EPA/635/R-10/006
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

BENZO[a]PYRENE

(CAS No. 50-32-8)

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

June 2011

NOTICE

This document is an ***Interagency Science Consultation*** draft. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS TOXICOLOGICAL REVIEW OF BENZO[a]PYRENE (CAS No. 50-32-8)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

1-OH-Py	1-hydroxypyrene
3-MC	3-methylcholanthrene
8-OHdG	8-hydroxydeoxyguanosine
ADAFs	age-dependent adjustment factors
AFC	antibody forming cells
Ah	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
AhR	Ah receptor
AhRE	AhR-responsive element
AhRR	AhR repressor
AIC	Akaike's Information Criterion
AKR	aldo-keto reductase
ALT	alanine aminotransferase
Arnt	Ah receptor nuclear translocator
AST	serum aspartate transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
benzo[a]pyrene	benzo[a]pyrene
BeP	benzo[e]pyrene
BMD	benchmark dose
BMDL	benchmark dose, 95% lower bound
BMDS	Benchmark Dose Software
BMR	benchmark response
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide
BPQ	benzo[a]pyrene-7,8-quinone
BRCA	breast cancer antigen
BrdU	bromodeoxyuridine
BSM	benzene-soluble matter
BUN	blood urea nitrogen
CA	chromosomal aberrations
CASRN	Chemical Abstracts Service Registry Number
CAT	chloramphenicol acetyltransferase
CB	carbon black
CDK	cyclin-dependent kinase
CFU-GM	colony forming unit-granulocyte macrophage
CHL	Chinese hamster lung cells
CHO	Chinese hamster ovary cells
CI	confidence interval
CNS	central nervous system
Con A	Concanavalin A
CONSAAM	Conversational SAAM
COX	cyclooxygenase
CPDB	Cancer Potency Database
cSt	centi-Stoke
CTPV	coal tar pitch volatiles
CYP	cytochrome

CYP450	cytochrome P450
dG	deoxyguanosine
dG-N^2-BPDE	10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydro-benzo[a]pyrene
DHH	dihydrodiol dehydrogenase
DMBA	7,12-dimethylbenzanthracene
DMSO	dimethyl sulfoxide
DNCB	2,4-dinitrochlorobenzene
DRE	dioxin-responsive element
ED	effective dose
EGFR	epidermal growth factor receptor
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
EPC	endothelial progenitor cells
EpRE	electrophile (or antioxidant) response element
ER	estrogen receptor
EROD	7-ethoxyresorufin-O-deethylase
ETS	environmental tobacco smoke
Fe_2O_3	ferrous oxide
FEL	frank effect level
Ga_2O_3	gallium oxide
GD	gestational day
GGT	γ-glutamyl transferase
GI	gastrointestinal
GJIC	gap junctional intercellular communication
GLP	good laboratory practice
GM-CSF	granulocyte-macrophage colony stimulating factor
GNMT	glycine N-methyltransferase
GP	glycophorin
GSH	reduced glutathione
GST	glutathione-S-transferase
hAR	human androgen receptor
HED	human equivalent dose
HF	human fibroblasts
HFC	high-frequency cells
HL	human lymphocytes
HPLC	high-performance liquid chromatography
hprt	hypoxanthine guanine phosphoribosyl transferase
Hsp	heat shock protein
IARC	International Agency for Research on Cancer
IC_{50}	half maximal inhibitory concentration
IFN	interferon
Ig	immunoglobulin
IGF	insulin-like growth factor
IHD	ischemic heart disease
IL	interleukin
γ-INF	gamma-interferon
i.p.	intraperitoneal
IRIS	Integrated Risk Information System

i.v.	intravenous
KLH	keyhole limpet hemocyanin
ko	knock-out
LALN	lung-associated lymph nodes
LC-MS	liquid chromatography-mass spectrometry
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MAP	mitogen-activated protein
MCHC	mean cell hemoglobin concentration
MCP	monocyte-chemoattractant protein
M-CSF	macrophage colony stimulating factor
MGP	manufactured gas plant residue
MLR	mixed lymphocyte response
MMAD	mass median aerodynamic diameter
MN	micronucleus
MOA	mode of action
MPO	myeloperoxidase
NADH	nicotinamide adenine dinucleotide phosphate
NAT	N-acetyl transferase
NER	nucleotide excision repair
NF	naphthoflavone
NK	natural-killer
NMDA	N-methyl-D-aspartate
NO	nitrous oxide
NOAEL	no-observed-adverse-effect level
NQO	NADPH:quinone oxidoreductase
NSAID	non-steroidal anti-inflammatory drug
NTP	National Toxicology Program
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PBMC	peripheral blood mononuclear cell
PBPK	physiologically-based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PHA	phytohemagglutinin
PHS	prostaglandin H synthase
PMN	polymorphonuclear leukocyte
PND	postnatal day
p.o.	per os
POD	point of departure
Py	pyrene
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RN	reaction network
ROS	reactive oxygen species
RR	relative risk

RT-PCR	real-time or reverse transcriptase PCR
s.c.	subcutaneous
SAAM	Simulation, Analysis and Modeling
SAM	S-adenosylmethionine
SCC	squamous cell carcinoma
SCE	sister chromatid exchanges
SCE-H	SCE heterogeneity index
SD	standard deviation
SEM	standard error of the mean
SIR	standardized incidence ratio
SLRL	sex-linked recessive lethal
SMR	standardized mortality ratio
SNP	single nucleotide polymorphisms
SPF	specific pathogen-free
SRBC	sheep red blood cell
SSB	single strand break
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	toxicity (or toxic) equivalency factor
TGF	transforming growth factor
TK	thymidine kinase
TNF	tumor necrosis factor
TPA	12-O-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
UDP	uridine diphosphate
UDPGA	UDP glucuronic acid
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UGT	UDP-dependent glucuronosyltransferase
Vmax	maximum substrate turnover velocity
vSMC	vascular smooth muscle cell
WBC	white blood cells
WHO	World Health Organization
WT	wild type
WTC	World Trade Center
XP	xeroderma pigmentosum
XPA	xeroderma pigmentosum group A

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to benzo[a]pyrene (benzo[a]pyrene). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of benzo[a]pyrene.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Kathleen Newhouse
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

CONTRIBUTORS

Christine Cai
Gene Ching-Hung Hsu
Glinda Cooper
Martin Gehlhaus
Scott Glaberman
Karen Hogan
Margaret Pratt
John Schaum
John Stanek
Suryanarayana Vulimiri
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

CONTRACTING SUPPORT

Heather Carlson-Lynch
Peter McClure
Kelly Salinas
Joe Santodonato
Julie Stickney
SRC, Inc.
North Syracuse, NY

George Holdsworth
Lutz W. Weber
Oak Ridge Institute for Science and Education
Oak Ridge, TN

Janusz Z. Byczkowski
Consultant
Fairborn, OH

Andrew Maier
Lynne Haber
Bernard Gadagbui

Toxicology Excellence for Risk Assessment
Cincinnati, OH

Glenn Talaska
University of Cincinnati
Cincinnati, OH

REVIEWERS

This document has been provided for review to EPA scientists.

INTERNAL EPA REVIEWERS

Stephen Nesnow
National Health and Environmental Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, NC

Rita Schoeny
Office of Water
U.S. Environmental Protection Agency
Washington, DC

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of benzo[a]pyrene (benzo[a]pyrene). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action (MOA). The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a plausible inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

There is evidence in humans and animal studies, demonstrating an increased incidence of skin tumors with increasing dermal exposure to polycyclic aromatic hydrocarbons (PAHs) mixtures including benzo[a]pyrene or to benzo[a]pyrene alone. Thus this assessment for benzo[a]pyrene derives a dermal slope factor; a quantitative risk estimate that is a plausible upper bound on the estimate of risk per μg/day of dermal exposure.

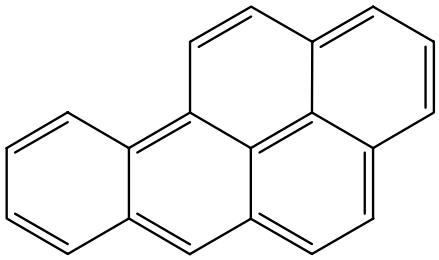
Development of these hazard identification and dose-response assessments for benzo[a]pyrene has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include

the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through December, 2010.

2. CHEMICAL AND PHYSICAL INFORMATION

Benzo[a]pyrene is a five-ring polycyclic aromatic hydrocarbon (PAH) (Figure 2-1). It is a pale yellow crystalline solid with a faint aromatic odor. It is relatively insoluble in water and has low volatility. Benzo[a]pyrene is released to the air from both natural and anthropogenic sources and removed from the atmosphere by photochemical oxidation; reaction with nitrogen oxides, hydroxy and hydroperoxy radicals, ozone, sulfur oxides, and peroxyacetyl nitrate; and dry deposition to land or water. In air, benzo[a]pyrene is predominantly adsorbed to particulates but may also exist as a vapor at high temperatures (NLM, 2010). The structural formula is presented in Figure 2-1. The physical and chemical properties of benzo[a]pyrene are shown in Table 2-1.



Benzo[a]pyrene

Figure 2-1. Structural formula of benzo[a]pyrene.

There is no known commercial use for benzo[a]pyrene and it is only produced as a research chemical. It is found ubiquitously in the environment primarily as a result of incomplete combustion emissions. It is released to the environment via both natural sources (such as forest fires) and anthropogenic sources including stoves/furnaces burning fossil fuels (especially wood and coal), motor vehicle exhaust, cigarettes, and various industrial combustion processes (ATSDR, 1995). Benzo[a]pyrene is also found in soot and coal tars. Mahler et al. (2005) has reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for the majority of PAHs in many watersheds (Mahler *et al.*, 2005). Occupational exposure to PAHs occurs primarily through inhalation and skin contact during the production and use of coal tar and coal tar-derived products, such as roofing tars, creoste and asphalt (IARC, 2010). Chimney sweeping can result in exposure to benzo[a]pyrene contaminated soot (ATSDR, 1995). As shown below in Table 2-2, benzo[a]pyrene exposure can also occur to workers involved in the production of aluminum, coke, graphite, and silicon carbide.

Table 2-1. Physical properties and chemical identity of benzo[a]pyrene

CASRN 50-32-8		
Synonyms	Benzo[d,e,f]chrysene; 3,4-benzopyrene, 3,4-benzpyrene; benz[a]pyrene; benzo[a]pyrene; BP	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM
Melting point	179–179.3°C	O'Neil et al. (2001)
Boiling point	310–312°C at 10 mm Hg	O'Neil et al. (2001)
Vapor pressure, at 20°C	5×10^{-7} mm Hg	Verschueren (2001)
Density	1.351 g/cm^3	IARC (1973)
Flashpoint (open cup)	No data	
Water solubility at 25°C	$1.6–2.3 \times 10^{-3}$ mg/L	ATSDR (1995); Howard and Meylan (1997)
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	$C_{20}H_{12}$	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM

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

Inhalation

ATSDR (1995) reports average indoor concentrations of benzo[a]pyrene as 0.37 to 1.7 ng/m^3 for smokers and 0.27 to 0.58 ng/m^3 for non-smokers. Naumova et al. (2002) measured PAHs in 55 nonsmoking residences in three urban areas during June 1999-May 2000. Mean indoor benzo[a]pyrene levels ranged from 0.02 to 0.078 ng/m^3. They also reported outdoor benzo[a]pyrene levels ranging from 0.025 to 0.14 ng/m^3. They concluded that indoor levels of the 5-7 ring PAHs (such as benzo[a]pyrene) were dominated by outdoor sources and observed an average indoor/outdoor ratio of approximately 0.7. Mitra and Wilson (1992) measured benzo[a]pyrene air levels in Columbus, OH and found elevated indoor levels in homes with smokers. They measured an average outdoor air concentration of 1.38 ng/m^3 and indoor concentrations of 0.07 ng/m^3 for homes with electrical utilities, 0.91 ng/m^3 for homes with gas utilities, 0.80 ng/m^3 for homes with gas utilities and a fireplace, 2.75 ng/m^3 for homes with gas utilities and smokers, and 1.82 ng/m^3 for homes with gas utilities, smokers, and a fireplace. Mitra and Ray (1995) evaluated data on benzo[a]pyrene air levels in Columbus, OH and reported an average of 0.77 ng/m^3 inside homes and 0.23 ng/m^3 outdoors. Park et al. (2001) measured ambient levels of benzo[a]pyrene in Seabrook, TX during 1995-1996. Based on continuous measurements over this period, they found an average of 0.05 ng/m^3 (vapor plus particulate). Parke et al. (2001) also reports average ambient air levels in ng/m^3 from other studies conducted earlier as 1.0 for Chicago, 0.19 for Lake Michigan, 0.01 for Chesapeake Bay and 0.02 for Corpus Christie, TX. Petry et al. (1996) conducted personal air sampling during 1992 at 5 workplaces in

Switzerland: carbon anode production, graphite production, silicon carbide production, bitumen paving work, and metal recycling. These data are summarized in Table 2-2.

Santodonato et al. (1981) estimated adult daily intake from inhalation as ranging from 9 to 43 ng/day. EC (2002) reported the following benzo[a]pyrene air levels in Europe during the 1990's: rural areas: 0.1 – 1 ng/m^3 and urban areas: 0.5 – 3 ng/m^3. They estimated the mean intake via inhalation for an adult non-smoker as 20 ng/day. The data from exposure studies by Naumova et al. (2002) suggest typical inhalation intakes may be lower (probably due in part to the focus on nonsmoker residences). These data suggest that air exposures are typically less than 0.14 ng/m^3 which would result in 2 ng/day assuming a 13 m^3/day inhalation rate (adult average based on USEPA, 1997).

Table 2-2. Benzo[a]pyrene Concentrations in Air

Setting	Year	n	Concentration (ng/m^3)	reference
Outdoor - Urban				
Los Angeles, CA	1999-2000	19	0.065	Naumova et al., 2002
Houston, TX	1999-2000	21	0.025	Naumova et al., 2002
Elizabeth, NJ	1999-2000	15	0.14	Naumova et al., 2002
Seabrook, TX	1995-1996	NA	0.05	Park et al. 2001
Columbus, OH	1986-1987	8	0.23	Mitra and Ray, 1995
Indoor Residential				
Los Angeles, CA	1999-2000	19	0.078	Naumova et al., 2002
Houston, TX	1999-2000	21	0.020	Naumova et al., 2002
Elizabeth, NJ	1999-2000	15	0.055	Naumova et al., 2002
Columbus, OH	1986-1987	8	0.77	Mitra and Ray, 1995
Columbus, OH		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 – 672 0 8000	ATSDR, 1995 Petry et al., 1996
Carbon anode production - Switzerland	1992	30	1100	Petry et al., 1996
Graphite production - Switzerland	1992	16	83	Petry et al., 1996
SiC production - Switzerland	1992	14	36	Petry et al., 1996
Metal recovery - Switzerland	1992	5	14	Petry et al., 1996
Bitumen paving - Switzerland	1992	9	10	Petry et al., 1996

NA = Not Available

Airborne intake of benzo[a]pyrene in the environment predominantly occurs via inhalation of insoluble carbonaceous particles (e.g. soot, diesel particles) to which organic compounds, such as PAHs, are adsorbed. Reliable, quantitative measurements of the percent

absorption of benzo[a]pyrene from insoluble particles are not available; however, studies in experimental animals indicate that benzo[a]pyrene is readily absorbed from carbonaceous particles following inhalation exposure (Gerde et al., 2001; Hood et al., 2000).

Oral

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

Kazerouni et al. (2001) measured benzo[a]pyrene in a variety of commonly consumed foods collected from grocery stores and restaurants in Maryland (analyzed as a composite from 4-6 samples of each food type). The foods were tested after various kinds of cooking. These results are reported in Table 2-3. The concentrations were combined with food consumption data to estimate intake. The intakes of the 228 subjects ranged from approximately 10 to 160 ng/d with about 30% in the 40 to 60 ng/day range. The largest contributions to total intake were reported as bread cereal and grain (29%) and grilled/barbecued meats (21%).

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

From the surveys conducted in six EU countries, the mean or national-averaged dietary intake of benzo[a]pyrene for an adult person was estimated in the range 0.05 to 0.29 μg/day (European Commission[EC], 2002). In the UK, average intakes on a ng kg^{-1} day^{-1} basis were estimated for the following age groups: adults – 1.6, 15 to 18 years – 1.4, 11 to 14 years – 1.8, 7 to 10 years – 2.6, 4 to 6 years – 3.3 and toddlers 3.1 – 3.8. The major contributors were the oils and fats group (50%), cereals (30%) and vegetables (8%) (EC, 2002). The contribution from grilled foods appeared less important in Europe than the U.S. because grilled foods are consumed less often (EC, 2002).

Table 2-3. Benzo[a]pyrene Levels in Food

	Concentration (ng/g)
Meat	
Fried or broiled beef	0.01 – 0.02
Grilled beef	0.09 – 4.9
Fried or broiled chicken	0.08 – 0.48
Grilled chicken	0.39 – 4.57

Fish	0.01 – 0.24
Smoked fish	0.1
Bread	0.1
Breakfast Cereals	0.02 – 0.3
Vegetable Oil	0.02
Eggs	0.03
Cheese	<0.005
Butter	<0.005
Milk	0.02
Fruit	0.01 – 0.17

Source: Kazerouni et al., 2001

Estimates of oral bioavailability from animal studies range from about 30-70% (Ramesh et al, 2001b; Cavret et al., 2003; Hecht et al., 1979). Direct information regarding absorption of benzo[a]pyrene in humans is limited. One study indicated near 100% absorption of benzo[a]pyrene in eight subjects exposed to benzo[a]pyrene through the ingestion of charbroiled meat (Hecht et al., 1979). Other dietary factors likely influence the oral absorption of benzo[a]pyrene. In experimental animals, a high fat diet appears to increase absorption of benzo[a]pyrene whereas a high fiber or protein rich diet appears to decrease absorption (Kawamura et al., 1988; O'Neill et al., 1991; Mirvish et al., 1981).

Dermal

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

PAHs are commonly found in all types of soils. ATSDR (1995) reported benzo[a]pyrene levels in soil for a variety of settings: 2-1,300 µg/kg in rural areas, 4.6 – 900 µg/kg in agricultural areas and 165-220 µg/kg in urban areas and 14,000-159,000 µg/kg at contaminated sites (before remediation). The soil levels for all land uses appear highly variable. The levels are affected by proximity to roads/combustion sources, use of sewage sludge derived amendments on agricultural lands, particle size and organic carbon content. Wilke (2000) reports that PAH levels in soils have generally increased during the 1900's and that sediment studies suggest some declines may have occurred since the 1970's. An illustration of benzo[a]pyrene levels in soil is presented in Table 2-4.

Table 2-4. Levels of benzo[a]pyrene in Soil

Reference	Location	Land Type	Concentration Mean (µg/kg)
Butler et al, 1984	UK	Urban	1165
Vogt et al. 1987	Norway	Industrial	321
	Norway	Rural	14
Yang et al. 1991	Australia	Residential	363

Maliszewska, 1996	Poland	Agricultural	22
Trapido, 1999	Estonia	Urban	106
	Estonia	Urban	398
	Estonia	Urban	1113
	Estonia	Urban	1224
	Estonia	Rural	6.8
	Estonia	Rural	15
	Estonia	Rural	27
	Estonia	Rural	31
Nam et al., 2008	UK	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, 2009	Poland	Agricultural	30
Wilkce, 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

A number of studies have measured dermal absorption of benzo[a]pyrene from soil (Turkall et al. 2008; Moody et al, 2007; Roy and Singh, 2001; Roy et al, 1998; Wester et al, 1990; Yang et al, 1989). These studies utilize in vitro and in vivo testing in a variety of animal species and in vitro testing with human skin samples. . The absorption percentages of benzo[a]pyrene from soil, as tested in vitro for human skin, ranged from 0.9 to 15% (Moody et al., 2007; Roy et al., 1998; Wester et al., 1990). However, major methodological differences between these studies exist including whether the amount of benzo[a]pyrene left in the skin depot was included as part of the absorbed fraction or whether only benzo[a]pyrene or its metabolites passing into the receptor fluid was quantified.

These studies of benzo[a]pyrene absorption from soil suggest that reduced absorption of benzo[a]pyrene occurs with increasing organic carbon content and clay content of the soils. They also indicate that dermal absorption increases as soil aging decreases (ie. contact time between soil and chemical).

3. TOXICOKINETICS

Benzo[a]pyrene is one of the most extensively studied PAH compounds. Numerous primary reports and secondary reviews are present in the scientific literature that describe the toxicokinetics of benzo[a]pyrene following oral, inhalation, and dermal exposures. Benzo[a]pyrene is absorbed following exposure by inhalation, oral, and dermal routes. The rate and extent of absorption are dependent upon the exposure medium. For example, bioavailability of benzo[a]pyrene is dependent on vehicle characteristics and adsorption to particles. 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 indicate wide 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, 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.

3.1. ABSORPTION

3.1.1. Inhalation Exposure

3.1.1.1. *Inhalation Exposure in Humans*

The absorption of benzo[a]pyrene is frequently assessed by identification of benzo[a]pyrene metabolites in the urine of people exposed to emissions from combustion processes. Because of the nature of these processes, oral and dermal exposures are likely to accompany exposure through the inhalation route, rendering estimates of inhalation-only exposure rather imprecise. For these reasons, quantitative estimates of absorption via the respiratory tract cannot be derived from these studies. Nevertheless, the observation of benzo[a]pyrene metabolites (as well as DNA adducts) in tissues and excreta of exposed humans provides qualitative evidence for benzo[a]pyrene absorption, at least some of which is likely to be via the respiratory tract.

Becher and Bjørseth (1983) studied urinary excretion of 11 PAHs in highly exposed Norwegian aluminum smelter workers (11 exposed: 7 smokers, 4 nonsmokers; 9 controls: 5 smokers, 4 nonsmokers). The authors compared urinary excretion of parent compound to that of hydroxy-metabolites (determined as parent compound following a chemical reduction procedure) and found that, in the case of benzo[a]pyrene, 92% of the total excreted were

metabolites. These values differed widely for the other 10 PAHs, several of which could be detected only following the reduction procedure. Exposed nonsmoking workers excreted 0.104 ± 0.154 μg total benzo[a]pyrene per mmol creatinine (range: 0.002–0.37; parent compound plus metabolites) in urine (0.0153 ± 0.016 without one extreme outlayer), while smoking workers excreted 0.025 ± 0.016 μg/mmol creatinine (range: 0.01–0.063); the difference was not significant. Total PAH excretion in the urine of aluminum plant workers was also higher in nonsmokers than in smokers (6.61 ± 3.59 vs. 5.65 ± 2.31 μg/mmol creatinine). Air concentrations of PAHs in the aluminum reduction plant were typically 100 $\mu g/m^3$. The authors concluded that neither high occupational exposure to PAHs nor smoking status provided accurate determinants for PAH body burdens and that interindividual differences in absorption or metabolism played a major role.

Grimmer et al. (1994) measured PAH metabolites in the 24-hour urines of four coke oven workers whose exposure to PAHs had been monitored with personal air samplers on 4 consecutive workdays. They observed a correlation between benzo[a]pyrene amounts extracted from the sampler filters and benzo[a]pyrene-9,10-dihydrodiol concentrations in urine. Urinary concentrations following similar levels of exposure, however, varied by a factor of about 5 among the four workers, which the authors attributed to differences in genetically determined metabolism. One of the central findings in that study was that only a very small fraction of the inhaled benzo[a]pyrene (0.013%) was recovered from urine, suggesting poor pulmonary absorption, poor metabolism, or that urine is not a major route for excretion of benzo[a]pyrene. In the case of phenanthrene and pyrene (Py), percentages recovered from urine were at least fivefold higher.

Gündel et al. (2000) studied the urinary excretion of metabolites of eight PAHs, among them benzo[a]pyrene, in 19 workers at a fireproof stone manufacturing plant in Germany, and provided concentrations in the air to which the workers were exposed. In the case of benzo[a]pyrene, the median for personal air samplers was 1.07 $\mu g/m^3$ (range: 0.043–2.96), and the median for stationary air sampling was 1.31 $\mu g/m^3$ (range: 0.63–5.41). Other PAH air concentrations ranged from 0.11 $\mu g/m^3$ (dibenz[a,h]anthracene) to 4.85 $\mu g/m^3$ (chrysene). The median for urinary excretion of 3-OH-benzo[a]pyrene (the only benzo[a]pyrene metabolite evaluated) was 1.58 ng/mmol creatinine (range: 0.34–22.6). This was by far the lowest level of PAH metabolites found in the urine of exposed workers; for comparison, phenanthrene, which showed almost the same median concentration as benzo[a]pyrene in personal air samplers (1.08 $\mu g/m^3$), produced a total of 679 ng/mmol creatinine in the form of metabolites (range: 205–4,700). (The author's values were given as μg/g creatinine and recalculated using a mol. wt. of 113.12 for creatinine. Values for phenanthrene metabolites were obtained by addition of urinary concentrations of four metabolites.) The authors pointed out that they were not able to detect a correlation between the levels of individual PAH exposures and urinary excretion of related metabolites.

Wu et al. (2002) found a statistically significant correlation between *trans-anti*-benzo[a]pyrene-tetrol in the urine of coke oven workers and PAH concentrations in benzene extracts obtained from personal air monitoring devices. These workers were exposed to a variety of PAHs, including benzo[a]pyrene. The results were not influenced by smoking or alcohol consumption habits. However, genetic factors had some influence on urinary *trans-anti*-benzo[a]pyrene-tetrol levels (e.g., workers homozygous for the cytochrome (CYP)1A1 *MspI* variant displayed 27% higher urinary tetrol levels than did workers heterozygous or wild type [WT] for this variant). There was also a statistically significant correlation between urinary levels of *trans-anti*-benzo[a]pyrene-tetrol and 1-hydroxypyrene (1-OH-Py), a metabolite not derived from benzo[a]pyrene, but from Py metabolism that is frequently used for assessment of PAH exposure.

Hecht et al. (2003) attempted to establish a procedure for the assessment of PAH exposure by measurement of urinary metabolites of phenanthrene. They compared levels of r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene to those of 1-OH-Py or r-7,t-8,9,c-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (*trans-anti*-benzo[a]pyrene tetrol) in the urine of psoriasis patients treated with coal tar, of coke oven workers, and of smoking or nonsmoking unexposed control persons. They demonstrated statistically significant correlations among all three metabolites in the urine of coke oven workers but not in psoriasis patients, despite the fact that the latter had 13- to 94-fold higher urinary metabolite concentrations. In controls, only *trans-anti*-PheT vs. 1-OH-Py was assessed, and the correlation was statistically significant. The authors emphasized that urinary concentrations of *trans-anti*-benzo[a]pyrene tetrol were 8,000–19,000 times lower than those of r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene. In a similar attempt, Ariese et al. (1994) tried to establish a correlation between urinary 1-OH-Py and 3-OH-benzo[a]pyrene and found a significant correlation in unexposed controls but not in exposed coke oven workers, who displayed significantly elevated 1-OH-Py levels but no corresponding elevation of 3-OH-benzo[a]pyrene.

The available data from human exposure studies provide qualitative evidence of benzo[a]pyrene absorption via the respiratory tract and also indicate that, in comparison with other PAHs, benzo[a]pyrene is absorbed in an unpredictable fashion. Most authors appear to assume that, in occupational settings, exposure occurs predominantly by the inhalation route. Occupational inhalation exposure to air contaminated with high levels of benzo[a]pyrene does not necessarily result in correspondingly elevated excretion of benzo[a]pyrene metabolites. These qualitative observations in humans are supported by inhalation and instillation toxicokinetic studies in animals.

3.1.1.2. *Inhalation Exposure in Animals*

Gerde et al. (1993a) conducted a series of studies on the disposition of benzo[a]pyrene in the respiratory tract of dogs. Seven-year-old female beagle dogs (n = 3) were exposed to a bolus

of 77 mg aerosolized benzo[a]pyrene crystals (particle size <1 μm) injected directly into the trachea of the animals during a single breath. Blood levels of benzo[a]pyrene in the ascending aorta and right atrium were monitored to evaluate the rate of appearance of benzo[a]pyrene in the systemic circulation. Benzo[a]pyrene concentrations in the blood built up rapidly and peaked at 1.8 minutes. Over the 15-minute time period after dosing, approximately 92% of the administered benzo[a]pyrene was cleared from the lungs. The half-life for lung clearance was 2.4 minutes. Comparing lung clearance rates for benzo[a]pyrene and the less lipophilic phenanthrene, which is cleared more quickly, the authors concluded that the clearance rate was limited by diffusion through the alveolar septa for highly lipophilic compounds such as benzo[a]pyrene. A half-life of 2 hours was estimated for clearance of absorbed benzo[a]pyrene from the blood. These data demonstrate that absorption of benzo[a]pyrene from the pulmonary portion of the respiratory tract of animals (as opposed to the tracheal, bronchial, and bronchiolar portions) is nearly complete and very rapid.

In a second study in this series, Gerde et al. (1993b) determined the disposition of benzo[a]pyrene in the conducting airways by instilling benzo[a]pyrene (dissolved in 20 μL saline and administered as a mist) into a main stem bronchus or distal portion of the trachea of female beagle dogs (n = 4). Benzo[a]pyrene retained in mucus was determined from lavage samples to assess the degree to which benzo[a]pyrene was transferred from the mucus into the bronchial epithelium. Roughly 34% of the benzo[a]pyrene was retained in the mucus collected 1 minute after instillation. Benzo[a]pyrene in the mucus cleared at the same rate as inert particles (90 mm/minute), suggesting transport via the mucociliary escalator. The estimated half-life for clearance of benzo[a]pyrene from the mucus to the respiratory epithelium was 9.5 minutes. Therefore, the mucociliary escalator acted like a rather shallow pool for benzo[a]pyrene. Absorption into the airway walls was evaluated by instilling benzo[a]pyrene solutions into the upper bronchial tree and measuring benzo[a]pyrene and metabolites in tissues. Approximately 20% of the benzo[a]pyrene dose penetrated into the epithelium of the left and right bronchus, respectively, within 45 minutes and cleared from the main stem bronchi with a half-time of 1.4 hours. Benzo[a]pyrene metabolites were also measured in main stem airway segments (trachea and bronchi were dissected into approximately 1-cm-long segments). The percent of benzo[a]pyrene recovered as parent compound was 62 and 33% after 45 minutes and 1.5 hours, respectively. The pattern of metabolites varied across these segments. Benzo[a]pyrene tetrols ranged from 9 to 37% and benzo[a]pyrene-9,10-diol ranged from 3 to 18% of the administered dose. About 8% of the administered dose was covalently bound to tissues. The authors concluded that the significantly higher retention time in the bronchi, as compared to pulmonary epithelium, made the conducting portion of the respiratory tract a possible target of benzo[a]pyrene toxicity.

In the third paper of the series, Gerde et al. (1993c) used the results from the previous two studies to evaluate benzo[a]pyrene dosimetry in the respiratory tract. The authors concluded

that benzo[a]pyrene uptake occurs via diffusion-limited transport through the lung epithelium. Uptake by the alveolar epithelium took only a few minutes while uptake by the airway epithelium took hours, due to the much thicker air/blood barrier in the conducting airways. This would result in longer residence time in the respiratory tract tissues, where benzo[a]pyrene can be metabolized to reactive metabolites, making the conducting airways an important target for benzo[a]pyrene.

This series of studies by Gerde et al. (1993a, b, c) assessed the absorption of benzo[a]pyrene in the respiratory tract using an aerosolized solution of benzo[a]pyrene in saline. However, other studies in animals assessed the bioavailability of benzo[a]pyrene in the lung using benzo[a]pyrene adsorbed onto particle substrates. Gerde et al. (2001) evaluated the bioavailability of diesel soot-adsorbed benzo[a]pyrene in three 1-year-old beagle dogs (n = 3). Soot particles were denuded by toluene extraction and benzo[a]pyrene was adsorbed onto the soot as a surface coating. The dogs were exposed to a single, 220 mL bolus of aerosolized benzo[a]pyrene-coated diesel soot (mass median aerodynamic diameter [MMAD] 1.3 ± 0.2 μm), followed by 90 mL of clean air to facilitate delivery of benzo[a]pyrene to the alveolar region, and arterial and venous blood samples were taken at intervals over a 1-hour period. In separate tests, the amount of benzo[a]pyrene deposited was determined to be 36 ± 20 μg (n = 6). The concentration of benzo[a]pyrene in the blood peaked at about 2 minutes and the first half-life of absorption was approximately 4 minutes. Although one dog received approximately seven times the dose of benzo[a]pyrene than the other two, probably due to variability in the aerosol generation technique, the fractional retention of benzo[a]pyrene in the lung was similar in all three dogs, indicating first-order absorption kinetics. The initial absorption was rapid; <10% of the dose remained in the lung after 30 minutes. However, there was a small fraction of benzo[a]pyrene that remained adsorbed to the soot even after 5.6 months in the lungs, when the fraction of material coating the particles had decreased to approximately 16% of what would be a monolayer of benzo[a]pyrene molecules deposited on the soot particles. The authors suggested that the small portion of tightly adsorbed benzo[a]pyrene reflected limited high-energy binding sites that cover only a fraction of the soot particle surface. Benzo[a]pyrene was further released from particles transported to the lymph nodes to approximately 10% of a monolayer coating, which may reflect the more reactive chemical environment provided by alveolar macrophages. Only 30% of the benzo[a]pyrene that remained bound to particles was present as parent compound. Based on these results, most of the adsorbed benzo[a]pyrene was readily released from diesel soot into the systemic circulation, mostly as parent benzo[a]pyrene (with only a minor portion as metabolites), while a small fraction was released from the soot at a much slower rate.

Ramesh et al. (2001a) conducted a toxicokinetic study of inhaled benzo[a]pyrene in F344 rats. The rats were exposed for a single 4-hour period via nose-only inhalation to aerosol concentrations of 0.1, 1.0, or 2.5 mg/m^3 of benzo[a]pyrene adsorbed to carbon black (CB)

particles. The particle size distribution was monodisperse and largely in the respirable range (the reported MMAD was 1.7 μm with a geometric standard deviation [SD] of 0.085 μm), suggesting that the results reflect absorption from the entire lung, since particles of the size distribution used here are expected to be deposited in all respiratory tract regions. Plasma and lung tissue concentrations of benzo[a]pyrene and metabolites were evaluated at 30, 60, 120, and 240 minutes postexposure. The plasma benzo[a]pyrene concentration peaked at 1 hour postexposure, and 65% of the inhaled aerosol was cleared from the lung at 2 hours postexposure, presumably at all dose levels, though this was not stated explicitly in the study. There was a significant difference in the time course of plasma levels between male and female rats. Female plasma benzo[a]pyrene levels were about one-third lower at 30 minutes, about 28% higher at 1 hour, and marginally lower than the male levels at later time points. This study is limited by the fact that administered aerosol concentration was reported instead of deposited dose, plasma samples were not collected during the 4-hour exposure period, and the study could not distinguish between absorption from the respiratory tract and mucociliary clearance followed by absorption from the gut.

Rapid absorption through the lungs was also shown following intratracheal administration of 1 μg/kg body weight [^{3}H]-benzo[a]pyrene dissolved in triethylene glycol in male Sprague-Dawley rats (Weyand and Bevan, 1986). Elimination of radiolabel from the lung was biphasic with half-lives of 5 and 116 minutes. The highest levels of liver radiolabel, equivalent to 21% of the administered dose, were found within 10 minutes after exposure, suggesting rapid absorption from the upper respiratory tract. The authors noted that it was unlikely that the appearance of radiolabel in organs was due to GI tract absorption after mucociliary clearance because the tracheal cannula was left in place for the entire experiment and levels of radioactivity in the stomach increased only slowly. Based on a comparison of benzo[a]pyrene concentrations in the blood following intratracheal administration versus intravenous (i.v.) dosing, the authors calculated the pulmonary bioavailability of benzo[a]pyrene as 57%. A significant degree of metabolism occurred in the lungs (as measured by the concentration of metabolites in lung), suggesting that benzo[a]pyrene absorption into the systemic circulation is limited by first-pass metabolism in the lung.

