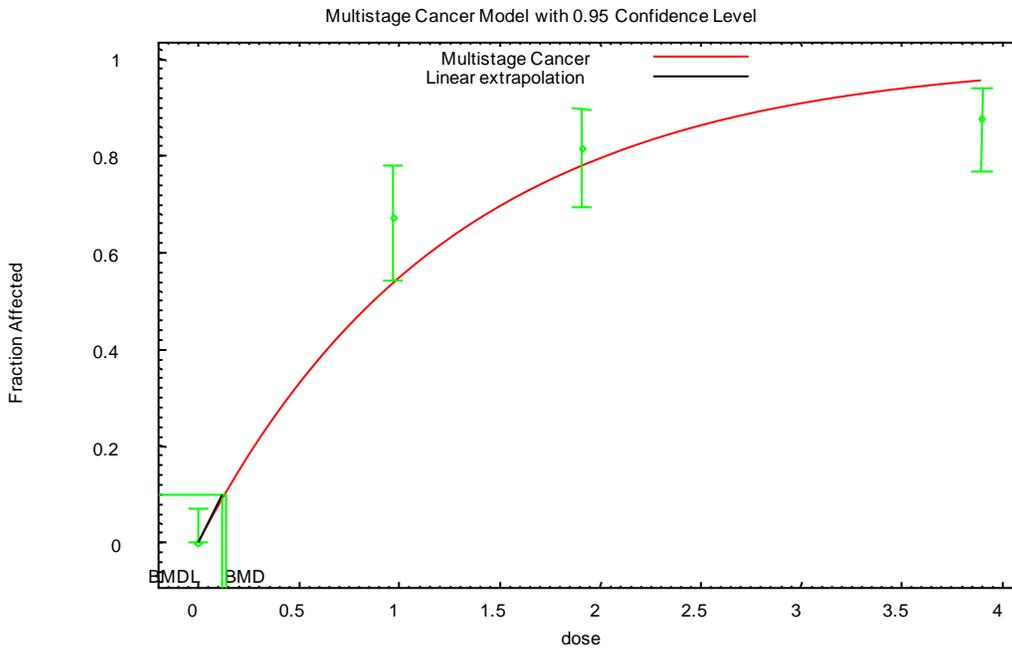


Figure E-32. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984).

```

=====
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:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est. Prob.	Expected	Observed	Size	Scaled 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

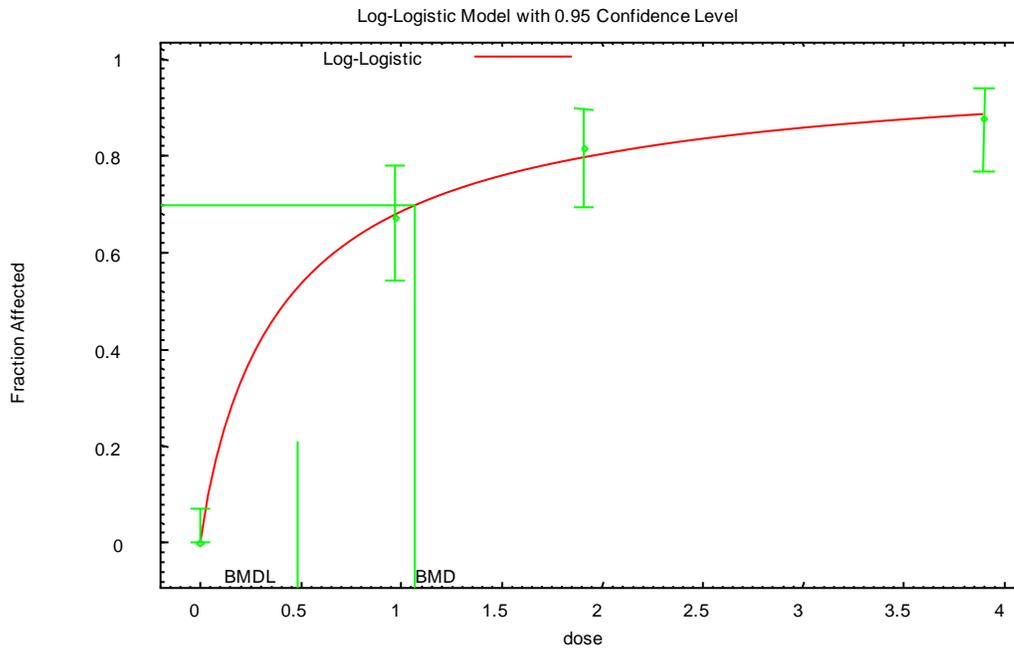
BMD = 0.132272

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



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Figure E-33. 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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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				

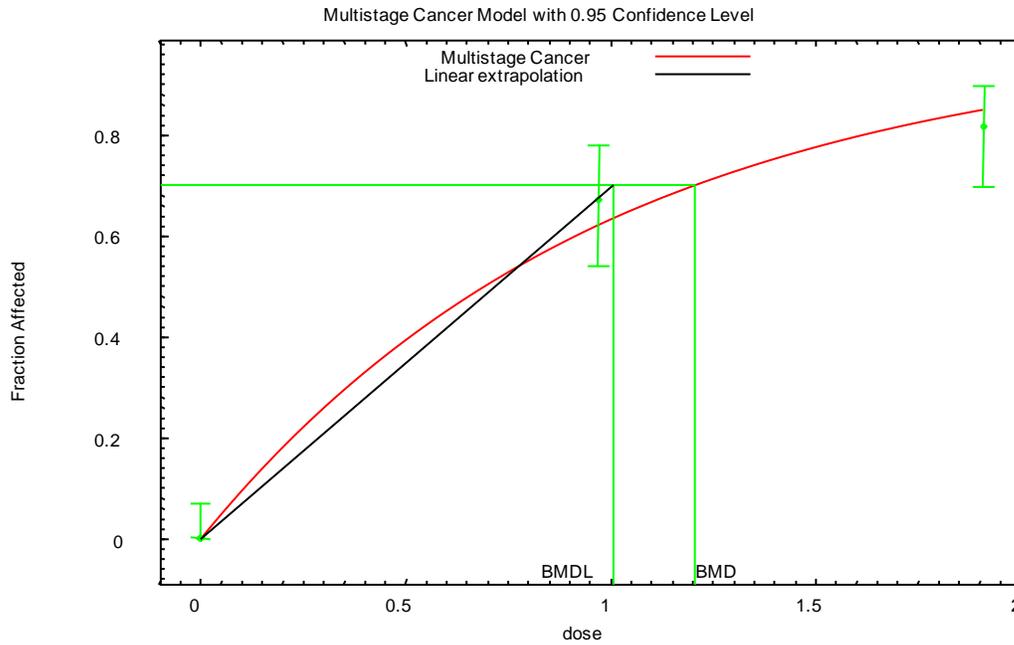
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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



1
2 **Figure E-34. Fit of multistage model to skin tumors in female CFLP mice**
3 **exposed dermally to benzo[a]pyrene ([Grimmer et al., 1984](#)), highest dose**
4 **dropped.**

```
5 =====
6 Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)
7 Input Data File: C:/Usepa/_BaP/msc_BaP_Grimmer1984_drophidose_MultiCanc1_0.7.(d)
8 Gnuplot Plotting File: C:/Usepa/_BaP/msc_BaP_Grimmer1984_drophidose_MultiCanc1_0.7.plt
9 =====
```

```
10 [add_notes_here]
11 ~~~~~
```

12
13 The form of the probability function is:

$$14 P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

15
16 The parameter betas are restricted to be positive

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18
19 Dependent variable = NumAff
20 Independent variable = LADD

21
22 Total number of observations = 3
23 Total number of records with missing values = 0
24 Total number of parameters in model = 2
25 Total number of specified parameters = 0
26 Degree of polynomial = 1

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28
29 Maximum number of iterations = 250
30 Relative Function Convergence has been set to: 1e-008
31 Parameter Convergence has been set to: 1e-008

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33  
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36  
37  
38 Default Initial Parameter Values
39 Background = 0.0806622
40 Beta(1) = 0.88595
```

```
41  
42  
43 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

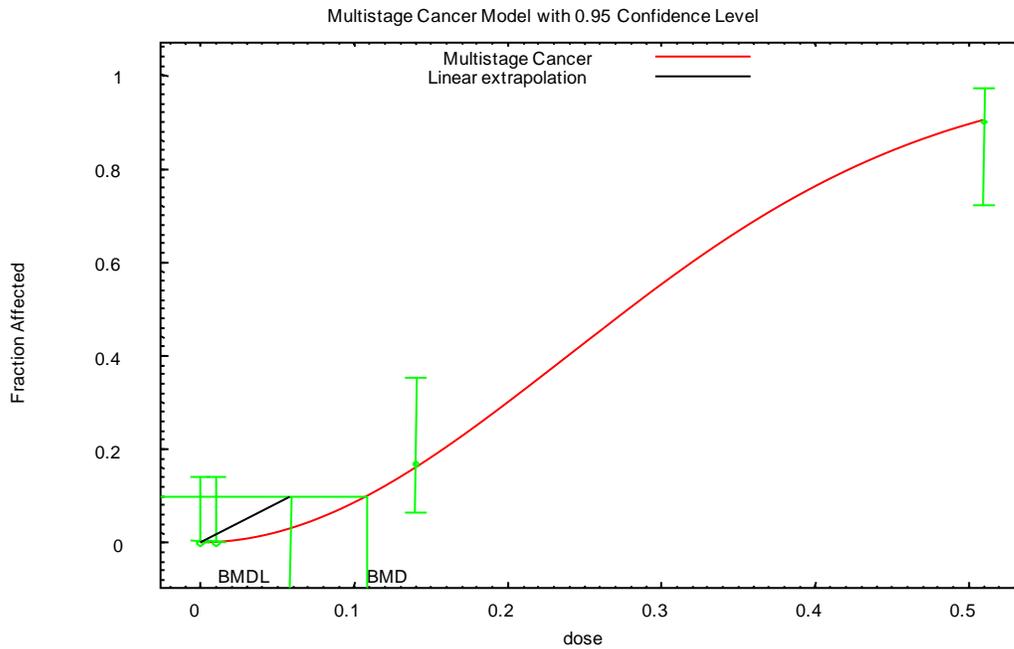


Figure E-35. Fit of multistage model to skin tumors in male CeH/HeJ mice exposed dermally to benzo[a]pyrene (Sivak et al., 1997).