Petridou-Fischer et al. (1988) applied 10 μL aliquots of [^{14}C]-benzo[a]pyrene in a gelatin:saline solution over a 2-hour period to the ethmoid and maxillary nasal turbinates of two female cynomolgus monkeys and four male beagle dogs to assess differences in benzo[a]pyrene disposition in portions of the nose. The dose of benzo[a]pyrene was not provided, but using the total radioactivity administered (93 μCi per animal) and the specific activity of the radiolabeled benzo[a]pyrene that was used (39 mCi/mmol), a total administered dose of 0.6 mg/animal can be calculated. (98 μCi × 1 mmol/39 mCi × 1 mCi/1,000 μCi × 252 mg benzo[a]pyrene/mmol = 0.6 mg/animal.) No radioactivity was found in blood (collected over 2 hours in dogs and 3 hours in monkeys), and very little radioactivity was identified in excreta. Urinary excretion reached a

maximum of 0.69% of the administered dose in dogs and 0.07% in monkeys, while in feces a maximum of 6.42% of the administered dose was recovered in dogs and 1.17% in monkeys over a period of 48 hours. These results suggest only limited systemic absorption of benzo[a]pyrene from the nasal turbinates under the test conditions used. The results of this study are in contrast to more traditional inhalation or intratracheal instillation experiments, which have demonstrated significant absorption via other portions of the respiratory tract following inhalation or intratracheal instillation. Little radioactivity was recovered from the mucus, blood, or excreta, suggesting that benzo[a]pyrene and its metabolites were sequestered in the nasal tissues.

Several studies demonstrated rapid desorption of benzo[a]pyrene bound to particles. However, the adsorption matrix can impact the bioavailability of inhaled benzo[a]pyrene. Leung et al. (1988) reported that benzo[a]pyrene adsorbed on diesel soot particles and suspended in buffer was transferred to microsomes in vitro far less efficiently than free benzo[a]pyrene. The authors concluded that benzo[a]pyrene transfer to microsomes depends on the lipid content of the particles rather than on protein in the medium. Microsomes may enhance the slow transfer of benzo[a]pyrene from particles, which may become an important source of exposure with long retention times. No metabolism of benzo[a]pyrene adsorbed to particles was detected in this study, suggesting that particle-bound benzo[a]pyrene serves as a slow release source of benzo[a]pyrene to the respiratory tract. These findings are consistent with the report by Gerde et al. (2001) that a slow-release phase follows the initial rapid desorption of benzo[a]pyrene from diesel soot. Furthermore, Gerde and Scholander (1989) found in an in vitro study that the release from carrier particles was the rate-limiting step in the absorption of benzo[a]pyrene by the bronchial epithelium.

The absorption of inhaled benzo[a]pyrene may also be affected by the size of the particle to which it is adsorbed. Elimination of benzo[a]pyrene from the lungs of mice was investigated following intratracheal administration of benzo[a]pyrene crystals (0.5–1.0 μm in size) or benzo[a]pyrene-coated carbon particles (0.5–1.0 μm or 15–30 μm) (Creasia et al., 1976). Approximately 50% of the benzo[a]pyrene crystals were cleared within 1.5 hours and >95% were cleared within 24 hours of treatment. In contrast, benzo[a]pyrene clearance was approximately 50% after 36 hours following exposure to benzo[a]pyrene absorbed onto small carbon particles. With larger carbon particles, desorption was even slower, requiring 4–5 days to release 50% of bound benzo[a]pyrene. The difference in absorption rate for small versus large carbon particles suggests an influence of particle area surface on the rate of desorption.

The deposition, retention, and bioavailability of benzo[a]pyrene as a pure aerosol or adsorbed onto gallium oxide ($^{67}Ga_2O_3$) particles was investigated by Sun et al. (1982). Male and female F344 rats were exposed nose-only for 30 minutes to atmospheres containing 0.6 mg/m^3 [^{3}H]-benzo[a]pyrene absorbed onto $^{67}Ga_2O_3$ or to 1.0 μg/L neat (i.e., the pure chemical) [^{3}H]-benzo[a]pyrene (MMADs were reported as approximately 0.1 μm in both cases). Radiolabel was detected in the esophagus, stomach, small and large intestines, cecum, liver, kidney and blood;

however, the time to reach peak tissue concentrations differed considerably between the two exposure regimens. Based on the total amount excreted, 22% of the inhaled dose of benzo[a]pyrene on Ga_2O_3 was released over 16 days, but only 10% of the inhaled dose of pure benzo[a]pyrene was released. Since the amount excreted can reflect differences in absorption, i.e., uptake via pulmonary epithelium vs. ingestion of cleared particles, and hence alternative tissue distribution and metabolism, this result cannot be used quantitatively to estimate bioavailability of inhaled benzo[a]pyrene. The study established that benzo[a]pyrene adsorbed to particles had a longer respiratory tract retention period. For benzo[a]pyrene coated on Ga_2O_3, 1 day was required to clear 90% of the [^{3}H]-benzo[a]pyrene lung and trachea burdens that were present 30 minutes after exposure. In contrast, only 1.5 and 4 hours were required to clear 90% of pure benzo[a]pyrene burdens from the lung and trachea, respectively. A different effect was observed in the nose, where clearance of 90% benzo[a]pyrene coated on Ga_2O_3 required 7 hours as compared to 20 hours for pure benzo[a]pyrene aerosol. Thereafter, clearance curves for particle-bound and neat benzo[a]pyrene were similar; the authors attributed this to the absence of a mucociliary escalator in the nose. Inhalation of benzo[a]pyrene on Ga_2O_3 also increased the dose of the compound and its metabolites to the stomach, liver, and kidneys, which the authors suggest may have resulted from mucociliary clearance with subsequent ingestion.

benzo[a]pyrene absorption from the respiratory tract may also be affected by the characteristics of the vehicle. Following intratracheal administration in hydrophilic triethylene glycol, approximately 70% of the benzo[a]pyrene administered was excreted within 6 hours by male Sprague-Dawley rats (Bevan and Ulman, 1991). In contrast, 58.4% and 56.2% of administered benzo[a]pyrene were excreted within a 6-hour period when the lipophilic solvents ethyl laurate and tricaprylin, respectively, were the vehicles.

Pregnant Wistar rats were exposed head-only for 95 minutes on gestational day (GD) 17 to 200, 350, 500, 650, or 800 mg/m^3 of a [^{3}H]-benzo[a]pyrene microcondensate generated from heated pure material (Withey et al., 1993). Particle sizes ranged from 0.61 to 0.88 μm MMAD. Immediately following exposure (no time estimate was provided), blood radiolabel concentrations varied >eightfold over the fourfold dose range (2.66 ± 0.51 vs. 21.96 ± 1.37 μg/g at lowest and highest dose, respectively). Six hours after exposure, blood radiolabel concentrations had decreased two- to fourfold from the earlier observation but retained a >10-fold difference over the dose range (0.74 ± 0.12 vs. 9.56 ± 2.1 μg/g). Therefore, the difference in the ratio of blood levels to dose range was not likely due to an initial rapid phase of benzo[a]pyrene absorption only at high exposures. However, the study authors did not provide an explanation for this finding. Radiolabel was detected at 0 and 6 hours in all maternal tissues and in fetuses examined, indicating that systemic absorption had occurred.

Hood et al. (2000) exposed male and timed pregnant female Sprague-Dawley rats to 100 μg/m^3 benzo[a]pyrene-CB aerosol (nose only for 4 hours on GD 15), and collected blood was analyzed at 30, 60, 120, 180, and 240 minutes for concentrations of benzo[a]pyrene. The

benzo[a]pyrene aerosol particle distribution was trimodal with a significant portion of particles <1 μm in size. The particle size distribution was expected to result in deposition across all regions of the respiratory tract, including the pulmonary region. Following exposure, blood benzo[a]pyrene levels peaked at 30 minutes (the first time point reported), with females exhibiting approximately a 1.7-fold higher peak concentration than males. At later time points, the female benzo[a]pyrene blood concentrations were similar to those observed in males. By 240 minutes postexposure benzo[a]pyrene in blood had diminished to <5% of the peak level. The authors did not report a mass balance to allow for the determination of the percentage of dose that was absorbed. Furthermore, benzo[a]pyrene metabolites were not measured. Nevertheless, the appearance of benzo[a]pyrene in the blood at the earliest time point measured is consistent with the conclusion that benzo[a]pyrene is rapidly absorbed from the respiratory tract. Although the peak came earlier than in the Ramesh et al. (2001a) study, the same trend of gender differences was observed, with females displaying higher peak blood levels than males.

In summary, although quantitative estimates of human lung absorption are not available, existing toxicity and biological monitoring studies suggest that benzo[a]pyrene is absorbed in the respiratory tract, albeit rather poorly, following inhalation exposure of humans. The evidence suggests, however, that in humans it is difficult to establish a relationship between benzo[a]pyrene exposure and urinary excretion of its metabolites due to large interindividual variation, most likely the result of different genetic makeups and varying background exposures. Numerous controlled studies indicate that benzo[a]pyrene is well absorbed in animals following inhalation or intratracheal instillation. In general, the animal studies show that benzo[a]pyrene is absorbed rapidly (within minutes) and extensively. The rate of absorption varies across regions of the respiratory tract, with more rapid absorption in the pulmonary regions and slower absorption in the conducting airways and nose. In some studies in rats, blood benzo[a]pyrene peak levels at early exposure time points differed between females and males, but leveled out at later time points. Quantitative estimates of benzo[a]pyrene absorption from the respiratory tract are difficult to derive because the contribution of absorption from the GI tract following mucociliary clearance and metabolism of benzo[a]pyrene in the respiratory tract itself are often difficult to determine. Another complication in interpreting these studies is that benzo[a]pyrene absorption from the lung depends on the characteristics of the exposure vehicle or the nature of the particle to which benzo[a]pyrene is adsorbed. In general the data indicate that benzo[a]pyrene is released to a greater extent from hydrophilic vehicles than from lipophilic solvents; particle-bound benzo[a]pyrene is released more slowly than the neat compound; and desorption from large particles is slower than from smaller particles. Because much of the environmental benzo[a]pyrene is adsorbed onto particles of other materials, the effect of the carrier particle is highly relevant to environmental exposures.

3.1.2. Oral Exposure

3.1.2.1. *Oral Exposure in Humans*

In a study with eight volunteers who ingested broiled meat containing approximately 8.6 μg of benzo[a]pyrene, the concentration of benzo[a]pyrene in feces was below detection limits (<0.1 μg/person) (Hecht et al., 1979). Although the analytical method used in this study assessed only parent compound, not fecal metabolites, the result can be interpreted as indicating that the ingested benzo[a]pyrene was absorbed completely from the GI tract. In addition, studies were conducted to assess DNA adduct levels or benzo[a]pyrene metabolites in humans exposed to PAHs by the oral route. In general, these human dietary studies are not adequate to develop quantitative estimates of oral bioavailability; in one case no measurable relationship between benzo[a]pyrene intake and internal dose measure was found (Scherer et al., 2000).

3.1.2.2. *Oral Exposure in Animals*

The bioavailability of benzo[a]pyrene was evaluated in F344 rats dosed by gavage with 100 mg/kg benzo[a]pyrene dissolved in peanut oil and sacrificed 0, 0.5, 1.0, 2.0, 4.0, 8.0, 24, 48, or 72 hours after dosing (Ramesh et al., 2001b). Blood, liver, reproductive tissues, urine and feces were analyzed for benzo[a]pyrene and metabolites. Plasma benzo[a]pyrene levels peaked at 8 hours and fecal levels at 2.4 hours postexposure. Lipophilic metabolites of benzo[a]pyrene peaked at 2 hours in liver, 8 hours in feces, 24 hours in blood and lung, and 48 hours postexposure in urine. Water-soluble metabolites reached their maxima at 4 hours in liver and lung, 8 hours in blood and feces, and 48 hours in urine. Some of the disposition patterns displayed minor peaks at earlier time points. Based on comparison with plasma levels following i.v. injection, the oral bioavailability was estimated by the authors as approximately 40%; however, the details of this determination were not presented.

Foth et al. (1988) conducted several experiments to determine the oral bioavailability of benzo[a]pyrene. In male Sprague Dawley rats, the areas under the blood concentration-time curve (AUCs) following oral versus i.v. bolus doses of benzo[a]pyrene (dissolved in Krebs Ringer buffer with 4% bovine serum albumin) were compared. The oral bioavailability was estimated as 7.8 and 11.5% for doses of 3.2 and 4.0 nmol [^{3}H]-benzo[a]pyrene per rat (approximately 1.8–2.7 and 2.2–3.4 μg/kg, respectively, calculated as 3.2–4 nmol/rat × 0.252 μg/nmol ÷ (0.30–0.46) kg body weight as stated by the authors). This result may reflect limited systemic absorption of benzo[a]pyrene at low doses. In contrast to this result, analysis of benzo[a]pyrene concentrations in arterial blood and bile after continuous intraduodenal infusion of radiolabeled benzo[a]pyrene (in the same vehicle as above) showed that approximately 40% of the administered dose was absorbed by the duodenum over a 240-minute period (Foth et al., 1988). In a similar experimental design, bile- and duodenum-cannulated male Sprague-Dawley rats were given [^{3}H]-benzo[a]pyrene in corn oil with and without exogenous bile (Rahman et al., 1986). The absorption of benzo[a]pyrene was estimated from the cumulative recovery of radioactivity in the bile and urine over 24 hours. This study showed that absorption of

benzo[a]pyrene was enhanced by bile as absorption in the presence of endogenous bile only was 22.9% of that when exogenous bile was administered.

Cavret et al. (2003) reported that absorption of benzo[a]pyrene (as measured by the percentage of orally administered radioactivity appearing in the portal blood in 24 hours) was 30.5% in pigs fed 1 L of 4% fat milk containing 235 μg/L [^{14}C]-benzo[a]pyrene (this corresponds to an oral dose of approximately 6 μg/kg based on the reported body weight of 40 kg). The level of radioactivity increased rapidly between 1 and 6 hours, with maximum uptake between 3 and 6 hours after dosing.

In male F344 rats administered [^{14}C]-benzo[a]pyrene in peanut oil via gavage at doses from 0.04 to 4.0 μmol/rat (approximately 0.03–0.04 mg/kg and 3.4–4 mg/kg, respectively, calculated as 0.04–4 μmol/rat × 0.252 mg/μmol ÷ 0.25–0.30 kg body weight as stated by the authors) approximately 85% of the radiolabel was recovered in the feces and 1–3% in the urine after 168 hours (Hecht et al., 1979). Because radiolabel in feces may represent unabsorbed as well as absorbed parent compound that is subsequently eliminated via biliary excretion, bioavailability cannot be estimated from this study. However, the percent of radioactivity recovered as parent benzo[a]pyrene was small (ranging from 6 to 13% of the administered radioactivity), suggesting that a minimum of 73% of the administered dose was absorbed (i.e., total in urine + total in feces – feces as benzo[a]pyrene).

Dietary matrices may have an impact on the absorption of benzo[a]pyrene from the GI tract. For example, intestinal absorption of benzo[a]pyrene was enhanced in rats when the compound was solubilized in lipophilic compounds such as triolein, soybean oil, and high-fat diets, as compared with fiber- or protein-rich diets (O'Neill et al., 1991; Kawamura et al., 1988). This may be relevant for the absorption of benzo[a]pyrene from charbroiled meats and other fatty foods.

O'Neill et al. (1990) assessed the intestinal absorption of [^{14}C]-benzo[a]pyrene given to rats by gavage in olive oil with regular rat chow or with low-fat diets high or low in either fiber or beef protein (used to represent human diets). Benzo[a]pyrene and its metabolites were recovered from feces, where they had been trapped by microcapsules given by gavage 2 hours prior to benzo[a]pyrene. Benzo[a]pyrene was absorbed from rat chow differently than from representative human diets. Dietary fiber decreased the availability of benzo[a]pyrene, as evidenced by the appearance of lower metabolite amounts in the GI tract, while the beef-enriched diet affected absorption to result in increased formation of 1,6- and 3,6-benzo[a]pyrene diones. Urinary excretion of benzo[a]pyrene was decreased in rats given the high fiber diet but not the beef-enriched diet. The total amount of benzo[a]pyrene excreted in feces and the feces/urine ratio were increased by the high-fiber diet but not by the beef-enriched diet. These results indicated that bioavailability of benzo[a]pyrene from the GI tract is affected by the type of diet and that bioavailability studies in animals, using typical laboratory animal chow, may not appropriately model the situation with varied human diets.

The potential influence of diet on PAH bioavailability was investigated also by Wu et al. (1994). Female mice were fed either gel or powder diets containing coal tar with detectable levels of benzo[a]pyrene, Py, and other PAHs. Urine samples were collected on the first, seventh, and fourteenth day of treatment. Measurement of the amount of the Py metabolite 1-OH-Py in the urine showed that the diet matrix did not influence the bioavailability of the PAHs. Stavric and Klassen (1994) administered radiolabeled benzo[a]pyrene dissolved in various vehicles (water, corn oil, liquid paraffin, or 50% ethanol) by gavage and monitored intestinal absorption in bile-cannulated rats. The animals were fed diets with or without added carbon particles and typical food components, such as quercetin or chlorogenic acid. They observed that aqueous vehicles, quercetin, chlorogenic acid, or carbon particles reduced biliary excretion of benzo[a]pyrene, while lipid media such as corn oil increased it strongly. The authors postulated that absorption of benzo[a]pyrene from food was affected by its solubility in the vehicle, by physical adsorption, and/or by adduction of benzo[a]pyrene to certain food ingredients. On the other hand, Mirvish et al. (1981) observed that varying the corn oil content of a synthetic diet containing 100 μg/g benzo[a]pyrene had little influence on fecal excretion of unmetabolized benzo[a]pyrene. However, addition of 5% wheat bran to the synthetic diet, or using standard lab chow, increased the fecal excretion of parent compound 13-fold. They suggested that the insoluble dietary fiber sequestered benzo[a]pyrene in the GI tract.

In summary, absorption of ingested benzo[a]pyrene was demonstrated qualitatively in exposed humans by the excretion of metabolites or the presence of DNA adducts. However, these studies are not sufficient to determine the rate and extent of absorption from the GI tract in humans. Animal studies have produced variable results, in part due to different study designs. Standard approaches in animal studies suggest that the oral bioavailability ranges from 10 to 40%. However, some studies have indicated that the standard diets of laboratory animals may not model the human oral exposure to benzo[a]pyrene appropriately. No data on species or gender-based differences in absorption were identified.

3.1.3. Dermal Exposure

Several studies in humans and experimental animals have investigated the dermal absorption of benzo[a]pyrene. Benzo[a]pyrene metabolites or DNA adducts were measured in humans exposed dermally to benzo[a]pyrene-containing mixtures in biological monitoring studies. These studies provide only qualitative support for assessing the rate and degree of dermal absorption of benzo[a]pyrene through human skin. However, some studies provide quantitative information on the degree of benzo[a]pyrene absorption through the skin in volunteers or in explanted viable skin samples from tissue donors.

3.1.3.1. *Dermal Exposure in Humans*

Van Rooij et al. (1993) demonstrated differences in absorption rates of PAHs in coal tar ointment at various skin sites in volunteers. A dose of 2.5 mg/cm^2 of coal tar ointment, which consisted of 10% coal tar in a vehicle of zinc oxide paste, was applied to 24 cm^2 of skin at the forehead, shoulder, volar forearm, palmar side of the hand, groin, or ankle and allowed to stand for 45 minutes before removing the residue. Dermal absorption of PAH was determined through measuring the disappearance of PAH fluorescence from the skin surface. Absorption rate constants ranged from 0.036 to 0.135/hour, across application sites, suggesting that 20–56% of the dose would be absorbed within 6 hours. A 69% difference in dermal absorption rates between anatomical sites was reported, while only a 7% difference between individual volunteers was observed. The skin on the shoulder absorbed the greatest dose of PAH, followed by the forehead, forearm, and groin, with the ankles and palms absorbing the least amount. There was, however, no correlation between anatomical site of exposure and excretion of 1-OH-Py in urine. The authors estimated that 0.3–1.4% of the PAH dose (assessed as 1-OH-Py) became systemically available, although systemic measurement of benzo[a]pyrene or its metabolites was not reported.

Quantitative comparisons of dermal penetration of human skin and other mammalian species have been conducted. Kao et al. (1985) compared benzo[a]pyrene permeation through skin in short-term organ cultures using skin harvested from mice, rats, rabbits, guinea pigs, marmosets, and human donors. Two test systems were used, a dynamic one where skin samples were held on top of chambers flushed with fresh organ culture medium at a constant rate and a static one where skin samples were incubated on a filter disk in a culture dish over culture medium at 36°C. The culture medium was a modified minimal essential medium with Earle's salts and 10% fetal calf serum. In addition, all experiments were conducted with fresh, metabolically viable skin and with metabolically nonviable skin (previously frozen or poisoned with cyanide). Using the static system, [^{3}H]-benzo[a]pyrene dissolved in acetone was applied to full-thickness skin samples (2.5 μg/cm^2) and medium collected after 24 hours to be assayed for [^{3}H]-benzo[a]pyrene and metabolites. The overall penetration rate of benzo[a]pyrene was about 2.6% of the dose in 24 hours from viable human skin and only about 0.5% from nonviable skin. After 24 hours penetration through viable skin, 52% of the radioactivity in medium consisted of water-soluble benzo[a]pyrene metabolites and 18% was parent compound. By contrast, after penetrating through nonviable skin, 90% of the recovered radioactivity was parent compound, and the ratio of water- to lipid-soluble metabolites was much lower. Results in marmoset, rat, and rabbit were similar. Skin from the mouse allowed more than 10% of the dose to penetrate, while that of guinea pig let only a negligible percentage of the dose penetrate. In all species, metabolism was an important determinant of permeation, with very low rates observed in nonviable skin.

Using the dynamic test system, Kao et al. (1985) studied the influence of dermal metabolism on the rate of penetration of benzo[a]pyrene in more detail. Comparing rat to mouse

skin they again found a 10-fold difference in the amount penetrating in 16 hours through viable skin, approximately 0.7% of the dose in rat skin vs. approximately 7% in murine skin, but most of this species difference disappeared when previously frozen skin was used or potassium cyanide was added to the medium to destroy metabolic capability. An additional factor was responsiveness to aryl hydrocarbon (Ah)-receptor agonists: dermal penetration in responsive mice given an inducing dose of 3-methylcholanthrene (3-MC) was more than twice that in noninduced Ah-responsive mice, and the latter was similar to nonresponsive mice (see Section 3.3 for further discussion of Ah-responsiveness). These results show that functional enzyme systems facilitate penetration of benzo[a]pyrene or its metabolites.

Wester et al. (1990) compared skin penetration of [^{14}C]-benzo[a]pyrene dissolved in acetone versus benzo[a]pyrene adsorbed onto soil using skin from human cadavers. Skin was dermatomed to 500 μm and stored in medium at 4°C to preserve viability. The authors used a dynamic system with undiluted human serum as the receptor fluid and 24-hour exposure. Radiolabeled benzo[a]pyrene was applied to the skin, and concentrations of radioactivity in the receptor fluid, skin, and surface skin wash were determined. For benzo[a]pyrene applied in acetone, 23.7% of the applied dose was recovered in the skin, 0.09% was recovered in the receptor fluid, and 53.0% was recovered in the surface wash. The amount of radiolabel recovered from exposure to benzo[a]pyrene in soil was 1.4% of the dose in skin and 0.01% in receptor fluid, indicating that the exposure matrix greatly impacted dermal absorption. The considerable difference in penetration through human skin observed in this study (only 0.09% of the dose per 24 hours) and that of Kao et al. (1985) (2.6% of the dose) is at least in part a result of differences in experimental design, such as full thickness (approximately 1.5 mm) vs. dermatomed (0.5 mm) skin and synthetic receptor fluid with 10% serum vs. pure human serum. Together, these studies suggest that benzo[a]pyrene is absorbed into human skin but does not penetrate through the skin rapidly.

In a second part of the same study, Wester et al. (1990) also evaluated the dermal penetration of benzo[a]pyrene in female rhesus monkeys in vivo. Radiolabeled benzo[a]pyrene was applied to a 12 cm^2 area of the abdominal skin that was protected by a nonocclusive cover. The material was maintained on the skin for 24 hours, after which time the skin area was washed. Urine was collected from the animals during the initial 24-hour exposure period and 6 days after. Skin penetration was determined as the ratio of urinary radiolabel for topically exposed animals compared to monkeys administered the same dose by i.v. injection. When benzo[a]pyrene was dissolved in acetone, 51% of the applied dose was absorbed as compared to 13.2% when benzo[a]pyrene was applied in soil. These results further stress the fact that the dermal absorption of benzo[a]pyrene depends on the vehicle of administration (pure substance vs. contaminated environmental material).

Also using human tissues, van der Bijl and van Eyk (1999) compared the mean flux of benzo[a]pyrene across vaginal and buccal mucosa samples from human donors. No significant

difference between the two tissue types was observed, and steady-state flux was very low, approximately 0.01% of the dose per cm^2/minute (400 cpm/cm^2/minute in a figure in the paper, or 4,000 dpm [assuming 10% [^{3}H]-liquid scintillation counting efficiency] out of a 17.33 μCi dose, or 3.8×10^7 dpm, equals about 0.01% of the dose/cm^2/minute). These results indicate that benzo[a]pyrene can be absorbed from and metabolized by the female reproductive tract. However, the results cannot be compared to those obtained with human skin. First, there was the difference in absorbing surface, namely, the quasi-dry, mainly lipophilic stratum corneum vs. fresh epithelium. Second, there were also fundamental differences in experimental design: although van der Bijl and van Eyk (1999) used a dynamic system, the tissues had been frozen previously, benzo[a]pyrene was administered in aqueous solution in a large compartment on top of the tissue sample, receptor fluid was a simple buffer, and incubation was performed at 20°C.

Potter et al. (1999) also demonstrated that the exposure matrix can affect the dermal absorption of benzo[a]pyrene in mice in vivo as well as in human skin in vitro. In particular, the uptake of benzo[a]pyrene decreased as the viscosity of the oil product used as vehicle increased. Although mouse skin absorbed more radiolabel than human skin, the trend of decreasing absorption with increasing viscosity found in the mouse was also present in human skin. Vehicles tested included mineral oils (32–198 centi-Stoke [cSt], measure of viscosity), residual aromatic extracts (5,160–5,400 cSt), and bitumens ($0.65–69 \times 10^6$ cSt). Most likely, differences in diffusion coefficients for benzo[a]pyrene in the various vehicles would adequately describe the differences in dermal absorption.

3.1.3.2. *Dermal Exposure in Animals*

Yang et al. (1989) compared dermal absorption of benzo[a]pyrene in crude oil adsorbed to soil (particle size of 150 μm or less) versus benzo[a]pyrene in the crude oil alone. In the in vivo portion of the study, test materials spiked with 100 ppm [^{3}H]-benzo[a]pyrene were placed over a 7-cm^2 portion of shaved dorsal skin of female Sprague-Dawley rats. Urine and feces were collected daily over 4 days, and at the end of the experiment animals were sacrificed and radioactivity in tissues was determined. The total percent of the absorbed dose recovered in excreta was greater for benzo[a]pyrene in crude oil (35.3%) than oil adsorbed to soil (approximately 9.2%). While the latter number refers to soil applied to the skin as a monolayer, when six times the amount of soil, and thus a sixfold dose, was applied, the absolute amount of benzo[a]pyrene absorbed remained virtually the same. The authors also found that using viable skin samples in vitro gave almost identical results to those obtained in vivo.

Ng et al. (1992) examined the percutaneous absorption of radiolabeled benzo[a]pyrene in the hairless guinea pig. A single dose of 28 μg benzo[a]pyrene dissolved in 50 μL acetone was applied to 4 cm^2 of dorsal skin and covered with a protective pad for 24 hours. Urine and feces were collected at 6 and 12 hours and then daily for the next 7 days postexposure. Approximately 34% of the radiolabel was absorbed and eliminated in 24 hours. Most excretion had occurred by

day 3 and then continued at a lower rate to reach about 73% by day 7. Benzo[a]pyrene in the skin wash accounted for 11% of the applied dose. Comparison with the amount excreted following intramuscular injection (which reached about 85% in 7 days) suggested that the dermal bioavailability of benzo[a]pyrene was high (about 85%). Ng et al. (1992) noted that, in an in vitro study, only metabolites were present in the receptor solution, indicating that metabolism in the skin preceded systemic absorption. This is consistent with the findings of Kao et al. (1985) that dermal absorption may be aided by a high metabolic capacity of the skin but leaves open the question how much of the reactive metabolites escape from the dermal compartment.

In an earlier study, Kao et al. (1984) used viable mouse skin to study the dose dependence of dermal absorption of benzo[a]pyrene. They administered 1, 2, 4, and 6 μg benzo[a]pyrene per cm^2 full-thickness, shaved skin incubated at 36°C for 24 hours. The percentage of absorbed benzo[a]pyrene dose decreased with increasing dose: 24, 17, 12, and 7% with 1, 2, 4, and 6 $\mu g/cm^2$, respectively. The authors also found that, with doses of 4 and 6 $\mu g/cm^2$, the total amount of penetrated benzo[a]pyrene-derived radioactivity remained similar, indicating saturation of the skin's absorption and metabolic capacity. When the donor mice were pretreated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to induce cytochrome P450 (CYP450) enzymes in the skin, amounts of penetrated benzo[a]pyrene increased to 38, 33, 30, and 19% of the dose, respectively. In addition to the increased rate of absorption, a shift in metabolic pattern was observed among the benzo[a]pyrene metabolites in the medium. CYP450 induction caused a large increase in metabolite conjugates at the expense of polar metabolites and benzo[a]pyrene diols, and a strong increase in covalent binding of benzo[a]pyrene to skin DNA.

Kao et al. (1988) also investigated the influence of skin hair on the rate of benzo[a]pyrene penetration. Using the same dynamic system, six strains of haired and two strains of hairless mice were administered a dose of 1 $\mu g/cm^2$ benzo[a]pyrene. After 16 hours of incubation, between 4.4 and 9.4% of the dose had penetrated through viable skin of haired mice but only 2–2.9% of the dose through skin of hairless mice. Since the authors found that permeation of testosterone, which was used as a comparison compound with similar physiochemical properties as benzo[a]pyrene, was the same in haired and hairless mice, 70% of the dose, they concluded that skin appendages have considerable metabolic capacity for benzo[a]pyrene. Extending these findings to humans implies that in the assessment of dermal absorption of benzo[a]pyrene, presence of dense hair may play an additional role.

Morse and Carlson (1985) compared in vivo dermal absorption of benzo[a]pyrene in BALB/c and SENCAR mice to find an explanation for the higher sensitivity of the latter strain toward benzo[a]pyrene-induced skin tumors. Animals were treated with 50 mg/kg benzo[a]pyrene dissolved in acetone. Levels of benzo[a]pyrene in skin of BALB/c mice did not reach higher peak levels, but stayed up to three times higher than in SENCAR mice between 6 and 24 hours postexposure. The levels of benzo[a]pyrene-derived radioactivity in liver, lung,

and stomach also peaked at comparable levels, but stayed at up to twice the levels in SENCAR mice between 6 and 48 hours posttreatment, compared with BALB/c mice. There were no strain differences in these tissues when benzo[a]pyrene was administered orally. The metabolic capacity of the skin was assessed by measuring DNA binding of benzo[a]pyrene in skin. Six hours after exposure DNA-binding of benzo[a]pyrene was higher in SENCAR than in BALB/c mice, but by 24 hours after topical administrations, DNA adducts were higher in BALB/c than in SENCAR mice, possibly indicating differences in metabolism. Kao et al. (1988) had included the same two strains of mice in one of their studies and found comparable results, with 9.2% of the dose penetrating over 16 hours in BALB/c and 4.4% in SENCAR mice. However, the results of Morse and Carlson (1985) did not provide the expected toxicokinetic explanation for the difference in skin tumor sensitivity between the two mouse strains.

In summary, due to the use of coal tar products for medicinal purposes, quantitative data on dermal absorption of benzo[a]pyrene in humans are more abundant than for other routes of exposure. The animal-to-human differences are significant. Generally, mice have greater absorption than humans, followed by rats and rabbits. The 24-hour penetration values range from 1 to 3% in viable human skin; much greater amounts of material are absorbed into the skin but do not readily permeate through it. Dermal absorption of PAH is strongly dependent on anatomical site (69% difference across six sites), while inter-individual variation is much smaller (7% difference across nine volunteers). Furthermore, dermal absorption is highly dependent on underlying metabolic activity of the skin. The vehicle of exposure also impacts dermal absorption, with vehicles that absorb benzo[a]pyrene, such as soil, or vehicles with low diffusion coefficients (high viscosity) decreasing the rate and degree of absorption.

3.1.4. Other Types of Exposure

Ewing et al. (2006) used isolated, perfused rat lungs and delivered benzo[a]pyrene coated onto silica carrier particles (average size: 3.5 μm) at mean doses of 2.2, 36, and 8,400 ng to each lung within <1 minute. Perfusate was collected for 77 minutes thereafter. Lungs and perfusates were analyzed for benzo[a]pyrene and metabolites. Absorption was strongly dose-dependent: at the low and mid exposure levels benzo[a]pyrene concentration increased rapidly in the perfusate to reach a maximum within <5 minutes, then decreased over the remaining observation period. At the high dose, benzo[a]pyrene in the perfusate reached the maximum at about 30 minutes after exposure and stayed at a constant level from there on, i.e., the absorption of benzo[a]pyrene proceeded at zero order until all deposited solid benzo[a]pyrene was dissolved. The mass balances for benzo[a]pyrene equivalents in lung vs. perfusate were lung ca. one-third perfusate at the low dose, lung = perfusate at the mid dose, and lung about twice that of perfusate at the high dose. At the low exposure level metabolism was apparently able to convert most of the parent compound, while at the highest exposure level most of the absorbed benzo[a]pyrene remained unmetabolized even at the end of the experiment. The results suggest that, at low doses (2.2

ng/lung) benzo[a]pyrene is absorbed very efficiently in rat lung (two-thirds absorbed in 77 minutes), while at higher doses the rate of absorption decreases markedly, either because of diffusion limitation, or by saturation of local metabolism.

3.2. DISTRIBUTION

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

3.2.1. Inhalation Exposure

Numerous studies evaluated the disposition of benzo[a]pyrene or its metabolites following inhalation or instillation in the respiratory tract in animals. The distribution of benzo[a]pyrene following inhalation exposure was shown to be similar in various species. Male and female F344 rats were exposed nose-only for 30 minutes to atmospheres containing 0.6 mg/m^3 [^{3}H]-benzo[a]pyrene adsorbed onto $^{67}Ga_2O_3$ or to 1.0 mg/m^3 neat [^{3}H]-benzo[a]pyrene (Sun et al., 1982). There were qualitative differences in time and amount of absorption between the exposure regimens. With either exposure regimen, high tissue levels of radiolabel were found in the small and large intestines and cecum, followed by liver, kidney, and blood. Levels in the upper GI tract (esophagus and stomach) differed, with significant levels of radioactivity in the stomach following exposure to [^{3}H]-benzo[a]pyrene adsorbed onto $^{67}Ga_2O_3$ but only minimal levels in the stomach following exposure to neat benzo[a]pyrene aerosol. Twelve hours after exposure, the highest tissue concentrations of radiolabel were found in the lower GI tract for both regimes. The differences in distribution of radiolabel likely reflect the relative contribution of two mechanisms for the delivery of benzo[a]pyrene-related radioactivity: for particle-adsorbed benzo[a]pyrene, mucociliary clearance followed by ingestion may be a significant factor, while aerosolized benzo[a]pyrene is likely to be more readily absorbed in the respiratory tract only.

Other inhalation studies measured levels of administered radioactivity as benzo[a]pyrene parent compound or metabolites in whole blood, plasma or lung, but not in any other tissues (Ramesh et al., 2001a; Gerde et al., 1993a, b). The rapid appearance of benzo[a]pyrene and metabolites in the blood is consistent with the conclusion that benzo[a]pyrene is readily bioavailable following exposure by the inhalation route. The degree to which absorbed benzo[a]pyrene or metabolites was delivered to target tissues was not determined in these studies.

Weyand and Bevan (1986) examined benzo[a]pyrene disposition and metabolism in male Sprague-Dawley rats following intratracheal instillation of 1 μg/kg [^{3}H]-benzo[a]pyrene dissolved in triethylene glycol. The amount of radioactivity in various organs was determined at timed intervals between 5 and 360 minutes after dosing. Peak levels of radioactivity as the percentage of the administered dose per organ/tissue as well as time profiles were as follows:

- Early peak levels: lungs (59.5% at 5 minutes, declining 10-fold over 360 minutes); blood (3–4% between 5 and 15 minutes, declining only twofold by 360 minutes); spleen (0.5% at 5 minutes, declining very slowly); liver (20.8% at 10 minutes, gradually declining); heart/thymus (1.6% at 10 minutes, barely declining from 0.5% after 30 minutes).

- Medium term peak levels: carcass (27.1% at 60 minutes, mostly stable at around approximately 25% between 10 and 90 minutes and at approximately around 22% thereafter); kidney (2.4% at 90 minutes but rather stable at approximately around 2% between 10 and 360 minutes); testes (1.3% at 90 minutes but rather stable at approximately around 1% between 15 and 360 minutes).

- Late peak levels: stomach (6.9% at 120 minutes, decreasing slowly); intestinal contents (44.7% at 360 minutes, increasing over time period); intestines (14.9% at 360 minutes, continually increasing).

These profiles are consistent with rapid uptake and delivery of benzo[a]pyrene to well-perfused tissues, followed by clearance from the tissues via metabolism and excretion (particularly in the feces). The profile of radioactivity appearing in the GI tract suggests partial removal of benzo[a]pyrene from the lungs via mucociliary escalator. Total recovery of radiolabel at 5 minutes was 96% of the dose and 104% at 360 minutes, indicating complete recovery from the tissues.

Weyand and Bevan (1986) also investigated benzo[a]pyrene metabolites in tissues. Quinones were at highest concentrations in both lung and liver 5 minutes after instillation, accounting for 12 and 7% of radiolabeled extractable material, respectively. Benzo[a]pyrene disposition was also investigated in male rats with and without biliary cannulas. Distribution patterns among organs were similar, though the amount excreted in bile and intestinal contents was 77% of the administered dose in cannulated rats and 53% in animals that were not cannulated. The intestinal contents carried lower fractions of the administered dose as thioether and glucuronic acid conjugates than the bile, indicating enterohepatic recirculation of benzo[a]pyrene metabolites.

A comparative intratracheal instillation study with Sprague-Dawley rats, Gunn rats, guinea pigs, and hamsters gave results (Weyand and Bevan, 1987) qualitatively similar to those reported by Weyand and Bevan (1986). Doses of 0.16 or 350 μg [^{3}H]-benzo[a]pyrene per animal were administered intratracheally, and the distribution of benzo[a]pyrene-derived radioactivity was determined in various tissues. Relative amounts of radiolabel recovered per gram of tissue

at 6 hours were: lung (1.7%) > kidney (0.76%) ≈ liver (0.67%) > testes (0.21%) = spleen (0.21%) = heart (0.21%) ≈ GI tract (0.19%) > stomach (0.13%) > carcass (0.071%). This pattern of distribution was qualitatively similar among all species tested at both doses, but the relative values differed between species. For example, in Sprague-Dawley rats the liver burden represented 0.67% of the recovered radiolabel/gram of tissue, while this value was 2.16% for hamsters, 0.35% for guinea pigs, and 1.02% for Gunn rats after the 0.16 μg/animal dose. The study did not include the intestine or its contents, where likely the majority of radioactivity was. When benzo[a]pyrene was instilled intratracheally into mice, Schnizlein et al. (1987) found that radioactivity in the lung declined steadily throughout the 144-hour investigation period, while levels in liver, spleen, and intestine peaked at 8 hours after dosing, decreased slowly until 24 hours, and declined rapidly thereafter. It was also noted that as the lung burden of the radiolabel decreased, radioactivity increased in lung-associated lymph nodes (LALN) over the 6-day study, suggesting distribution of benzo[a]pyrene or its metabolites via the lymph.

Bevan and Ulman (1991) administered 1 μg/kg [^{3}H]-benzo[a]pyrene intratracheally to male Sprague-Dawley rats in three different liquid vehicles. At 6 hours after dosing, 56.2, 58.4, and 70.5% of the dose delivered from tricaprylin, ethyl laurate, and triethylene glycol, respectively, were recovered from bile. Recovery from whole lung after 6 hours was 13.0% of the administered dose for tricaprylin and 15.6% for ethyl laurate but only 2.6% for triethylene glycol, indicating that pulmonary absorption of benzo[a]pyrene was more efficient from a less hydrophobic vehicle than from a highly hydrophobic one. Among the other organs, kidney and liver retained rather high levels of radioactivity (around 2 and 5%, respectively, for the whole organs). Two percent or less of the administered dose was recovered from intestine and its contents in this study.

Pregnant Wistar rats were exposed for 95 minutes on GDs 17 to 200, 350, 500, 650, or 800 mg/m^3 [^{3}H]-benzo[a]pyrene generated as a microcondensate from heated pure material (Withey et al., 1993). Immediately (time not specified) following exposure, the ranking of benzo[a]pyrene concentrations was maternal lung > blood > liver > kidney > fat > fetus. When total metabolites (as measured by detection of radiolabel) were measured immediately following dosing, the ranking was maternal lung > blood > liver > kidney > fetus > fat. Six hours after exposure, benzo[a]pyrene concentrations were fat > lung > kidney > liver > blood > fetus, while total metabolite concentrations were lung = fat > kidney > liver = blood > fetus. Concentrations of benzo[a]pyrene and metabolites in the GI tract were not reported. This study is consistent with other studies in showing wide tissue distribution of benzo[a]pyrene. In addition, the results also demonstrated placental transfer of benzo[a]pyrene and its metabolites.

3.2.2. Oral Exposure

Saunders et al. (2002) evaluated the neurotoxicity of benzo[a]pyrene in male F344 rats following a single gavage dose in peanut oil at doses of 0, 25, 50, 100, or 200 mg/kg body

weight (10/sex/dose). Benzo[a]pyrene and metabolite concentrations were monitored for up to 96 hours after administration in plasma, cerebellum, and cerebral cortex. Unmetabolized benzo[a]pyrene was observed in brain tissue only at the two highest doses, peaking at 2 to 4 hours and then gradually decreasing. Total metabolite concentrations in plasma as well as brain tissue peaked at 2 hours, remained at similar levels until 6 hours and then gradually decreased. By 96 hours after dosing benzo[a]pyrene or its metabolites had dropped to trace levels. The distribution of metabolites shifted over the observation period, with diol metabolites (4,5-, 7,8-, and 9,10-benzo[a]pyrene diols) predominating for the first 12 hours and hydroxy metabolites (3-OH, 9-OH-benzo[a]pyrene) predominating at later time points. The distribution of metabolites was similar in plasma and brain.

Neubert and Tapken (1988) administered a single 12 mg/kg oral dose of [^{14}C]-benzo[a]pyrene to groups of five pregnant NMRI:Han-mice on GDs 11, 12, and 13 to determine whether placental transfer occurred. Six, 24, and 48 hours after treatment radiolabel was found in the maternal lung, liver, and kidney (between 5 and 17% of the dose were recovered per gram tissue at 6 hours post dosing, decreasing to 0.5–1.3% by 48 hours). Radiolabel was also found in the placenta and embryonic liver at one to two orders of magnitude less than that found in maternal tissues. Similar results were found with five pregnant albino rats that received a single oral dose of 200 mg/kg benzo[a]pyrene in sunflower oil (Shendrikova and Aleksandrov, 1974). Three hours after treatment, fetal levels of radiolabel were approximately 10% of the maternal concentration.

Taken together it is apparent that, in rats, benzo[a]pyrene can penetrate the blood barrier, and in pregnant mice or rats it crosses the placental barrier and reaches the fetus. Otherwise, distribution data on benzo[a]pyrene following oral administration are insufficient to establish a cogent picture of organ or tissue distribution.

3.2.3. Dermal and Other Exposures

Some studies have evaluated the distribution of benzo[a]pyrene and its metabolites following dermal or other dose routes. Morse and Carlson (1985) sought to determine whether differences in toxicokinetic parameters could explain the difference in tumor response between SENCAR (high susceptibility) and BALB/c mice (low susceptibility). The mice were administered radiolabeled benzo[a]pyrene via dermal application or orally, and the time course of radioactivity levels was assessed in several organs. Following topical application of benzo[a]pyrene, organ levels of radioactivity were generally 1.5–4 times higher in SENCAR than in BALB/c mice. Radioactivity levels were liver > lung ≈ stomach, with an approximately twofold difference. DNA adduct levels were liver > lung > stomach, with a threefold difference. Following oral dosing, tissue radioactivity levels differed little between the two strains and essentially followed the same pattern of distribution. However, DNA adduct levels following oral dosing were much higher than after topical administration, approximately 6 times higher in

stomach and liver and 10 times higher in lung. RNA and protein binding showed patterns similar to DNA binding. This study seems to indicate that the route of administration exerts little influence on the tissue distribution of benzo[a]pyrene.

Moir et al. (1998) also measured the toxicokinetics of benzo[a]pyrene in male Wistar rats dosed i.v. with 2, 6, or 15 mg/kg of [^{14}C]-benzo[a]pyrene dissolved in an emulphor/saline emulsion. The concentrations of both benzo[a]pyrene-derived radioactivity and parent compound were determined in blood, adipose tissue, lung, liver, kidney, heart, spleen, brain, and testes as well as in urine and feces. The concentrations in all tissues examined except lung appeared to follow a similar pattern of smoothly increasing and decreasing curves, while the lung data were rather erratic (this pattern may reflect temporary trapping of lipid vesicles from the benzo[a]pyrene emulsion in the fine lung vessels). The authors also extracted selected tissues and determined parent compound levels by high-performance liquid chromatography (HPLC). Peak concentrations of [^{14}C]-benzo[a]pyrene equivalents and of parent compound and time to peak for the 2 mg/kg dose group are given in Table 3-1. Similar patterns were observed at higher doses, with notable exceptions noted below.

Table 3-1. Distribution of benzo[a]pyrene in selected tissues of male rats following i.v. dosing with 2 mg/kg

Tissue/organ[a]	Blood	Adipose[b]	Kidney[c]	Liver	Lung[d]
Total tissue radioactivity					
Peak level (μg/g tissue)	4.27 ± 0.25	2.31 ± 0.87	8.94 ± 1.21	20.55 ± 2.20	40.54 ± 4.85
Peak time (min)	5	120	5 and 20	5	5 and 120
Parent compound					
Peak level (μg/g tissue)	2.64 ± 0.84	3.96 ± 1.92	7.68 ± 1.23	11.33 ± 4.66	5.17 ± 1.15
Peak time (min)	5	120	5 to 20	5	5

[a]Mean ± SD, n = 3–4.
[b]Adipose tissue levels showed broad maxima between 20 and 480 min; the highest values are shown.
[c]Total radioactivity levels in kidney changed little between 30 and 480 min.
[d]Parent compound lung had another maximum of 14.65 ± 1.67 μg/g at 120 min; a second maximum at this time point was observed at the higher doses, too, but those did not exceed the value of the first maximum at 5 min.

Source: Moir et al. (1998).

At all exposure levels blood maintained the lowest initial concentrations (with some exceptions in white adipose tissue discussed below). Half-lives for benzo[a]pyrene parent compound in blood after the 2 and 6 μg/kg doses were 36 and 25 minutes, respectively, while no parent compound could be detected at time points >480 minutes. Following the 15 μg/kg dose, blood showed an initial benzo[a]pyrene decline similar to the lower doses, but benzo[a]pyrene could be recovered up to the end of the study at 32 hours post dosing, and with these additional data a second elimination phase half-life for benzo[a]pyrene was estimated at 408 minutes. The

final half-lives for elimination of benzo[a]pyrene "metabolites" (total benzo[a]pyrene-derived radioactivity minus parent compound) from blood were 666, 848, and 179 minutes for the 2, 6, and 15 mg/kg doses, respectively. It is fair to speculate that the second phase of elimination is the result of redistribution of parent compound (and subsequent metabolism) from a large, deep compartment such as adipose tissue and/or enterohepatic circulation.

Similar to the results in the lung, erratic patterns of radioactivity occurred in adipose tissue. Peaks and dips in radioactivity levels in lung occurred roughly with a pattern opposite to that in adipose tissue. This might suggest occasional redistribution of benzo[a]pyrene parent compound between a pool of vesicles trapped in the lung and adipose tissue. Data derived from these tissues should therefore be viewed with caution. Moir et al. (1998) noted that, as a general rule, increasing tissue radioactivity levels following administration of [^{14}C]-benzo[a]pyrene reflected metabolite accumulation. Kinetics models were fit to the data to derive rate constants for clearance of benzo[a]pyrene. Many organs displayed rapid uptake phases for benzo[a]pyrene: liver (uptake complete at the first time point, 5 minutes) kidney, brain, testis, and adipose tissue, which was followed by a bi-exponential decline (except in adipose tissue). Half-lives for benzo[a]pyrene parent compound elimination changed with the dose administered (half-life in minutes at doses of 2, 6, and 15 μg/kg, respectively): liver (28, 163, and 281), kidney (498, 456, and 389), and adipose tissue (239, 945, and 781). The following organs were evaluated for the 2 μg/kg dose only: brain (2,326 minutes), heart (25 minutes), and spleen (38 minutes); the latter, most likely due to nondetectable radioactivity levels at later time points, reflect the initial elimination phase only. Elimination half-lives of benzo[a]pyrene metabolites in liver and several other organs were in the range of 10–15 hours and independent of dose, suggesting first-order elimination. Overall, the results of Moir et al. (1998) suggest a pattern consistent with initial wide distribution determined by blood flow, with lipophilicity and rates of metabolism contributing to temporal patterns of organ levels after the initial distribution period.

Some studies showed that reactive metabolites of benzo[a]pyrene are transported in the blood and may be distributed to tissues incapable of benzo[a]pyrene metabolism. Ginsberg and Atherholt (1989) evaluated DNA adduct formation after intraperitoneal (i.p.) administration of benzo[a]pyrene in mice. Serum of benzo[a]pyrene-treated mice incubated with splenocytes or salmon sperm DNA resulted in adduct formation suggesting that reactive benzo[a]pyrene metabolites were systemically distributed and available for interaction with target tissues. DNA adduct levels formed in vivo were highest in liver, lung, and spleen, with levels in kidney and stomach significantly lower.

Taken together, the limited human data and few toxicokinetic studies in animals demonstrate that benzo[a]pyrene and its metabolites are widely distributed throughout the body. Inhalation of particle-bound or pure benzo[a]pyrene results in significant levels of benzo[a]pyrene and metabolites in numerous tissues. Distribution of inhaled benzo[a]pyrene and its metabolites to the GI tract is a result of both mucociliary clearance of particulates from the

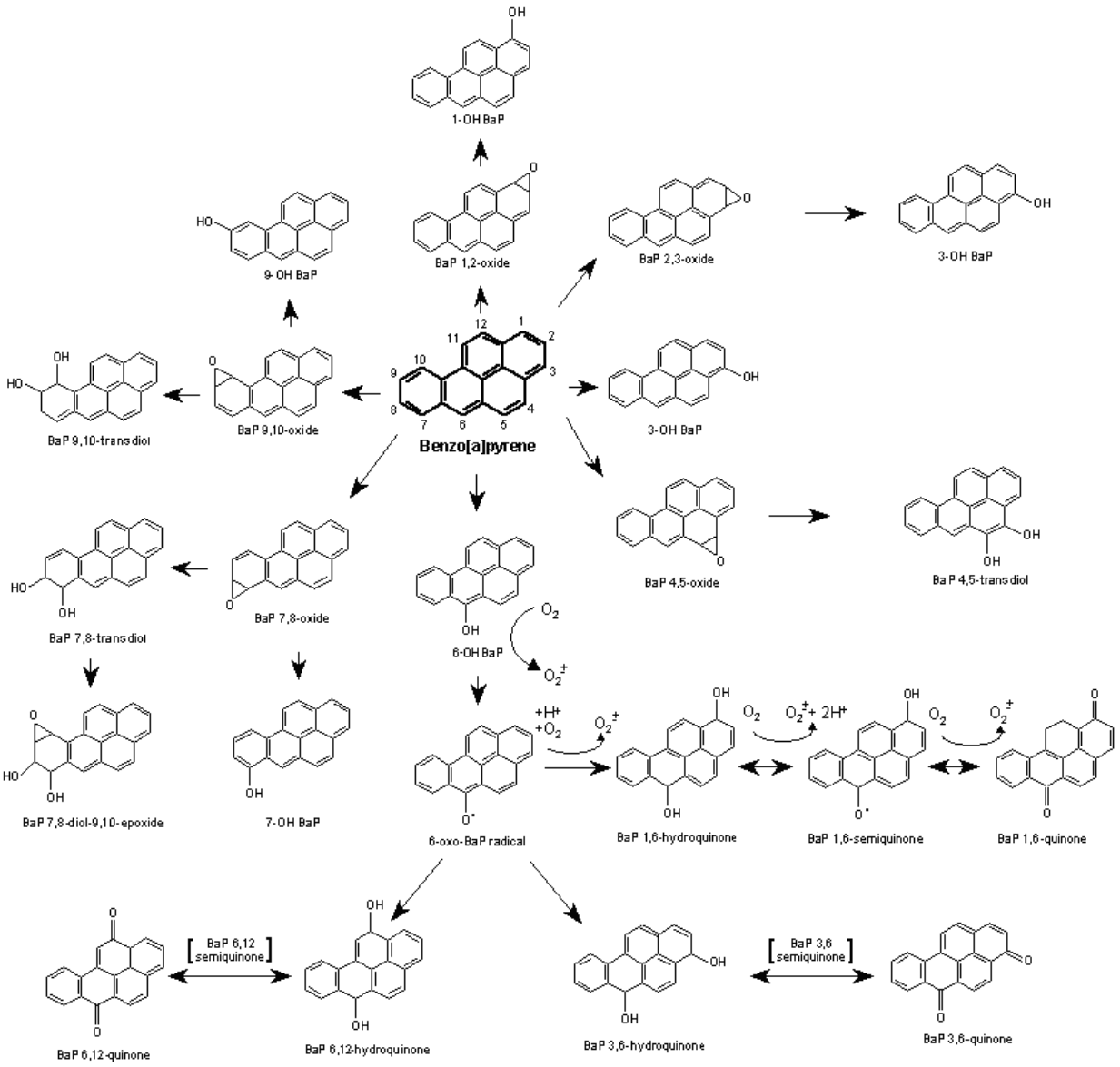
lung and of biliary excretion following metabolism. Following absorption, benzo[a]pyrene and metabolites are initially found predominantly in the highly perfused tissues, such as the lung, liver, and kidneys. Lower amounts are distributed to other tissues, including the male reproductive organs, central nervous system, and adipose tissue. Despite its high lipophilicity benzo[a]pyrene has no specific affinity for lipid-rich tissues, most likely because of its rapid metabolism to more hydrophilic compounds. There is some indication that the distribution of benzo[a]pyrene is not much influenced by the route of administration. Reactive benzo[a]pyrene metabolites are also distributed via blood where they may form protein adducts or reach tissues themselves unable to form reactive benzo[a]pyrene metabolites. Benzo[a]pyrene or metabolites are also transferred to the fetus at concentrations one to two orders of magnitude lower than those found in maternal tissues (Withey et al., 1993; Neubert and Tapkin, 1988; Shendrikov and Aleksandrov, 1974).

3.3. METABOLISM

The metabolism of benzo[a]pyrene is a critical aspect of the assessment of its potential toxicity because for many endpoints reactive metabolites are likely to contribute to the toxic response. Numerous reviews on the metabolism of benzo[a]pyrene are available (Miller and Ramos, 2001; WHO, 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.

benzo[a]pyrene is metabolized extensively by Phase I reactions to form numerous oxidative or reactive metabolites that are targets for further metabolism through diverse phase II reactions. Many of the enzymes involved in benzo[a]pyrene metabolism are isoenzymes within gene families, the members of which have varying metabolic specificities. Many of these enzymes are encoded by genes that show functional polymorphism. Many of the critical enzymes are inducible by a variety of agents, including benzo[a]pyrene itself, and, therefore, studies that evaluate benzo[a]pyrene kinetics following single short-term exposures may not be representative of the kinetics of benzo[a]pyrene following longer-term exposure conditions or exposure to mixtures. Metabolism of benzo[a]pyrene is species-, strain-, and organ-system-specific. There are age- and gender-related differences in the expression of many of the key enzymes that must be considered.

The metabolism of benzo[a]pyrene has been extensively studied using both in vivo and in vitro models, and a schematic representation of metabolic pathways is provided in Figure 3-1. Only Phase I reaction products are shown. Phase II reactions include glutathione conjugation of diol epoxides, sulfation and glucuronidation of phenols, and reduction of quinones by NADPH:quinone oxidoreductase (NQO)with subsequent conjugation.



Source: Miller and Ramos (2001).

Figure 3-1. Metabolic pathways for benzo[a]pyrene.

Some enzymes involved in the metabolism of benzo[a]pyrene are highly inducible (although benzo[a]pyrene itself is a relatively weak inducer compared to other environmental pollutants, such as certain dioxins and polychlorinated biphenyls). The cellular mechanisms underlying the inducibility of these enzymes, as well as the relative potency of various inducers, has been reviewed in detail (Miller and Ramos, 2001; Whitlock, 1999; Nebert et al., 1993). Inducibility of genes that metabolize benzo[a]pyrene is termed genetic responsiveness and the genetic locus that imparts inducibility is designated the Ah locus, so named for the enzyme activity aryl hydrocarbon hydroxylase (AHH) (now known to be catalyzed by enzyme activities from the CYP1 family. The Ah gene encodes a cytosolic receptor that regulates the inducible

expression of genes that encode Phase I (CYP1A and CYP1B isoforms) enzymes and may interact coordinately in regulating genes that encode isoforms of the Phase II enzymes uridine diphosphate (UDP)-dependent glucuronosyltransferases [UGTs], GSTs, NQO1) that metabolize the products of Phase I metabolism. The role of these enzyme systems in benzo[a]pyrene metabolism is discussed below. Due to the inducibility of benzo[a]pyrene metabolism, interpretation of toxicity studies should consider whether the studies were conducted in species and strains that have inducible metabolism, whether duration of exposure was sufficient to induce benzo[a]pyrene metabolism, and whether or not known inducers were administered.

3.3.1. Phase I Metabolism

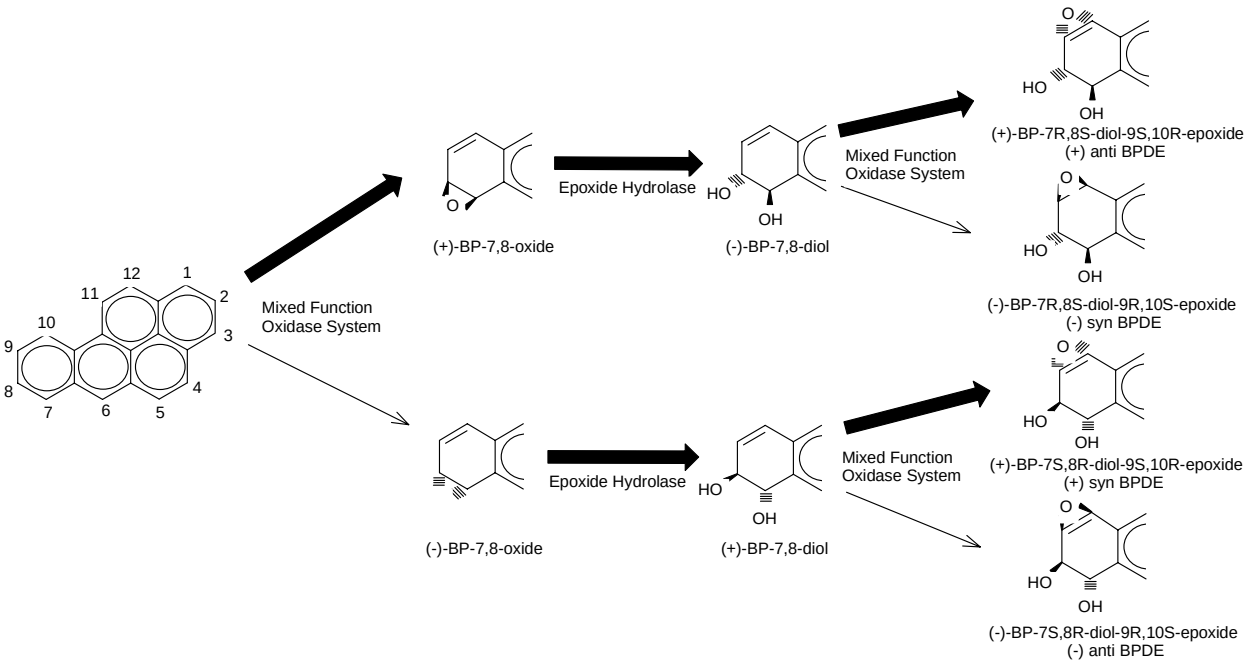
3.3.1.1. *CYP450-dependent Reactions*

Phase I reactions are catalyzed primarily by the mixed function oxidase system of enzymes associated with CYP450 to form arene oxides. This initial phase I metabolic reaction of benzo[a]pyrene is carried out primarily by the inducible activities of CYP1A1 or CYP1B1 and the constitutively expressed and somewhat inducible CYP1A2, depending on the tissue. Other CYP isoforms may also catalyze the initial oxidation reactions. NAPDH:CYP reductase is an important cofactor for this reaction as a supplier of redox equivalents (Byczkowski and Gessner, 1989). In addition to the CYP450 enzymes there are also a number of oxidoreductases that are not typically considered Phase I enzymes, yet can play an important role in the oxidative metabolism of benzo[a]pyrene (see below).

The isomerization of the arene oxides to their respective phenol metabolites is thought to be a nonenzymatic process; however, physical/chemical studies have shown that rearrangement is susceptible to catalysis by amines (Johnson and Bruice, 1975). This would suggest that rearrangements in vivo could be catalyzed by the amino or sulfhydryl groups of proteins. Typically, a single phenolic isomer tends to be produced and the direction of regioselective ring opening is predictable based on the relative stability of the two possible cationic intermediates (Fu et al., 1978). In accordance with these predictions, exclusively 3-OH-benzo[a]pyrene but not the other possible phenol metabolite, 2-OH-benzo[a]pyrene, is formed from the 2,3-oxide of benzo[a]pyrene (Yang et al., 1977). The other monophenol metabolites of benzo[a]pyrene include 1-, 6-, 7-, and 9-OH-benzo[a]pyrene. Benzo[a]pyrene epoxide formation may yield both phenols and dihydrodiols. Arene oxides that are poor substrates for epoxide hydrolase (EH) or the less stable ones that rearrange rapidly, such as benzo[a]pyrene 2,3-oxide, are less likely to yield dihydrodiols.

The arene oxides can be hydrolyzed by EH to form dihydrodiols (Oesch, 1980). The dihydrodiols may be further metabolized by CYPs to form diol epoxides, which are the DNA-reactive metabolites that have been the subject of most studies. In particular, much of the study of oxidative products of benzo[a]pyrene metabolism has been done for the 7,8-oxide, since it is a precursor to the potent DNA-binding metabolites. The metabolism of benzo[a]pyrene, as well as

of PAHs in general, proceeds with a high degree of stereoselectivity. Since most aromatic bonds are prochiral, their epoxidation catalyzed by CYPs often results in optically active products. Liver microsomes from rats stereospecifically oxidize the 7,8-bond of benzo[a]pyrene to yield almost exclusively the (+)-benzo[a]pyrene-(7,8)-oxide (see Figure 3-2). Each enantiomer of the 7,8-oxide is stereospecifically converted by EH to a different dihydrodiol via attack of water at the 8-position. The (+)-benzo[a]pyrene-7,8-oxide gives rise to the (-)-benzo[a]pyrene-7,8-dihydrodiol, while the (-)-benzo[a]pyrene-7,8-oxide yields the (+)-benzo[a]pyrene-7,8-di-



hydrodiol.

Source: Grover (1986).

Figure 3-2. The stereospecific activation of benzo[a]pyrene.

Further metabolism of the (-)-benzo[a]pyrene-7,8-dihydrodiol enantiomer by rat CYP enzymes preferentially yields (+)-benzo[a]pyrene-7R,8S-diol-9S,10R-epoxide [(+)-anti-BPDE], which is believed to be the most potent carcinogen among the four stereoisomers (Figure 3-2). Formation of these stereoisomers does not occur at equimolar ratios, and the ratios differ between biological systems. For example, in a study with rabbit livers, purified microsomes oxidized the (-)-benzo[a]pyrene-7,8-dihydrodiol to isomeric diol epoxides in a ratio ranging from 1.8:1 to 11:1 in favor of the (+)-anti-BPDE isomer (Deutsch et al., 1979).

Another important factor in evaluating variability in the metabolic activation of benzo[a]pyrene is the degree to which functional polymorphisms plays a role. Schwarz et al. (2001) used recombinant CYP1A1 allelic variants to determine catalytic activity in vitro. Catalytic activity of the variant allele 1A1.4 was 70%, and that of variant allele 1A1.2 was only

50% of the WT allele, CYP1A1.1. Km values were generally lower for variants than for the WT. Each variant produced BPDE, with the activity of CYP1A1.1 > 1.2 > 1.4. The formation of diol epoxides was stereospecific, with the allelic variants producing about three times the amount of (±)-anti-BPDE isomers, the suspected ultimate carcinogens, as compared to the noncarcinogenic stereoisomers (±)-syn-BPDE. Wu et al. (2002) found no relationship between benzo[a]pyrene metabolite formation and the CYP1A1 *MspI* polymorphism. The identification and characterization of CYP polymorphisms has been the subject of numerous reviews (e.g., Wormhoudt et al., 1999).

Several studies have attempted to clarify the question of which CYP isozyme is predominantly responsible for the metabolism of benzo[a]pyrene. The studies used knock-out (ko) animals in which either one of the isozymes in question, CYP1A1 1A2, or 1B1, or the Ah receptor (AhR) had been removed or inactivated (CYP 1A1 and 1B1 levels respond to AhR induction, while 1A2 is expressed constitutively). Kleiner et al. (2004) measured DNA adduct formation in the epidermis of $1B1^{-/-}$, $1A2^{-/-}$, $AhR^{-/-}$, and WT mice. Six [^{3}H]-PAHs were administered topically in one dose of 10–2000 nmol and animals were sacrificed after 24 hours. Absence of CYP1A2 had very little effect on benzo[a]pyrene adduct formation; in $1B1^{-/-}$ mice adducts were about 150% of WT (not significant), while in $AhR^{-/-}$ they were only 27% of WT. These findings differed considerably with other PAHs. The benzo[a]pyrene-DNA adduct was identified as being derived from (+)-anti-BPDE. The authors concluded that 1A1 was the primary CYP to metabolize benzo[a]pyrene, and that 1B1 rather serves detoxification.

Sagredo et al. (2006) conducted a similar experiment, but with various types of AhR knock out mice. $AhR^{+/+}$, $^{+/-}$, and $^{-/-}$ mice were treated once with 100 mg/kg benzo[a]pyrene by gavage. Twenty-four hours after treatment gene expression for CYP1A1, 1B1, and AhR was measured in lung, liver, spleen, kidney, heart, and blood by real-time or reverse transcriptase PCR (RT-PCR). CYP1A1 expression was increased following benzo[a]pyrene treatment in $^{+/+}$ and $^{+/-}$ mice (generally higher in heterozygotes), but $^{-/-}$ mice expressed no 1A1. There was a low level of basic 1B1 expression in all three genotypes that was inducible by benzo[a]pyrene in lung, but not in liver in $AhR^{+/+}$ and $^{+/-}$, but not at all in either tissue of $AhR^{-/-}$ mice. Expression of 1A1 was 25–40 times that of 1B1. There was an AhR gene-dose-response relationship for the basal CYP1A1 expression, i.e., +/+ > +/– > –/–, but no such dependence was seen for 1B1. Protein adduct levels were spleen > liver > lung > heart > plasma > kidney. The tissue levels of adducts showed an inverse relationship with AhR gene dose, i.e., –/– > +/– > +/+. Similarly, the levels of unmetabolized benzo[a]pyrene and of free benzo[a]pyrene-tetrol metabolites were higher in all organs of AhR^- mice, as compared with AhR^+ mice. Also, in the livers of AhR^- mice the levels of the less carcinogenic tetrol II were much higher than those of the tetrol I, opposite to the situation in AhR^+ mice. The authors suggested that the high levels of free benzo[a]pyrene metabolites in $AhR^{-/-}$ mice were the result of delayed bioactivation, and that a powerful AhR-independent pathway for benzo[a]pyrene metabolism must exist. The authors

explained the very high levels of benzo[a]pyrene adducts in organs outside the liver as the result of slow detoxification of the agent in the liver of AhR^{-} mice, allowing high concentrations of the parent compound to reach distant tissues. These findings establish important roles in benzo[a]pyrene metabolism for both CYP1A1 and 1B1, but they do not clarify which enzyme is responsible for biological activation, and which for detoxification.

Uno et al. (2006) investigated the finding that CYP1A1 ($1A1^{-/-}$) knock out mice are more sensitive than WT animals to the toxic effects of orally administered benzo[a]pyrene. They produced a series of C57BL/6-based single- or double knock out mice, $1A2^{-/-}$, $1B1^{-/-}$, $1A1/1B1^{-/-}$ and $1A2/1B1^{-/-}$. Benzo[a]pyrene was administered in the feed at 1.25, 12.5, or 125 mg/kg for 18 days (this dose is well tolerated by WT C57BL/6 mice for 1 year, but lethal within 30 days to the $1A1^{-/-}$ type). Steady-state blood levels of benzo[a]pyrene, reached within 5 days of treatment, were ~25 times higher in $1A1^{-/-}$ and ~75 times higher in $1A1/1B1^{-/-}$ than in WT mice, while in the other knock out types clearance differed little from that in WT animals. Pretreatment of the animals with TCDD to induce the CYP1 enzyme family resulted in decreased benzo[a]pyrene peak blood levels and AUCs in WT, but doubled peak blood levels and vastly increased AUCs in the two $1A1^{-/-}$ types. A lower-than-WT benzo[a]pyrene clearance was observed only in the two $1A1^{-/-}$ types. DNA adduct levels, measured by [^{32}P]-postlabeling in liver, spleen, and bone marrow, were highest in the $1A1^{-/-}$ mice at the higher doses, and in the $1A1/1B1^{-/-}$ mice at the mid dose only. Only $1A1^{-/-}$ mice, but not the other genotypes, showed signs of severe toxicity. In conclusion, the authors of the study painted a rather complex picture of how the three CYP1 family enzymes affect the toxicokinetics of benzo[a]pyrene. Detoxification of benzo[a]pyrene is mostly achieved by 1A1, probably not only in liver, but also in the intestine. Second, in spleen and bone marrow 1B1 brings about metabolic activation of benzo[a]pyrene, which, in the absence of 1A1, results in damage to the immune system. The authors suggested that tissue-specific expression of 1A1 and 1B1, respectively, determine an organism's susceptibility to benzo[a]pyrene toxicity and, possibly, carcinogenicity.