```

=====
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:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2)]$$

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

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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

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

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

13 Because there is no established methodology for cross-species extrapolation of dermal
 14 toxicity, several alternative approaches were evaluated. Each approach begins with the POD of
 15 0.066 µg/day that was based on a 10% extra risk for skin tumors in male mice. Based on the
 16 assumptions of each approach, a dermal slope factor for humans is calculated. The discussion of
 17 these approaches uses the following abbreviations:

18

19 DSF = dermal slope factor

20 POD_M = point of departure (for 10% extra risk) from mouse bioassay, in µg/day21 BW_M = mouse body weight = 0.035 kg (assumed)22 BW_H = human body weight = 70 kg (assumed)23 SA_H = total human surface area = 19,000 cm² (assumed)24 SA_M = total mouse surface area = 100 cm² (assumed)

25 **Approach 1. No Interspecies Adjustment to Daily Applied Dose (POD) in Mouse Model**

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

29

30
$$DSF = 0.1 / POD_M$$

31
$$DSF = 0.1 / 0.060 \text{ µg/day} = \mathbf{1.7 \text{ (µg/day)}^{-1}}$$

32

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

35 **Approach 2. Cross-Species Adjustment Based on Whole-Body Surface-Area Scaling**

36 Under this approach, animals and humans are assumed to have equal lifetime cancer risk
 37 with equal average whole-body exposures in loading units (µg/cm²-day). As long as doses are low

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

3

$$4 \quad \text{POD } (\mu\text{g}/\text{cm}^2\text{-day}) = \text{POD}_{\text{M/SA}} (\mu\text{g}/\text{cm}^2\text{-day}) = \text{POD}_{\text{M}} (\mu\text{g}/\text{day}) / \text{SA}_{\text{M}} (\text{cm}^2)$$

$$5 \quad \text{POD} = (0.060 \mu\text{g}/\text{day}) / 100 \text{ cm}^2 = 0.00060 \mu\text{g}/\text{cm}^2\text{-day}$$

$$6 \quad \text{DSF} = 0.1/(0.00060 \mu\text{g}/\text{cm}^2\text{-day}) \approx \mathbf{170 (\mu\text{g}/\text{cm}^2\text{-day})}^{-1}$$

7

8 *Assumptions:* Mouse and human slope factors are equipotent if total dermal dose is
 9 averaged over equal fractions of the entire surface area. Tumor potency of benzo[a]pyrene is
 10 assumed to be related to overall dose and not dose per unit area. For example, a human exposed to
 11 0.01 $\mu\text{g}/\text{day}$ on 10 cm^2 would be assumed to have the same potential to form a skin tumor as
 12 someone treated with 0.01 $\mu\text{g}/\text{day}$ over 19,000 cm^2 (assumed human surface area).

13 **Approach 3. Cross-Species Adjustment Based on Body Weight**

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

16

$$17 \quad \text{POD}_{\text{M}} / \text{BW}_{\text{M}} = 0.060 \mu\text{g}/0.035 \text{ kg-day} = 1.7 \mu\text{g}/\text{kg-day}$$

$$18 \quad \text{DSF} = 0.1/1.7 \mu\text{g}/\text{kg-day} = \mathbf{0.058 (\mu\text{g}/\text{kg-day})}^{-1}$$

19

20 *Assumptions:* The potency of point of contact skin tumors is related to body weight, and
 21 humans and mice would have an equal likelihood of developing skin tumors based on a dermal dose
 22 per kg basis.

23

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

26 **Approach 4. Cross-Species Adjustment Based on Allometric Scaling Using Body Weight to the** 27 **3/4 Power**

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

34

$$35 \quad \text{POD } (\mu\text{g}/\text{day}) = \text{POD}_{\text{M}} (\mu\text{g}/\text{day}) \times (\text{BW}_{\text{H}} / \text{BW}_{\text{M}})^{3/4}$$

$$36 \quad \text{POD } (\mu\text{g}/\text{day}) = 0.060 \mu\text{g}/\text{day} \times (70 \text{ kg} / 0.035 \text{ kg})^{3/4} = 17.9 \mu\text{g}/\text{day}$$

$$37 \quad \text{DSF} = 0.1/(17.9 \mu\text{g}/\text{day}) \approx \mathbf{0.0056 (\mu\text{g}/\text{day})}^{-1}$$

38

39 *Assumptions:* Risk at low doses of benzo[a]pyrene is dependent on absolute dermal dose
 40 and not dose per unit of skin, meaning that a higher exposure concentration of benzo[a]pyrene

1 contacting a smaller area of exposed skin could carry the same risk of skin tumors as a lower
2 exposure concentration of benzo[a]pyrene that contacts a larger area of skin.

3

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

6 ***Synthesis of the Alternative Approaches to Cross-Species Scaling***

7 A comparison of the above approaches is provided in Table E-25. The lifetime risk from a
8 nominal human dermal exposure to benzo[a]pyrene over a 5% area of exposed skin (approximately
9 950 cm²), estimated at 1×10^{-4} µg/day, is calculated for each of the approaches in order to judge
10 whether the method yields risk estimates that are unrealistically high.

11 ***Other Potential Interspecies Adjustments***

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

- 17 a. Quantitative information on interspecies differences in partitioning from exposure medium
18 to the skin and absorption through the skin
- 19 b. Thickness of the stratum corneum between anatomical sites and between species
- 20 c. Thickness of epidermal layer
- 21 d. Skin permeability
- 22 e. Metabolic activity of skin
- 23 f. Formation of DNA adducts in skin

Table E-25. 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 (no adjustment)	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	1.7 per µg/d	7 × 10 ⁻⁴
2. Surface-area scaling	Equal mass per day (µg/d), if applied to <u>equal fractions</u> 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	170 per µg/cm ² -d	4 × 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/d) and body weight (kg) that have the same result when divided will result in the same risk.	µg/kg-day	0.058 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 <u>metabolism</u> 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	0.0056 per µg/d	2 × 10 ⁻⁶

^aCalculated 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 law³. The report language included direction to the U.S. Environmental Protection Agency (EPA) for the Integrated Risk Information System (IRIS) Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde⁴. 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

³Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.

⁴([ENREF 351](#)). Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

1 focused on assessments near the end of the development process and close to final posting. The
 2 IRIS benzo[a]pyrene assessment is in Phase 2 and represents a significant advancement in
 3 implementing the NRC recommendations shown in Table F-1 below. The Program is implementing
 4 all of these recommendations, but recognizes that achieving full and robust implementation of
 5 certain recommendations will be an evolving process with input and feedback from the public,
 6 stakeholders, and external peer review committees. Phase 3 of implementation will incorporate
 7 the longer-term recommendations made by the NRC as outlined below in Table F-2, including the
 8 development of a standardized approach to describe the strength of evidence for noncancer effects.
 9 In May 2014, the NRC released their report reviewing the IRIS assessment development process.
 10 As part of this review, the NRC reviewed current methods for evidence-based reviews and made
 11 several recommendations with respect to integrating scientific evidence for chemical hazard and
 12 dose-response assessments. In their report, the NRC states that EPA should continue to improve its
 13 evidence-integration process incrementally and enhance the transparency of its process. The
 14 committee did not offer a preference but suggests that EPA consider which approach best fits its
 15 plans for the IRIS process. The NRC recommendations will inform the IRIS Program’s efforts in this
 16 area going forward.

17 **Table F-1. The EPA’s implementation of the National Research Council’s**
 18 **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

National Research Council recommendations that EPA is implementing in the short term	Implementation status
	eliminate any redundancies or inconsistencies.
<p>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 reference concentrations (RfCs) and unit risk estimates.</p>	<p>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.</p> <p>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.</p>
<p>3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriate tables, long text descriptions of studies could be moved to an appendix.</p>	<p>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).</p>
<p>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.</p>	<p>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.</p>
<p>5. The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate</p>	<p>Implemented. The Dose-Response Analysis section of the new document structure provides a clear explanation of the rationale used to select and advance studies that</p>

National Research Council recommendations that EPA is implementing in the short term	Implementation status
<p>RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.</p>	<p>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 (PODs) 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 RfDs. Such values have been developed previously on a case-by-case basis and will be developed in future assessments, where the data allow.</p>
<p>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.</p>	<p>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 (Section 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.</p>

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 are 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 HERO database, to capture study information and relevant quantitative data.	
Evidence Evaluation: Hazard Identification and 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-	Implemented. Standardized tables have been developed 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

National Research Council recommendations that EPA is implementing in the short term	Implementation status
evidence, and utility as a basis for deriving reference values and unit risks.	addition, exposure-response arrays are utilized in the assessment to provide a graphical representation of PODs 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.
<p>Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165)</p> <p>17. Establish clear guidelines for study selection.</p> <p>a) Balance strengths and weaknesses.</p> <p>b) Weigh human vs. experimental evidence.</p> <p>c) Determine whether combining estimates among studies is warranted.</p>	<p>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.</p> <p>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.</p>
<p>Calculation of Reference Values and Unit Risks (pp. 165–166)</p> <p>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 PODs (such as benchmark dose [BMD], NOAEL, and LOAEL), and assessment of the analyses that underlie the PODs.</p>	Implemented. The rationale for the selection of the PODs for the organ/system-specific oral reference values is provided in Section 2.1. The rationale for the selection of the POD and the inhalation dosimetry modeling (for the approximation of a HEC) for the derivation of the inhalation reference value is transparently described in Section 2.2. The BMD 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 biologic model fit to the data) that are used to develop a unit risk estimate.	Implemented. The risk-estimation modeling processes used to develop cancer risk estimates for benzo[a]pyrene 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	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

National Research Council recommendations that EPA is implementing in the short term	Implementation status
<p>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.</p>	<p>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 appendixes. Detailed modeling analyses are presented in the appendixes.</p>

1 **Table F-2. National Research Council recommendations that the EPA is**
 2 **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
<p>Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (p. 165)</p> <ol style="list-style-type: none"> 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. 	<p>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 announced⁶ 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.</p>
<p>Calculation of Reference Values and Unit Risks (pp. 165–166)</p> <ol style="list-style-type: none"> 7. Assess the sensitivity of derived estimates to model assumptions and endpoints 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. 	<p>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 endpoints.</p>

⁶EPA Announces NAS’ Review of IRIS Assessment Development Process (www.epa.gov/iris).

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

EPA's Response to Selected Major Public Comments on the Public Comment Draft (August 2013) of the IRIS Toxicological Review of Benzo[a]pyrene

Purpose: The Integrated Risk Information System (IRIS) assessment development process of May 2009, includes release of the draft IRIS assessment for public review and comment and independent expert peer review (Step 4). During this step, EPA holds a public meeting to discuss the draft assessment and draft peer review charge. As part of enhancements to the IRIS assessment development process announced in July 2013, in some cases, the IRIS Program may decide to revise the draft assessment and peer review charge after hearing the public's comments about these materials. For a complete description of the IRIS process, visit the IRIS website at www.epa.gov/iris.

The following are EPA's responses to the scientific issues raised in the public comments received on the draft IRIS Toxicological Review of Benzo[a]pyrene (dated August 2013). The comments have been synthesized and paraphrased, and are organized to follow the order of the Toxicological Review. Editorial changes were incorporated in the document as appropriate and are not discussed further. All public comments provided were taken into consideration in revising the draft assessment prior to posting for external peer review. The complete set of public comments are available on the docket at <http://www.regulations.gov> (Docket ID No. EPA-HQ-ORD-2011-0391).

Background: The Toxicological Review of Benzo[a]pyrene was released for a 60-day public comment period on August 21, 2013. The comment period was subsequently extended to 90 days, ending on November 21, 2013. During this period, public comments on the assessment were submitted to EPA by the Utility Solid Waste Activities Group (USWAG), CDM Smith Inc, Pavement Coating Technology Council, Electric Power Research Institute (EPRI), Duke Energy, CH2M Hill, Gradient, American Chemistry Council (ACC), Agnes Francisco, Melanie Nembhard, and by Arcadis on behalf of American Coke and Coal Chemicals Institute, American Fuels and Petrochemical Manufacturers, American Petroleum Institute, Asphalt Institute, Association of American Railroads, Beazer East, Inc. and Pavement Coatings Technology Council. In addition, a public meeting was held in December 2013 to provide the public an opportunity to engage in early discussions on the draft IRIS Toxicological Review of Benzo[a]pyrene and the draft charge to the peer review panel prior to release for external peer review.

RESOLUTION OF PUBLIC COMMENTS ON THE DRAFT TOXICOLOGICAL REVIEW (dated August 2013)

1 CONSIDERATION OF ADDITIONAL LITERATURE

2
3 **Comment:** *Inclusion of supporting data for critical study used to derive the dermal slope factor.* The
4 August 2013 public comment draft derived the dermal slope factor from points of departure from
5 two lifetime dermal cancer bioassays that were deemed to be of similar quality ([Sivak et al., 1997](#);
6 [Poel, 1959](#)). Gradient identified a report ([Arthur D Little, 1989](#)) that presents the original results,
7 including the individual animal data, for the NIOSH dermal study publication ([Sivak et al., 1997](#)). In
8 addition, Gradient also provided an additional reference, [Clark et al. \(2011\)](#), for consideration in the
9 development of the dermal slope factor.

10
11 **EPA Response:** EPA has revised the dose response analysis for the derivation of the dermal slope
12 factor to incorporate the individual animal survival data identified by the public commenter
13 (Section 2.5.2 of the Toxicological Review and Appendix E.2.3 of the Supplemental Information).
14 This allowed EPA to utilize the MultiStage-Weibull model, a model that incorporates dose and the
15 time at which death with tumor occurred. Use of this model accounts for competing risks
16 associated with decreased survival times and other causes of death, including other tumors.

17
18 Previously, the PODs from the [Sivak et al. \(1997\)](#) and [Poel \(1959\)](#) studies were considered to be of
19 similar quality and thus were averaged to derive the dermal slope factor. In consideration of these
20 additional supporting data, EPA selected the NIOSH dermal study ([Sivak et al., 1997](#); [Arthur D Little,](#)
21 [1989](#)) as the best available study for dose-response analysis and extrapolation to lifetime cancer
22 risk following dermal exposure to benzo[a]pyrene.

23
24 The publication by [Clark et al. \(2011\)](#), reported results of a lifetime dermal cancer bioassay which
25 primarily tested polycyclic aromatic hydrocarbon (PAH) mixtures, and included one high dose
26 group of benzo[a]pyrene as a positive control. EPA considered this study, but did not present [Clark](#)
27 [et al. \(2011\)](#) in the revised assessment due to the availability of several dermal lifetime cancer
28 bioassays for benzo[a]pyrene with multiple doses which enable greater characterization of the
29 dose-response relationship, especially in the low dose range.

30
31 **Comment:** *Further consideration of human skin graft mouse bioassay studies.* Arcadis, EPRI and
32 CDM Smith recommended increased consideration of studies of PAH exposure in murine models
33 with human skin xenografts ([Atilasoy et al., 1997](#); [Soballe et al., 1996](#); [Urano et al., 1995](#); [Graem,](#)
34 [1986](#)). These studies transplanted human skin onto the backs of immunodeficient mice and after
35 grafts had been established, treated the skin with carcinogens, including PAHs, and mice did not
36 develop tumors. The commenters stated that these papers demonstrate that human skin is
37 resistant to the skin tumorigenesis that is seen in mouse skin with benzo[a]pyrene and other PAHs.

38
39 **EPA Response:** Several studies identified by the commenters used the potent carcinogen 7,12-
40 dimethylbenz(a)anthracene (DMBA) ([Soballe et al., 1996](#); [Urano et al., 1995](#); [Graem, 1986](#)) and a
41 single study tested benzo[a]pyrene ([Urano et al., 1995](#)). In the studies using benzo[a]pyrene or
42 DMBA alone, tumors were identified at the graft border, and judged to be of mouse skin origin, but
43 no tumors were identified as originating from the human skin graft. The ability of this model
44 system to predict hazard for human skin cancer risk from dermally active procarcinogens is
45 unclear. Though some studies indicate that the skin grafts maintain some metabolic function ([Das](#)
46 [et al., 1986](#)), it is unclear whether the human skin grafts (some obtained from cadavers) maintain
47 the same viability, vascularization, and full metabolic capacity as human skin in vivo ([Kappes et al.,](#)
48 [2004](#)). Potent mutagenic carcinogens such as DMBA, methylcholanthrene,
49 methylnitronitrosoguanidine also fail to produce skin tumors in this model system ([Soballe et al.,](#)

1 [1996](#); [Urano et al., 1995](#); [Graem, 1986](#)). In addition, the treatment time and the period of
2 observation was quite short in all identified studies. The mice with the benzo[a]pyrene-treated
3 human skin grafts in [Urano et al. \(1995\)](#) died within six months after the initial benzo[a]pyrene
4 treatment. In addition, the PAH-treated skin graft mice in [Graem \(1986\)](#) and [Soballe et al. \(1996\)](#)
5 were only followed for an average of 4-7 months from the start of treatment. While six months is
6 generally sufficient for the development of skin tumors in mouse skin (depending on dose level),
7 human latency for squamous cell carcinoma in PAH-exposed occupational cohorts is thought to be
8 greater than 20 years ([Young et al., 2012](#); [Voelter-Mahlknecht et al., 2007](#); [Everall and Dowd, 1978](#)).
9 Therefore, the single available study ([Urano et al., 1995](#)) evaluating the carcinogenicity of
10 benzo[a]pyrene in this model system was considered in the Toxicological Review, but was regarded
11 with low confidence.