Van Lipzig et al. (2005) conducted experiments concerning potential estrogenic activity of mono- and dihydroxylated metabolites of benzo[a]pyrene. Estrogen receptor (ER) affinity and estrogenic activity were tested in T47D human breast adenocarcinoma cells (for experimental details see Section 4.5.2). Benzo[a]pyrene metabolite mixtures were generated using β-naphthoflavone (β-NF)-induced rat liver microsomes. Several hydroxylated benzo[a]pyrene metabolites had measurable estrogenic activity. In an attempt to identify enzymes involved in benzo[a]pyrene hydroxylation, the researchers added a specific CYP1A2 inhibitor to the metabolic activation mixture and found a ~15-fold increased estrogenic activity of the benzo[a]pyrene metabolite mix. They suggested that 1A2 inhibition drove the metabolism of benzo[a]pyrene towards formation of more estrogenic metabolites. These findings impart a role to CYP1A2 in the metabolism of benzo[a]pyrene.

To summarize, there is experimental evidence to suggest that three members of the CYP1 family, 1A1, 1A2, and 1B1, contribute to the metabolism of benzo[a]pyrene. However, the available data do not attribute precise roles to either of these enzymes. There is evidence that their expressions, and thus activities, are organ- or tissue-specific and that local isozyme activities determine not only the ratio of toxification vs. detoxification, but also the pattern of highly toxic vs. less toxic metabolites.

3.3.1.2. *Non-CYP-related metabolic pathways*

Other bioactivation processes independent of CYP-mediated diolepoxide formation have been demonstrated for benzo[a]pyrene. One-electron oxidation (via CYPs or peroxidases) can generate radical cations, which, in turn, can generate benzo[a]pyrene quinones (see bottom portion of Figure 3-1). These metabolites may generate DNA damage through redox cycling or the formation of depurinating adducts (McCoull et al., 1999; Cavalieri et al., 1990). Kim et al. (2000) treated male Sprague-Dawley rats with 20 mg/rat benzo[a]pyrene by i.p. injection and reported that the pattern of lipid peroxidation and increase in antioxidant enzymes correlated with formation of quinone metabolites of benzo[a]pyrene. Direct i.v. injection of benzo[a]pyrene and a series of metabolites confirmed the quinone metabolites indeed to be associated with the observed increase in lipid peroxidation. Secondary oxidation of 6-OH-benzo[a]pyrene can generate hydroquinone and quinone metabolites at the 1,6-, 3,6-, or 6,12-positions. Quinone formation may be catalyzed by dihydrodiol dehydrogenases (DHHs), such as aldo-keto reductase (AKR) (Penning et al., 1999). Tsuruda et al. (2001) showed in vitro that the expression of rat DHH in human breast cancer MCF-7 cells generated 7,8-benzo[a]pyrene-diones from benzo[a]pyrene-7,8-diol.

Mallet et al. (1991) treated tetradecanoylphorbol acetate-stimulated human polymorphonuclear leukocytes (PMNs) with (±)-trans-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene. They found that the cells were able to transform the benzo[a]pyrene-diol into the diolepoxide and tetrols with a stereochemical *anti/syn* ratio of six. The kinetics of the reaction suggested that hydrogen peroxide or a ferryl-oxygen-transfer were involved. Because myeloperoxidase (MPO) uses hydrogen peroxide in its reaction, the authors inhibited this enzyme specifically with azide and found that the formation of tetrols from benzo[a]pyrene-diol was reduced. Thus, MPO is able to execute the metabolic activation of benzo[a]pyrene.

Byczkowski and Kulkarni (1990) observed that benzo[a]pyrene diol can be cooxygenated during lipid peroxidation to form the diolepoxide. To reduce interference from CYP-catalyzed reactions they used term human placental microsomes, which are low in CYP450. Peroxidative conditions were created by a redox cycling system comprised of partially chelated ferrous ions and NADPH:CYP450 oxidase; this system quadrupled the formation of malonic dialdehyde (a measure of lipid peroxidation) compared with placental microsomes alone. The peroxidative system increased overall metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-diol by 27–

28%, compared with placental microsomes alone. The amounts of individual metabolites were changed to various extents in the presence of peroxidative conditions; the most striking observation was the more than doubled formation of trans-anti-benzo[a]pyrene-tetrol (the proximate carcinogen) and an almost tripled binding to protein. There was a highly significant correlation ($p < 0.0005$) between malondialdehyde and trans-anti-benzo[a]pyrene-tetrol formation. The authors pointed out that by means of this metabolic pathway the human fetus could be exposed to BPDE despite the absence of pronounced CYP450 activity in term placenta. Similarly, a redox cycling system based on vanadate-IV ions was able to increase formation of trans-anti-benzo[a]pyrene-tetrol five- to sixfold in the presence of term human placental microsomes (Byczkowski and Kulkarni, 1992).

Flowers et al. (1996) investigated the role of radical and reactive oxygen species (ROS) formation in benzo[a]pyrene-quinone-induced redox cycling, a reaction involving DHH, which can oxidize BP-diol to benzo[a]pyrene-7,8-dione (BPQ). BPQ is mutagenic and genotoxic. In isolated rat hepatocytes BPQ was incorporated covalently into DNA (30 ± 17 adducts/10^6 base pairs) while extensive DNA fragmentation took place. DNA fragmentation was also observed in hepatocytes treated with BP-diol; the effect was partially abolished when an inhibitor of DHH was added to the reaction. Hepatocytes treated with either BP-diol or BPQ produced superoxide anion radical, formation of which could be blocked by DHH inhibitors. In an in vitro experiment it was shown that BPQ at 0.05–10 μM caused DNA strand scission in the presence of NADPH and $CuCl_2$, suggesting that redox-cycling took place. DNA strand scission was prevented by catalase and hydroxyl radical scavengers but not by superoxide dismutase. The authors concluded that DHH metabolizes (+/-)-anti-BPDE to BPQ, which in turn causes extensive DNA fragmentation via the generation of ROS.

In a subsequent study, Flowers et al. (1997) provided more detail on the redox cycling reaction of BPQ. They showed that the reaction required the presence of NADPH (1 mM) and Cu^{2+} (10 μM). During the reaction superoxide anion radicals, benzo[a]pyrene semiquinone radicals, hydroxyl radicals, and H_2O_2 were formed. Hydroxyl radical scavengers, such as mannitol, sodium benzoate, or formic acid prevented the redox cycling (as assessed by DNA strand scission), as did the Cu^+ chelators bathocuproine or neocuproine. The results were interpreted as indicating that redox cycling of benzo[a]pyrene quinone involves a Cu^+-catalyzed Fenton reaction.

Other peroxidases such as prostaglandin H synthase (PHS) may also generate radical cations from benzo[a]pyrene (Parman and Wells, 2002). Marnett (1990) reviewed the role of PHS in benzo[a]pyrene metabolism. Peroxy radicals epoxidize the procarcinogen benzo[a]pyrene-7,8-diol via PHS to the epoxide BPDE. Stereochemical experiments have allowed the distinction between peroxide-mediated and CYP450-mediated epoxide formation from benzo[a]pyrene. Thus, peroxy radical-dependent epoxidation of benzo[a]pyrene-7,8-diol occurs in rat liver microsomes, mouse skin homogenates, cultured fibroblasts, cultured hamster

trachea, and freshly isolated mouse epidermal cells. Peroxy radical-generated metabolites are predominant in uninduced animals, while in ß-NF-induced animals the CYP450-produced metabolites prevail. There are other pathways by which peroxides can oxidize benzo[a]pyrene-7,8-diol because inhibition of PGH synthase with non-steroidal anti-inflammatory drug (NSAIDs) does not prevent BPDE formation.

Redox-active quinones are formed through the oxidative metabolism of benzo[a]pyrene, particularly at the 6-position (see Figure 3-1). NQO1 is an important enzyme for the detoxification of reactive quinones. Joseph and Jaiswal (1998) reported that NQO1 expression inhibited the formation of benzo[a]pyrene-quinone adducts with DNA and mutagenicity in vitro. There are also potential ring opening mechanisms for benzo[a]pyrene. Stansbury et al. (2000) used activated polymorphonuclear monocytes or a reconstituted in vitro system to generate a ring-opening dialdehyde metabolite from the benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) that can react with DNA to generate unique DNA adducts that are different from the ones formed by BPDE.

Nordling et al. (2002) identified a novel benzo[a]pyrene metabolite (7-oxo-benz[d]anthracene-3,4-dicarboxylic acid anhydride) in the urine of benzo[a]pyrene-treated rats. Follow-up in vitro experiments with this compound found it to be weakly mutagenic in salmonella strain TA102, able to induce DNA stand breaks in HT-29 cells, capable of inducing cytotoxicity via an apoptotic mechanism, and increasing gene expression through a cyclooxygenase (COX)-2 promoter in HCT 116 cells. These results demonstrate that novel benzo[a]pyrene metabolites have toxicological properties distinct from those of the better studied BPDE.

The roles of PHS-2 (now mostly called COX-2), MPO, NQO1, and other enzymes in the oxidative metabolism of benzo[a]pyrene may be crucial, but not enough data are as yet available to attempt a quantitative comparison with the CYP-mediated pathways. This may be of some importance in the assessment of cancer risks because not only CYP450 isozymes, but also MPO and NQO1 exhibit gene polymorphism. This is an area where much more research is necessary.

3.3.2. Phase II Metabolism

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

The diol epoxides formed from benzo[a]pyrene metabolism are not usually found as urinary metabolites, rather they are detected as adducts of nucleic acids or proteins if not further metabolized. Detoxification of the diol epoxide metabolites of benzo[a]pyrene is through

rearrangement to form tetrols or via conjugation with glutathione. Early work by Gozukara et al. (1981) demonstrated that exogenous EH added to benzo[a]pyrene-treated human monocytes reduced DNA binding, suggesting a role for this enzyme in the inactivation of diol epoxides. Furthermore, nonenzymatic hydration of diol epoxides proceeds rapidly in aqueous media in the absence of EH to yield tetrol products via cis or trans addition. A second mechanism for the detoxification of reactive diol epoxides formed from benzo[a]pyrene is through glutathione (GSH) conjugation. This Phase II reaction is catalyzed by GSTs. GSTs are a family of enzymes with varying substrate specificity and distribution among tissues (reviewed in Hayes and Strange, 2000; Eaton and Bammler, 1999; Hayes and Pulford, 1995). Primary isoforms of relevance for conjugation of BPDE include the α, μ, π, and θ isoforms (GSTA, GSTM, GSTP, GSTT, respectively).

Numerous studies using human GSTs expressed in mammalian cell lines have demonstrated the ability of GST to metabolize benzo[a]pyrene diol epoxides. For example, Dreij et al. (2002) demonstrated that GST isozymes, including alpha class GSTA1-1, GSTM1-1, and GSTP1-1 isoforms, had significant catalytic activity toward benzo[a]pyrene-derived diol epoxides. Robertson et al. (1986) incubated isolated human GST isoforms with GSH and (±)anti-BPDE to assess differences in their catalytic properties. Maximum substrate turnover velocity (V_{max}) values for α, μ, and π were 38, 570, and 825 nmol mg^{-1} $minute^{-1}$, and K_m values were 28, 27, and 54 μM, respectively. Rojas et al. (1998) reported that no BPDE adducts were formed in GSTM1-positive cells, but adducts were present in GSTM1-negative cells. This body of in vitro studies suggests that GST is an important detoxification mechanism for benzo[a]pyrene-derived epoxides. This compelling evidence for a role of GSTs in protecting against reactive benzo[a]pyrene metabolites has triggered several molecular epidemiology studies. However, recent studies on the impact of polymorphism on adduct levels in PAH-exposed human populations did not succeed in showing clear relationships between CYP1A1, EH, or GSTM1 polymorphisms and DNA (Hemminki et al., 1997) or blood protein adduct formation (Pastorelli et al., 1998).

Conjugation with glucuronide is another important detoxification mechanism for oxidative benzo[a]pyrene metabolites. Most of the phenolic metabolites of benzo[a]pyrene are further metabolized by glucuronidation or sulfation, and significant portions of total metabolites in excreta or tissues can be recovered in this form. Bevan and Sadler (1992) administered a single 2 μg/kg benzo[a]pyrene dose by intratracheal instillation to male Sprague-Dawley rats and assessed the benzo[a]pyrene metabolite profiles in bile after 6 hours. Identified metabolites were 31.2% quinol diglucuronides, 30.4% thioether conjugates, 17.8% monoglucuronide, 6.2% sulfate conjugates, and 14.4% unconjugated metabolites.

The UGTs are a family of enzymes that catalyze the conjugation of UDP-glucuronide with endogenous substrates (e.g., bilirubin) as well as xenobiotics (reviewed in Guillemette, 2003). UGT isoforms as well as their allelic variants show different patterns of tissue

distribution and catalytic activity toward benzo[a]pyrene-derived phenols and diols. For example, Fang and Lazarus (2004) assessed the ability of the allelic variants UGT1A1 and 1A9 in human liver to catalyze glucuronidation of benzo[a]pyrene-7,8-diol. Microsomes from subjects homozygous for the UGT1A1*28 variant (present in approximately 12% of the Caucasian population) had approximately twofold lower UGT1A1 protein levels than liver microsomes from individuals with the WT allele. Addition of a UGT1A9 inhibitor to the incubation decreased benzo[a]pyrene-glucuronide formation three- to sixfold, suggesting that UGT1A9 also has significant catalytic activity toward benzo[a]pyrene-7,8-diols. With UGT1A9 activity blocked, glucuronide formation in UGT1A1*28 homozygotes was significantly lower than in WT and heterozygous individuals. The apparent Km for this reaction did not differ among microsomes from allelic variants.

UGT activity also shows significant interindividual variability. Hu and Wells (2004) evaluated glucuronidation of benzo[a]pyrene metabolites in human lymphocytes (HL) in vitro. The degree to which glucuronide conjugates were formed varied over 200-fold (percent of metabolites as glucuronide conjugates ranged from 0.01 to 5% of total benzo[a]pyrene metabolites). Incubation of lymphocytes with benzo[a]pyrene, benzo[a]pyrene-7,8-diol, or benzo[a]pyrene-4,5-diol resulted in covalent binding to protein with, in the case of benzo[a]pyrene, a 220-fold inter-individual variability. Addition of the UGT substrate, UDP glucuronic acid (UDPGA), lowered the inter-individual variability to 143-fold. For benzo[a]pyrene or its diols there was a statistically highly significant relationship between increase in glucuronidation and decrease in protein binding. Cytotoxicity also was inversely correlated to conjugation of diols and diones, suggesting that glucuronidation is an important pathway for protection from chemically reactive benzo[a]pyrene metabolites.

Sulfation, normally a defoliation process, can produce a DNA-damaging intermediate in the case of benzo[a]pyrene. It was shown that in rat or mouse liver cytosolic sulfotransferase (in the presence of 3'-phosphoadenosine 5'-phosphosulfate) catalyzes formation of sulfates of benzo[a]pyrene-7,8,9,10-tetrahydro-7-ol, benzo[a]pyrene-7,8-dihydrodiol, and benzo[a]pyrene-7,8,9,10-tetrol. All three sulfates were tested for their ability to bind to DNA, but only the benzo[a]pyrene-7,8,9,10-tetrahydro-7-ol-sulfate formed DNA adducts (Surh and Tannenbaum, 1995).

Although not specific for benzo[a]pyrene, there is now considerable evidence that genetic polymorphisms of the GST, UGT, and EH genes impart an added risk to humans for developing cancer. Of some significance to the assessment of benzo[a]pyrene may be that smoking, in combination with genetic polymorphism at several gene loci (for detail, see Section 4.8.3), increases the risk for bladder cancer (Moore et al., 2004; Choi et al., 2003; Park et al., 2003) and lung cancer (Alexandrie et al., 2004; Lin et al., 2003). Also, Leng et al. (2004), according to the English abstract of a paper in Chinese, showed that coke oven workers (who are exposed to PAHs, including benzo[a]pyrene) homozygous at the P187S site of the NQO1 gene or carrying

the null variant of the GSTM1 gene had a significantly increased risk of chromosomal damage in peripheral blood lymphocytes, while the risk was much lower than controls in subjects with a variant allele at the H113Y site of the EH gene.

3.3.3. Tissue-specific Metabolism

3.3.3.1. *Respiratory Tract Tissues*

benzo[a]pyrene treatment has been associated with the induction of respiratory tract tumors. This finding is consistent with the ability of the lung to metabolize benzo[a]pyrene, which has been demonstrated in numerous studies. Ewing et al. (2006) investigated the hypothesis that lung cancer following PAH induction may be a result of slow absorption and extensive metabolism in the thick respiratory epithelia. The researchers used isolated, perfused rat lung to investigate these processes. Benzo[a]pyrene was coated onto 3.5 μm silica carrier particles at concentrations to deliver an average of 2.2, 36, and 8,400 ng to each lung within <1 minute. Perfusate was collected for 77 minutes thereafter. Both perfusates and lungs were analyzed for benzo[a]pyrene and metabolites. Absorption and metabolism were both strongly dose-dependent: at the low and mid exposure levels benzo[a]pyrene concentration increased rapidly in the perfusate to a maximum after <5 minutes, then decreased over the remaining observation period. At the high dose, benzo[a]pyrene in perfusate reached the maximum at about 30 minutes after exposure and stayed at a constant level from there on, i.e., the absorption of benzo[a]pyrene proceeded at zero order until all deposited solid benzo[a]pyrene was dissolved. The mass balances for benzo[a]pyrene equivalents in lung vs. perfusate were lung ca. one-third perfusate at the low dose, lung = perfusate at the mid dose, and lung about twice that of perfusate at the high dose. At the low exposure level metabolism was apparently able to convert most of the parent compound, while at the highest exposure level most of the absorbed benzo[a]pyrene remained unmetabolized even at the end of the experiment. The authors pointed out that these findings may explain why many attempts have failed to inducer lung cancer in animals using high-dose particle inhalation protocols. The results further confirm that benzo[a]pyrene metabolism is organ specific.

Autrup et al. (1980) compared the metabolic capacity of tracheobronchial tissues in culture among several species, including humans, mice, rats, hamsters and bovines. Results from this study are summarized in Table 3-2. Benzo[a]pyrene was metabolized extensively in tissues from all species tested, with lower amounts of metabolites identified in rats and nonresponsive mice. Patterns of metabolism differed among the species but showed formation of a complex array of metabolites, including phenols, diols, tetrols, and quinones. Data summarized from the study suggest that under the conditions tested: (1) upper respiratory tract tissues for all species were able to metabolize benzo[a]pyrene, (2) the degree of phase II conjugation products was greatest in humans, followed by hamsters, Ah-responsive mice, bovines, rats, and Ah-nonresponsive mice, (3) multiple phase II conjugation pathways were operative in tissues of all

species, although the relative proportions of conjugate formation varied, and (4) conducting airway tissues from all species were able to metabolize benzo[a]pyrene into DNA-reactive metabolites, with DNA binding greatest in hamster trachea followed by human and bovine bronchus.

DNA binding in human, rat, and mouse tissue was similar but considerably higher in hamster (Table 3-2). The results were quite variable among individuals, a 33-fold difference in human bronchus, a fivefold variation in human trachea, and a threefold difference in bovine bronchus, but minimal variation among individuals of the laboratory animal species. Overall, these results show that human lung tissue metabolizes benzo[a]pyrene in a manner that is qualitatively similar to that of species that are susceptible to lung tumors, although some quantitative differences in specific metabolic pathways are observed (Autrup et al., 1980).

Table 3-2. Species differences in tracheobronchial benzo[a]pyrene metabolism

Species	Total metabolites[a]	Ratio organic/water soluble metabolites	Percent water soluble metabolites as sulfate esters, glucuronides, and glutathione conjugates	DNA binding[b]
C57B1/6N mouse	1.00 ± 0.25	1.4	31/30/39	10
DBA/2N mouse	0.40 ± 0.13	0.5	28/27/44	10
CD rat	0.65 ± 0.10	0.5	31/13/56	10
Syrian golden hamsters	1.34 ± 0.13	1.2	20/17/63	26
Bovine bronchus	0.93 ± 0.12	0.12	24/29/38	16
Human trachea	1.09 ± 0.48	2.5	56/12/32	11
Human - main-stem bronchus	1.33 ± 0.72	1.7	44/7/51	16
Human secondary and tertiary bronchus	1.75 ± 0.82	2.3	44/6/51	16

[a]Mean ± SD in pmol/μg DNA.
[b]Mean ± SD in pmol/mg DNA; results are from trachea in mouse, rat, and hamster.

Source: Autrup et al. (1980).

In vitro studies with human bronchial epithelial and lung tissue showed that benzo[a]pyrene is metabolized to the 7,8- and 9,10-diols and, to a lesser extent, to the 4,5-diol and 3-OH metabolites (Autrup et al., 1978; Cohen et al., 1976). The metabolites were identified as glutathione and sulfate conjugates; no glucuronide metabolites were found. The ability of human tissues to metabolize benzo[a]pyrene has also been demonstrated in lung-derived cell lines. Kiefer et al. (1988) demonstrated that benzo[a]pyrene was metabolized in vitro in the human lung cancer cell line NCI-H322. These cells were also able to form benzo[a]pyrene-7,8-diol, suggesting that human lung cells are able to generate carcinogenic metabolites of benzo[a]pyrene. Approximately 30% of the detected metabolites were water-soluble, about 30%

of which were glutathione conjugates. Sulfates, but not glucuronide conjugates, were also detected.

A complement of enzymes for the oxidative metabolism of benzo[a]pyrene in the lungs has been identified in both humans and animal tissues, and these activities are inducible with prior exposure. Wei et al. (2001) evaluated CYP1A1 levels in fresh lung tissue from nine human donors. CYP1A1 and CYP1A2 were present at variable levels in lung tissues based on mRNA, protein levels, enzyme activity, and ability of S9 fractions to induce mutagenicity in an Ames assay. CYP1B1 was not identified. The authors emphasized that, in contrast to some previous studies, they were able to identify CYP1A2 in human lung (Wei et al., 2001). In a subsequent study, Wei et al. (2002) lung tissue from 27 human donors was evaluated for CYP1A status. CYP1A1 and CYP1A2 transcripts were present at variable levels in nearly all samples and were inducible by benzo[a]pyrene treatment. Mean inducible CYP1A2 levels were roughly four times lower than CYP1A1. Microsomes prepared from these tissues pretreated with benzo[a]pyrene resulted in a threefold increase in DNA adduct formation, while pretreatment with the potent AhR ligand TCDD increased benzo[a]pyrene-DNA adduct formation to 24-fold over controls. This result shows that that CYP1A activity was highly inducible via the AhR pathway in human lung tissues.

These results using human donor tissues are consistent with the body of literature demonstrating the induction of benzo[a]pyrene metabolism in lungs of rodents. For example, Vainio et al. (1976) compared the metabolism of intratracheally-instilled benzo[a]pyrene in the isolated perfused rat lung of both control and rats induced with 3-MC. Pretreated rat lungs had increased covalent binding of [^{3}H]-benzo[a]pyrene to lung tissue, decreased appearance of unmetabolized benzo[a]pyrene in perfusion liquid, and increased formation of water soluble metabolites. Bompart and Clamens (1990) assessed AHH activity in male Sprague-Dawley rats given 2 mg/kg benzo[a]pyrene by i.p. injection weekly for 30 weeks. Every third week, five animals were sacrificed and lung and liver microsomes were prepared for determination of AHH activity (as measured by formation of the 3-OH metabolite of benzo[a]pyrene). Control levels of AHH in lung were much lower than in liver. AHH activity in the lung increased with repeated benzo[a]pyrene doses until approximately week 15, when it reached levels approximately eightfold over controls. Even after this induction, lung AHH activity was still approximately 30-fold lower than in liver. Benzo[a]pyrene treatments had no effect on AHH activity in the liver in this study.

Petridou-Fischer et al. (1988) instilled radiolabeled benzo[a]pyrene into the nasal turbinates (ethmoid and maxillary) of monkeys and dogs. Metabolic activity in these tissues was demonstrated by the formation of diverse metabolic products (phenols, diols, tetrols, and quinones). No region-specific metabolism was identified. Even though ethmoid versus maxillary turbinates contain different CYP activities, the pattern of metabolites was qualitatively

similar. Differences were not compared quantitatively due to the small number of animals used (four dogs and two monkeys).

Bond et al. (1988) showed differential levels of Phase I and Phase II activities in respiratory tract regions of dogs. Benzo[a]pyrene metabolism was greater in nasal tissue than in lung tissue. Metabolic activities towards benzo[a]pyrene in various areas of the upper respiratory tract ranged from 5 to 15 pmol/mg protein/minute; however, in the ethmoid turbinates they reached approximately 45 pmol/mg-minute. The was no difference in the regional patterns of metabolite formation. Although CYP isozyme activity was similar in liver and lung, benzo[a]pyrene metabolism in respiratory tract tissues was about one-tenth that in liver (one-third for the ethmoid turbinates). EH activity was highest in the lower generations of the conducting airways, followed by liver then nasal tissues. GST activity was highest in liver, followed by nasal tissues. UGT activity was more evenly distributed among lung regions, and was similar to levels in the liver. These data show that in dogs the nasal region and lungs have greater metabolic capability for benzo[a]pyrene and its metabolites than the conducting airways, and levels of metabolism are generally similar to those observed in the liver.

Dahl et al. (1985) evaluated benzo[a]pyrene metabolism in the respiratory tract tissues from Syrian hamsters. All regions of the respiratory tract had metabolic activity as assessed by the formation of benzo[a]pyrene metabolites. Activity was highest in the nasal tissues on a per gram tissue basis, with similar activities observed in esophagus, forestomach, trachea, larynx, and lungs. Total metabolism on a per organ basis was highest for the lung and trachea. Similar results were obtained for lung and nasal tissue of rats that had inhaled benzo[a]pyrene (Wolff et al., 1989).

Persson et al. (2002) showed that [^{3}H]-benzo[a]pyrene instilled nasally in female Sprague- Dawley rats was taken up in nasal structures (sustenacular cells and Bowman's glands). Transport of benzo[a]pyrene or metabolites (as determined by radiography) via axons of olfactory neurons to the olfactory bulb was identified, indicating uptake into nasal structures and transport to central nervous system (CNS) structures via neurons.

Weyand and Bevan (1986) examined benzo[a]pyrene disposition and metabolism in male Sprague-Dawley rats following intratracheal instillation of 1 μg/kg body weight [^{3}H]-benzo[a]pyrene. The overall concentration of benzo[a]pyrene metabolites in lung and liver decreased over the 360-minute period, with a shift from predominately lipid-soluble metabolites to an increasing component of water-soluble metabolites at later times. In the lung, benzo[a]pyrene metabolites represented 47.7% of the administered dose in the organic and 11.9% in the aqueous fraction, respectively, at 5 minutes, while at 360 minutes the corresponding fractions were 2.16 and 1.83%, respectively. The metabolite profile determined in the organic phase at 360 minutes was as follows: conjugates or polyhydroxylated compounds, 16.3%; 9,10-diol, 4.95%; 4,5-diol, 2.60%; 7,8-diol, 3.11%; 1,6-quinone, 2.99%; 3,6-quinone, 4.09%; 6,12-quinone, 2.23%; 9-OH, 2.26%; 3-OH, 4.59%; and benzo[a]pyrene, 20.0%. Over the 360-minute

period, notable shifts in the relative proportion of benzo[a]pyrene and metabolite levels in the lung indicated a net increase in the contribution of conjugates and tetrols, stable to decreasing levels of quinones, increasing levels of diols and phenols, and decreasing levels of parent benzo[a]pyrene. A similar pattern was observed for concentrations of benzo[a]pyrene metabolites in the liver, except that quinone levels also increased in this tissue over the 6-hour period. This identification of significant levels of quinone metabolites early in the lungs is consistent with in vitro studies, such as that of Autrup et al. (1980) in which rat lung had a high capacity to form quinones originating from the oxidation of benzo[a]pyrene at the 6 position.

Most of the metabolism studies have focused on the role of the CYP1 isoforms on benzo[a]pyrene metabolism in the lung. However, other CYPs may also be important for benzo[a]pyrene metabolism among species. For example, Shultz et al. (2001) reported that benzo[a]pyrene was metabolized using recombinant CYP2F2 from mouse lung in a cell-free in vitro assay, although metabolic capability was less than that of other isoforms. CYP2F2 expression in mouse lung airways was greater than that in tracheal parenchyma, showing region-specific metabolic differences.

3.3.3.2. *GI Tract and Liver Tissues*

Fontana et al. (1999) reported that in healthy volunteers fed diets enriched with char-grilled meat, CYP1A1 and 1A2 activities were induced in the liver and CYP1A1 protein levels were increased in small intestine biopsies. No change in CYP3A4 or 3A5 levels was observed. DNA adducts in peripheral blood cells were inversely correlated to CYP1A levels in enterocytes and CYP1A2 levels in liver. These findings point to the presence of AhR ligands, such as benzo[a]pyrene, in char-grilled meat and lend further support to the complexity of benzo[a]pyrene metabolism by CYP isozymes.

In a human hepatoma cell line (HepG2) incubated with [^{3}H]-benzo[a]pyrene, radiolabeled metabolites were recovered primarily in the medium (88.4% of the radioactive material) (Diamond et al., 1980). Sixty-four percent of the metabolites were unidentified water-soluble metabolites. Chloroform extractable metabolites (36% of the radiolabel) included 7,8-diol, 9,10-diol, quinones, 3-OH (16% combined) metabolites, and unmetabolized benzo[a]pyrene (20%). The cell lysate contained the same metabolites, but the proportions of the 3-OH metabolite and parent compound were relatively higher. Enzymatic treatment for conjugate formation did not change recovery of radioactivity, suggesting that at least this tumor cell line did not extensively form phenol products. The authors noted that the HepG2 cell line did not utilize the major phenol detoxification pathway of rodent cell cultures.

Similar results were obtained with human hepatocytes in culture (Monteith et al., 1987). Following incubation for 24 hours, the primary metabolites of [^{3}H]-benzo[a]pyrene were unidentified highly polar, water-soluble conjugates. The next four most prevalent metabolites consisted of 3-OH-benzo[a]pyrene and the 4,5-, 9,10- and 7,8-diols. As the dose of

benzo[a]pyrene was increased from 10 to 100 μmol, the amount of metabolites increased in a linear fashion, suggesting that the capacity of human hepatocytes to metabolize benzo[a]pyrene was not saturated at benzo[a]pyrene concentrations up to 100 μmol. 3-MC induced rat microsomes convert benzo[a]pyrene to the BPDE approximately 10 times faster than microsomes from uninduced rats, likely through the induction of CYP1A1 (Keller et al., 1987). The rate-limiting step appeared to be competition for CYP1A1 binding sites between parent benzo[a]pyrene and the benzo[a]pyrene-7,8-diol.

The metabolism of benzo[a]pyrene in the GI tract and liver has been studied. Zheng et al. (2002) determined the expression of UGT isozyme levels and activity toward benzo[a]pyrene from human tissue samples of the liver, lung, and regions of the aerodigestive tract (tongue, tonsil, floor of the mouth, larynx, esophagus). Glucuronidation of phenolic benzo[a]pyrene metabolites was detected in all aerodigestive tract tissues examined and, of the isoforms tested, activity was identified for UGT1A7, 1A8, and 1A10. No UGT expression or glucuronidation activity was detected in lung tissue.

Bentsen-Farmen et al. (1999a) measured CYP1A1 induction and DNA adduct levels in Wistar rats given i.p. doses of 3-MC and followed by a single dose of benzo[a]pyrene, or a single benzo[a]pyrene dose without pretreatment. 3-MC pretreatment increased CYP1A1 activity in the liver and DNA adduct levels in both liver and lung, with significant correlation to CYP1A1 activity in lung but not in liver. The results indicate the difficulty in using DNA adducts as a biomarker in short-term exposures. The authors further reported that the study results were highly dependent on the analytical technique used.

Ramesh et al. (2001b) exposed F344 rats for up to 90 days to benzo[a]pyrene in the diet at doses of 0, 5, 50, or 100 mg/kg-day. AHH activity (as measured by formation of the 3-OH-benzo[a]pyrene) in the liver was increased in both males and females (approximately twofold higher in females at the end of the study) in a dose- and duration-dependent manner.

Granberg et al. (2000) treated female NMRI mice with [^{3}H]-benzo[a]pyrene by i.v. injection and identified tissue-bound radiolabel in the lung, liver, and cardiovascular endothelial cells. The levels of tissue-bound radioactivity were correlated to CYP1A1, as determined by 7-ethoxyresorufin-O-deethylase (EROD) activity. Pretreatment with the AhR ligand β-NF increased tissue binding in lung and heart cells.

3.3.3.3. *Skin*

Indirect evidence for metabolism of benzo[a]pyrene in skin is plentiful and includes numerous studies of dermally exposed humans or animals with subsequent detection of benzo[a]pyrene metabolites in tissues or excreta. These types of studies provide qualitative evidence for dermal metabolism but are confounded by potential contribution of metabolism in other tissues following systemic circulation. However, direct measurements of benzo[a]pyrene metabolism have been made in in vitro models using human skin or skin cells.

Hall and Grover (1988) also showed that human skin samples can metabolize benzo[a]pyrene. In skin samples from 11 subjects, the majority of benzo[a]pyrene-7,8-diol formed from benzo[a]pyrene was recovered as the (-) enantiomer. However, the stereospecific formation of tetrol metabolites was highly variable, suggesting significant inter-individual variability in the relative formation of the DNA reactive epoxide metabolites from benzo[a]pyrene. Bowman et al. (1997) assessed levels of the 7,8,9,10-tetrol metabolite in the urine of 43 psoriasis patients treated with coal tar medication. Urinary benzo[a]pyrene metabolites were detected in 40% of patients vs. 10% of matched controls. Amounts of metabolites detected were highly variable, but precise measures of applied dose were not available. These studies show that human skin can metabolize benzo[a]pyrene to diverse products, the metabolism of benzo[a]pyrene is inducible and variable with regard to stereospecific formation of downstream metabolites, and there may be significant interindividual variation.

Merk et al. (1987) used hair follicles from volunteers to study benzo[a]pyrene metabolism and the effect of coal-tar-containing shampoos. Hair follicles were able to form numerous metabolites of benzo[a]pyrene. The results with coal-tar-exposed individuals showed that AHH activity was inducible in these cells and inhibition of CYP activity decreased the metabolism of benzo[a]pyrene and the formation of DNA binding activity. Alexandrov et al. (1990) demonstrated that hair follicles from human subjects (10 healthy female smokers and 10 healthy female nonsmokers) were able to generate benzo[a]pyrene tetrols from diol epoxide precursors. There was stereospecific metabolism with most of the tetrol formed from (-)-benzo[a]pyrene-7,8-diol consistent with extensive formation of (±)-anti-BPDE rather than the (±)-syn-BPDE metabolite. Agarwal et al. (1991) reported that human melanocytes treated in culture formed diverse benzo[a]pyrene metabolites, including dihydrodiols, hydroxyl compounds, quinones, and their glucuronide and sulfate conjugates.

Kao et al. (1985) investigated the metabolism of benzo[a]pyrene in skin from several species, including humans. In the case of human skin, TCDD induction could not be studied, but the authors investigated the influence of metabolic viability of the skin on metabolism. Skin samples were treated with 2 μg/cm^2 [^{14}C]-benzo[a]pyrene in acetone and were incubated for 24 hours. Medium under the skin was then extracted with ethyl acetate but was not subjected to hydrolysis to identify conjugate formation. Fifty-two percent of the radioactivity in culture medium below viable human skin was composed of water-soluble metabolites, 8% were lipid-soluble polar metabolites, 17% were diols, 1.2% were monophenols, 2.5% were quinones, and 18% were parent compound. By contrast, previously frozen, nonviable skin allowed mostly parent compound to pass (50% of radioactivity in the medium) and considerably smaller portions of water-soluble and polar metabolites and diols to pass, but, surprisingly, relatively increased portions of monophenols and quinines to pass. Results for skin from other species—marmoset, rabbit, rat, mouse—were similar: water-soluble metabolites varied between 55 and 77% of the

radioactivity in the medium; polar metabolites, 5.8–13.2%; diols, 7.9–15.2%; monophenols, 0.7–1.8%; quinones, 0.6–2.1%; and parent compound, 0% (mouse), to 6.7% (rabbit). Metabolites found below nonviable skin from animal species were not reported.