12
13 Additional text to clarify these points has been added to Section 1.1.5 of the Toxicological Review.

14
15 **Comment:** *Inclusion of studies of patients therapeutically treated with coal tar.* Arcadis, EPRI, and
16 CDM Smith suggested the inclusion of epidemiologic studies of skin cancer risk in eczema and
17 psoriasis patients treated therapeutically with a dermatological formulations containing coal tar (a
18 PAH mixture). These commenters suggested that the available epidemiological studies of coal tar
19 treated patients clearly demonstrate that benzo[a]pyrene does not cause skin cancer in humans.

20
21 **EPA Response:** In addition to the comments described above, EPA received comments from the
22 American Petroleum Institute in March 2013 listing references related to therapeutic coal tar use.
23 EPA reviewed the references noted in these comments, as well as those identified through
24 additional review of citations contained in the identified studies and related reviews. Studies that
25 included a measure of coal tar exposure in relation to a measure of skin cancer risk were
26 considered and discussed in the Toxicological Review and Supplemental Information drafts dated
27 August 2013. Specifically, Table D-6 of the Supplemental Information presents a summary of the
28 methodological features as well as the results of these studies. A discussion of this database was
29 also included in Section 1.1.5 of the Toxicological Review and Appendix D.3.3 of the Supplemental
30 Information. Case reports and studies that did not include a measure of coal tar exposure were not
31 included in the August 2013 draft assessment.

32
33 EPA noted considerable limitations to this body of literature, particularly relating to the level of
34 detail pertaining to exposure measures, length of follow-up, and ability to address effects
35 attributable to other types of therapies. A single population-based case-control study was
36 identified ([Mitropoulos and Norman, 2005](#)); this study examined self-reported use of coal
37 tar/dandruff shampoo and the association with increased incidence of dermal squamous cell
38 carcinomas. EPA considered this exposure measure to be highly susceptible to misclassification.
39 Other epidemiological studies of patients with specific types of skin conditions (e.g., psoriasis,
40 eczema) were limited by the quality of the exposure data and inability to examine variation in
41 exposure level (i.e., duration of use), sample size and duration of follow-up, and choice of referent
42 rates and differences in disease ascertainment between cases and the reference population. In
43 addition, clinic-based studies focused on the commonly used regimen of coal tar in conjunction with
44 ultraviolet (UV) B therapy (e.g., the Goeckerman treatment), and could not distinguish effects of
45 coal tar alone.

46
47 EPA does not consider the identified studies to adequately address the question of the potential
48 association between coal tar treatments and skin cancer due to limitations in design and conduct.

1 Thus, EPA disagrees with the commenters' view that these studies demonstrate that
2 benzo[a]pyrene does not cause skin cancer in humans.

3
4 Although EPA does not consider the available studies sufficient to evaluate the potential association
5 between use of coal tar therapies and risk of skin cancer, acute studies of coal tar treated patients
6 provide in vivo evidence of benzo[a]pyrene-specific genotoxicity (increased BPDE-DNA adducts) in
7 human skin ([Godschalk et al., 2001](#); [Rojas et al., 2001](#); [Zhang et al., 1990](#)), an early key event in the
8 carcinogenic mode of action of benzo[a]pyrene (Figure 1-6 of Section 1.1.5 of the Toxicological
9 Review).

10
11
12 COMMENTS ON THE WEIGHT OF EVIDENCE AND MODE OF ACTION FOR CANCER

13
14 **Comment:** *Use of epidemiological studies to support the weight of evidence for cancer.* Arcadis and
15 EPRI commented that the draft benzo[a]pyrene assessment has mischaracterized the evidence
16 supporting an association between benzo[a]pyrene exposure and lung and skin cancers in humans.
17 They further state that the human studies that have been presented are studies of worker groups
18 who were exposed to complex mixtures and it is not possible to attribute the effects of the mixture
19 to one component.

20
21 **EPA Response:** EPA agrees that benzo[a]pyrene exposure in the environment occurs as a complex
22 mixture with many components including other PAHs, and notes in Section 1.1 of the Toxicological
23 Review that accordingly, there are few epidemiologic studies designed to investigate the effects of
24 benzo[a]pyrene, though there are many that have investigated the effects of exposure to PAH
25 mixtures as a whole. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005](#)) emphasize
26 the importance of weighing all of the evidence in reaching conclusions about human carcinogenic
27 potential. Specifically, the guidelines describe that the descriptor of "carcinogenic to humans" can
28 be used when the following conditions are met: (a) there is strong evidence of an association
29 between human exposure and either cancer or the key precursor events of the agent's mode of
30 action but not enough for a causal association, (b) there is extensive evidence of carcinogenicity in
31 animals, (c) the mode or modes of carcinogenic action and associated key precursor events have
32 been identified in animals, and (d) there is strong evidence that the key precursor events that
33 precede the cancer response in animals are anticipated to occur in humans and progress to tumors,
34 based on available biological information. In Section 1.2.2 and Table 1-18 of the Toxicological
35 Review the data for benzo[a]pyrene supporting these four conditions are presented.

36
37 Extensive evidence of carcinogenicity in animal bioassays along with strong and consistent
38 mechanistic data for a mutagenic mode of action support conditions (b) and (c) above. Numerous
39 studies demonstrate the carcinogenicity of benzo[a]pyrene in multiple species by all tested routes
40 of administration. In addition, mechanistic studies provide strong evidence that links the
41 metabolism of benzo[a]pyrene to DNA reactive agents with key mutational events in genes that can
42 lead to tumor development. These events include the formation of specific DNA adducts and
43 characteristic mutations in oncogenes and tumor suppressor genes.

44
45 Several human exposure studies are available supporting both conditions (a) and (d) above.
46 Specifically, epidemiological studies evaluating exposure to PAH mixtures (both occupational
47 exposures and tobacco smoke) containing benzo[a]pyrene demonstrate an association with cancer.
48 Furthermore, multiple studies have reported benzo[a]pyrene-specific DNA adducts as well as

1 characteristic mutations in oncogenes and tumor suppressor genes in humans exposed to PAH
2 mixtures.

3
4 EPA recognizes that benzo[a]pyrene is one of many PAHs that could contribute to the observed
5 increases in cancer in humans exposed to PAH mixtures. However, the combination of strong and
6 consistent human, animal, and mechanistic evidence for the carcinogenicity of benzo[a]pyrene
7 provides the basis for characterizing benzo[a]pyrene as “carcinogenic to humans”.

8
9
10 COMMENTS ON THE DERIVATION OF THE REFERENCE DOSE AND REFERENCE CONCENTRATION

11
12 **Comment:** *Metric used to characterize results in the elevated plus maze.* Dose-related differences in
13 number of open arm entries, increased time spent in the open arms, and decreased closed arm
14 entries were observed in the study by [Chen et al. \(2012\)](#). The number of entries into the open arms
15 was used for benchmark dose modeling to extrapolate a POD for the RfD. Arcadis commented that
16 according to [Hogg \(1996\)](#), the preferred way to express results from the elevated plus maze is as
17 percentage of open arm entries (among total arm entries) or percentage of time spent in open arms,
18 to correct for overall changes in exploration and reduce activity-induced artifacts.

19
20 **EPA Response:** EPA agrees that the optimal way to express elevated plus maze data is in relation
21 to the total number of arm entries or to the total amount of time spent in the arms of the maze. The
22 reason for presenting the information in such a way is to account for potential differences due to
23 changes in general locomotor or exploratory behaviors, rather than changes in anxiety (which is the
24 critical effect). While [Chen et al. \(2012\)](#) did not present the data in this manner (EPA’s attempts to
25 obtain the raw data have been unsuccessful), the authors did present enough information for EPA
26 to arrive at the conclusion that general locomotor or exploratory behaviors were not affected by
27 exposure. Specifically, as a measure of total locomotor activity and exploration in the elevated plus
28 maze test, the *total* number of arm entries between control and benzo[a]pyrene-treated animals
29 were calculated from the graphically reported results provided for open and closed arm entries;
30 total arm entries were unchanged with treatment (e.g., the number of closed arm entries was
31 decreased with exposure). Therefore, it is unlikely that the results observed were confounded by a
32 general increase in locomotor activity in the benzo[a]pyrene treated animals.

33
34 Two additional studies which tested the effects of benzo[a]pyrene exposure in the elevated plus
35 maze ([Bouayed et al., 2009a](#); [Grova et al., 2008](#)) reported findings consistent with [Chen et al.](#)
36 [\(2012\)](#), and similarly did not observe an increase in general locomotor activity with treatment.
37 Although they tested higher doses of benzo[a]pyrene than [Chen et al. \(2012\)](#), both [Bouayed et al.](#)
38 [\(2009a\)](#) and [Grova et al. \(2008\)](#) observed statistically significant effects of exposure on percentage
39 of open arm entries and no effects on the total number of arm entries, the former following oral
40 exposure during lactation and the latter following i.p. injection in adulthood. See Section 1.1.1 and
41 Section 2.1.1 for discussion of the consistency of the decreased anxiety-like effects in the evidence
42 database.

43
44 Although EPA concluded that the elevated plus maze data presented by [Chen et al. \(2012\)](#) is
45 appropriate for use in dose-response modeling, EPA also conducted a sensitivity analysis using the
46 metric proposed by [Hogg \(1996\)](#), expressing the data as a fraction of the open arm entries over the
47 total arm entries. The data for this endpoint were only available as group means and standard
48 deviations for the open and closed arm entries (separately), presented graphically; the group
49 means and standard deviations for total arm entries were not reported, and would ideally be

1 calculated from individual animal data. However, it was feasible to derive inputs for dose-response
2 analysis by summing the group means for open and closed arm entries to yield total arm entries,
3 and by using Monte Carlo simulations (assuming a normal distribution) to estimate the variance of
4 the ratio of open arm entries to total arm entries. The resulting BMD and BMDL for a one standard
5 deviation decrease in mean percentage of open arm entries among total entries were 0.09 and 0.05
6 mg/kg-day, respectively. Compared to the BMD and BMDL (POD) of 0.16 and 0.09 mg/kg-day
7 based on the [Chen et al. \(2012\)](#) data, the analysis incorporating total arm entries suggests a lower
8 POD for this effect; however, EPA notes that the two sets of results overlap substantially. EPA
9 considers the results corroborative of the POD for this effect based on the published data.

10
11 **Comment:** *Use of decreased anxiety-like effects as a critical effect.* Arcadis and ACC questioned why
12 the behavioral effect of “decreased anxiety” observed in rodents tested in the elevated plus maze
13 was regarded as an adverse effect given that this test is used in pharmacology to evaluate the
14 efficacy of anxiety reducing drugs.

15
16 **EPA Response:** A normal level of anxiety is a protective function of the nervous system. A decrease
17 in anxiety, a clear change in nervous system function, can impair an organism’s ability to react to a
18 potentially harmful situation. The decreased ability of an organism to adapt to the environment is
19 considered to be an adverse effect ([U.S. EPA, 1998](#)). In addition, any functional alteration resulting
20 from developmental exposure is considered biologically significant ([U.S. EPA, 1991c](#)). Additional
21 discussion of the significance of this endpoint has been added in Section 2.1.1 of the Toxicological
22 Review.

23
24 In contrast to environmental exposure, exposure to drugs is intended to occur at a controlled
25 dosage and with a defined periodicity and duration, and drugs are only administered to a subset of
26 the population with an underlying, clinically-identified neurological dysfunction that requires
27 treatment.

28
29 **Comment:** *Consideration of additional studies for the derivation of the RfC.* Arcadis commented that
30 the RfC should quantitatively consider the NOAELs and LOAELs from [Archibong et al. \(2002\)](#) and
31 [Wu et al. \(2003a\)](#) to develop the RfC based on decreased pup survival. They state that the
32 publication by {Archibong, 1007602} reported a LOAEL for this effect as 25 µg/m³, but that another
33 publication, from the same laboratory, [Wu et al. \(2003a\)](#), reported that this exposure concentration
34 was a NOAEL. They suggest that both studies can be used quantitatively to establish the point of
35 departure for the RfC.

36
37 **EPA Response:** The publications by [Archibong et al. \(2002\)](#) and [Wu et al. \(2003a\)](#) were generated
38 by the same laboratory and used identical exposure methods. As both publications reported
39 similar results for fetal survival, it appears possible that both reported effects on the same group of
40 exposed dams. EPA notes that reporting for the endpoint of decreased fetal survival met a higher
41 standard in [Archibong et al. \(2002\)](#) compared with [Wu et al. \(2003a\)](#), with [Archibong et al. \(2002\)](#)
42 reporting means and variances for implantation sites, pups per litter, and % litter survival and [Wu](#)
43 [et al. \(2003a\)](#) reporting decreased survival graphically. Although the focus of this study was on
44 metabolic activation in the liver and brain of exposed offspring, [Wu et al. \(2003a\)](#) did report that
45 “the number of resorptions was more at 75 and 100 µg/m³ compared to 25 µg/m³ and was
46 statistically significant,” but did not report whether the apparent decrease in birth index at 25
47 µg/m³ was statistically significant compared to the vehicle control group. This decrease in fetal
48 survival at 25 µg/m³ is consistent with the lowest exposure level being a LOAEL rather than a
49 NOAEL as suggested by the commenters. Due to incomplete reporting on this endpoint, the dataset
50 reported by [Wu et al. \(2003a\)](#) was inadequate for the derivation of a candidate RfC. These points

1 have been clarified in the discussion of [Wu et al. \(2003a\)](#) in Section 1.1.1 and 2.2.1 of the
2 Toxicological Review.
3
4

5 GENERAL COMMENTS ON THE QUANTITATIVE CANCER ASSESSMENT 6

7 **Comment:** *Apparent threshold in animal cancer bioassays.* Arcadis, CDM Smith, and USWAG
8 commented that the animal carcinogenicity studies of benzo[a]pyrene used as the bases of the oral
9 slope factor ([Beland and Culp, 1998](#)), inhalation unit risk ([Thyssen et al., 1981](#)), and dermal slope
10 factor ([Sivak et al., 1997](#); [Poel, 1959](#)), demonstrate threshold exposures for benzo[a]pyrene. They
11 state that plots resulting from EPA’s Benchmark Dose Modeling Software show that the bioassay
12 data exhibit threshold responses near the origin and that the tumor incidence of 0% at the low
13 doses demonstrate evidence of a threshold below which no cancer effects are seen.
14

15 **EPA Response:** EPA disagrees that the cited studies establish thresholds. Although animal
16 bioassays may seem to suggest thresholds, even the largest studies that are feasible to conduct have
17 insufficient power to detect risk levels of concern for public health. For example, a response of
18 1/50, or 2%, is the lowest response that can be observed in typical carcinogenicity bioassays. Such
19 a study cannot demonstrate a response of 0.1% (1/1000), say, often considered a high level of
20 cancer risk in a human population. If the true cancer rate at a particular exposure level is 0.1%, the
21 experimental outcome in a group of 50 will be 0% about 95% of the time.
22

23 Evidence for thresholds is more solidly determined through consideration of modes of action and
24 toxicokinetic pathways. Without consideration of such information, lack of response at low
25 exposures in animal bioassays cannot be distinguished from lack of statistical power.
26
27

28 COMMENTS ON THE INHALATION UNIT RISK 29

30 **Comment:** *Availability of an additional benzo[a]pyrene inhalation study.* Arcadis and EPRI
31 commented that an abstract for an unpublished study by [Pauluhn et al. \(1985\)](#) contradicts the
32 findings of carcinogenicity in [Thyssen et al. \(1981\)](#), and recommended that EPA acquire the raw
33 data.
34

35 **EPA Response:** The study referred to by the commenters, “Long-term inhalation study with
36 benzo[a]pyrene and SO₂ in Syrian golden hamsters,” assesses the effects of combined SO₂ and
37 benzo[a]pyrene (abbreviated as BP in the abstract) exposure in hamsters. It is unclear how this
38 study provides negative or contradictory evidence because the abstract indicates a carcinogenic
39 response: “[i]n hamsters which were exposed to 2 or 10 mg BP/m³ air without SO₂, a few
40 neoplastic alterations were found”. While it would be useful to have another study of inhalation
41 exposure, no further information is available in the published literature, and EPA’s attempts to
42 obtain the raw data have been unsuccessful.
43

44 **Comment:** *Exceedance of maximum tolerated dose in Inhalation Unit Risk study.* In comments
45 submitted by Arcadis and EPRI, it was stated that the exposure concentrations in the inhalation
46 study by [Thyssen et al. \(1981\)](#) likely caused particle overload, exceeding the maximum tolerated
47 dose (MTD), and according to [U.S. EPA \(2005\)](#), data that exceed the MTD should not be used for
48 quantitative risk assessment purposes.
49

1 **EPA Response:** In [Thyssen et al. \(1981\)](#), hamsters received time-weighted, average daily
2 concentrations of 0.25, 1.01, and 4.29 mg/m³ benzo[a]pyrene condensed onto sodium chloride
3 particles (calculated based on weekly exposure chamber measurements). Animals in the low and
4 mid benzo[a]pyrene concentration groups had comparable survival to the vehicle control group.
5 The study authors reported that decreased survival in the high concentration group was associated
6 with increased incidence of tumors in the larynx and pharynx (affecting approximately half of the
7 high dose group). The study authors also concluded that particle clearance mechanisms of the
8 respiratory epithelium remained intact since the tumors of the upper respiratory and digestive
9 tract occurred relatively late in life.