Animal studies are consistent with the metabolism data in human skin. Ng et al. (1992) used an in vitro culture system with skin from female hairless guinea pigs and concluded that the degree of penetration of benzo[a]pyrene was dependent on metabolism, since the collection of the administered radioactivity was much greater for viable than nonviable skin. At the lowest dose tested (32.1 nmol/cm^2), 37% of the applied radioactivity was recovered in the receptor fluid within 24 hours, 84% of which were metabolites, including OH derivatives, dihydrodiol diones, and conjugates. The tetrol metabolite of the DNA-reactive epoxide accounted for 2.56% of the administered dose. MacNicoll et al. (1980) assessed metabolism of [^{3}H]-benzo[a]pyrene in Swiss mouse skin maintained in short-term organ culture. After incubation for 24 hours, radioactivity derived from benzo[a]pyrene consisted of 3 benzo[a]pyrene-equivalents of lipid-soluble metabolites, 147 benzo[a]pyrene-equivalents water-soluble metabolites, and 5 benzo[a]pyrene-equivalents bound to skin. The high proportion of water-soluble metabolites indicates that benzo[a]pyrene was readily metabolized.

Kao et al. (1984) evaluated metabolism of benzo[a]pyrene as a factor in the dermal penetration of benzo[a]pyrene in mouse skin. Skin samples formed predominantly polar metabolites and diols (approximately 20% of the radioactivity each), with small shares (approximately 1% of the penetrated material) of conjugates, monophenols, and quinones. Parent compound was recovered at about 1.5%. TCDD induction changed the portion of conjugates to almost 8%, at the expense of polar metabolites, diols, and parent compound, but did not affect monophenol or quinone formation. Investigation of the radioactivity left in the skin revealed that it was mostly parent compound (almost 50% of the dose in uninduced and a little more than 30% in induced skin), with about 12–16% water-soluble metabolites and polar metabolites, diols, monophenols, and quinones decreasing in that order from ~5% of the dose to <2%.

3.3.3.4. *Reproductive Tissues and Fetal Metabolism*

Several toxicity studies have demonstrated the ability of benzo[a]pyrene to impair reproductive function in male and female rodents as well as induce developmental toxicity. Therefore, the ability of benzo[a]pyrene to be metabolized in tissues that affect reproduction or development is of interest. Williams et al. (2000) reported the presence of CYP1A1, CYP1A2, and CYP1B1 transcripts in prostate tissue from human donors exposed in short-term organ culture and the activation of benzo[a]pyrene as indicated by DNA adduct formation. Primary human prostate cells have also been demonstrated to metabolize benzo[a]pyrene in vitro (Martin et al., 2002).

Bao et al. (2002) exposed a human endometrium epithelial cell line, which expresses low constitutional levels of EROD activity (used as a measure of CYP1 activity), to benzo[a]pyrene (1 mM) in vitro and observed a 12-fold induction of CYP1A1. Specific inhibitors of CYP1B1 and CYP1A2 had no effect on EROD activity, suggesting little metabolic contribution from these two isoforms in endometrial tissues, while the CYP1A1 inhibitor α-NF (100 nM) inhibited EROD activity more than 60%. Melikian et al. (1999) measured DNA adducts in cervical epithelial and stromal tissues in smokers and nonsmokers. Increased levels of adducts in the tissues of smokers suggested that delivery of benzo[a]pyrene to the cervical tissues with subsequent metabolism may occur, although transport of reactive metabolites generated in other tissues was not specifically ruled out.

Ramesh et al. (2003, available only as abstract) investigated benzo[a]pyrene metabolism in multiple organ systems in rats exposed by nose-only inhalation to 75 $\mu g/m^3$ benzo[a]pyrene adsorbed on CB 4 hours/day for 60 days. AHH activity and benzo[a]pyrene metabolism were reported for various tissues. A diverse spectrum of metabolites was identified, including dihydrodiols and 3- and 9-monophenols. The concentrations of BPDE were highest in testes, providing mechanistic support for previously observed effects of benzo[a]pyrene on male reproductive parameters in animal toxicity studies.

Exposure to reactive benzo[a]pyrene metabolites may be a concern both in utero and during lactation. Wu et al. (2003) measured the generation of benzo[a]pyrene metabolites in F1 generation pups and determined the mRNA development profile for the AhR in the absence and presence of subacute exposure concentrations of benzo[a]pyrene in preweaning rats. Pregnant F344 rats were exposed to 25, 75, or 100 $\mu g/m^3$ benzo[a]pyrene aerosols via nose-only inhalation, 4 hours/day for 10 days (GDs 11–21). Benzo[a]pyrene metabolites and mRNA and protein expression profiles of AhR and CYP1A1 were analyzed in the cerebral cortex, hippocampus, liver, and plasma. Plasma and cerebral cortex benzo[a]pyrene levels on postnatal day (PND) 0 changed with the dose. Benzo[a]pyrene decreased steadily with time in these tissues, always reflecting the administered dose, reaching nondetectable levels by PND 30. In plasma, diols represented approximately 60% of the total metabolites over the period of 20 PNDs. In the cerebral cortex, diols represented close to 80% of metabolites soon after parturition, decreasing to approximately 40% by PND 20. Benzo[a]pyrene-7,8-diol represented a maximum of approximately 25% of the recovered metabolites in plasma and approximately 30% of recovered metabolites in the cerebral cortex.

There was a statistically significant ($p < 0.05$) up-regulation of AhR mRNA, a subsequent induction of CYP1A1 mRNA, and a significant increase in CYP1A1 protein levels in pup livers at 100 $\mu g/m^3$ compared to unexposed controls (data were presented only for PND 60 and the 100 $\mu g/m^3$ concentration). The AhR mRNA expression profile in the developing cerebral cortex and hippocampus indicated up-regulation of AhR during the first 3 postnatal weeks at all concentrations, although these differences were not statistically significant due to large

individual variation. At the high concentration, AhR mRNA abundance was more than twice that of controls by PND 30. However, this up-regulation of AhR mRNA was not accompanied by a concomitant up-regulation of CYP1A1 mRNA in the CNS. In fact, expression of CYP1A1 in these tissues was very low or absent during the 1-month postnatal period after exposure to 100 $\mu g/m^3$ of benzo[a]pyrene (the only dose tested). Based on these findings, the authors suggested that the up-regulation of the AhR may increase the potential for benzo[a]pyrene neurotoxicity via the activation of CYP450 in liver and the subsequent deposition of toxic metabolites in the developing CNS. The results do not suggest that benzo[a]pyrene metabolism by CYP1A1 is induced in the developing CNS.

Pregnant Swiss Webster mice were administered radiolabeled benzo[a]pyrene by i.v. injection, and maternal and fetal levels of benzo[a]pyrene and radiolabel were determined. Fetal tissue levels of radiolabel increased while maternal tissues decreased. The level of benzo[a]pyrene decreased in fetal tissue during this period, suggesting the accumulation of benzo[a]pyrene metabolites. Increasing the capacity of maternal plasma to bind benzo[a]pyrene by administering a benzo[a]pyrene antiserum decreased fetal accumulation of radiolabel, suggesting that bioavailability had decreased (McCabe and Flynn, 1990). Other investigators have also demonstrated the ability of benzo[a]pyrene to be transported to the fetus (Neubert and Tapken, 1988; Shendrikova and Aleksandrov, 1974). Martin et al. (2000) exposed exfoliated cells from breast milk of human donors to benzo[a]pyrene in vitro. Treated cells showed increased DNA single strand breaks (SSB) relative to controls, indicating that these cells can activate benzo[a]pyrene. Taken together, the results from these various experiments suggest that placental and lactational transfer of benzo[a]pyrene and active metabolites may be of concern.

The developmental expression patterns of metabolizing enzymes can be an important determinant of childhood susceptibility. Numerous in vivo mechanistic tumor screening assays have been conducted in newborn mice (see Section 4.4.1 on mechanistic cancer studies), and the observed increases in tumors provide evidence for the ability of young animals to metabolize benzo[a]pyrene to DNA reactive metabolites. Melikian et al. (1989) administered [^{3}H]-benzo[a]pyrene or [^{3}H]-BPDE via i.p. injection to CD-1 mice on PND 1, 8, or 15 and formation of benzo[a]pyrene metabolites was determined in lung and liver over time periods ranging from 2 minutes to 24 hours after the last dose. In the lung, metabolites included diones, quinones, and phenols, and metabolite levels in the lung were higher on day 1 than on days 8 or 15. In liver, a different spectrum of metabolites was observed, dominated by unidentified polar metabolites. The percentage of radiolabel as metabolites was increased on days 8 and 15, perhaps reflecting greater inducibility of benzo[a]pyrene metabolism in liver versus the lung. Formation of glucuronide or sulfate conjugates was greater than glutathione conjugates in both tissues. The total amount of benzo[a]pyrene metabolized by Phase II enzymes was greater in liver than in lung on day 1 but was similar on days 8 and 15. Age-dependent expression of genes that encode

metabolizing enzymes has been the subject of reviews (e.g., Cresteil, 1998) that are addressed in Section 4.4.

3.3.3.5. *Other Tissues*

Moore et al. (1982) exposed organ cultures of human and rat bladder tissues to [^{3}H]-benzo[a]pyrene for 24 hours and evaluated metabolite profiles. Benzo[a]pyrene was metabolized by bladder tissues of both species. Total mean amount of metabolites formed was higher in human than rat bladders (twofold). A similar spectrum of diverse metabolites was generated, although relative proportions varied. Formation of benzo[a]pyrene-7,8-diol was similar in both species and similar levels of DNA binding was observed. These results indicate that bladder tissues have metabolic capability for benzo[a]pyrene and that the metabolic capacity is similar between humans and rats.

Several other target tissues for benzo[a]pyrene toxicity have been found to have the capacity to metabolize benzo[a]pyrene. For example, Moorthy et al. (2003) treated mouse aortic smooth muscle cells with benzo[a]pyrene, 3-OH-benzo[a]pyrene, or benzo[a]pyrene-3,6-quinone in vitro. Several DNA adducts were identified that were attributed to the 3-OH and BPQ metabolites. Benzo[a]pyrene treatment increased CYP1B1 but not CYP1A1 in these cells. The authors suggest that CYP1B1 activates benzo[a]pyrene to 3-OH and BPQ metabolites, which induce the DNA damage responsible for changes that are important precursors for atherosclerosis.

3.4. ELIMINATION

3.4.1. Inhalation Exposure

Human studies of benzo[a]pyrene exposure have generally been limited to individuals exposed to coke oven emissions, coal tars, or other products containing a mixture of PAHs and are of limited value in assessing urinary benzo[a]pyrene exposure biomarkers. Numerous studies have evaluated 1-OH pyrene in urine as a general marker for PAH exposure, but, because 1-OH pyrene is not a metabolite of benzo[a]pyrene, these data are not directly useful in evaluating the toxicokinetics of benzo[a]pyrene. Some studies have unsuccessfully attempted to quantify exposure to benzo[a]pyrene via measurement of parent compound or its metabolites. Bentsen-Farmen et al. (1999b) compared air concentrations of PAHs to PAH metabolites in the urine of 17 electrode paste plant workers and detected 1-OH-Py but no benzo[a]pyrene metabolites despite the fact that benzo[a]pyrene in personal air samples showed mean exposure levels of 0.3 μg/m^3. Waidyanatha et al. (2003) also attempted to measure PAH exposure via urinary metabolite levels of coke oven workers; several PAHs but no benzo[a]pyrene were detected. In other cases, exposure and urinary metabolites were positively correlated (Hecht et al., 2003; Wu et al., 2002; Gündel et al., 2000; Grimmer et al., 1993; Becher and Bjørseth, 1983).

Wu et al. (2002) reported a correlation between benzo[a]pyrene-tetrols and total PAH exposure in coke oven workers.

Several studies evaluated the elimination of benzo[a]pyrene in animals following exposure via the respiratory tract. Petridou-Fischer et al. (1988) applied 10 μL aliquots of [^{14}C]-benzo[a]pyrene in a gelatin:saline solution over a 2-hour period to the ethmoid and maxillary nasal turbinates of monkeys and dogs to assess differences in benzo[a]pyrene disposition in portions of the nose. Urine levels reached a maximum of 0.69% of the administered dose in dogs and 0.07% in monkeys, while fecal levels reached a maximum of 6.42% of the dose in dogs and 1.17% in monkeys over 48 hours. The pattern of metabolites in the excreta was consistent with results from other respiratory tract deposition studies, showing that benzo[a]pyrene is excreted preferentially in the feces. Wolff et al. (1989) investigated the effects of nose-only exposure of male and female F344 rats to unlabeled benzo[a]pyrene for 4 weeks, followed by a single exposure to [^{14}C]-benzo[a]pyrene. Four weeks after treatment with the radiolabel, the rats had eliminated approximately 96% of the total dose in the feces. The mean half-life was calculated as 22 hours for feces and 28 hours for urine. Sun et al. (1982) exposed male and female F344 rats via nose-only inhalation for 30 minutes to atmospheres containing 0.6 μg/L [^{3}H]-benzo[a]pyrene absorbed onto 67Ga2O3 or to 1.0 μg/L neat [^{3}H]-benzo[a]pyrene and measured levels of radioactivity in tissues and excreta. With either exposure, benzo[a]pyrene excretion declined rapidly over the first 3 days after exposure, with little additional excretion occurring by 5 days postexposure. Excretion in feces was much greater than in urine for either exposure regimen. Following exposure of rats to neat benzo[a]pyrene, feces accounted for 86% of total excreted radioactivity and urine for 14%. Metabolite profiles were not evaluated. Wang et al. (2003) exposed B6C3F$_1$ mice for 10 days to asphalt fumes in an inhalation chamber at a concentration of approximately 180 mg/m^3; the benzo[a]pyrene content of the fumes was not reported. Benzo[a]pyrene metabolites measured in the urine of exposed mice were in the ng/100 mL range and were identified at the following mass ratios (with benzo[a]pyrene arbitrarily set as 1): 7,8,9,10-benzo[a]pyrene-tetrol, 14; benzo[a]pyrene-7,8-diol epoxides, 17; benzo[a]pyrene-7,8-diol, 3.6; 3-OH-benzo[a]pyrene, 10.

In a study investigating the disposition of benzo[a]pyrene, 1 μg/kg of the [^{3}H]-labeled compound dissolved in triethylene glycol was instilled into the trachea of male Sprague-Dawley rats (Weyand and Bevan, 1986). Approximately 60% of the administered dose was associated with the intestine and intestinal contents, and 2.2% of the radiolabel was recovered in the urine 6 hours after dosing. The relative contribution of water- and lipid-soluble metabolites in the intestinal contents was 18.2 and 26.5% of the administered dose, respectively. The amounts of metabolites determined in the organic phase 6 hours after the end of exposure were as follows (percent of total in the organic phase): conjugates or polyhydroxylated compounds (24.1%), 9,10-diol (7.75%), 4,5-diol (8.43%), 7,8-diol (5.73%), 1,6-quinone (7.13%), 3,6-quinone (7.85%), 6,12-quinone (7.10%), 9-OH (2.24%), 3-OH (4.66%), benzo[a]pyrene (1.55%). The

total amounts of metabolites in feces and urine were not reported in this study, and, due to enterohepatic circulation, the intestinal content concentrations may not reflect the final amounts of each metabolite in the excreta. Bevan and Sadler (1992) administered a single intratracheal instillation of benzo[a]pyrene (2 μg/kg) to male Sprague-Dawley rats and assessed metabolite profiles in bile after 6 hours. Relative metabolite levels were 31.2% diglucuronides, 30.4% thioether conjugates, 17.8% monoglucuronides, 6.2% sulfate conjugates, and 14.4% unconjugated metabolites.

Weyand and Bevan (1987) studied the differences in benzo[a]pyrene elimination among rats, hamsters, and guinea pigs. Male Sprague-Dawley and Gunn rats (the latter a strain genetically deficient in bilirubin glucuronidation), male Syrian golden hamsters, and male Dunkin-Hartley guinea pigs were given single intratracheal doses of 0.16 μg or 350 μg [^{3}H]-benzo[a]pyrene per animal. Tissue levels and the amount of radiolabel excreted into the urine and bile 6 hours after treatment are given in Table 3-3. Urinary excretion of benzo[a]pyrene metabolites amounted only to a small fraction of biliary excretion in all tested species. The results suggested that the metabolic capacity for benzo[a]pyrene became saturated in guinea pigs and Sprague-Dawley and Gunn rats but not in hamsters at the 350 μg/animal dose (note: rats weighed 200–250 grams, average high dose 1.56 mg/kg; hamsters 100–140 grams, 2.92 mg/kg; and guinea pigs 600–850 grams, 0.48 mg/kg), indicating that hamsters, who received about 6 times the dose of guinea pigs, command a metabolic system that handles benzo[a]pyrene very well. Moir et al. (1998) administered benzo[a]pyrene i.v. to Wistar rats and recovered, at 8 hours after a 2 mg/kg dose, 4.27% of the dose in urine but only 0.06% in feces, confirming the fact of enterohepatic circulation of benzo[a]pyrene metabolite conjugates (Hirom et al., 1983; Weyand and Bevan, 1986).

Table 3-3. Excretion of benzo[a]pyrene metabolites in several animal species

Dose	Route of excretion	Gunn rat[a]	Sprague-Dawley rat[a]	Syrian golden hamster[a]	Dunkin-Hartley guinea pig[a]
0.16 μg/animal	Urine	0.98 ± 0.16	2.21 ± 1.1	3.74 ± 1.2	2.22 ± 1.2
	Bile	59.4 ± 1.07	70.3 ± 2.0	54.6 ± 3.6	71.7 ± 1.9
350 μg/animal	Urine	1.44 ± 1.82	1.68 ± 0.6	2.53 ± 0.4	1.27 ± 0.4
	Bile	30.8 ± 1.51	55.0 ± 2.0	52.9 ± 2.7	47.9 ± 4.9

[a]Values are percent of the applied dose at 6 hours after intratracheal instillation.

Source: Weyand and Bevan (1987).

The pattern of metabolites in bile was also reported; results are compiled in Table 3-4. At each dose thioether conjugates predominated but to a varying extent in each species. Guinea pigs evidently used preferentially thioether conjugation for biliary benzo[a]pyrene elimination, with the other conjugates (or nonconjugated metabolites) making up a small portion of biliary

excretion. At the high dose a shift in the relative proportion of metabolites was observed, with thioethers decreasing, glucuronides and sulfates increasing, and total nonconjugated remaining unchanged in both rat strains and hamster. The data also indicate that glucuronidation and sulfation became saturated at the high dose only in guinea pigs, while thioether formation capacity became overwhelmed by the high dose in all species. Glucuronide levels in the bile of Gunn rats were about one-half those of Sprague-Dawley rats; this strain also excreted fewer nonconjugated metabolites and appeared to have a generally lower metabolic capacity for benzo[a]pyrene.

Table 3-4. Biliary excretion of benzo[a]pyrene metabolites in several species

Metabolite group	Dose (μg/animal)	Gunn rat[a]	Sprague-Dawley rat[a]	Syrian golden hamster[a]	Dunkin-Hartley guinea pig[a]
Nonconjugated	0.16	4	11.5	4.1	1.7
	350	2	8.3	4.2	1.3
Glucuronides	0.16	7	12.9	11.9	4.5
	350	9	18.7	18.1	0.2
Sulfate conjugates	0.16	8	3	3.7	1.9
	350	8	5.9	6.9	1.1
Thioether conjugates	0.16	40	42.9	33.8	54.1
	350	12	22.1	23.7	45.4

[a]Values are percent of the applied dose at 6 hours after intratracheal instillation.

Source: Weyand and Bevan (1987).

Following intratracheal administration of 1 μg/kg body weight [^{3}H]-benzo[a]pyrene to male Sprague-Dawley rats in hydrophilic triethylene glycol, 70.5% of the administered benzo[a]pyrene was excreted into bile within 6 hours (Bevan and Ulman, 1991). In contrast, benzo[a]pyrene excretion was 58.4 and 56.2% in the same time period when the lipophilic solvents ethyl laurate and tricaprylin, respectively, were the vehicles. Benzo[a]pyrene (in triethylene glycol) excretion in bile was described as biphasic with half-lives of 31 and 100 minutes, respectively. Individual metabolite concentrations were not monitored.

3.4.2. Oral Exposure

Only limited data were identified on the elimination of benzo[a]pyrene following exposure by the oral route. Although the dietary/oral route is likely to be the predominant route of exposure for the general population not occupationally exposed, it has received very little attention (Stavric and Klassen, 1994). In the only study investigating this issue in humans, the concentration of benzo[a]pyrene was below detection limits (<0.1 μg/person) in the feces of

eight volunteers who had ingested broiled meat containing approximately 8.6 μg of benzo[a]pyrene (Hecht et al., 1979).

Ramesh et al. (2001b) evaluated benzo[a]pyrene disposition in F344 rats dosed via gavage with 100 mg/kg benzo[a]pyrene dissolved in peanut oil. Recovery of unmetabolized benzo[a]pyrene from feces reached a maximum of 35% of the dose at 24 hours but was very low at 48 hours and thereafter. Lipid-soluble metabolites in feces reached approximately 28% of all metabolites by 8 hours after dosing then declined sharply. Lipid-soluble metabolites in urine reached 35–40% of total by 48 hours then declined to approximately 10% by 72 hours. Diol metabolites were most numerous in feces, while phenols predominated in urine.

Hecht et al. (1979) conducted a study of fecal excretion of benzo[a]pyrene and its metabolites in rats. In male F344 rats administered [^{14}C]-benzo[a]pyrene via gavage (0.04, 0.4, or 4 μmol/animal), approximately 85% of radiolabel was recovered in feces, and 1–3% in urine after 168 hours. The portion of benzo[a]pyrene recovered from feces as parent compound ranged from 13 to 6% of the administered dose within 48 hours. In rats fed charcoal-broiled hamburger containing 52.7 μg benzo[a]pyrene/kg meat, 11% of the benzo[a]pyrene was excreted unchanged in feces.

3.4.3. Dermal Exposure and Other Exposure Routes

Bowman et al. (1997) detected benzo[a]pyrene-tetrols in 40% of the urine samples from psoriasis patients treated with coal tar medication, as compared to only 10% of those of controls. No specific measures of applied dose were available.

In a dermal absorption study, Yang et al. (1989) evaluated the recovery of [^{3}H]-benzo[a]pyrene, 100 ppm in crude oil applied topically, in urine and feces of female Sprague-Dawley rats. Total recovery of applied radioactivity over 96 hours was 5.3% in urine and 27.5% in feces. Individual metabolite concentrations were not measured. Ng et al. (1992) examined the percutaneous absorption of radiolabeled benzo[a]pyrene in the hairless guinea pig. Following a single application of 28 μg benzo[a]pyrene (dissolved in 50 μL acetone applied to 4 cm^2 of dorsal skin), approximately 34% of the administered radiolabel was eliminated within 24 hours. Most excretion had occurred by day 3 and continued slowly to reach 73% by day 7. Relative amounts of benzo[a]pyrene or metabolites in urine versus feces were not reported.

Moir et al. (1998) dosed male Wistar rats i.v. with 2, 6, or 15 mg/kg of [^{14}C]-benzo[a]pyrene and examined excretion in urine and feces over 32 hours. At 8 hours after injection, urinary excretion was 4.3, 2.6, and 3.2% of the dose, while fecal excretion was only 0.06, 5.6, and 0.43% of the 2, 6, and 15 mg/kg doses, an indication of enterohepatic circulation. The amount of radioactivity excreted in the urine after 32 hours was similar in each dose group (6–7% of the administered dose). However, the proportion of the administered dose in the feces was dose-dependent. At the low dose (2 mg/kg) fecal excretion accounted for 26% of the dose,

while at the mid- and high-doses, fecal excretion accounted for 56 and 50% of the dose, respectively. No measurement of specific metabolites or whole body clearance was conducted.

Following i.v. administration of 3 μmol [^{14}C]-benzo[a]pyrene to male New Zealand white rabbits, approximately 30% of the dose was recovered in the bile and 12% in the urine within 6 hours after treatment (Chipman et al., 1982). Excretion in the bile was biphasic, with estimated half-lives of 0.27 and 4.62 hours. Further analysis of metabolite profiles in bile and urine were conducted. Treatment of bile or urine with β-glucuronidase or aryl sulfatase increased the amount of radioactivity recovered in ethyl acetate extracts, suggesting that sulfate and glucuronide conjugation are important contributors to benzo[a]pyrene metabolites in excreta. Analysis of these extracts revealed the primary metabolite as 9,10-diol, with lower amounts of numerous other metabolites identified (diols, quinones, and monophenols).

Likhachev et al. (1992) measured the excretion of benzo[a]pyrene-7,8-diol and 3-OH-benzo[a]pyrene in L10 rats given a single i.p. dose of 200 mg/kg benzo[a]pyrene. Urine and feces were collected over a 15-day period and then again at 30 days after exposure for another 5 days. Both metabolites were excreted in feces at two to three times the amount excreted in urine. Metabolites in urine and feces decreased steadily over the 15-day postexposure period, but no more metabolites were detected at 30 days. A comparative study of metabolism in male *Macaca fascicularis* monkeys (five animals) and male L10 rats (four animals) was also performed. Both species were given a single i.p. dose of 100 mg/kg benzo[a]pyrene, and levels of benzo[a]pyrene metabolites in feces were evaluated over 8 days. Total benzo[a]pyrene metabolites were significantly lower in monkeys than in rats, but the results were hampered by infrequent feces collection in monkeys. Monkeys had an approximately fourfold higher benzo[a]pyrene-7,8-diol to 3-OH-benzo[a]pyrene ratio in feces than rats. Together these data suggest that monkeys have lower excretion of benzo[a]pyrene (and perhaps lower metabolism), and formation of the proximate carcinogenic metabolite is higher than in rats.

In another part of this study (Likhachev et al., 1992), benzo[a]pyrene metabolism and excretion were assessed following multiple exposures. L10 rats were given i.p. injections of 1 mg/kg benzo[a]pyrene every 11th day for 10 treatments. Benzo[a]pyrene metabolites were measured in excreta for 8 days after 1, 5, and 10 treatments. After the single dose, excretion in feces was favored over urine, and benzo[a]pyrene-7,8-diol was the predominant metabolite. The rate of metabolite excretion was decreased considerably after 5 and 10 doses, respectively. The authors attributed this finding to age-related changes in metabolic capacity or "exhaustion of enzymes." They also compared metabolite profiles for individual rats with their tumor responses and based on the results suggested a link between tumor latency and elimination of benzo[a]pyrene metabolites (although small sample size and variability limited the power of the analysis). A direct correlation between benzo[a]pyrene-7,8-diol excreted in urine and tumor latency was observed. It was postulated that higher excretion of reactive benzo[a]pyrene

metabolites caused fewer of these metabolites to bind to DNA targets and thus increased tumor latency.

In addition to benzo[a]pyrene and its metabolites, adducts of benzo[a]pyrene with nucleotides have also been identified in feces and urine of animals but only as a small fraction of the administered dose. Autrup and Seremet (1986) administered i.p. doses of 0, 10, 50, or 100 μg/kg tritiated benzo[a]pyrene to male Wistar rats and collected urine over 24-hour periods for 72 hours. The level of BPDE adducts with guanine detected in urine was dose-dependent. The authors reported that, at the high dose, 0.15% of the administered benzo[a]pyrene dose was excreted in the urine as this adduct within 48 hours. Rogan et al. (1990, only study abstract was available for review) reported that 0.02% of a benzo[a]pyrene dose was excreted as an adduct with guanine in urine and feces over 5 days.

Overall, the data on benzo[a]pyrene elimination in humans are too limited to estimate quantitative rates of elimination. The situation is further complicated by the existence of multiple pathways in which many of the key enzymes exhibit polymorphisms. In the context of biomonitoring studies, benzo[a]pyrene metabolites have been detected in the urine of exposed humans, but the fecal excretion has not been investigated in any detail. The animal data are consistent in showing that feces are the primary route of elimination of benzo[a]pyrene, while urinary excretion plays a lesser role. A diverse array of metabolites, as well as parent benzo[a]pyrene, is found in the excreta. Enterohepatic circulation of benzo[a]pyrene metabolite conjugates has been demonstrated in animals and may exist in humans as well, but its impact on the toxicokinetics of benzo[a]pyrene is not understood.

Similar considerations apply to the toxicokinetics of benzo[a]pyrene as a whole. A few animal studies that have attempted comprehensive assessments of benzo[a]pyrene metabolism. Although a lot is known from ex vivo studies about which humans tissue can metabolize benzo[a]pyrene to what extent, comprehensive studies to estimate overall metabolic capacities or the balance between Phase I and Phase II metabolism in humans have not been conducted. There are also no comprehensive data on the tissue distribution of benzo[a]pyrene in humans, not counting a number of studies that have reported post mortem levels in a few tissues in the absence of any exposure assessments. Thus, despite the fact the humans are universally exposed to benzo[a]pyrene, there is a great need for more knowledge concerning its toxicokinetics.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS

Several toxicokinetic or pharmacokinetic models of benzo[a]pyrene have been developed for rodents (rat and hamster), but none has been calibrated in humans. Bevan and Weyand (1988) performed compartmental pharmacokinetic analysis of distribution of radioactivity in male Sprague-Dawley rats, following the intratracheal instillation of benzo[a]pyrene to normal and bile duct-cannulated animals (Weyand and Bevan, 1987, 1986). The authors used the Simulation, Analysis and Modeling (SAAM) and Conversational SAAM (CONSAAM)

computer programs to model the disposition of labeled benzo[a]pyrene and its metabolites. A good fit to the experimental data was achieved with 10 assumed compartments corresponding to lung, blood, liver, other tissues (reflecting the sum of radioactivity in kidney, stomach, testis, spleen, heart, and thymus), carcass (reflecting the sum of radioactivity in skin, fat, bones, muscle, and blood in those organs), and several additional hypothetical compartments that linked the central compartment with intestines (including their contents) and urine. Enterohepatic circulation and intestinal secretion were both included in the model. The model allowed calculation of linear rate constants for moving radioactivity among compartments (in $minute^{-1}$), representing the probability per unit time that radioactivity from one compartment would be transferred to another. The model adequately simulated disposition of benzo[a]pyrene and its metabolites, measured as total radioactivity in blood, organs and excreta, under the assumption that the kinetics of benzo[a]pyrene and its metabolites are the same. The authors emphasized differences between their approach in building the implicit SAAM model and the approach used to build explicit PBPK models. Roth and Vinegar (1990) reviewed the capacity of the lung to impact the disposition of chemicals and used benzo[a]pyrene as a case study. A PBPK model was presented based on data from Wiersma and Roth (1983a, b) and was evaluated against tissue concentration data from Schlede et al. (1970). The model was structured with compartments for arterial blood, venous blood, lung, liver, fat, and slowly as well as rapidly perfused tissues. Metabolism in liver and lung was estimated using kinetic data from control rats and rats pretreated with 3-MC to induce benzo[a]pyrene metabolism. Benzo[a]pyrene binding was accounted for in blood, liver, and lung, but only one binding component was used, which, as the authors suggested, resulted in a rather poor fit for liver. The model was built based on blood and tissue concentrations measured over 5 hours in rats given a dose of 117 nmol/kg by i.v. injection into the arterial circulation or the venous blood supply of the liver. The model was tested against the data of Schlede et al. (1970). The number of data points was limited (six time points for the venous circulation, liver, and fat and only a single time point for the lung). The model predicted the data of Schlede et al. (1970) reasonably well, although deviations were apparent for several compartments. Most notably, the model overpredicted concentrations in the induced lung, uninduced and induced liver, and induced fat compartments. The results of PBPK simulations showed that induction of metabolizing enzymes increased the amount of benzo[a]pyrene cleared by the lungs relative to the liver.

An interesting result of the Roth and Vinegar (1990) modeling study was that even though the metabolic clearance of benzo[a]pyrene in the lungs was low in comparison to the liver, under simulated pathological conditions of reduced hepatic blood flow, a substantial metabolic clearance was carried on by well perfused lungs. This illustrates that changes in tissue perfusion can shift the organ pattern of benzo[a]pyrene metabolic clearance in vivo, and consequently, might also shift the organ pattern of benzo[a]pyrene-induced disease, such as cancer. The authors emphasized that their PBPK model with the results of simulations should

not be taken as a definite model for benzo[a]pyrene but that it could be used for designing further studies of benzo[a]pyrene metabolic clearance. They also suggested that it would be appropriate to incorporate into the PBPK model a description of the appearance of various metabolites of benzo[a]pyrene.

Moir et al. (1998) conducted a pharmacokinetic study on benzo[a]pyrene to obtain data for model development. Rats were injected with [^{14}C]-benzo[a]pyrene at doses of 2, 6, or 15 mg/kg and blood, liver, fat, and richly perfused tissue were sampled at 15 time points from 5 minutes to 32 hours after dosing. Moir (1999) then described a model for lung, liver, fat, richly and slowly perfused tissues, and venous blood, with saturable metabolism occurring in the liver. The fat and richly perfused tissues were modeled as diffusion-limited, while the other tissues were flow-limited. The model was developed using the 15 mg/kg dose group data from the Moir et al. (1998) study and validated using the 2 and 6 mg/kg data. The model predicted the blood benzo[a]pyrene concentrations well, although it overestimated the 6 mg/kg results at longer times (>100 minutes). The fat and richly perfused tissue benzo[a]pyrene levels were reproduced by the model fairly well at the two highest dose levels but underestimated at the 2 mg/kg dose. The model produced a poor fit to the liver data, underestimating benzo[a]pyrene tissue levels at the two lowest doses. At the high dose the model overestimated the liver benzo[a]pyrene concentration at times <200 minutes but afterwards underestimated them increasingly more. It appeared that the model could not accommodate a slow elimination phase at times beyond 200 minutes for the mid and high dose levels. The model simulations were also compared to data of Schlede et al. (1970) who had injected rats with 0.056 mg/kg body weight of benzo[a]pyrene. Again the model predicted blood and fat benzo[a]pyrene concentrations quite well but underestimated liver benzo[a]pyrene concentrations.

Moir (1999) suggested that the poor prediction of fat benzo[a]pyrene concentrations after injection of 2 mg/kg benzo[a]pyrene in their own study (Moir et al., 1998) was due to analytical error, causing the model to fail. This explanation is credible because the measured fat benzo[a]pyrene concentrations following injection of 2 mg/kg benzo[a]pyrene were indistinguishable from those obtained following injection of 6 mg/kg benzo[a]pyrene (Moir et al., 1998). The author also speculated that the poor fit to liver benzo[a]pyrene concentration data was due to binding of benzo[a]pyrene to receptors in the liver. The model predicted blood and fat benzo[a]pyrene concentrations fairly accurately over a wide range of doses (0.056–15 mg/kg), but it was limited by the failure to accurately predict liver benzo[a]pyrene concentrations. The model included only one saturable metabolic pathway, and only parent chemical concentrations were used to establish the model. Metabolites were not modeled.An attempt to scale the rodent PBPK model to humans, relevant to risk assessment of oral exposures to benzo[a]pyrene, was presented by Zeilmaker et al. (1999a, b). The PBPK model for benzo[a]pyrene was derived from an earlier model for TCDD in rats (Zeilmaker and van Eijkeren, 1997). The structure of the mainly perfusion-limited PBPK model included

compartments for blood, adipose tissue (with diffusion limitation), slowly and richly perfused tissues, and the liver (Figure 3-3). However, there was no separate compartment for the lung. The liver compartment featured the AhR-dependent CYP450 induction mechanism and DNA adduct formation as a marker for formation of genotoxic benzo[a]pyrene metabolites. It was assumed that DNA adduct formation and the bulk benzo[a]pyrene metabolism were mediated by two different metabolic pathways. The model was experimentally calibrated in rats with the data for EROD and formation of DNA adducts in the liver after i.v. administration of a single dose and per oral (p.o.) administration of a single or repeated doses of benzo[a]pyrene (Zeilmaker et al., 1999a).