10
11 EPA's Guidelines for Carcinogen Risk Assessment ([U.S. EPA, 2005](#)) distinguish between cancer and
12 other effects when assessing toxicity, e.g.: "If adequate data demonstrate that the effects are solely
13 the result of excessive toxicity *rather than carcinogenicity* [emphasis added] of the tested agent *per*
14 *se*, then the effects may be regarded as not appropriate to include in assessment of the potential for
15 human carcinogenicity of the agent." No evidence supports that the tumors noted by [Thyssen et al.](#)
16 [\(1981\)](#) in the upper respiratory and digestive tract were solely the result of excessive toxicity
17 rather than carcinogenicity. The decreased survival observed in the high dose group appears to be
18 a direct effect of the early occurrence of tumors in this group. Therefore, the [Thyssen et al. \(1981\)](#)
19 study was judged to support the derivation of a unit risk.

20
21 **Comment:** *Apparent discrepancies in cancer incidence data used in the derivation of the inhalation*
22 *unit risk.* In comments submitted by Arcadis and EPRI, it was noted that the total number of
23 animals at risk and the total number of tumors observed in each treatment group from the [Thyssen](#)
24 [et al. \(1981\)](#) study varied between that publication, a secondary analysis ([U.S. EPA, 1990](#)), and
25 study design descriptions and tables (D-13, D-14, E-17, and dose-response model output
26 summaries) presented in the Toxicological Review.

27
28 **EPA Response:** Concerning the number of tumor-bearing animals, [Thyssen et al. \(1981\)](#)
29 summarized only the animals with malignant tumors without identifying them as such; EPA's
30 review ([U.S. EPA, 1990](#)) of the individual animal pathology reports provided by the investigators
31 ([Clement Associates, 1990](#)) showed that totals reported in the publication matched the incidence of
32 malignant tumors, and that there were benign tumors as well. Table D-13 of the Supplemental
33 Information has been revised to reflect the incidence of benign and malignant tumors.

34
35 Concerning the numbers of animals at risk, the totals in [Thyssen et al. \(1981\)](#), [U.S. EPA \(1990\)](#), and
36 the draft Toxicological Review differed because the number of animals examined for histopathology
37 varied for different tissues:

- 38 • [Thyssen et al. \(1981\)](#) reported the overall number of animals evaluated for histopathology,
39 but did not clarify when individual tissues were not available for examination.
- 40 • The draft Toxicological Review (August 2013) relied on the summaries in [U.S. EPA \(1990\)](#)
41 augmented by details from the histopathology reports ([Clement Associates, 1990](#)), such as
42 clarifications that some tumors were metastases or types not likely related to squamous
43 neoplasia. Upon re-review of the histopathology reports, EPA determined that results
44 were not available for five low-exposure animals included in the [U.S. EPA \(1990\)](#) analysis.
45 EPA has revised the incidence summaries and the dose-response modeling and has
46 omitted data for these animals.

1 **Comment:** *Exposure variability in the study used to derive inhalation unit risk.* Arcadis and EPRI
2 stated that [Thyssen et al. \(1981\)](#) is inappropriate for dose response modeling given concerns
3 regarding variability in exposure concentrations.
4

5 **EPA Response:** As discussed in Sections 2.4.1 and 2.4.4 of the Toxicological Review, the lifetime
6 inhalation bioassay by [Thyssen et al. \(1981\)](#), reported weekly averages of chamber concentrations
7 of benzo[a]pyrene which varied two- to fivefold from the overall average for each group, which
8 exceeds the limit for exposure variability of <20% for aerosols recommended by [OECD \(2009\)](#).
9 Continuous time weighted group average concentrations were calculated for use in dose response
10 modeling under the assumption that equal cumulative exposures are expected to lead to similar
11 outcomes. For risk assessment purposes, EPA generally assumes that cancer risk is proportional to
12 cumulative exposure, and therefore to lifetime average exposure as estimated here, when there is
13 no information to the contrary. Under this assumption, the variability of the chamber
14 concentrations has little impact on the estimated exposure-response relationship.
15

16 COMMENTS ON THE DERMAL SLOPE FACTOR

17

18 **Comment:** *Consideration of nonlinear MOAs for dermal carcinogenicity.* USWAG commented that
19 EPA failed to consider nonlinear dose-response models to extrapolate from dermal exposure
20 studies to predict cancer risk. USWAG cited the availability of a dermal exposure study in mice (and
21 follow up study) that measured DNA adducts, necrosis, and inflammation after 5 weeks of exposure
22 and observed the tumor response at approximately 8 months of treatment ([Albert et al., 1996](#);
23 [Albert et al., 1991](#)). This commenter stated that the observed tumor response in this study was
24 “remarkably nonlinear, with pronounced tumor formation at 35 weeks observed only in the high
25 dose group” and that this study “demonstrated definitively, and quantitatively, that pervasive
26 dermal tissue injury was induced by all benzo[a]pyrene dose levels investigated in this study.” They
27 also go on to state that “[t]he studies by ([Albert et al. \(1996\)](#); [Albert et al. \(1991\)](#)) demonstrate that
28 benzo[a]pyrene-induced inflammation, cell killing, and cell replication was highly likely to have
29 occurred at tumorigenic benzo[a]pyrene dose levels used in every bioassay that EPA relied on to
30 calculate a DSF.”
31

32 **EPA Response:** EPA agrees that the studies by ([Albert et al. \(1996\)](#); [Albert et al. \(1991\)](#)) appear to
33 indicate that at high doses of benzo[a]pyrene, inflammation promotes the formation of tumors.
34 [Albert et al. \(1991\)](#) treated animals with 16, 32, or 64 µg of benzo[a]pyrene once per week and
35 reported the number of tumors per mouse after 8 months (current standardized rodent cancer
36 bioassays treat animals for 18 to 24 months ([U.S. EPA, 2005](#))). The mice in the 16 and 32 µg dose
37 groups had an average of 1 tumor/animal, whereas the animals in the highest dose group had
38 approximately 8 tumors/animal. A follow up to this study, by the same authors, measured DNA
39 adducts, necrosis, and inflammation in treated mice (at the site of exposure) after 5 weeks of
40 dermal exposure. In the 64 µg/week dose group, statistically elevated levels of DNA adducts,
41 inflammation, and necrosis were reported; however, in the lower dose group (16 µg/week), DNA
42 adducts were statistically significantly elevated without increases in inflammation and necrosis.
43

44 In comparison, the lifetime dermal cancer bioassay in mice used for the derivation of the dermal
45 slope factor, [Sivak et al. \(1997\)](#), used much lower exposure concentrations of benzo[a]pyrene (0.1,
46 1 or 10 µg/week). Even so, after two years of exposure, animals in highest dose group had a tumor
47 incidence of 27/30 with dermal scabs and sores reported in 80% of animals. However, the next
48 dose level down reported tumors in 5/30 animals with no elevation of cytotoxic lesions.
49

1 Cytotoxicity and mutation are not mutually exclusive modes of action; some observed effects can be
2 consistent with more than one mode of action. A mutagen at high doses can cause cytotoxicity and
3 regenerative proliferation that is a secondary response to massive DNA damage. As discussed in
4 Section 1.1.5 of the Toxicological Review, benzo[a]pyrene is a complete carcinogen; the
5 contributing roles of other processes involved in the promotion and progression of
6 benzo[a]pyrene-induced tumors, including cytotoxicity, inflammation, and regenerative cell
7 proliferation, are acknowledged within the MOA discussion. However, there is insufficient evidence
8 that these mechanisms act independently of DNA damage and mutation to produce
9 benzo[a]pyrene-induced tumors.

10
11 EPA has revised the assessment to provide additional discussion of the ([Albert et al. \(1996\)](#); [Albert
12 et al. \(1991\)](#)) studies in the cancer MOA section with particular attention to the observed
13 quantitative and temporal relationship between DNA adducts and indicators of cytotoxicity
14 (including inflammation and necrosis).

15
16 **Comment:** *Potential for different mode of action for benzo[a]pyrene-induced tumors in mouse skin
17 versus human skin.* Arcadis and EPRI commented that PAH-induced mouse skin tumors have a
18 different genetic signature than tumors in human skin. They cited [Balmain and Harris \(2000\)](#) as
19 support for their hypothesis that PAH-induced tumors in mouse skin have an H-ras mutation
20 signature whereas human skin cancers have a p53 mutation signature, therefore showing that
21 PAHs are not causally related to human skin cancers.

22
23 **EPA Response:** The review article cited above ([Balmain and Harris, 2000](#)) does not discuss PAH-
24 induced skin tumors in mouse skin or human skin. This article reviews the association between
25 benzo[a]pyrene exposure from tobacco smoke and p53 mutations in human lung cancer, the
26 association between sun exposure and p53 mutations in human skin cancer, and the association
27 between dietary aflatoxin B1 exposure and p53 mutations in human liver cancer.

28
29 Benzo[a]pyrene has been shown to be a complete carcinogen in multiple animal species. Skin
30 tumors in mice, rats, rabbits, and guinea pigs have been associated with repeated application of
31 benzo[a]pyrene to skin in the absence of exogenous promoters ([Sivak et al., 1997](#); [Grimmer et al.,
32 1984](#); [Habs et al., 1984](#); [Grimmer et al., 1983](#); [Habs et al., 1980](#); [Schmähl et al., 1977](#); [Schmidt et al.,
33 1973](#); [Roe et al., 1970](#); [Poel, 1959](#)). The proposed mutagenic MOA for benzo[a]pyrene involves the
34 bioactivation of benzo[a]pyrene to DNA-reactive metabolites, direct DNA damage by reactive
35 metabolites, formation and fixation of DNA mutations, and clonal expansion of mutated cells. These
36 key events have been observed in animals and humans and by multiple routes of exposure (see
37 Table 1-17 of the Toxicological Review). Benzo[a]pyrene specific skin adducts have been observed
38 in vivo in both benzo[a]pyrene-treated mouse skin and human skin exposed to PAH-mixtures
39 ([Godschalk et al., 2001](#); [Rojas et al., 2001](#); [Zhang et al., 1990](#)). In addition, studies of dermal
40 benzo[a]pyrene exposure in mice have shown increased mutations in several gene targets including
41 the tumor suppressor p53 ([Ruggeri et al., 1993](#)), the proto-oncogene H-Ras ([Wei et al., 1999](#);
42 [Chakravarti et al., 1995](#)) and the lacZ transgene ([Miller et al., 2000](#)). Studies which examine
43 mutational spectra in human skin tumors thought to be related to PAH exposure are not available in
44 the literature.

45
46 The lack of specific H-ras mutational evidence in humans (due to lack of human studies) or
47 suggestive evidence of p53 mutations in UV-induced human skin tumors does not preclude the fact
48 that benzo[a]pyrene can initiate DNA damage via DNA adducts. If not adequately repaired, this
49 damage can form mutations and mutagenesis is a well-established cause of carcinogenesis.

1
2 Text in the mode of action analysis for carcinogenicity (in Section 1.1.5) has been modified to
3 include studies observing additional target gene mutations (in addition to H-ras) in dermally
4 exposed mice ([Miller et al., 2000](#); [Ruggeri et al., 1993](#)).

5
6 **Comment:** *Exceedance of maximum tolerated dose in studies used to derive the dermal slope factor.*
7 Arcadis and EPRI disagreed with the study that EPA selected for characterizing the dermal slope
8 factor, [Sivak et al. \(1997\)](#), stating that this study included exposure levels that exceeded the
9 maximum tolerated dose (MTD) by causing significant skin toxicity and excessive mortality, and
10 therefore is not suitable for the derivation of the dermal slope factor.

11
12 **EPA Response:** While lower lifetime exposures are preferred for cancer risk estimation, EPA
13 disagrees that studies demonstrating carcinogenicity, with or without causing mortality, should
14 automatically be excluded from cancer risk assessment. EPA's Guidelines for Carcinogen Risk
15 Assessment ([U.S. EPA, 2005](#)) distinguish between cancer and other effects when assessing toxicity
16 at exposures at or above the maximum tolerated dose, e.g.: "If adequate data demonstrate that the
17 effects are solely the result of excessive toxicity *rather than carcinogenicity* [emphasis added] of the
18 tested agent *per se*, then the effects may be regarded as not appropriate to include in assessment of
19 the potential for human carcinogenicity of the agent." Therefore, EPA concluded it is justified in
20 excluding individual *dose groups* with exposure above the MTD, but not necessarily the entire study.

21
22 Some skin toxicity was noted in the highest dose group with an 80% incidence of scabs and sores.
23 However, the next lower dose group did not produce notable non-cancer skin lesions. An analysis
24 excluding the high dose group from [Sivak et al. \(1997\)](#) (not shown in the assessment) showed no
25 impact on the resulting dermal slope factor (within one significant figure), because the slope factor
26 reflects the dose-response relationship at the lower exposure levels. When reported by study
27 authors, text to clarify incidence of any non-cancerous skin lesions has been added to Section 1.1.5.

28
29 **Comment:** *Cross-species extrapolation of dermal slope factor.* CDM Smith, Arcadis and EPRI
30 commented that differences between mouse and human skin should be recognized and accounted
31 for in the calculation of the dermal slope factor for skin cancer. Specifically, the commenters stated
32 mouse skin is thinner and more permeable to benzo[a]pyrene and produces more benzo[a]pyrene-
33 diol epoxide metabolites than humans. They also stated that aryl hydrocarbon hydroxylase
34 inducibility is higher in mice than humans and that DNA-adducts are formed at higher rates in
35 mouse skin and repaired at lower rates relative to human skin. Another commenter, CH2M Hill
36 recommended increased discussion of the uncertainty regarding the method for interspecies
37 scaling of the dermal slope factor.

38
39 **EPA Response:** The key events in the mutagenic mode of action of benzo[a]pyrene are well
40 conserved between species and tissues (see Section 1.1.5 and Table 1-17 of the Toxicological
41 Review). However, the quantitative differences in processes that may presumably affect the
42 carcinogenicity of benzo[a]pyrene are not well known. These processes likely include species (and
43 inter-individual) differences in rates of absorption, metabolism into reactive metabolites, de-
44 activation of reactive metabolites, DNA repair, and DNA replication. As mentioned in Section E of
45 the Supplemental Information, biological information is not currently comprehensive or detailed
46 enough to develop robust models for cross-species extrapolation. This assessment evaluated
47 several alternative approaches in Appendix E of the Supplemental Information. Of these potential
48 approaches, allometric scaling using body weight to the $\frac{3}{4}$ power was selected based on observed
49 species differences in the rate of dermal absorption and metabolism of benzo[a]pyrene. Using this

1 approach, rodents and humans exposed to the same daily dose of a carcinogen, adjusted for $BW^{3/4}$,
2 would be expected to have equal lifetime risks of cancer. Several assumptions are made in the use
3 of this scaling method. First, it is assumed that the toxicokinetic processes in the skin will scale
4 similarly to interspecies differences in whole-body toxicokinetics. Secondly, it is assumed that the
5 risk at low doses of benzo[a]pyrene is linear. A charge question on the method used for
6 interspecies scaling of the dermal slope factor has been included in the charge to the external peer
7 reviewers.

8
9 **Comment:** *Uncertainties regarding the implementation of the dermal slope factor.* CH2M Hill
10 suggested that the IRIS assessment for benzo[a]pyrene could be strengthened with increased
11 discussion of uncertainties in assessing risk from exposure to benzo[a]pyrene in soil. In addition,
12 they noted that EPA's Risk Assessment Guidelines for Superfund part E ([U.S. EPA, 2004](#))
13 recommend an absorption factor of 13% for benzo[a]pyrene exposure through soil, but that this is
14 for systemic absorption and may not be consistent with the mode of action of benzo[a]pyrene in the
15 skin. Another commenter, USWAG, stated that expressing skin cancer risk for benzo[a]pyrene in
16 terms of $(\mu\text{g}/\text{d})^{-1}$ is not appropriate because of the implicit assumption that the surface area over
17 which exposure occurs is irrelevant.

18
19 **EPA Response:** The lifetime dermal cancer bioassays available for benzo[a]pyrene reported the
20 total dose applied to skin and reported the general area treated (i.e. dorsal or interscapular area),
21 but did not quantify the actual cm^2 of skin treated. For this reason, the draft dermal slope factor
22 expresses risk of skin tumors from benzo[a]pyrene dermal exposure as risk per $\mu\text{g}/\text{d}$. The
23 assumption of this dose metric is that risk at low doses of benzo[a]pyrene is dependent on absolute
24 dermal dose and not dose per unit of skin, meaning that a higher exposure concentration of
25 benzo[a]pyrene contacting a smaller area of exposed skin could carry the same risk of skin tumors
26 as a lower exposure concentration of benzo[a]pyrene that contacts a larger area of skin. The skin
27 surface area exposed to benzo[a]pyrene contaminated media is an important variable in the
28 exposure assessment calculation of the absolute dermal dose of benzo[a]pyrene. An increase in
29 skin area exposed to benzo[a]pyrene contaminated media would result in an increased absolute
30 dermal dose, and therefore an associated increase in risk. Example equations for calculating the
31 absolute dermal dose of benzo[a]pyrene are included below.

32
33 The dermal slope factor is based on applied dose of benzo[a]pyrene in solvent. As environmental
34 dermal exposure is assumed to be predominantly through contaminated soil, it is recommended
35 that exposure assessment include an adjustment for exposure through soil. In the attached
36 example exposure calculations for exposure to benzo[a]pyrene through soil, a soil to skin transfer
37 coefficient (K_{soil}) of 0.25 was estimated based on a study in monkeys ([Wester et al., 1990](#)) which
38 measured dermal absorption of benzo[a]pyrene from soil as 13% and from acetone as 51%. In the
39 acetone vehicle, all of the benzo[a]pyrene contacts the skin after the acetone evaporates, implying
40 that 51% of the benzo[a]pyrene contacting the skin is absorbed. The soil experiment found that
41 13% was absorbed, so dividing this by 0.51 indicates that 25% of the benzo[a]pyrene in soil must
42 have contacted the skin. This may be a high estimate because [Wester et al. \(1990\)](#) used a low
43 carbon soil (0.9%).