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

Even though the model of Zeilmaker et al. (1999b) represents a major improvement in predictive modeling of benzo[a]pyrene toxicokinetics, the results of modeling, which included interspecies extrapolation, bear significant uncertainties. As emphasized by the authors, the conversion of benzo[a]pyrene to its mutagenic and carcinogenic metabolites could not be explicitly modeled in human liver because no suitable experimental data were available. According to the authors, to improve the model would require direct measurements of basal activities of CYP1A1 and CYP1A2 and formation of benzo[a]pyrene-DNA adducts in human liver. Moreover, despite the prior results of the study by Roth and Vinegar (1990), the metabolic clearance of benzo[a]pyrene in the lungs was not addressed. Also, the toxicokinetic modeling by Zeilmaker et al. (1999b) addressed only one pathway of benzo[a]pyrene metabolic activation, a single target organ (the liver), and one route of administration (oral).

For modeling and predicting of the health outcomes of exposures to benzo[a]pyrene, a mechanistically accurate PBPK model needs to follow, over time and in several target organs, the rate of accumulation of benzo[a]pyrene-DNA adducts and/or the distribution and fate of benzo[a]pyrene metabolites (e.g., BPDE) that bind to DNA and other macromolecules. Alternatively, a stable derivative of the "ultimately carcinogenic" metabolite (e.g., benzo[a]pyrene trans-anti-tetrol) may be used as an internal dose surrogate. Calibration of such a model requires quantitation of these benzo[a]pyrene metabolites in biological samples, which,

in turn, would require refined and sufficiently sensitive analytical methods. Therefore, while the metabolic pattern of benzo[a]pyrene has been relatively well characterized qualitatively in animals, both in vitro and in vivo, the quantitative kinetic relationships between overlapping and sometimes competing metabolic reactions in potential target organs, essential for meaningful PBPK modeling, are yet not well defined.

Furthermore, the potential for scaling this model to humans without complete reparameterization is questionable. According to Liao (2004), the recombinant human CYP1A1 has a higher activity for the formation of benzo[a]pyrene-hydroxy metabolites and a lower activity for the formation of benzo[a]pyrene-diones, when compared with recombinant rat CYP1A1. The RN-PBPK model overestimated the benzo[a]pyrene concentration in the liver, but after 4 hours, it underestimated benzo[a]pyrene concentration in fat. In contrast to the findings of Zeilmaker et al. (1999a), the adipose tissue compartment was modeled only as perfusion-limited. Tissue-blood partition coefficients for benzo[a]pyrene and its metabolites used in the RN-PBPK model were estimated but not validated experimentally or otherwise. Also, no adjustment was made for using in vitro enzyme activities, especially when measured in recombinant proteins as was done in the calibration of this model, although they typically differ from those in vivo.

The published PBPK models for benzo[a]pyrene were evaluated to determine whether the existing models could be used to extrapolate from rats to humans or for a route to route extrapolation from oral exposure to the inhalation route. The focus was concentrated on models for the inhalation route since several well conducted studies by the oral route exist from which to derive toxicity values. No full PBPK model for the inhalation route was identified. It was concluded that at present, none of the published models allow for computation of the resulting internal doses used in the only cancer bioassay available for the inhalation route (Thyssen et al. 1981), nor is there a model for humans that simulates the typical inhalation exposure to benzo[a]pyrene on poorly soluble carbonaceous particles.

4. HAZARD IDENTIFICATION

4.1. HUMANS STUDIES

4.1.1. Sources of Human Exposure

Although it has no commercial production or significant uses, benzo[a]pyrene is nevertheless a ubiquitous environmental contaminant resulting from the incomplete combustion of organic matter. Essentially all humans experience repeated exposure to benzo[a]pyrene, which can begin in utero and continue throughout life. The magnitude of benzo[a]pyrene exposure depends on several factors related to lifestyle (e.g., diet, tobacco smoking), occupation, and living conditions (e.g., urban versus rural setting, domestic heating and cooking methods). A distinguishing feature concerning benzo[a]pyrene exposure is that environmental sources always occur as complex mixtures, which may consist of numerous PAHs, including heterocyclic and nonheterocyclic forms as well as aza arenes and nitro-substituted PAHs (reviewed in Bostrom et al., 2002; WHO, 1998; ATSDR, 1995; IARC, 1973). Many of these complex mixture components are carcinogens, and some can exceed the carcinogenic potency of benzo[a]pyrene, as observed in animal bioassays.

With the exception of certain occupational exposure sources such as aluminum production and the conversion of coal to coke and coal tar, the major contributor to total PAH (and thus benzo[a]pyrene) exposure in nonsmokers is the diet (Cogliano et al., 2008; Straif et al., 2005; Bostrom et al., 2002; Cenni et al., 1993; Andersson et al., 1983; Bjorseth et al., 1978a, b). In particular, charbroiled and grilled meats, and certain grain products, are important determinants of PAH exposure in most populations. In very limited populations receiving clinical coal tar treatment of the skin for conditions such as psoriasis, acute dermal exposure to benzo[a]pyrene may greatly exceed that received from both occupational and nonoccupational sources (Godschalk et al., 2001; Pavanello et al., 1999).

4.1.2. Biomonitoring of Benzo[a]pyrene Exposure and Effects In Humans

Quantitative exposure assessment becomes very challenging for complex mixture components such as benzo[a]pyrene, where individual exposures vary depending on background concentrations in the environment, lifestyle factors, and occupation. An alternative to measuring concentrations in various environmental media, human biomonitoring focuses on biomarkers of internal dose which may also serve as mechanistic indicators of early biological response following exposure to a genotoxic agent. Carcinogen exposure biomarkers are generated by uptake and metabolic processes which may result either in detoxification and excretion or in bioactivation to reactive forms that can bind covalently with DNA and other macromolecules. Following exposure to complex PAH mixtures, biomonitoring of benzo[a]pyrene uptake can involve evaluation of urinary metabolites of benzo[a]pyrene or surrogates, DNA adducts in

peripheral blood cells such as leukocytes and lymphocytes, protein adducts in hemoglobin and serum albumin, and cytogenetic damage in lymphocytes (Gyorffy et al., 2008; Godschalk et al., 2003; Bostrom et al., 2002; Scherer et al., 2000). Biomarkers offer advantages over traditional benzo[a]pyrene exposure concentration monitoring because they can be quantitative indicators of an individual's environmental exposure and internal carcinogen dose irrespective of time, route of exposure, and inter-individual metabolic differences. Associations between increased cancer risk and levels of specific biomarkers, such as benzo[a]pyrene-DNA adducts in target or surrogate tissues, also provide important mechanistic information in studies of human disease etiology.

Considerable progress has been achieved in the application of biomarker methods for human biomonitoring of carcinogens (Vineis and Perera, 2007; Perera and Weinstein, 2000; Poirier et al., 2000). However, further validation is needed before using biomarkers to routinely predict disease risk. Successful application of biomarkers in benzo[a]pyrene exposure assessment currently depends on several assumptions: 1) urinary metabolites, whether derived from benzo[a]pyrene or surrogate PAH compounds such as Py or phenanthrene, provide information about recent benzo[a]pyrene exposures; 2) DNA and protein adduct measurements in easily accessible tissues serve as surrogate biomarkers of long-term benzo[a]pyrene exposure and internal dose for less accessible target tissues (e.g., lung); 3) and cytogenetic biomarkers reflect early biological effects that correlate with relevant preclinical events occurring at target sites, and with biomarkers of biologically effective benzo[a]pyrene dose. Evidence is accumulating to support these assumptions for carcinogenic PAHs, in general, and for benzo[a]pyrene, in particular, although conflicting and variable results among some studies limit their application for dose-response assessment. Important issues to address include differences in biomarker assay sensitivity and specificity, acquired and inherited variations in PAH bioactivation, detoxification, and DNA repair, and uncertain carcinogen intake(Gyorffy et al., 2008; Divi et al., 2002; Poirier et al., 2000; Scherer et al., 2000; Santella et al., 1994).

4.1.2.1. *Urinary Excretion of Benzo[a]pyrene*

Based on animal studies, urinary excretion of benzo[a]pyrene is a minor pathway of elimination compared with elimination in the feces. The hydroxylated metabolites of benzo[a]pyrene, 3-hydroxy-benzo[a]pyrene (3-OH-benzo[a]pyrene) and 9-hydroxy-benzo[a]pyrene, have been identified in the urine of humans exposed to PAH, although they are often not detectable in human urine despite known exposure to benzo[a]pyrene (Rossella et al., 2009; Hecht, 2002; Bentsen-Farmen et al., 1999b). In numerous human studies, the hydroxylated metabolite of Py, 1-OH-Py, is typically used as a surrogate indictor of internal exposure to carcinogenic PAH mixtures based on the assumption that levels of 1-OH-Py in urine correlate with exposure to individual PAH compounds of higher molecular weight, including benzo[a]pyrene. Although urinary 1-OH-Py appears suitable as a sensitive biomarker for total

PAH exposure, results are variable for the correlation of 1-OH-Py with the benzo[a]pyrene metabolite, 3-OH-benzo[a]pyrene, in urine (Forster et al., 2008; Godschalk et al., 1998a). In a study of urinary PAHs and their hydroxylated metabolites in 55 coke oven workers, 1-OH-Py was found in 100% of the urine samples, whereas 3-OH-benzo[a]pyrene was always below the quantification limit (Rossella et al., 2009). In addition, reported correlations between urinary 1-OH-Py levels and PAH-DNA adducts in white blood cells (WBCs) are conflicting, although in one study, 3-OH-benzo[a]pyrene levels in urine were significantly correlated with specific benzo[a]pyrene diol epoxide adducts in skin DNA following dermal application of coal tar ointments (Gyorffy et al., 2008; Godschalk et al., 1998a).

Buckley et al. (1995) found that excretion of benzo[a]pyrene metabolites in residents (non-smokers not employed in high PAH occupational environments) of Phillipsburg, New Jersey, correlated better with ingestion of benzo[a]pyrene from food rather than with environmental inhalation exposures. Benzo[a]pyrene metabolites in urine were measured following "reverse metabolism," a procedure that involves enzymatic hydrolysis of benzo[a]pyrene conjugates followed by chemical conversion of all hydroxylated benzo[a]pyrene metabolites back to the parent compound for subsequent thin-layer chromatography and scanning spectrofluorometry. Buckley et al. (1995) estimated pulmonary uptake at 11 and 2.3 ng benzo[a]pyrene/person/day for winter and summer, respectively, based on 24-hour personal air measurements. The median intake of benzo[a]pyrene from the diet was estimated at 176 ng/day. The median urinary excretion of benzo[a]pyrene and metabolites was 121 and 129 ng/day in winter and summer, respectively. Based on multiple regression analyses of estimated inhaled and ingested doses, change in urinary excretion of benzo[a]pyrene was only marginally predictive of benzo[a]pyrene exposure. Most of the variation in urinary benzo[a]pyrene excretion was explained by the ingested dose. These results confirm an earlier study of occupationally exposed (aluminum plant) workers using the same reverse metabolism procedure to measure urinary PAH elimination (Becher and Bjørseth, 1983). Although a significant difference in urinary PAH excretion was seen in nonoccupationallly exposed smokers versus nonsmokers, PAH metabolite levels in urine of aluminum plant workers did not reflect the large difference in inhalation exposure relative to controls (Becher and Bjørseth, 1983).

Gündel et al. (2000) investigated PAH exposure and urinary metabolite excretion in 19 workers from a fireproof stone factory. Along with other PAH metabolites in urine, they measured 3-OH-benzo[a]pyrene levels following airborne exposures to benzo[a]pyrene ranging from 0.043 to 5.41 $\mu g/m^3$ based on personal and stationary air sampling. The study authors failed to identify a correlation between benzo[a]pyrene inhalation exposure concentration and urinary 3-OH-benzo[a]pyrene excretion, nor between 1-OH-Py and 3-OH-benzo[a]pyrene levels in urine.

4.1.2.2. *Adduct Formation with DNA and Protein*

A large body of literature supports DNA and protein adducts as biomarkers of a biologically effective dose (ED) for exposures to some DNA reactive human carcinogens (Gyorffy et al., 2008; Vineis and Perera, 2007; Hecht, 2004; Godschalk et al., 2003; Bostrom et al., 2002; Perera and Weinstein, 2000; Poirier et al., 2000). DNA adduct formation is considered to be a necessary early event in tumor formation for many such compounds, particularly large, bulky carcinogens such as benzo[a]pyrene and PAHs in general. In PAH exposed populations, there is evidence demonstrating the formation of DNA adducts with tobacco-derived PAH including benzo[a]pyrene, not only sites directly exposed, but also in distant organs and the peripheral blood (see Table 4-1).

Table 4-1. Studies of PAH-DNA adducts in human populations or tissues exposed to PAHs

Reference	Study description
Arnould et al. (1998)	DNA adducts from leukocytes from heavy smokers
Arnould et al. (1999)	DNA adducts in leukocytes from workers from plant producing carbon electrodes
Arnould et al. (2000)	DNA adducts in leukocytes of coke oven workers
Assennato et al. (1993)	DNA adducts in peripheral blood leukocytes in coke oven workers
Bartsch et al. (1999)	PAH DNA adduct levels in lung parenchyma of coke oven workers and smokers
Bartsch et al. (1999)	DNA adduct formation in smokers, tobacco chewers, coke oven workers
Bhattacharya et al. (2003)	benzo[a]pyrene-DNA adducts in human urine
Casale et al. (2001)	benzo[a]pyrene adducts in urine of cigarette smokers and women exposed to household smoke
Galati et al. (2001)	DNA adducts in sera of humans exposed to PAHs
Gallagher et al. (1993)	DNA adducts in blood cells, placental syncytial nuclei, placental tissue homogenates, and lung cells following exposure to cigarette or coal smoke
Godschalk et al. (1998a)	DNA adducts in alveolar macrophages and subpopulations of white blood cells in smokers
Godschalk et al. (1998b)	DNA adducts in biopsies of treated skin and in WBCs along with levels of urinary 1-OH-pyrene in psoriasis patients being treated with coal tar
Hemminki et al. (1997	DNA adduct formation in exposed humans
Izzotti et al. (1991, 1992)	DNA adducts in pulmonary alveolar macrophages following exposure to cigarette smoke
Li et al. (2001)	DNA adduct levels in human peripheral blood lymphocytes of SCC patients and controls
Li et al. (2002)	BPDE-DNA adducts in human breast tissues
Lodovici et al. (1998)	benzo[a]pyrene levels in autoptic lungs of smokers and nonsmokers
Lodovici et al. (1999)	DNA adduct formation in human white blood cells from smokers and nonsmokers
Mancini et al. (1999)	DNA adducts in cervical cells from cigarette smokers
Melikian et al. (1999)	DNA adducts in epithelial and stromal cervical tissue samples from women smokers and self-reported nonsmokers after hysterectomy for nonmalignant conditions
Paleologo et al. (1992)	BPDE-DNA adducts in the white blood cells of patients treated with coal tar preparations
Pavanello et al. (1999)	DNA adducts in mononuclear white blood cells of coke oven workers and chimney sweeps
Rojas et al. (2000)	PAH DNA adduct levels in lung parenchyma of coke oven workers and smokers
Scherer et al. (2000)	benzo[a]pyrene adducts of hemoglobin and albumin in smokers and

Reference	Study description
	nonsmokers
Schoket et al. (1993)	DNA adducts in lymphocytes in aluminum plant workers
Schwartz et al. (2003)	DNA analysis (content, damage, cell cycle, and apoptosis) in smokers and nonsmokers
Shinozaki et al. (1999)	DNA adduct formation in aging smokers and non-smokers
Van Delft et al. (1998)	WBC-DNA adducts in workers in a carbon electrode manufacturing facility
Wiencke et al. (1990)	benzo[a]pyrene DNA adducts and SCEs in human lymphocytes
Zenzes et al. (1999a)	DNA adducts in sperm cells from smokers and nonsmokers
Zenzes et al. (1999b)	DNA adducts in embryos from smoking couples
Zhang et al. (1995)	DNA adduct levels in oral mucosa cells from nonsmokers and smokers

Measurement of DNA adducts in target tissues (e.g., lung) can be difficult and invasive; however, readily accessible nucleated tissue (e.g., WBCs, sperm cells, cervical cells) and proteins (e.g., hemoglobin and albumin in blood) can serve as surrogate biomarkers of exposure. DNA adducts in WBCs reflect exposure over a relatively long period, and are indicative of the individual's metabolic and DNA repair capability, both of which are genetically influenced. Although protein adducts are not thought to be mechanistic intermediates in PAH-initiated carcinogenesis, they offer several advantages as biomarkers. For example, hemoglobin and albumin are much more abundant than DNA; their adducts are not subject to removal by enzymatic repair, and they can integrate exposures over the protein lifespan of days to weeks.

In humans, PAH albumin and hemoglobin adducts have often been used to investigate internal dosimetry of direct tobacco and environmental tobacco smoke (ETS) exposures. Elevated benzo[a]pyrene adducts are often reported in smokers versus nonsmokers and in children exposed to ETS from their smoking mothers (Hecht, 2004; Philips, 2002). Benzo[a]pyrene-protein adduct levels in smokers are typically increased twofold compared with nonsmokers (Scherer et al., 2000; Sherson et al., 1990).

4.1.2.2.1. DNA adduct measures in blood

Major advances in methodology have greatly increased the sensitivity and specificity (Himmelstein et al., 2009; Jarabek et al., 2009) of DNA adducts measurements. The various techniques utilized include immunoassays and immunohistochemistry, [^{32}P]- and [^{33}P]-postlabelling of modified nucleotides paired with thin layer or high performance liquid chromatography (TLC and HPLC, respectively), fluorescence and phosphorescence spectroscopy, electrochemical detection, and mass spectrometry (Arlt et al., 2007; Poirier et al., 2000). [^{32}P]-postlabelling assays and immunological methods are the most commonly used. The immunoassays employ an antiserum generated against benzo[a]pyrene-DNA adducts. In addition to DNA adducts formed by benzo[a]pyrene, the antibody cross reacts with DNA adducts formed by other carcinogenic polycyclic aromatic hydrocarbons (PAHs) (Weston, et al, 1989) but has no affinity for DNA or PAHs alone. DNA adducts detected in non-laboratory samples using this technique are therefore referred to as "PAH-DNA" adducts. The [^{32}P]-postlabelling method is highly sensitive in detecting bulky DNA adducts, including but not limited to benzo[a]pyrene- and PAH-DNA adducts, enabling quantitation down to 1 DNA adduct/10^9 normal nucleotides, and achieving high resolution of individual adducts particularly when combined with HPLC. Unless specific chromatographic standards are available to assist in identification, adducts detected using this technique are referred to in general as "bulky DNA adducts". Accelerator mass spectrometry is a relatively new method for adduct determination that has demonstrated even greater sensitivity. Most studies do not identify the specific DNA adduct structures, but instead report total "PAH-DNA," "bulky-DNA," "hydrophobic-DNA," or

"aromatic-DNA" adducts (Alexandrov et al., 2002). Further investigations are needed to examine correlations between DNA adduct levels determined by different methods, and correlations between DNA adducts and urinary PAH metabolites.

benzo[a]pyrene-DNA and PAH-DNA adducts have been measured in WBCs in several PAH-exposed human groups and found to be associated with, and predictive of, elevated cancer risks (Vineis and Perera, 2007; Pavanello et al., 1999). Coke oven workers experience especially high exposures to PAHs and demonstrate increased lung cancer rates. Pavanello and coworkers (2005) studied a group of 67 highly exposed coke oven workers for genetic factors that can modulate individual responses to carcinogenic PAHs. Levels of BPDE-DNA adducts in mononuclear WBCs (lymphocyte plus monocyte fraction) were associated with workplace PAH exposure as indicated by urinary 1-OH-Py excretion. The authors concluded that the elevated levels of BPDE-DNA adducts reflected both exposure and individual variation in expression of genes involved with glutathione conjugation activity and DNA excision repair capacity.

In a similar study, Pavanello et al. (2006) screened 585 Caucasian municipal workers (52% males, 20–62 years old) from northeast Italy for BPDE-DNA adduct formation in peripheral lymphocytes. Forty-two percent of the participants had elevated anti-BPDE-DNA adduct levels, defined as >0.5 adducts/10^8 nucleotides (mean, 1.28 ± 2.80 adducts/10^8 nucleotides). Comparison of adduct levels with questionnaire responses indicated that smoking, frequent consumption of PAH-rich meals (>52 times/year vs. <52 times/year), and long time periods spent outdoors (>4 hours/day vs. <4 hours/day) were risk factors as all increased BPDE-DNA adduct levels significantly. Exposure to indoor combustion sources (use of fireplace, coal- or wood stove >5 times/year) significantly increased the frequency of subjects positive for BPDE-DNA adducts. Exposure to heavy traffic did not alter lymphocyte BPDE-DNA adduct levels. Smoking and high-PAH diets were associated with increased BPDE-DNA adduct levels. In nonsmokers, high-PAH diets and extended time spent indoors associated with increased BPDE-DNA adduct formation, while in smokers, personal cigarette smoking was the only factor that was positively correlated with adduct levels. These results demonstrate the potential utility of BPDE-DNA adduct measurement as a biomarker of PAH exposure not only in heavily PAH-exposed occupations and tobacco smokers, but also in the general population, including nonsmokers.

Several studies investigated the level of DNA adducts in the WBCs of foundry workers (Perera et al., 1988) or coke oven workers (Arnould et al., 2000; Assennato et al., 1993a, b) using an immunoassay. The antibody's cross-reactivity with adducts originating from PAHs other than benzo[a]pyrene generally results in higher reported adduct levels than the [^{32}P]-postlabelling method which can isolate benzo[a]pyrene-DNA adducts. All three studies involved several levels of potential benzo[a]pyrene exposure as assessed by environmental sampling: 0.02, 5, 25, and 45 μg/m^3 (Arnould et al., 2000); <0.05, 0.05–0.2, and >0.2 μg/m^3 (Perera et al., 1988); and 0.03–12.6 μg/m^3 (Assennato et al., 1993a, b). Adduct levels ranged from 0.05 to 7 fmol PAH-

DNA per μg DNA, and variation within exposure groups was high; however, all three studies reported statistically significant correlations between exposure and adduct levels. However, there are a large number of studies, many of them conducted by the same laboratories cited here, that did not report positive correlations between benzo[a]pyrene exposure and DNA adduct formation in people exposed occupationally or to cigarette smoke (e.g., van Delft et al., 2001; Arnould et al., 1999; Pan et al., 1998; Peluso et al., 1998; Lewtas et al., 1997; Assennato et al., 1993a, b; Kriek et al., 1993; Paleologo et al., 1992; Herbert et al., 1990). These studies illustrate that attempting to correlate DNA adducts with benzo[a]pyrene exposure by a single route of exposure (e.g., inhalation), or by occupation alone, may produce highly variable or misleading results.

Perera et al. (2005a) measured BPDE-DNA adduct levels in maternal and umbilical cord blood obtained following normal delivery from 329 nonsmoking pregnant women exposed to emissions from fires during the 4 weeks following the collapse of the World Trade Center (WTC) building in New York City on 09/11/2001. BPDE-DNA adduct levels were highest in study participants who lived within 1 mile of the WTC, with inverse correlation between cord blood levels and distance from WTC. For the group of participants that resided within 1 mile of the WTC, maternal levels were 0.30 ± 0.16 adducts/10^8 nucleotides, umbilical cord levels were 0.28 ± 0.08; for the group employed within 1 mile of WTC, the corresponding values were 0.25 ± 0.11 and 0.24 ± 0.12, and for the unexposed referent group, the values were 0.22 ± 0.10 and 0.23 ± 0.10. Differences between referent and exposed subjects were marginally significant ($0.1 > p > 0.05$); however, the trend for adducts in maternal blood decreasing with increasing distance from the WTC was statistically significant ($p = 0.02$) as was the finding that the percentage of participants with detectable BPDE-DNA adducts increased significantly (trend $p = 0.05$) with increasing time and proximity to WTC–from 52.6% (reference group) to 66.7% (employed group) to 80.0% (resident group).

Tuntawiroon et al. (2007) evaluated airborne PAH concentrations and internal biomarker levels in 115 Thai school boys (8–12 years old) attending schools adjacent to high-density traffic areas in Bangkok (high exposure group), compared with 69 boys (9–13 years old) attending schools located in a provincial area (low exposure group). Ambient air concentrations (roadside and school areas) and personal breathing zone air concentrations of 10 particulate PAHs were measured in Bangkok and in the provincial location. Peripheral blood samples were collected from the school boys for [^{32}P]-postlabeling determination of bulky DNA adducts in lymphocytes. Based on toxic equivalency factors (TEFs, Nisbet and LaGoy, 1992) for PAH, benzo[a]pyrene equivalent exposures from personal breathing zone measurements in Bangkok children were about 3.5-fold greater than in rural provincial children ($p < 0.001$). Interestingly, although bulky DNA adducts were increased fivefold in Bangkok children compared with provincial children ($p < 0.001$), adduct levels were negatively correlated with total PAH and benzo[a]pyrene equivalent exposures.

4.1.2.2.2. Adduct measures in reproductive tissues

Zenzes et al. (1998) used immunostaining to detect PAH-DNA adducts in ovarian granulosa-lutein cells from women undergoing procedures for reproductive assistance. The 32 women in the sample were separated by smoking status and consisted of 14 active smokers (1-20 cigarettes per day), 7 passive smokers (nonsmoker but husband smoked), and 11 non-smokers with a non-smoking partner. The collected cells were fixed and stained with the anti-BP-DNA antibody which is known to cross-react with several carcinogenic PAH-DNA adducts. In this assay, darker nuclear staining results from a greater concentration of PAH-DNA adducts and is assigned a higher score. The proportion of nuclei exhibiting respective intensities of staining were combined to obtain an overall intensity score for the sample. The observed staining intensity was related to smoking status, with a mean ± SE of 1.91 ± 0.10 among active smokers, 1.21 ± 0.20 among passive smokers, and 0.62 ± 0.17 among nonsmokers ($p < 0.0001$). The authors concluded that smoking-related seminal DNA adducts could be a potential source of transmissible zygotic DNA damage.

In another study using the same immunostaining assay, Zenzes et al. (1999a) investigated the occurrence of PAH-DNA adducts in semen in relation to tobacco use. The study included 23 men (11 smokers and 12 nonsmokers), mean age 37 years, recruited through couples attending an in vitro fertilization – embryo transfer clinic in Toronto. Among smokers, the mean amount smoked was 20.6 cigarettes per day. The PAH-DNA adduct staining intensity was higher in the sperm samples from smokers (mean ± SE, 1.73 ± 0.09) when compared with nonsmokers (0.93 ± 0.10) ($p < 0.0001$); 5.7% and 30.9% of the sperm from smokers and nonsmokers, respectively, exhibited negative staining and 21.2% and 3.8%, respectively, exhibited strong staining. The authors concluded that smoking-related DNA adducts in semen could potentially be a source of zygotic DNA damage.

A related study examined the presence of PAH-DNA adducts in 112 blastomere cells from 22 pre-implantation embryos available through an in vitro fertilization – embryo transfer clinic (Zenzes et al., 1999b). The donated embryos were grouped with respect to the maternal and paternal smoking status (n = 8 both parents smoked, n = 12 father smoked but mother did not smoke, and n=7 neither parent smoked). Five of the embryos (4 from the father-only smoking and 1 from a non-smoking couple) were very fragmented and were not used in the analysis of PAH-DNA staining intensity. Among the smoking parents, women smoked less per day than men (mean ± SE, 13.0 ± 3.0 and 16.8 ± 1.5 cigarettes per day for women and men, respectively). The intensity score was higher for embryos with at least one parent who smoked (1.40 ± 0.28) compared with embryos from nonsmoking parents (0.38 ± 0.14) ($p = 0.015$), but there was little difference between embryos with two compared with one smoking parent (1.34 ± 0.30 and 1.48 ± 0.55 for both smokers and one smoker, respectively). Similar results were seen using the

proportion of embryos exhibiting any staining rather than a mean staining measure, and with the proportion of blastomere cells exhibiting staining. Intensity score was correlated with the amount smoked by the father ($p = 0.020$) but not by the mother. Analysis of the presence of PAH-DNA adducts in sperm cells also revealed an increased proportion and staining intensity in samples from smokers compared with nonsmokers ($p < 0.0001$ for categorical analysis of negative, weak, moderate and strong staining intensity). The authors noted that the demonstrated presence of these adducts in the embryos, reflecting most strongly a paternal origin, may affect the viability of the pregnancy.

4.1.2.3. *Benzo[a]pyrene-Induced Cytogenetic Damage*

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

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

Perera and coworkers assessed DNA damage in Finnish iron foundry workers in two separate studies and using three methodologies. Based on results from personal sampling and stationary monitoring in both studies, three levels of benzo[a]pyrene air concentrations were defined: low (<5 ng/m^3 benzo[a]pyrene), medium (5–12 ng/m^3), and high (>12 ng/m^3). (Perera et al., 1994, 1993). In the first study, involving 48 workers, several biomarkers were analyzed for dose-response and interindividual variability (Perera et al., 1993). PAH-DNA adducts were determined in WBCs using an immunoassay as described in Section 4.1.2.2.1 and enzyme-linked immunosorbent assay (ELISA) with fluorescence detection. Mutations at the hprt locus were also measured in WBC DNA. The latter assay is based on the fact that each cell contains only one copy of the hprt gene, which is located on the X-chromosome. While male cells have only one X-chromosome, female cells inactivate one of the two X-chromosomes at random. The gene is highly sensitive to mutations such that in the event of a crucial mutation in the gene, enzyme

activity disappears completely from the cell. In addition, mutations at the GPA gene locus were measured in red blood cells (RBCs). The GPA mutation frequency was not correlated with either benzo[a]pyrene exposure or PAH-DNA adduct formation. However, both PAH-DNA adduct levels and hprt mutation frequency increased with increasing benzo[a]pyrene exposure. In addition, there was a highly significant correlation between incidence of hprt mutations and PAH-DNA adduct levels ($p = 0.004$).

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

Kalina et al. (1998) studied several cytogenetic markers in 64 coke oven workers and 34 controls employed at other locations within the same plant. Airborne benzo[a]pyrene and seven other carcinogenic PAHs were collected by personal air samplers, which showed ambient benzo[a]pyrene concentrations ranging widely from 0.002 to 50 μg/m^3 in coke oven workers and from 0.002 to 0.063 μg/m^3 in controls. CAs, SCEs, high-frequency cells (HFCs), and SCE heterogeneity index (SCE-H) were all significantly increased with benzo[a]pyrene exposure. Except for increases in HFCs, no effect of smoking was observed. Consistent with studies of PAH-DNA adduct formation, reduced cytogenetic response at high exposure levels produced a nonlinear dose-response relationship. The authors also evaluated the potential influence of polymorphisms in enzymes involved in the metabolism of benzo[a]pyrene. Glutathione S-transferase M1 (GSTM-1) and N-acetyl transferase (NAT)-2 polymorphisms were studied and no evidence of the two gene polymorphisms having any influence on the incidence of cytogenetic damage was found.

Motykiewicz et al. (1998) conducted a similar study of genotoxicity associated with benzo[a]pyrene exposure in 67 female residents of a highly polluted industrial urban area of Upper Silesia, Poland, and compared the results to those obtained from 72 female residents of another urban but less polluted area in the same province of Poland. Urinary mutagenicity and 1-OH-Py levels, PAH-DNA adducts in oral mucosa cells (detected by immunoperoxidase staining), SCE, HFC, CA, bleomycin sensitivity, and GSTM-1 and CYP1A1 polymorphisms in blood lymphocytes were investigated. High volume air samplers and gas chromatography were used to quantify ambient benzo[a]pyrene levels which during the summer were 3.7 ng/m^3 in the polluted area and 0.6 ng/m^3 in the control area. During winter, levels rose to 43.4 and 7.2 ng/m^3 in the two areas, respectively. The cytogenetic biomarkers (CA and SCE/HFC), urinary

mutagenicity, and urinary 1-OH-Py excretion were significantly increased in females from the polluted area, and differences appeared to be more pronounced during winter time. PAH-DNA adduct levels were significantly increased in the study population, when compared to the controls, only in the winter season. No difference in sensitivity to bleomycin-induced lymphocyte chromatid breaks was seen between the two populations. As with the study by Kalina et al. (1998), genetic polymorphisms assumed to affect the metabolic transformation of benzo[a]pyrene were not associated with any difference in the incidence of DNA damage.

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

4.1.3. Epidemiologic Findings in Humans

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

4.1.3.1. *Molecular Epidemiology and Case-Control Cancer Studies*

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

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

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

Collectively, the results of molecular epidemiology studies with lung and bladder cancer patients indicate that individuals with a defective ability to repair BPDE-DNA adducts are at increased risk for cancer and, moreover, that specific genes linked to tumorigenesis pathways may be molecular targets for benzo[a]pyrene and other carcinogens.

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

4.1.3.2. *Cohort Cancer Studies*

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

4.1.3.2.1. ***Cancer incidence in aluminum and electrode production plants.***

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

Spinelli et al. (2006) reported a 14-year update to a previously published historical cohort study (Spinelli et al., 1991) of Canadian aluminum reduction plant workers. The results confirmed and extended the findings from the earlier epidemiology study. The study surveyed a total of 6,423 workers with ≥3 years of employment at an aluminum reduction plant in British Columbia, Canada, between the years 1954 and 1997, and evaluated all types of cancers. The focus was on cumulative exposure to coal tar pitch volatiles, measured as BSM and as benzo[a]pyrene. Benzo[a]pyrene exposure categories were determined from the range of predicted exposures over time from statistical exposure models. There were 662 cancer cases, of which approximately 98% had confirmed diagnoses. The overall cancer mortality rate (standardized mortality ratio [SMR] 0.97; CI 0.87–1.08) and cancer incidence rate (standardized incidence ratio [SIR] 1.00; CI 0.92–1.08) were not different from that of the British Columbia general population. However, this study identified significantly increased incidence rates for cancers of the bladder (SIR 1.80; CI 1.45–2.21) and the stomach (SIR 1.46; CI 1.01–2.04). The lung cancer incidence rate was only slightly higher than expected (SIR = 1.10; CI 0.93–1.30). Significant dose-response associations with cumulative benzo[a]pyrene exposure were seen for bladder cancer (p trend <0.001), stomach cancer (p trend <0.05), lung cancer (p trend <0.001), non-Hodgkin lymphoma (p trend <0.001), and kidney cancer (p trend <0.01), although the overall incidence rates for the latter three cancer types were not significantly elevated versus the general population. Similar cancer risk results were obtained using BSM as the exposure measure; the cumulative benzo[a]pyrene and BSM exposures were highly correlated (r = 0.94).

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

In a study covering 11,103 workers and 272,554 person × years of PAH exposure, cancer incidence was evaluated in six Norwegian aluminum smelters (Romundstad et al., 2000a, b). Reported estimates of PAH exposure concentrations reached a maximum of 3,400 μg/m^3 PAH (680 μg/m^3 benzo[a]pyrene). The overall number of cancers observed in this study did not differ significantly from control values (SIR 1.03; CI 1.0–1.1). The data from this study showed

significantly increased incidences for cancer of the bladder (SIR 1.3; CI 1.1–1.5), and elevated, but not significant, SIRs for larynx (SIR 1.3; CI 0.8–1.9), thyroid (SIR 1.4; CI 0.7–2.5), and multiple myeloma (SIR 1.4; CI 0.9–1.9). Incidence rates for bladder, lung, pancreas, and kidney cancer (the latter three with SIRs close to unity) were subjected to a cumulative exposure-response analysis. The incidence rate for bladder cancer showed a trend with increasing cumulative exposure and with increasing lag times (up to 30 years) at the highest exposure level. The incidence of both lung and bladder cancers was greatly increased in smokers. The authors reported that using local county rates rather than national cancer incidence rates as controls increased the SIR for lung cancer (SIR 1.4; CI 1.2–1.6) to a statistically significant level.

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

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

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

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

40 cases were observed compared with 31.0 expected based on four studies (pooled RR 1.30; 95% CI 0.95–1.77).

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

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

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

4.1.3.2.3. *Cancer incidence in asphalt workers and roofers.*

These groups encompass different types of work (asphalt paving vs. roofing) and also different types of historical exposure that have changed from using PAH-rich coal tar pitch to the

use of bitumen or asphalt, both of which are rather low in PAHs due to their source (crude oil refinery) and a special purification process. Increased risks for lung cancer were reported in large cohorts of asphalt workers and roofers; evidence for increased bladder cancer risk is weak (Burstyn et al., 2007; Partanen and Boffetta, 1994; Chiazze et al., 1991; Hansen, 1991, 1989; Hammond et al., 1976). In an analysis of pooled data from two cohorts of asphalt workers, 822 lung cancer cases were observed versus 730.7 expected (pooled RR 1.14; CI 1.07–1.22) (Bosetti et al., 2007). In two cohorts of roofers, analysis of pooled data indicated that 138 lung cancer cases were observed, compared with 91.9 expected (pooled RR 1.51; CI 1.28–1.78) (Bosetti et al., 2007).

4.1.3.3. *Noncancer Disease Caused by Benzo[a]pyrene*

Because accumulating evidence indicates that PAH exposure is a risk factor for ischemic heart disease (IHD), Burstyn et al. (2005) investigated 418 cases of fatal IHD in a cohort of 12,367 asphalt paving workers exposed to low-level PAH from bitumen and coal tar. The follow-up started in 1953 and ended in 2000, with an average exposure of 17 ± 9 years (minimum: one work season), resulting in 193,889 person-years of observation. Previous analyses of this cohort indicated no association between PAH exposure and excess mortality from cancer or by all causes. Quantitative estimates of exposure to benzo[a]pyrene were obtained for paving operations on the basis of previously available personal exposure measurements from workers in the asphalt industry (but not necessarily from cohort members). Exposures were calculated as average (0–68 [reference], 68–105, 106–146, 147–272, and 273+ ng/m^3) and cumulative (0–189 [reference], 189–501, 502–931, 932–2,012, and 2,013+ ng/m^3-years), respectively.

Cumulative and average exposure indices for benzo[a]pyrene were positively associated with mortality from IHD; the highest RR coincided with an average exposure to benzo[a]pyrene of 273 ng/m^3 or higher (RR = 1.64; CI = 1.13–2.28). A similar risk was observed for the highest cumulative benzo[a]pyrene exposure group (>2,013 ng/m^3-years) (RR = 1.58; CI = 0.98–2.55). Length of employment had no influence on this result. A dose-response was evident for IHD, but not for other types of cardiovascular disease. The RR remained elevated even with adjustment for smoking as a confounder; the RR was 1.24 under the extreme assumption of 0% never smokers, 30% former smokers, and 70% current smokers in the highest-exposed group. The authors discussed the possibility of bias because some of their study subjects might have been exposed to other IHD-causing factors that were not controlled for in their study, or might have been misclassified.

An occupational study of Canadian aluminum smelter workers investigated the effect of benzo[a]pyrene exposure on cardiopulmonary mortality (Friesen et al., 2010). Adjusted internal comparisons for smoking were conducted using Cox regression for male subjects (n = 6,423). Ischemic heart disease (IHD) was associated with cumulative benzo[a]pyrene exposure with a

hazard ratio of 1.62 (95% CI 1.06-2.46) in the highest benzo[a]pyrene exposure category. For active employment, the hazard ratio for IHD was 2.39 (95% CI 0.95-6.05) in the highest cumulative benzo[a]pyrene exposure category.

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

Perera et al. (2005b) compared various exposures—ETS, nutrition, pesticides, material hardship—with birth outcomes (length, head circumference, cognitive development). ETS exposure and intake of PAH-rich foods by pregnant women were determined by questionnaire. Levels of benzo[a]pyrene(BPDE)-DNA adducts were determined in umbilical cord blood collected at delivery. The study population consisted of Dominican or African-American nonsmoking pregnant women (n = 529; 24 ± 5 years old) free of diabetes, hypertension, HIV, and drug or alcohol abuse. Benzo[a]pyrene adducts, ETS, and dietary PAHs were not significantly correlated with each other. However, the interaction between benzo[a]pyrene-DNA adducts and ETS exposure was significantly associated with reduced birth weights (-6.8%; $p = 0.03$) and reduced head circumference (-2.9%; $p = 0.04$).

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

High cord blood adduct levels were significantly associated with reduced infant/child weight at 18 months ($\beta = -0.048$, $p = 0.03$), 24 months ($\beta = -0.041$, $p = 0.027$), and 30 months of age ($\beta = -0.040$, $p = 0.049$); decreased birth head circumference was marginally associated with DNA adduct levels ($\beta = -0.011$, $p = 0.057$). Maternal adduct levels were correlated neither with

cord blood adduct levels nor with fetal and child growth. Among female infants, cord blood adduct levels were significantly associated with smaller birth head circumference ($p = 0.022$) and with lower weight at 18 months ($p = 0.014$), 24 months ($p = 0.012$), and 30 months of age ($p = 0.033$), and with decreased body length at 18 months of age ($p = 0.033$). Among male infants, the corresponding associations were also inverse but statistically not significant.

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

Neal et al. (2008, 2007) examined levels of benzo[a]pyrene and other PAHs in follicular fluid and serum sample from 36 women undergoing in vitro fertilization at a clinic in Toronto, and compared the successful conception rate in relation to benzo[a]pyrene levels. The women were classified by smoking status, with 19 who were current cigarette smokers, 7 with passive or sidestream smoke exposure (i.e., non-smoker with a partner who smoked) and 10 non-smoked exposed. An early follicular phase blood sample and follicular fluid sample from the follicle at the time of ovum retrieval were collected and analyzed for the presence of benzo[a]pyrene, acenapthelene, phenanthrene, pyrene and chrysene using GC/MS (detection limit 5 pg/ml). The frequency of non-dectable levels of serum benzo[a]pyrene was highest in the non-smoking group (60.0%, 14.3%, and 21.0% below detection limit in non-smoking, sidestream smoke, and active smoking groups, respectively). A similar pattern was seen with follicular fluid benzo[a]pyrene (30.0%, 14.3%, and 10.5% below detection limit limit in non-smoking, sidestream smoke, and active smoking groups, respectively). In the analyses comparing mean values across groups, an assigned value of 0 was used for non-detectable samples. Follicular fluid benzo[a]pyrene levels were higher in the active smoking group (mean ± SE, 1.32 ± 0.68 ng/ml) than in the sidestream (0.05 ± 0.01 ng/ml) or non-smoking (0.03 ± 0.01 ng/ml) groups ($p = 0.04$). The between-group differences in serum benzo[a]pyrene levels were not statistically significant (0.22 ± 0.15, 0.98 ± 0.56, and 0.40 ± 0.13 ng/ml in non-smoking, sidestream smoke, and active smoking groups, respectively), and there were no differences in relation to smoking status. Among active smokers, the number of cigarettes smoked per day was strongly correlated with follicular fluid benzo[a]pyrene levels ($r= 0.7$, $p < 0.01$). Follicular fluid benzo[a]pyrene levels were significantly higher among the women who did not conceive (1.79 ng/ml ± 0.86) compared with women who did get pregnant (mean approximately, 0.10 ng/ml, as estimated from graph) ($p < 0.001$), but serum levels of benzo[a]pyrene were not associated with successful conception.

A small case-control study conducted between August 2005 and February 2006 in Lucknow city (Uttar Pradesh), India examined PAH concentrations in placental tissues (Singh et

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

Wu et al. (2010) conducted a study of benzo[a]pyrene-DNA adduct levels in relation to risk of fetal death in Tianjin, China. This case-control study included women who experienced a missed abortion before 14 weeks gestational age, that is a fetal death that remained in utero and so required surgical intervention. Cases were matched by age and gravidity to controls (women undergoing induced abortion due to an unplanned or unwanted pregnancy). The study excluded women who smoked, women with chronic disease and pregnancy complications, and women with occupational exposures to PAHs. Residency within Tianjin for at least one year was also an eligibility criterion. The participation rate was high: 81 of 84 eligible cases participanted and 81 of 89 eligible controls participated. Data pertaining to demographic characteristics, reproductive history, and factors relating to potential PAH exposure were collected using a structured interview, and samples from the aborted tissue were obtained. In two of the four hospitals used in the study, blood samples from the women (n=51 cases and 51 controls) were also collected. The presence of benzo[a]pyrene-BPDE adducts was assessed in the blood and tissue samples using HPLC. There was no correlation between blood and aborted tissue levels of benzo[a]pyrene adducts ($r = –0.12$ for the 102 blood-tissue pairs, $r = –0.02$ for the 51 case paris and $r = –0.21$ for the 51 control pairs). (The authors noted that there was little difference between women with and without blood samples in terms of the interview- based measures collected or in terms of the DNA-adduct levels in aborted tissue.) benzo[a]pyrene-adduct levels were similar but slightly lower in the aborted tissue of cases compared with controls (mean ± SD

4.8 ± 6.0 in cases and 6.0 ± 7.4 in controls, p = 0.29). In the blood samples, however, benzo[a]pyrene-adduct levels were higher in cases (6.0 ± 4.7 and 2.7 ± 2.2 in cases and controls, respectively, p < 0.001). In logistic regression analyses using a continuous adduct measure, the OR was 1.35 (95% CI 1.11 – 1.64) per adduct/10^8 nucleotide. These results adjusted for education and household income, but were very similar to the unadjusted results. Categorizing exposure at the median value resulted in an adjusted OR of 4.27 (95% CI 1.41 – 12.99) in the high compared with low benzo[a]pyrene-adduct group. There was no relation between benzo[a]pyrene-adduct levels in the aborted tissue and missed abortion in the logistic regression analyses using either the continuous (adjusted OR 0.97, 95% CI 0.93 – 1.02) or dichotomous exposure measure (adjusted OR 0.76, 95% CI 0.37 – 1.54). Associations between missed abortion and several interview-based measures of potential PAH exposure were also seen: adjusted OR 3.07 (95% CI 1.31 – 7.16) for traffic congestion near residence, 3.52 (95% CI 1.44 – 8.57) for commuting by walking, 3.78 (95% CI 1.11 – 12.87) for routinely cooked during pregnancy, and 3.21 (95% CI 0.98 – 10.48) for industrial site or stack near residence, but there was no association with other types of commuting (e.g., by bike, car, or bus).

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL, INHALATION, AND DERMAL

4.2.1. Oral

4.2.1.1. *Subchronic Studies*

De Jong et al. (1999) 35-day rat study

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

Body weight gain was decreased beginning at week 2 at the high dose of 90 mg/kg-day; there was no effect at lower doses (De Jong et al., 1999). Hematology revealed a dose-related decrease in RBC count, hemoglobin, and hematocrit at ≥10 mg/kg-day (Table 4-2). A minimal but significant increase in mean cell volume and a decrease in mean cell hemoglobin

concentration were noted at 90 mg/kg-day, and may indicate dose-related toxicity for the RBCs and/or RBC precursors in the bone marrow. A decrease in WBCs, attributed to a decrease in the number of lymphocytes (approximately 50%) and eosinophils (approximately 90%), was observed at 90 mg/kg-day; however, there was no effect on the number of neutrophils or monocytes. A decrease in the cell number in the bone marrow observed in the 90 mg/kg-day dose group was consistent with the observed decrease in the RBC and WBC counts at this dose level. In the 90 mg/kg-day dose group, brain, heart, kidney, and lymph node weights were decreased and liver weight was increased (Table 4-2). Decreases in heart weight at 3 mg/kg-day and in kidney weight at 3 and 30 mg/kg-day were also observed, but these changes did not show dose-dependent responses. Dose-related decreases in thymus weight were statistically significant at ≥10 mg/kg-day (Table 4-2).

Table 4-2. Exposure-related effects in male Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 5 weeks

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

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

Source: De Jong et al. (1999).

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

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

Knuckles et al. (2001) 90-day rat study

Knuckles et al. (2001) exposed male and female F344 rats (20/sex/dose group) to benzo[a]pyrene (98% purity) at doses of 0, 5, 50, or 100 mg/kg-day in the diet for 90 days. Food consumption and body weight were monitored, and the concentration of benzo[a]pyrene in the food was adjusted every 3–4 days to maintain the target dose. The authors indicated that actual intake of benzo[a]pyrene by the rats was within 10% of the calculated intake, and the nominal doses were not corrected to actual doses. Hematology and serum chemistry parameters were evaluated. Urinalysis was also performed. Animals were examined for gross pathology, and histopathology was performed on selected organs (stomach, liver, kidney, testes, and ovaries). Statistically significant decreases in RBC counts and hematocrit level (decreases as much as 10 and 12%, respectively) were observed in males at doses ≥50 mg/kg-day and in females at 100 mg/kg-day. A maximum 12% decrease (statistically significant) in hemoglobin level was noted in both sexes at 100 mg/kg-day. Blood chemistry analysis showed a significant increase in blood urea nitrogen (BUN) only in high-dose (100 mg/kg-day) males. Histopathology examination revealed an apparent increase in the incidence of abnormal tubular casts in the kidney in males at 5 mg/kg-day (40%), 50 mg/kg-day (80%) and 100 mg/kg-day (100%), compared to 10% in the controls. Only 10% of the females showed significant kidney tubular changes at the two high dose levels compared to zero animals in the female control group. The casts were described as molds of distal nephron lumen and were considered by the study authors

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

Kroese et al. (2001) 5-week rat study

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

A significant, but not dose-dependent, increase in food consumption in males at ≥1.5 mg/kg-day and a decrease in females at ≥5 mg/kg-day was observed (Kroese et al., 2001). Water consumption was statistically significantly altered in males only, a decrease at 1.5, 5, and 15 mg/kg-day and an increase at 50 mg/kg-day. Organ weights of lung, spleen, kidneys, adrenals, and ovaries were not affected by treatment. There was a dose-related, statistically significant decrease in thymus weight in males at 15 and 20 mg/kg-day (decreased by 28 and 33%, respectively) and a significant decrease in females at 50 mg/kg-day (decreased by 17%) (Table 4-3). In both sexes, liver weight was statistically significantly increased only at 50 mg/kg-day by about 18% (Table 4-3).

Table 4-3. Exposure-related effects in Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 5 weeks

Organ	Dose (mg/kg)				
	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[a]
Females	4.28 ± 0.11	4.40 ± 0.73	4.37 ± 0.11	4.67 ± 0.17	5.03 ± 0.15[a]
Thymus weight (mg; mean ± SD)					
Males	471 ± 19	434 ± 20	418 ± 26	342 ± 20[a]	317 ± 21[a]
Females	326 ± 12	367 ± 23	351 ± 25	317 ± 30	271 ± 16[a]
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[a]	7/10[a]

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

Source: Kroese et al. (2001).

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

Histopathology examination revealed a statistically significant increase in basal cell hyperplasia in the forestomach of females at doses ≥15 mg/kg-day (Kroese et al., 2001). The induction of liver microsomal EROD was not accompanied by any adverse histopathologic findings in the liver at the highest dose, 50 mg/kg-day, so the livers from intermediate-dose

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

Kroese et al. (2001) 90-day rat study

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

There were no obvious effects on behavior of the animals, and no difference was observed in survival or food consumption between exposed animals and controls (Kroese et al. 2001). Higher water consumption and slightly lower body weights than the controls were observed in males but not females at the high dose of 30 mg/kg-day. Hematological investigations showed only nonsignificant, small dose-related decreases in RBC count and hemoglobin level in both sexes. Clinical chemistry evaluation did not show any treatment-related group differences or dose-response relationships for alanine aminotransferase (ALT), serum aspartate transaminase (AST), lactate dehydrogenase (LDH), or creatinine, but a small dose-related decrease in γ-glutamyl transferase (GGT) activity was observed in males only. Urinalysis revealed an increase in urine volume in males at 30 mg/kg-day, which was not dose related. At the highest dose, both sexes showed increased levels of urinary creatinine and a dose-related increase in urinary protein. However, no further investigation was conducted to determine the underlying mechanisms for these changes. At necropsy, reddish to brown/gray discoloration of the mandibular lymph nodes was consistently noted in most rats; occasional discoloration was also observed in other regional lymph nodes (axillary). Statistically significant increases in liver weight were observed at 10 and 30 mg/kg-day in males only (15 and 29%) and a decrease in thymus weight in both sexes only at 30 mg/kg-day (17 and 33% decrease in females and males, respectively, compared with controls) (Table 4-4). At 10 mg/kg-day, thymus weight in males was decreased by 15%, but the decrease did not reach statistical significance.

Table 4-4. Means ± SD[a] for liver and thymus weights in Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 90 days

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

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

Source: Kroese et al. (2001).

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

The target organs of benzo[a]pyrene toxicity in this 90-day dietary study of Wistar rats were the forestomach, thymus, and liver. The LOAEL for forestomach hyperplasia, decreased

thymus weight, thymus atrophy, and increased liver weight was 30 mg/kg-day and the NOAEL was 10 mg/kg-day.

4.2.1.2. *Chronic Studies and Cancer Bioassays*

Kroese et al. (2001) 2-year rat study

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

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

Tumors were detected at significantly elevated incidences at several tissue sites in female and male rats at doses ≥10 and ≥3 mg/kg-day, respectively (Table 4-4; Kroese et al., 2001). The

tissue sites with the highest incidences of tumors were the liver (hepatocellular adenoma and carcinoma) and forestomach (squamous cell papilloma and carcinoma) in both sexes (Table 4-4). The first liver tumors were detected in week 35 in high-dose male rats. Liver tumors were described as complex, with a considerable proportion (59/150 tumors) metastasizing to the lungs. At the highest dose level, 95% of rats with liver tumors had malignant carcinomas (95/100; Table 4-4). Forestomach tumors were associated with the basal cell proliferation observed (without diffuse hyperplasia) in the forestomach of rats in the preliminary range-finding and 90-day exposure studies described previously in Section 4.2.1. At the highest dose level, 59% of rats with forestomach tumors had malignant carcinomas (60/102; Table 4-4). Other tissue sites with distinctly elevated incidences of tumors in the 30 mg/kg-day dose group included: the oral cavity (papilloma and squamous cell carcinoma [SCC]) in both sexes, and the jejunum (adenocarcinoma), kidney (cortical adenoma) and skin (basal cell adenoma and carcinoma) in male rats (Table 4-4). In addition, auditory canal tumors (carcinoma or squamous cell papilloma originating from pilo-sebaceous units including the Zymbal's gland) were also detected in both sexes at 30 mg/kg-day, but auditory canal tissue was not histologically examined in the lower dose groups and the controls (Table 4-4). Gross examination revealed auditory canal tumors only in the high-dose group.

Table 4-5. Incidences of exposure-related neoplasms in Wistar rats treated by gavage with benzo[a]pyrene, 5 days/week, for 104 weeks

	Dose (mg/kg-d)			
	0	**3**	**10**	**30[b]**
Site	**Females[a]**			
Oral cavity				
Papilloma	0/19	0/21	0/9	9/31[c]
SCC	1/19	0/21	0/9	9/31[c]
Basal cell adenoma	0/19	0/21	1/9	4/31
Sebaceous cell carcinoma	0/19	0/21	0/9	1/31
Oesophagus				
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[c]	25/52[c]
SCC	0/52	3/51	10/51[c]	25/52[c]
Liver				
Hepatocellular adenoma	0/52	2/52	7/52[c]	1/52
Hepatocellular carcinoma	0/52	0/52	32/52[c]	50/52[c]
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[c]
	Males[a]			
Oral cavity				
Papilloma	0/24	0/24	2/37	10/38[c]
Squamouse cell carcinoma	1/24	0/24	5/37	11/38[c]
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[c]	18/52[c]	17/52[c]
SCC	0/52	1/52	25/52[c]	35/52[c]
Jejunum				
Adenocarcinoma	0/51	0/50	1/51	8/49[c]
Liver				
Hepatocellular adenoma	0/52	3/52	15/52[c]	4/52
Hepatocellular carcinoma	0/52	1/52	23/52[c]	45/52[c]
Cholangiocarcinoma	0/52	0/52	0/52	1/52
Kidney				
Cortical adenoma	0/52	0/52	7/52[c]	8/52[c]
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[c]
Sebaceous cell adenoma	0/1	0/0	0/7	1/33

Table 4-5. Incidences of exposure-related neoplasms in Wistar rats treated by gavage with benzo[a]pyrene, 5 days/week, for 104 weeks

	Dose (mg/kg-d)			
	0	**3**	**10**	**30[b]**
Skin and mammary				
Basal cell adenoma	2/52	0/52	1/52	10/51[c]
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[c]
Fibrosarcoma	0/52	3/52	5/52	0/51
Fibrous histiocytoma (malignant)	0/52	0/52	1/52	1/52

[a]Incidences are for number of rats with tumors compared with number of tissues examined histologically. Auditory canal and oral cavity tissues were only examined histologically when abnormalities were observed upon macroscopic examination.
[b]This group had significantly decreased survival.
[c]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.

Source: Kroese et al. (2001).

Kroese et al. (2001) did not systematically investigate nonneoplastic lesions detected in rats sacrificed during the 2-year study, because the focus was to identify and quantitate tumor occurrence. However, incidences were reported for nonneoplastic lesions in tissues or organs in which tumors were detected (i.e., oral cavity, oesophagus, forestomach, jejunum, liver, kidney, skin, mammary, and auditory canal). The reported nonneoplastic lesions associated with exposure were the forestomach basal cell hyperplasia and clear cell foci of cellular alteration in the liver. Incidences for forestomach basal cell hyperplasia in the control through high-dose groups were: 1/52, 8/51, 13/51, and 2/52 for females and 2/50, 8/52, 8/52, and 0/52 in males. Incidences for hepatic clear cell foci of cellular alteration were 22/52, 33/52, 4/52, and 2/52 for females and 8/52, 22/52, 1/52, and 1/52 for males. These results indicate that the lowest dose group, 3 mg/kg-day, was a LOAEL for increased incidence of forestomach hyperplasia and hepatic histological changes in male and female Wistar rats exposed by gavage to benzo[a]pyrene for up to 104 weeks. The lack of an increase in incidence of these nonneoplastic lesions in the forestomach and liver at the intermediate and high doses (compared with controls) may be associated with increased incidences of forestomach and liver tumors at these dose levels (see Table 4-4).

As an adjunct study to the 2-year gavage study with Wistar rats, Kroese et al. (2001) sacrificed additional rats (6/sex/group) after 4 and 5 months of exposure (0, 1, 3, 10, or 30 mg/kg-day) for analysis of DNA adduct formation in WBCs and major organs and tissues. Additional rats (6/sex/time period) were exposed to 0.1 mg/kg-day benzo[a]pyrene for 4 and 5 months for analysis of DNA adduct formation. Using the [^{32}P]-postlabeling technique, five

benzo[a]pyrene-DNA adducts were identified in all of the examined tissues at 4 months (WBCs, liver, kidney, heart, lung, skin, forestomach, glandular stomach, brain). Only one of these adducts (adduct 2) was identified based on co-chromatography with a standard. This adduct, identified as dG-N^2-BPDE, was the predominant adduct in all organs of female rats exposed to 10 mg/kg-day, except the liver and kidney, in which another adduct (unidentified adduct 4) was predominant. Levels of total adducts (number of benzo[a]pyrene-DNA adducts per 10^{10} nucleotides) in examined tissues (from the single 10 mg/kg-day female rat) showed the following order: liver > heart > kidney > lung > skin > forestomach ≈ WBCs > brain. Mean values for female levels of total benzo[a]pyrene-DNA adducts (number per 10^{10} nucleotides) in four organs showed the same order, regardless of exposure group: liver > lung > forestomach ≈ WBCs; comparable data for males were not reported). Mean total benzo[a]pyrene-DNA adduct levels in livers increased in both sexes from about 100 adducts per 10^{10} nucleotides at 0.1 mg/kg-day to about 70,000 adducts per 10^{10} nucleotides at 30 mg/kg-day. In summary, these results suggest that total benzo[a]pyrene-DNA adduct levels in tissues at 4 months were not independently associated with the carcinogenic responses noted after 2 years of exposure to benzo[a]pyrene. The liver showed the highest total DNA adduct levels and a carcinogenic response, but total DNA adduct levels in heart, kidney, and lung (in which no carcinogenic responses were detected) were higher than levels in forestomach and skin (in which carcinogenic responses were detected).

Brune et al. (1981) 2-year rat study

Groups of Sprague-Dawley rats (32/sex/dose) were fed diets delivering a daily dose of 0.15 mg benzo[a]pyrene/kg body weight every 9^{th} day or 5 times/week (Brune et al., 1981). Other groups (32/ sex/dose) were given gavage doses of 0.15 mg benzo[a]pyrene (in aqueous 1.5% caffeine solution)/kg every 9^{th} day, every 3^{rd} day, or 5 times/week. The study included an untreated control group (to compare with the dietary exposed groups) and a gavage vehicle control group (each with 32 rats/sex). Rats were treated until moribundity or death occurred, with average annual doses are reported in Table 4-2 (mg/kg-year, calculated by Brune et al. [1981]). The following tissues were prepared for histopathological examination: tongue, larynx, lung, heart, trachea, esophagus, stomach, small intestine, colon, rectum, spleen, liver, urinary bladder, kidney, adrenal gland, and any tissues showing tumors or other gross changes. Survival was similar among the groups, with the exception that the highest gavage-exposure group showed a decreased median time of survival (Table 4-5). Increased incidences of portal-of-entry tumors (forestomach, esophagus, and larynx) were observed in all of the gavage-exposed groups and in the highest dietary exposure group (Table 4-5). Following dietary administration, all observed tumors were papillomas. Following gavage administration, two malignant forestomach tumors were found (one each in the mid- and high-dose groups) and the remaining tumors were benign. The data in Table 4-5 show that the carcinogenic response to benzo[a]pyrene was

stronger with the gavage protocol compared with dietary exposure, and that no distinct difference in response was apparent between the sexes. Tumors at distant sites (mammary gland, kidney, pancreas, lung, urinary bladder, testes, hematopoietic, and soft tissue) were not considered treatment-related as they were also observed at similar rates in the control group (data not provided). The study report did not address noncancer systemic effects.

Table 4-6. Incidences of alimentary tract tumors in Sprague-Dawley rats chronically exposed to benzo[a]pyrene in the diet or by gavage in caffeine solution

Average annual dose (mg/kg-yr)	Estimated average daily dose[a] (mg/kg-d)	Forestomach tumors[b]	Total alimentary tract tumors[c] (larynx, esophagus, forestomach)	Median survival time (wks)
benzo[a]pyrene by gavage in 1.5% caffeine solution				
0	0	3/64 (4.7%)	6/64 (9.4%)	102
6	0.016	12/64 (18.8%)[d]	13/64 (20.3%)	112
18	0.049	26/64 (40.1%)[e]	26/64 (40.6%)	113
39	0.107	14/64 (21.9%)[e]	14/64 (21.9%)	87
benzo[a]pyrene in diet				
0	0	2/64 (3.1%)	3/64 (4.7%)	129
6	0.016	1/64 (1.6%)	3/64 (4.7%)	128
39	0.107	9/64 (14.1%)[d]	10/64 (15.6%)	131

[a]Average annual dose divided by 365 d.
[b]No sex-specific forestomach tumor incidence data were reported by Brune et al. (1981).
[c]Sex-specific incidences for total alimentary tract tumors were reported as follows:
Gavage (control – high dose): Male: 6/32, 7/32, 15/32, 8/32
Female: 0/32, 6/32, 11/32, 6/32
Diet (control – high dose): Male: 3/32, 3/32, 8/32
Female: 0/32, 0/32, 2/32
[d]Significantly ($p < 0.1$) different from control using a modified χ^2 test that accounted for group differences in survival time.
[e]Significantly ($p < 0.05$) different from control using a modified χ^2 test that accounted for group differences in survival time.

Source: Brune et al. (1981).

Beland and Culp (1998; Culp et al., 1998) 2-year mouse study

In the other modern cancer bioassay with benzo[a]pyrene, female B6C3F$_1$ mice (48/dose group) were administered benzo[a]pyrene (98.5% purity) at concentrations of 0 (acetone vehicle), 5, 25, or 100 ppm in the diet for 2 years (Beland and Culp, 1998; Culp et al., 1998). This study was designed to compare the carcinogenicity of coal tar mixtures with that of benzo[a]pyrene and included groups of mice fed diets containing one of several concentrations of two coal tar mixtures. Benzo[a]pyrene was dissolved in acetone before mixing with the feed. Control mice received only acetone-treated feed. Female mice were chosen because they have a lower background incidence of lung tumors than male B6C3F$_1$ mice. Culp et al. (1998) reported

that the average daily intakes of benzo[a]pyrene in the 25- and 100-ppm groups were 104 and 430 μg/day, but did not report intakes for the 5-ppm group. Based on the assumption that daily benzo[a]pyrene intake at 5 ppm was one-fifth of the 25-ppm intake (about 21 μg/day), average daily doses for the three benzo[a]pyrene groups are estimated at 0.7, 3.3, and 16.5 mg/kg-day. Estimated doses were calculated using time-weighted average (TWA) body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group (estimated from graphically presented data). Food consumption, body weights, morbidity, and mortality were monitored at intervals, and lung, kidneys, and liver were weighed at sacrifice. Necropsy was performed on all mice that died during the experiment or survived to the end of the study period. Limited histopathologic examinations (liver, lung, small intestine, stomach, tongue, esophagus) were performed on all control and high-dose mice and on all mice that died during the experimental period, regardless of treatment group. In addition, all gross lesions found in mice of the low- and mid- dose groups were examined histopathologically.

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

Papillomas and/or carcinomas of the forestomach, esophagus, tongue, and larynx at elevated incidences occurred in groups of mice exposed to 25 or 100 ppm, but no exposure-related tumors occurred in the liver or lung (Table 4-6; Beland and Culp [1998]; Culp et al. [1998]). The forestomach was the most sensitive tissue, and demonstrated the highest tumor incidence among the examined tissues and was the only tissue with an elevated incidence of tumors at 25 ppm (Table 4-6). In addition, most of the forestomach tumors in the exposed groups were carcinomas, as 1, 31, and 45 mice had forestomach carcinomas in the 5-, 25-, and 100-ppm groups respectively. Nonneoplastic lesions were also found in the forestomach at significantly ($p < 0.05$) elevated incidences: hyperplasia at ≥5 ppm and hyperkeratosis at ≥25 ppm (Table 4-6). The esophagus was the only other examined tissue showing elevated incidence of a nonneoplastic lesion (basal cell hyperplasia, see Table 4-6). Tumors (papillomas and carcinonas) were also significantly elevated in the esophagus and tongue at 100 ppm (Table 4-6). Esophogeal carcinomas were detected in 1 mouse at 25 ppm and in 11 mice at 100 ppm. Tongue

carcinomas were detected in seven 100-ppm mice; the remaining tongue tumors were papillomas. Although incidences of tumors of the larynx were not significantly elevated in any of the exposed groups, a significant dose-related trend was apparent (Table 4-6).

Table 4-7. Incidence of nonneoplastic and neoplastic lesions in female B6C3F$_1$ mice fed benzo[a]pyrene in the diet for up to 2 years

Tissue and lesion	Incidence (%)			
	benzo[a]pyrene concentration (ppm) in diet[a]			
	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)[b]	1/48 (2)	3/47 (6)	36/46[b] (78)	46/47[b] (98)
Forestomach (hyperplasia)[b]	13/48 (27)	23/47[a] (49)	33/46[b] (72)	37/47[b] (79)
Forestomach (hyperkeratosis)[b]	13/48 (27)	22/47 (47)	33/46[b] (72)	38/47[b] (81)
Esophagus (papilloma and/or carcinoma)[b]	0/48 (0)	0/48 (0)	2/45 (0)	27/46[b] (59)
Esophagus (basal cell hyperplasia)[b]	1/48 (2)	0/48 (0)	5/45 (11)	30/46[b] (65)
Tongue (papilloma and/or carcinoma)[b]	0/49 (0)	0/48 (0)	2/46 (4)	23/48[b] (48)
Larynx (papilloma and/or carcinoma)[b]	0/35 (0)	0/35 (0)	3/34 (9)	5/38 (13)

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

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

Neal and Rigdon (1967; Rigdon and Neal 1969, 1966) mouse study

Neal and Rigdon (1967) fed BAP (purity not reported) at concentrations of 0, 1, 10, 20, 30, 40, 45, 50, 100 and 250 ppm in the diets of male and female CFW-Swiss mice. Corresponding doses (in mg/kg-day) were calculated[1] as 0, 0.2, 1.8, 3.6, 5.3, 7.1, 8, 8.9, 17.8, 44.4 mg/kg-day. The age of the mice ranged from 17-180 days old and the treatment time from 1-197 days; the size of the treated groups ranged from 9 to 73. There were 289 mice (number of

[1] Calculation: mg/kg-day = (ppm in feed x 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 (females) were used (U.S. EPA, 1988) and resulting doses were averaged between males and females.

mice/sex not stated) in the control group. No forestomach tumors were reported in the 0-, 0.2- and 1.8 mg/kg-day dose groups. The incidence of forestomach tumors in the 20-, 30-, 40-, 45-, 50-, 100- and 250-ppm dose groups (3.6, 5.3, 7.1, 8, 8.9, 17.8, 44.4 mg/kg-day) were 1/23, 0/37, 1/40, 4/40, 23/34, 19/23 and 66/73, respectively.

Other oral exposure cancer bioassays in mice

Numerous other oral exposure cancer bioassays in mice have limitations which restrict their usefulness for characterizing dose-response relationships between chronic-duration oral exposure to benzo[a]pyrene and noncancer effects or cancer, but collectively, they provide strong evidence that oral exposure to benzo[a]pyrene can cause portal-of-entry site tumors (see Table 4-8 for references).