44
45 **Comment:** *"Real world" validation of dermal slope factor.* Arcadis and EPRI recommended that EPA
46 perform calculations to determine if the proposed dermal slope factor is scientifically supportable.
47 These commenters stated that based on their calculations (details not provided to EPA) the current
48 dermal slope factor would indicate that benzo[a]pyrene and benzo[a]pyrene-toxic equivalents in
49 soil throughout the United States are the cause of 30% of all human skin cancers and that 100% of

1 users of pharmaceutical coal tar products should develop skin cancer due to exposure to
2 benzo[a]pyrene. The commenters recommended that EPA not derive a dermal slope factor in the
3 benzo[a]pyrene assessment, but stated that if a dermal slope factor was included a real world
4 validation should be included to determine if the value is scientifically supportable.

5
6 **EPA Response:** The commenters did not provide the exposure equation, benzo[a]pyrene soil
7 concentration, or assumptions used in their calculation of a 30% risk estimate. Without these
8 details, EPA could not reproduce the exposure and associated risk estimates presented in the
9 written comments.

10
11 EPA has used the proposed dermal slope factor to calculate average daily dermal doses and
12 associated risks at those dermal doses. A modified exposure equation, similar to equation 3.11 (for
13 dermal absorbed dose upon soil contact) in EPA's Risk Assessment Guidelines for Superfund part E
14 ([U.S. EPA, 2004](#)), was used to calculate the average-daily dose of benzo[a]pyrene absorbed into the
15 skin (not absorbed systemically, which is the original intent of the equation). Central tendency
16 exposures using benzo[a]pyrene soil concentrations of 100 ppb (a central estimate of several
17 published measurements of uncontaminated sites) and default exposure assumptions result in risk
18 estimates of approximately 6×10^{-6} for average lifetime exposure that occurs during childhood, and
19 1×10^{-6} for average lifetime exposure that occurs during adulthood.

20
21 Example exposure calculations for risk associated with a central tendency exposure to
22 benzo[a]pyrene throughout a lifetime (including childhood) are included below.
23

1

Example Calculations: Estimated Dermal Dose and Risk from Exposure to Benzo[a]pyrene in Soil

$$LADD_{\text{dermal}} = \frac{C_{\text{soil}} \times CF \times SA \times AF \times ED \times EF \times K_{\text{soil}}}{AT}$$

$$Risk_{\text{dermal}} = LADD_{\text{dermal}} \times SF_{\text{dermal}} \times ADAF$$

LADD_{dermal} = Lifetime Average Daily Dose (ug/day)

C_{soil} = Soil Concentration (ug/kg)

CF = Conversion Factor (kg/ug)

SA = Surface Area of Skin Exposed (cm²)

AF = Soil Adherence Factor (ug/cm²-day)

AT = averaging time, the period over which the exposure is averaged (days)

ED = Exposure Duration (years)

EF = Exposure Frequency (days/year)

K_{soil} = Soil to skin Transfer Coefficient for benzo[a]pyrene (unitless)

SF_{dermal} = Dermal Slope Factor for benzo[a]pyrene (ug/day)⁻¹

ADAF = Age Dependent Adjustment Factor (unitless)

2
3

Central Tendency Exposure (CTE) Dose: Dermal Contact with benzo[a]pyrene in Soil - Child age 1-<2 years

LADD _{dermal} ug/day	C _{soil} ug/kg	CF kg/ug	SA _{child 1-<6} cm ²	AF _{child 1-<6} ug/cm ² -day	ED _{child 1-<6} years	EF days/year	K _{soil} unitless	AT days
3.84E-05	100	1.00E-09	2800	40	1	350	0.25	25550

Risk _{dermal} unitless	LADD ug/day	SF _{dermal} (ug/day) ⁻¹	ADAF
2.30E-06	3.84E-05	0.006	10

4

Supplemental Information—Benzo[a]pyrene

Central Tendency Exposure (CTE) Dose: Dermal Contact with benzo[a]pyrene in Soil - Child age 2-<6 years

LADD_{dermal} ug/day	C_{soil} ug/kg	CF kg/ug	SA_{child 1-<6} cm²	AF_{child 1-<6} ug/cm²-day	ED_{child 1-<6} years	EF days/year	K_{soil} unitless	AT days
1.53E-04	100	1.00E-09	2800	40	4	350	0.25	25550

Risk_{dermal}	LADD	SF_{dermal}	ADAF
unitless	ug/day	(ug/day)⁻¹	
2.76E-06	1.53E-04	0.006	3

Central Tendency Exposure (CTE) Dose: Dermal Contact with benzo[a]pyrene in Soil - Child age 6-<10 years

LADD_{dermal} ug/day	C_{soil} ug/kg	CF kg/ug	SA_{child 6-<10} cm²	AF_{child 6-<10} ug/cm²-day	ED_{child 6-<10} years	EF days/year	K_{soil} unitless	AT days
7.81E-05	100	1.00E-09	5700	10	4	350	0.25	25550

Risk_{dermal}	LADD	SF_{dermal}	ADAF
unitless	ug/day	(ug/day)⁻¹	
1.41E-06	7.81E-05	0.006	3

Total CTE	
Risk_{child}	6.47E-06

1

Central Tendency Exposure (CTE) Dose: Dermal Contact with benzo[a]pyrene in Soil – Adult

LADD _{dermal} ug/day	C _{soil} ug/kg	CF kg/ug	SA _{adult} cm ²	AF _{adult} ug/cm ² -day	ED _{adult} years	EF days/year	K _{soil} unitless	AT days
1.76E-04	100	1.00E-09	5700	10	9	350	0.25	25550

Risk _{dermal} unitless	LADD ug/day	SF _{dermal} (ug/day) ⁻¹	ADAF
1.05E-06	1.76E-04	0.006	1

CTE Assumptions:

C_{soil} = an assumed concentration of 0.1 ppm or 100 ug/kg; central tendency value based on average of data for all sites (to 1 significant figure) in Table A-4 of Supplemental Information of the Public Comment Draft (dated August 2013) except the 3 high-end urban sites.

SA_{child 1-<6 years} = 2800 cm² for child residents; average of the 50th percentile values for males and females 1 to <6 years of age ([U.S. EPA, 2004, Exhibit 3-5](#)); the child resident was assumed to wear a short-sleeved shirt, and shorts (no shoes); therefore, the exposed skin surface is limited to the head, hands, forearms, lower legs, and feet.

SA_{child 6-<10 years} = 5700 cm²; adult value assumed based on ([U.S. EPA, 2004, Page 3-19](#)).

SA_{adult} = 5700 cm² for adults; average of the 50th percentile values for males and females greater than 18 years of age ([U.S. EPA, 2004, Exhibit 3-5](#)); assumed to wear a short-sleeved shirt, short pants and shoes; therefore, the exposed skin surface is limited to the head, hands, lower legs, and forearms.

AF_{child 1-<6 years} = 40 ug/cm²-day for children; based on the 50th percentile weighted AF for children playing at a daycare center ([U.S. EPA, 2004, Exhibit 3-3 and 3-5](#)); weighted based on body parts exposed.

AF_{child 6-<10 years} = 10 ug/cm²-day; adult value assumed based on ([U.S. EPA, 2004, Page 3-19](#)).

AF_{adult} = 10 ug/cm²-day for adult residents; based on the geometric mean weighted AFs for groundskeepers ([U.S. EPA, 2004, Exhibit 3-3 and 3-5](#)); weighted based on body parts exposed.

AT = averaging time (the period over which the exposure is averaged).

ED_{child 1-<2 years} = 1 year for a child resident.

ED_{child 2-<6 years} = 4 years for a child resident.

ED_{child 6-<10 years} = 4 years for a child resident based on a central tendency residency time of 9 years minus 5 years as a child age 1-<6 years at that residence.

ED_{adult} = 9 years for adults based on a central tendency residency time of 9 years.

EF = 350 days per year; assumes 2 weeks away from the contaminated area each year for vacation.

K_{soil} = 0.25; This estimate was based on a study in monkeys ([Wester et al., 1990](#)) which measured dermal absorption of benzo[a]pyrene from soil as 13% and from acetone as 51%. In the acetone vehicle, all of the benzo[a]pyrene contacts the skin after the acetone evaporates, implying that 51% of the benzo[a]pyrene contacting the skin is absorbed. The soil experiment found that 13% was absorbed, so dividing this by 0.51 indicates that 25% must have contacted the skin.

AT = assumed to be 70 years converted to days: 25550 days.

SF_{dermal} = 0.006 (ug/day)⁻¹

ADAF = 10 for children 1-<2 years; 3 for children 2-<10 years.

1
2

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