1

Table 4-8. Tumor incidence in oral exposure rodent cancer bioassays with limitations for describing dose-response relationships for lifetime exposure to benzo[a]pyrene

Species/strain	Exposure	Results	Comments	Reference
Mouse/HaICR	Groups of nine mice (9 wks old) were fed benzo[a]pyrene in the diet (0, 0.2, or 0.3 mg/g diet) for 12 wks and sacrificed. Estimated doses were 0, 27.3, or 41 mg/kg-d.	Incidence with forestomach tumors: Control 0/9 Low 6/9 High 9/9	Less than lifetime exposure duration; glandular stomach, lung, and livers from control and exposed mice showed no tumors.	Triolo et al., 1977
Mouse/HaICR	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/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 is 33 mg/kg-d, using an average body weight of 0.030 kg from reported data.	Incidence with forestomach tumors: Exposed 17/20 (85%) Controls 0/24	Less than lifetime exposure duration; only stomach were examined for tumors; tumors found only in forestomach.	El-Bayoumy, 1985
Mouse/BALB	25 mice (8 wks old) were given 0.5 mg benzo[a]pyrene 2 times/wk for 15 wks.	5/25 mice had squamous carcinomas of the forestomach; tumors were detected 28–65 wks after treatment.	Less than lifetime exposure duration; the following details were not reported: inclusion of controls, methods for detecting tumors, and body weight data.	Biancifiori et al., 1967
Mouse/C3H	19 mice (about 3 mo old) were given 0.3 mL of 0.5% benzo[a]pyrene in polyethylene glycol-400 by gavage, once/d for 3 d.	By 30 wks, 7/10 mice had papillomas; no carcinomas were evident	Less than lifetime exposure duration.	Berenblum and Haran, 1955
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: Dose (μg) Incidence Experiment 1 Experiment 2 Control 0/17 0/18 12.5 3/17 2/18 50 0/17 1/17 200 8/17 ------[a]	Less than lifetime exposure duration; GI tract examined for tumors with hand lens; body weight data not reported.	Field and Roe, 1965

Table 4-8. Tumor incidence in oral exposure rodent cancer bioassays with limitations for describing dose-response relationships for lifetime exposure to 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/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/white	Groups of 16–30 mice were given benzo[a]pyrene in triethylene glycol (0.001–10 mg) wkly for 10 wks and observed until 19 mo.	Tumors in stomach antrum Dose (mg) – Carcinoma - Papilloma 0.001 0/16 0/16 0.01– 0/26 2/26 0.1 0/24 5/24 1.0 11/30 12/30 10 16/27 7/27	Less than lifetime exposure duration.	Fedorenko et al., 1967,as cited in U.S. EPA, 1991a
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/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
Mouse/Swiss albino	Groups of mice (9–14 wks old) were given single doses of 0 or 0.05 mg benzo[a]pyrene in polyethylene glycol-400 by gavage. Surviving mice were killed at 18 mo of age and examined for macroscopic tumors.	Forestomach tumor incidence: Dose (μg) – Carcinoma – Papilloma 0 0/65 2/65 50 1/61 20/61	Less than lifetime duration of exposure; exposure-related tumors only found in forestomach.	Roe et al., 1970

Table 4-8. Tumor incidence in oral exposure rodent cancer bioassays with limitations for describing dose-response relationships for lifetime exposure to 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/CFW	Groups of mice (mixed sex) were fed benzo[a]pyrene in the diet (dissolved in benzene and mixed with diet) at 0, 1, 10, 20, 30, 40, 45, 50, 100, or 250 ppm in the diet.	ppm Exposure (d) Forestomach 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 three wks to 6 mo old at the start of dosing; only alimentary tract was examined for tumors; (see also Rigdon and Neal, 1969, 1967, 1966).	Neil and Rigdon, 1967
Rat/ Sprague-Dawley	Groups of Sprague-Dawley rats (32/sex/dose) were fed diets delivering a daily dose of 0.15 mg benzo[a]pyrene/kg body weight every 9th day or 5 times/week (Brune et al., 1981). Other groups (32/ sex/dose) were given gavage doses of 0.15 mg benzo[a]pyrene (in aqueous 1.5% caffeine solution)/kg every 9th day, every 3rd day, or 5 times/week.	Dose larynx, esopogus, and forestomach (gavage) tumors 0 6/64 0.016 13/64 0.049 26/64 0.107 14/64 (diet) 0 3/64 0.016 3/64 0.107 10/64	Doses are annual averages. Non-standard treatment protocol involved animals being treated for 5 days a week or fewer; relatively high control incidence compared to other gavage studies;	Brune et al., 1981
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 are 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

[a] ---- = not evaluated

4.2.2. Inhalation

4.2.2.1. *Short-term and Subchronic Studies*

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

4.2.2.2. *Chronic Studies and Cancer Bioassays*

Thyssen et al. (1981) conducted an inhalation study in which male Syrian golden hamsters were exposed to benzo[a]pyrene for their natural lifetime. Groups of 20–30 male Syrian golden hamsters (8 weeks old) were exposed by nose-only inhalation to NaCl aerosols (controls; 240 μg NaCl/m^3) or benzo[a]pyrene condensed onto NaCl aerosols at three nominal concentrations of 2, 10, or 50 mg benzo[a]pyrene/m^3 for 3–4.5 hours/day, 5 days/week for 1–41 weeks, followed by 3 hours/day, 7 days/week for the remainder of study (until hamsters died or became moribund). Thyssen et al. (1981) reported average measured benzo[a]pyrene concentrations to be 0, 2.2, 9.5, or 46.5 mg/m^3. More than 99% of the particles were between 0.2 and 0.5 μm in diameter, and over 80% had diameters between 0.2 and 0.3 μm. The particle analysis of the aerosols was not reported to modern standards (MMAD and geometric SD were not reported). Each group initially consisted of 24 hamsters; final group sizes were larger as animals dying during the first 12 months of the study were replaced.

Survival was similar in the control, low-dose, and mid-dose groups, but was significantly decreased in the high-dose group. Average survival times in the control, low-, mid-, and high-dose groups were 96.4 ± 27.6, 95.2 ± 29.1, 96.4 ± 27.8, and 59.5 ± 15.2 weeks, respectively. After the 60th week, body weights decreased and mortality increased steeply in the highest dose group. Histologic examination of organs (a complete list of organs examined histologically was not reported by Thyssen et al. [1981]) revealed a dose-related increase in tumors in the upper respiratory tract, including the nasal cavity, pharynx, larynx, and trachea and in the digestive tract in the mid- and high-dose groups (Table 4-8). A statistical analysis was not included in the Thyssen et al. (1981) report. No lung tumors were observed. Squamous cell tumors in the esophagus and forestomach were also observed in the high-dose group, presumably as a

consequence of mucociliary particle clearance. Tumors were detected in other sites, but none of these appeared to be related to exposure. The results indicated that the pharynx and larynx, including the epiglottis, were the main cancer targets (Table 4-8).

Table 4-9. Incidence of respiratory and upper digestive tract tumors in male hamsters treated for life with benzo[a]pyrene by inhalation

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

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

Source: Thyssen et al. (1981).

Under contract to the U.S. EPA, Clement Associates (1990) obtained the individual animal data (including individual animal pathology reports, time-to-death data, and exposure chamber monitoring data) collected by Thyssen et al. (1981). Re-analysis of the original data revealed several errors and omissions in the published report. The actual exposure protocol was as follows: 4.5 hours/day 5 days/week on weeks 1–12, 3 hours/day 5 days/week on weeks 13–29, 3.7 hours/day 5 days/week on week 30, 3 hours/day 5 days/week on weeks 31–41, and 3 hours/day 7 days/week for the reminder of the experiment. In addition, actual exposure concentrations varied widely from week to week. Because different animals were started at different times, each individual animal had an exposure history somewhat different than others in the same exposure group. In order to deal with this problem, Clement Associates (1990) used the orignial individual animal data to calculate average continuous lifetime exposures for each individual hamster. Group averages of individual average continuous lifetime exposure concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3 for the control through high-exposure groups.

For this assessment, the individual animal pathology reports prepared by Thyssen et al. (1981) and obtained by Clement Associates (1990) were examined to independently assess the numbers of hamsters with tumors in the larynx, pharynx, and nose in each group. Table 4-9 presents the number of animals with tumors in the larynx and pharynx and the numbers of animals in each exposure group. Numbers of animals with either laryngeal or pharyngeal tumors

are also noted in Table 4-10, since these two types of tumors arise in close anatomical proximity from similar cell types. Examination of the individual animal pathology reports also showed that all of the nasal, forestomach, esophageal, and tracheal tumors occurred in animals that also had either laryngeal or pharyngeal tumors, except for two animals in the mid-dose group that displayed nasal tumors (one malignant and one benign) without displaying tumors in the pharynx or larynx.

Table 4-10. Number of animals with pharynx and larynx tumors in male hamsters exposed by inhalation to benzo[a]pyrene for life

Average continuous benzo[a]pyrene concentration[a] (mg/m^3)	Number of hamsters in group[b]	Larynx[b]		Pharynx[b]		Larynx or pharynx, combined[c]	
		Malignant	All	Malignant	All	Malignant	All
Control	27	0	0	0	0	0	0
0.25	27	0	0	0	0	0	0
1.01	26	8	11	7	9	11	16
4.29	34	9	12	17	18	17	18

[a]As calculated by Clement Associates (1990) from air monitoring data collected by Thyssen and colleagues.
[b]As counted from information in Table D-1 in Appendix D, which was obtained from examination of individual animal pathology reports prepared by Thyssen and colleagues and obtained by Clement Associates.
[c]As counted from information in Table D-1 in Appendix D. Nasal, forestomach, esophageal, and tracheal tumors occurred in hamsters that also had tumors in the larynx or pharynx, except for two animals in the mid-dose group that displayed nasal tumors (one malignant and one benign) without displaying tumors in the pharynx or larynx.

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

4.2.3. Dermal Exposure

4.2.3.1. *Skin-Tumor Initiation-Promotion Assays*

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

4.2.3.2. *Carcinogenicity Dermal Bioassays*

Poel (1959)

Poel (1959) applied benzo[a]pyrene in toluene to shaved interscapular skin of groups of 13–56 male C57L mice at doses of 0, 0.15, 0.38, 0.75, 3.8, 19, 94, 188, 376, or 752 μg, 3 times/week for up to 103 weeks or until the appearance of a tumor by gross examination (3 times weekly). Some organs (not further specified) and interscapular skin in sacrificed mice were examined histologically. With increasing dose level, the incidence of mice with skin tumors increased and the time of tumor appearance decreased (see Table 4-10). Doses >3.8 μg were associated with 100% mortality after increasingly shorter exposure periods, none greater than 44 weeks. Poel (1959) did not mention the appearance of exposure-related tumors in tissues other than interscapular skin.

Table 4-11. Skin tumor incidence and time of appearance in male C57L mice dermally exposed to benzo[a]pyrene for up to 103 weeks

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

[a]Indicated doses were applied to interscapular skin 3 times/wk for up to 103 wks or until time of appearance of a grossly detected skin tumor.
[b]Carcinomas were histologically confirmed.

[c]Ranges reflect differing information in Tables 4 and 6 of Poel (1959).

Source: Poel (1959).

Poel (1960) applied benzo[a]pyrene in a toluene vehicle to shaved interscapular skin of groups of 14–25 male SWR, C3HeB, or A/He mice 3 times/week at doses of 0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 μg benzo[a]pyrene per application, until mice died or a skin tumor was observed. Time ranges for tumor observations were provided, but not times of death for mice without tumors, so it was not possible to evaluate differential mortality among all dose groups or the length of exposure for mice without tumors. With increasing dose level, the incidence of mice with skin tumors increased and the time of tumor appearance decreased (Table 4-11). The lowest dose level did not induce an increased incidence of mice with skin tumors in any strain, but strain differences in susceptibility were evident at higher dose levels. SWR and C3HeB mice showed skin tumors at doses ≥0.38 μg benzo[a]pyrene, whereas AH/e mice showed tumors at doses ≥19 μg benzo[a]pyrene (Table 4-11). Except for metastases of the skin tumors to lymph nodes and lung, Poel (1960) did not mention the appearance of exposure-related tumors in tissues other than interscapular skin.

Table 4-12. Skin tumor incidence and time of appearance in male SWR, C3HeB, and A/He mice dermally exposed to benzo[a]pyrene for life or until a skin tumor was detected

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

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

Source: Poel (1960).

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

Table 4-13. Tumor incidence in female Swiss mice dermally exposed to benzo[a]pyrene for up to 93 weeks

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

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

Source: Roe et al. (1970).

Schmidt et al. (1973) dermally administered benzo[a]pyrene in acetone to female NMRI mice (100/group) and female Swiss mice. Benzo[a]pyrene was applied to the shaved dorsal skin twice weekly with doses of 0, 0.05, 0.2, 0.8, or 2 μg until spontaneous death occurred or until an advanced carcinoma was observed. Skin carcinomas were identified by the presence of crater-

shaped ulcerations, infiltrative growth and the beginning of physical wasting (i.e., cachexia). Necropsy was performed for all animals, and histopathological examination of the dermal site of application and any other tissues with gross abnormalities was conducted. Skin tumors were observed at the two highest doses in both strains of female mice (see Table 4-14), with induction periods of 53.0 and 75.8 weeks for the 0.8 and 2.0 μg NMRI mice and 57.8 and 60.7 weeks for the Swiss mice, respectively. The authors indicated that the latency period for tumor formation was highly variable and significant differences among exposure groups could not be identified, but no further timing information was available, including overall survival. Carcinoma was the primary tumor type seen after lifetime application of benzo[a]pyrene to mouse skin.

Table 4-14. Skin tumor incidence in female NMRI and Swiss mice dermally exposed to benzo[a]pyrene

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

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

Source: Schmidt et al. (1973).

Schmähl et al. (1977) applied benzo[a]pyrene 2 times/week to the shaved dorsal skin of female NMRI mice (100/group) at doses of 0, 1, 1.7, or 3 μg in 20 μL acetone. The authors reported that animals were observed until natural death or until they developed a carcinoma at the site of application. The effective numbers of animals at risk was about 80% of the nominal group sizes, which the authors attributed to autolyis; no information was provided concerning when tumors appeared in the relevant groups, how long treatment lasted in each group, or any times of death. Necropsy was performed on all mice and the skin of the back, as well as any organs that exhibited macroscopic changes, were examined histopathologically. The incidence of all types of skin tumors was increased in a dose related manner compared to controls (see Table 4-15). Carcinoma was the primary tumor type observed following chronic dermal

exposure to benzo[a]pyrene, and skin papillomas occurred infrequently. Dermal sarcoma was not observed.

Table 4-15. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene

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

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

Source: Schmähl et al. (1977).

Habs et al. (1980) applied benzo[a]pyrene to the shaved interscapular skin of female NMRI mice (40/group) at doses of 0, 1.7, 2.8 or 4.6 μg in 20 μL acetone twice weekly, from 10 weeks of age until natural death or gross observation of infiltrative tumor growth. Latency of tumors, either as time of first appearance or average time of appearance of tumors, was not reported. Necropsy was performed on all animals, and the dorsal skin, as well as any organs showing gross alterations at autopsy, was prepared for histopathological examination. Age-standardized mortality rates, using the total population of the experiment as the standard population, were used to adjust tumor incidence findings in the study. Benzo[a]pyrene application was associated with a statistically significant increase in the incidence of skin tumors at each dose level (see Table 4-17).

Table 4-16. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene

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

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

Source: Habs et al. (1980).

Grimmer et al. (1983) and Grimmer et al. (1984) applied benzo[a]pyrene (in 0.1 mL of a 1:3 solution of acetone:dimethyl sulfoxide [DMSO]) to the interscapular skin of female CFLP mice (65–80/group) 2 times/week for 104 weeks. Doses were 0, 3.9, 7.7 and 15.4 μg in the 1983

experiment, and 0, 3.4, 6.7, and 13.5 μg in the 1984 experiment. Mice were observed until spontaneous death, unless an advanced tumor was observed or if animals were found moribund. Survival information was not provided; incidences reflect the number of animals placed on study. Necropsy was performed on all mice. Histopathological examination of the skin and any other organ showing gross abnormalities was performed. Chronic dermal exposure to benzo[a]pyrene produced a dose-related increase in skin tumor incidence and a decrease in tumor latency (see Table 4-17). Carcinoma was the primary tumor type observed and a dose-response relationship was evident for carcinoma formation and incidence of all types of skin tumors.

Table 4-17. Skin tumor incidence and time of appearance in female CFLP mice dermally exposed to benzo[a]pyrene for 104 weeks

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

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

Habs et al. (1984) applied benzo[a]pyrene (in 0.01 mL acetone) to the shaved interscapular skin of female NMRI mice at doses of 0, 2 or 4 μg, 2 times/week for life. Animals were observed twice daily until spontaneous death, unless an invasive tumor was observed. All animals were necropsied and histopathological examination was performed on the dorsal skin and any other organ with gross abnormalities. Chronic dermal exposure to benzo[a]pyrene did not affect body weight gain, but appeared to reduce survival at the highest dose with mean survival times of 691, 648, and 528 days for the 0, 2, and 4 μg/day groups, respectively. The total length of exposure for each group was not reported, but can be inferred from the survival data. Latency also was not reported. Benzo[a]pyrene application resulted in a dose-related increase the incidence of total skin tumors and skin carcinomas (see Table 4-18). Hematopoietic tumors (at 6/20, 3/20, and 3/20) and lung adenomas (at 2/20, 1/20, and 0/20) were observed in

the controls and in the benzo[a]pyrene treatment groups, but did not appear to be treatment related according to the study authors.

Table 4-18. Skin tumor incidence in female NMRI mice dermally exposed to benzo[a]pyrene for life

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

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

Source: Habs et al. (1984).

Higginbotham et al. (1993)

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

Sivak et al. (1997)

Sivak et al. (1997) designed a study to compare the carcinogenicity of condensed asphalt fumes (including benzo[a]pyrene and other PAHs) with several doses of benzo[a]pyrene alone. For the purposes of this assessment, the exposure groups exposed to PAH mixtures are not discussed. Groups of 30 male C3H /HeJ mice were treated dermally twice/week to 0, 0.0001, 0.001, or 0.01% (0, 0.05, 0.5, or 5 µg) benzo[a]pyrene in a 50 µl volume of cyclohexanone/acetone (1:1) for 104 weeks beginning at 8 weeks of age. All mice were necropsied, and skin samples from all as well as any grossly observed lesions were subjected to histopathological examination. The incidence of skin tumors and mean survival times for each

group are shown in Table 4-19. All high dose mice died before the final sacrifice. The extent of deaths prior to one year in each group was not provided, so that the reported incidence may underestimate the tumor rate of animals exposed long enough to develop tumors. However, the crude skin tumor rates show an increasing trend in incidence.

Table 4-19. Skin tumor incidence in male C3H /HeJ mice dermally exposed to benzo[a]pyrene for 24 months

Dose (μg)[a]	Skin tumor incidence (all types)	No. died before final sacrifice	Mean survival time, days
0 cyclohexanone/acetone (1:1)	0/30 (0%)	19	607
0.05	0/30 (0%)	15	630
0.5	5/30 (20%)	15	666
5.0	27/30 (90%)	30	449

[a]Indicated doses were applied twice/week to shaved dorsal skin.

Source: Sivak et al. (1997).

Albert et al. (1991)

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

No tumors were present in vehicle-treated or untreated control mice. In exposed groups, incidences of mice with skin tumors were not reported, but time-course data for cumulative number of tumors per mouse, corrected for deaths from nontumor causes, were reported. Tumors began appearing after 12–14 weeks of exposure for the mid- and high-dose groups and at 18 weeks for the low-dose group. At study termination (35 weeks after start of exposure), the mean number of tumors per mouse was approximately one per mouse in the low- and mid-dose groups and eight per mouse in the high-dose group; indicating that most, if not all, mice in each exposure group developed skin tumors and that the tumorigenic response was greatest in the highest dose group. The majority of tumors were initially benign, with an average time of 8 weeks for progression from benign papillomas to malignant carcinomas. Epidermal damage occurred in a dose-related manner (more severe in the high-dose group than in the low- and mid-dose groups) and included statistically significant increases (compared with controls) in: [^{3}H]-thymidine labeling and mitotic indices; incidence of pyknotic and dark cells (signs of apoptosis);

and epidermal thickness. Only a minor expansion of the epidermal cell population was observed. In the high-dose group, indices of epidermal damage increased to a plateau by 2 weeks of exposure. The early time course of epidermal damage indices was not described in the low- and mid-dose groups, since data for these endpoints were only collected at 20, 24, and 30 weeks of exposure. An increased level of BPDE-DNA adducts, compared with controls, was apparent in all exposed groups after 4 weeks of exposure in the following order: 64>32>16>8 μg/week. The time-course data indicate that benzo[a]pyrene-induced increases in epidermal damage indices and BPDE-DNA adducts preceded the appearance of skin tumors.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL, INHALATION, AND DERMAL

As discussed in Section 4.1.4.3, several studies of human cohorts have examined possible associations between lower body weights or head circumference in newborns or infants with benzo[a]pyrene-DNA adducts levels in cord blood and exposure to ETS (Tang et al., 2006; Perera et al., 2005a, b). Available studies of reproductive or developmental endpoints in animals exposed orally or by inhalation to benzo[a]pyrene are reviewed as follows in this section. No studies that evaluated reproductive or developmental effects following dermal exposure were identified.

4.3.1. Oral

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

Testicular atrophy was observed in the benzo[a]pyrene treatment groups, but was not statistically different than controls. Statistically significant reductions were observed in epididymal sperm counts of F0 and F1 generations treated with the high or low dose of benzo[a]pyrene. For F0 and F1 generations, epididymal sperm counts were reduced approximately 50% and 70%, respectively, in the low and high dose groups. Additionally, sperm motility was statistically significantly decreased in the high dose in the F0 and F1 generations. Sperm parameters of the F3 generation were not statistically different from controls. An in vitro

sperm penetration assay revealed statistically significantly reduced fertilization in F0 and F1 generations of the low and high dose groups. However, the value of this in vitro test is limited as it bypasses essential components of the intact animal system (US EPA, 1996). Based on decreased epididymal sperm counts of F0 and F1 generations, a LOAEL of 1 mg/kg-day was established from this study (no NOAEL was identified).

Xu et al., (2010) treated female Sprague-Dawley rats (6/group) to 0 (corn oil only), 5, or 10 mg/kg-day benzo[a]pyrene by gavage every other day for a duration of 60 days. This resulted in time weighted average doses of 0, 2.5, and 5 mg/kg-day over the study period of 60 days. Endpoints examined included ovary weight, estrous cycle, 17B-estradiol blood level, and ovarian follicle populations (including primordial, primary, secondary, atretic, and corpora leutea). Animals were observed daily for any clinical signs of toxicity and following sacrifice, gross pathological examinations were made and any findings were recorded. All animals survived to necropsy. A difference in clinical signs was not observed for the treated groups and body weights were not statistically different in treated animals (though they appear to be depressed 6% at the high dose). Absolute ovary weight was statistically significantly reduced in the both the low and high dose groups, 11 and 15% respectively (see Table 4-20). Animals treated with the high dose were noted to have a statistically significantly prolonged duration of the estrous cycle and non-estrus phase compared to controls. Animals in the high dose group also had statistically significantly depressed levels of estradiol (by approximately 25%) and decreased numbers of primordial follicles (by approximately 20%). This study also indicated a strong apoptotic response of ovarian granulosa cells as visualized through TUNEL labeling, however, the strongest response was seen at the low dose; decreased apoptosis was also observed at the high dose. Based on decreased ovary weight following 60 day oral exposure to benzo[a]pyrene, a LOAEL of 2.5 mg/kg-day was established from this study (no NOAEL was identified).

Table 4-20. Means ± SD for ovary weight in female SD-rats

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

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

Source: Xu et al. (2010).

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

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

Rigdon and Neal (1965) administered diets containing 1,000 ppm benzo[a]pyrene to pregnant mice (nine/group) on GDs 10–21 or 5–21. The pups were reported as appearing generally normal at birth, but cannibalism was elevated in the exposed groups. These results contrast with an earlier study (Rigdon and Rennels, 1964) in which rats (strain not specified) were fed diets containing benzo[a]pyrene at 1,000 ppm for approximately 28 days prior to mating and during gestation. In the earlier study, five of eight treated females mated with untreated males became pregnant, but only one delivered live young. The treated dam that delivered had two live and two stillborn pups; one dead pup was grossly malformed. In the remaining treated females, vaginal bleeding was observed on GDs 23 or 24. In the inverse experimental design, three of six controls mated to benzo[a]pyrene-treated males became pregnant and delivered live young. Visceral and skeletal examinations of the pups were not conducted. These studies were limited by the small numbers of animals, minimal evaluation of the pups, lack of details on days of treatment (food consumption, weight gain), and the occurrence of cannibalism.

Reproductive effects of in utero exposure via oral route

MacKenzie and Angevine (1981) conducted a two-generation reproductive and developmental toxicity study for benzo[a]pyrene in CD-1 mice. Benzo[a]pyrene was

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

No maternal toxicity was observed. The number of F0 females with viable litters at parturition at the highest dose was statistically significantly reduced by about 35% (Table 4-21), but progeny were normal by gross observation. Parturition rates of the low- and mid-dose groups were unaffected by treatment, and litter sizes of all treated groups were similar to the control group throughout lactation. However, body weights of the F1 pups in the mid-dose and high-dose groups were statistically significantly decreased on PND 20, by 7 and 13%, respectively, and in all treated pups on PND 42, 6, 6, and 10% for the low, mid, and high dose, respectively (Table 4-21). The number of F1 pups surviving to PNDs 20 and 42 was significantly reduced at the high dose ($p < 0.01$), by 8 and 16%, respectively. When F1 males were bred to untreated females and F1 females were mated with untreated males, a marked dose-related decrease in fertility of > 30% was observed in both sexes, starting at the lowest exposure. There were no treatment-associated gross abnormalities or differences in body weights in the F2 offspring.

Table 4-21. Reproductive effects in male and female CD-1 F1 mice exposed in utero to benzo[a]pyrene

Effect	Dose (mg/kg-d)[a]			
	0	10	40	160

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

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

Source: MacKenzie and Angevine (1981).

Exposure to benzo[a]pyrene caused a marked dose-related decrease in the size of the gonads. In F1 males, testes weights were statistically significantly reduced. Testes from animals exposed in utero to 10 and 40 mg/kg-day weighed approximately 60 and 18%, respectively, of the weight of testes from the control animals (no F2 offspring were produced in the high dose group). This was confirmed by histopathologic observation of atrophic seminiferous tubules in the 40 mg/kg-day group that were smaller than those of controls and were empty except for a basal layer of cells. The number of interstitial cells in the testes was also increased in this group. Males from the 10 mg/kg-day group showed limited testicular damage; although all exhibited evidence of tubular injury, each animal had some seminiferous tubules that displayed active spermatogenesis. Ovarian tissue was absent or reduced in F1 females such that organ weights were not possible to obtain. Examination of available tissue in these females revealed hypoplastic ovaries with few follicles and corpora lutea (10 mg/kg-day) or with no evidence of folliculogenesis (40 mg/kg-day). Ovarian tissue was not examined in highest-dose females.

The LOAEL in this study was 10 mg/kg-day, based on decreases in mean pup weight (<5%) at PND 42 of F1 offspring of dams treated with 10, 40, or 160 mg/kg-day benzo[a]pyrene, marked decreases in the reproductive capacity (as measured by fertility index) of both male and female F1 offspring exposed at all three treatment levels of benzo[a]pyrene (by approximately 30% in males and females), decreased litter size (by about 20%) in offspring of F1 dams, and also the dramatic decrease in size and alteration in anatomy of the gonads of both male and female F1 mice exposed to 10 and 40 mg/kg-day benzo[a]pyrene in utero. A NOAEL was not identified.

In another reproductive and developmental toxicity study, benzo[a]pyrene was administered by gavage in corn oil to nine female NMRI mice at a dose of 10 mg/kg-day on GDs 7–16; a group of nine controls received corn oil (Kristensen et al., 1995). Body weights were monitored. F0 females were kept with their offspring until after weaning (21 days after delivery). At 6 weeks of age, one F1 female from each litter (n = 9) was caged with an untreated male. The F2 offspring were inspected for gross deformities at birth, weight and sex were recorded 2 days after birth, and the pups were sacrificed. The F1 females were sacrificed after 6

months of continuous breeding. The effects of benzo[a]pyrene treatment on fertility, ovary weights, follicles, and corpora lutea were evaluated. F0 females showed no signs of general toxicity, and there was no effect on their fertility. F1 females had statistically significantly lower median numbers of offspring, number of litters, and litter sizes and a statistically significantly greater median number of days between litters as compared with the controls (Table 4-22). At necropsy, the F1 females from treated F0 females had statistically significantly reduced ovary weights; histologic examination of the ovaries revealed decreased numbers of small, medium, or large follicles and corpora lutea (Table 4-22). Only one dose group was used in this study, with decreased F1 female fertility observed following in utero exposure at the LOAEL of 10 mg/kg-day; no NOAEL was identified.

Table 4-22. Effect of prenatal exposure to benzo[a]pyrene on indices of reproductive performance in F1 female NMRI mice

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

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

Source: Kristensen et al. (1995).

Reproductive effects in adults and repeated oral exposure

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

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

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

Immunosuppression effects and in utero exposure via oral route

No studies were found that examined immune system endpoints following in utero exposure via the oral route. The abstract of a report by Holladay and Smith (1994) referred to gavage dosing in a study of immune endpoints in fetuses of pregnant mice exposed to benzo[a]pyrene on GDs 13–17, but the methods section of the report described an i.p. injection procedure in detail (see Section 4.4.2).

4.3.2. Inhalation

Reproductive toxicity and in utero exposure via inhalation

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

Archibong et al. (2002) reported that exposure of rats to benzo[a]pyrene caused biologically and statistically significant ($p \leq 0.05$) reductions in fetal survival compared with the two control groups; fetal survival rates were 78.3, 38.0, and 33.8% per litter at 25, 75, and 100 μg/m^3, respectively, and 96.7% with CB or 98.8% per litter in untreated controls (see Table 4-23). Consequently, the number of pups per litter was also decreased in a concentration-dependent manner. The decrease was ~50% at 75 μg/m^3 and ~65% at 100 μg/m^3, compared with sham-exposed and unexposed control groups. No effects on hormone levels were observed on GDs 15 or 17 at the low-dose. Biologically significant decreases in mean pup weights (expressed as g per litter) of >5% were observed at doses ≥75 μg/m^3 (14 and 16% decreases at 75 and 100 μg/m^3, respectively, $p < 0.05$). Exposure to benzo[a]pyrene did not affect crown-rump length (see Table 4-22).

Table 4-23. Pregnancy outcomes in female F344 rats treated with benzo[a]pyrene on GDs 11–21 by inhalation

	Administered concentration of benzo[a]pyrene (μg/m^3)				
Parameter[a]	0 (unexposed control)	0 (carbon black)	25	75	100
Implantation sites	8.6 ± 0.2	8.8 ± 0.1	8.8 ± 0.5	9.0 ± 0.2	8.8 ± 0.1
Pups per litter	8.5 ± 0.2	8.7 ± 0.2	7.4 ± 0.5[b]	4.2 ± 0.1[b]	3.0 ± 0.2[b]

Survival (litter %)	98.9 ± 1.1	96.7 ± 1.7	78.3 ± 4.1[b]	38.0 ± 2.1[b]	33.8 ± 1.3[b]
Pup weight (g/litter)	10.6 ± 0.1	8.8 ± 0.1	10.5 ± 0.2	9.1 ± 0.2[b]	8.9 ± 0.1[b]
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

aValues presented as means ± SEM.
bSignificantly different from controls at $p < 0.05$ by one-tailed post-hoc t-testing following ANOVA.

Source: Archibong et al. (2002).

benzo[a]pyrene exposure at 75 μg/m^3 caused a statistically significant decrease in plasma progesterone, estradiol, and prolactin on GD 17; these levels were not determined in the rats exposed to 100 μg/m^3 (Archibong et al., 2002). Plasma prolactin is an indirect measure of the activity of decidual luteotropin, a prolactin-like hormone whose activity is necessary for luteal maintenance during pregnancy in rats. Control levels of prolactin increased from GDs 15 to 17, but this increase did not occur in the rats exposed to 75 μg/m^3. Although the progesterone concentration at 75 μg/m^3 was significantly lower than in controls on GD 17, the authors thought that the circulating levels should have been sufficient to maintain pregnancy; thus, the increased loss of fetuses was thought to be caused by the lower prolactin levels rather than progesterone deficiency. The reduced circulating levels of progesterone and estradiol-17β among benzo[a]pyrene-treated rats were thought to be a result of limited decidual luteotropic support for the corpora lutea. The authors proposed the following mechanism for the effects of benzo[a]pyrene on fertility: benzo[a]pyrene or its metabolites decreased prolactin and decidual luteotropin levels, compromising the luteotropic support for the corpora lutea and thereby decreasing the plasma levels of progesterone and estradiol-17β. The low estradiol-17β may decrease uterine levels of progesterone receptors, thereby resulting in fetal mortality. Based on biologically and statistically significant decreases in pups/litter and percent fetal survival/per litter, the LOAEL was 25 μg/m^3; no NOAEL was identified.

Neurotoxicity and in utero exposure via inhalation

To evaluate the effects of benzo[a]pyrene on the developing central nervous system, Wormley et al. (2004) exposed timed-pregnant F344 rats (10/group) to benzo[a]pyrene:CB aerosols by nose-only inhalation on GDs 11–21 for 4 hours/day at a concentration of 100 μg/m^3. Results of particle size analysis of genenerated aerosols were reported by other reports from this laboratory (Ramesh et al., 2001a; Hood et al., 2000). Particle size analysis of a 100-μg/m^3 aerosol showed a trimodal distribution with averages of 95% cumulative mass with diameters <15.85 μm; 90% <10 μm; 67.5% <2.5 μm; and 66.2% <1 μm; the MMAD ± GSD for the latter fraction was 0.4 ± 0.02 μm (Hood et al., 2000). Dams were maintained to term and pups were weaned on PND 30. Benzo[a]pyrene reduced the number of live pups to one-third of control values without affecting the number of implantation sites. During PNDs 60–70 electrical stimulation and evoked field potential responses were recorded via electrodes implanted into the

brains of the animals. Direct stimulation of perforant paths in the entorhinal region revealed a diminution in long-term potentiation of population spikes across the perforant path-granular cell synapses in the dentate gyrus of the hippocampus of F1 generation benzo[a]pyrene-exposed animals; responses in exposed offspring were about 25% weaker than in control offspring. Additionally, NMDA receptor subunit 1 protein (important for synaptic functioning) was down-regulated in the hippocampus of benzo[a]pyrene exposed F1 pups. The authors interpreted their results as suggesting that gestational exposure to benzo[a]pyrene inhalation attenuates the capacity for long-term potentiation (a cellular correlate of learning and memory) in the F1 generation.

In a later study by this same group of investigators, Wu et al. (2003) evaluated the generation of benzo[a]pyrene metabolites in F1 generation pups, as well as the developmental profile for AhR and mRNA. In this study, confirmed pregnant F344 rats were exposed to benzo[a]pyrene:CB aerosols at 25, 75, or 100 $\mu g/m^3$ via nose-only inhalation, 4 hours/day, for 10 days (GDs 11–21). Control animals were exposed to CB (sham) to control for inert carrier effects or they remained untreated. Each benzo[a]pyrene concentration had its own set of controls (CB and untreated). Two randomly selected pups were sacrificed on each of PND 0, 3, 5, 10, 15, 20, and 30. Body, brain, and liver weights were recorded. Benzo[a]pyrene metabolites were analyzed in the cerebral cortex, hippocampus, liver, and plasma. A dose-related increase in plasma and cortex benzo[a]pyrene metabolite concentrations in pups was observed. Dihydrodiols (4,5-; 7,8-; 9,10-) dominated the metabolite distribution profile up to PND 15 and the hydroxy (3-OH-benzo[a]pyrene; 9-OH-benzo[a]pyrene) metabolites after PND 15 at 100 $\mu g/m^3$ (the only exposure concentration reported). Results indicated a dose-related decrease in the ratio of the total number of pups born per litter to the total number of implantation sites per litter. The number of resorptions at 75 and 100 $\mu g/m^3$, but not at 25 $\mu g/m^3$, was statistically significantly increased compared with controls.

Adult male reproductive effects and repeated inhalation exposure

Inyang et al. (2003) evaluated the effect of sub-acute exposure to inhaled benzo[a]pyrene on testicular steroidogenesis and epididymal function in rats. Male F344 rats (10/group), 13 weeks of age, were exposed to benzo[a]pyrene:CB aerosols at 25, 75, or 100 $\mu g/m^3$ via nose-only inhalation, 4 hours/day for 10 days. Control animals were either exposed to CB (sham) to control for exposure to the inert carrier, or they remained untreated. Each benzo[a]pyrene concentration had its own set of controls (CB and untreated). Aerosols showed a trimodal distribution with averages of 95% cumulative mass <15.85 μm; 89% <10 μm; 55% <2.5 μm; and 38% <1 μm (Inyang et al., 2003); an earlier report from this laboratory indicated that the 55% mass fraction had a MMAD ± GSD of 1.7 ± 0.085 (Ramesh et al., 2001a). Blood samples were collected at 0, 24, 48, and 72 hours after cessation of exposure to assess the effect of benzo[a]pyrene on systemic concentrations of testosterone and luteinizing hormone (LH),

hormones that regulate testosterone synthesis. Reproductive endpoints such as testis weight and motility and density of stored (epididymal) sperm were evaluated.

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

In a follow up study with longer exposure duration, adult male F344 rats (10 per group) were exposed to benzo[a]pyrene:CB aerosols at 75 $\mu g/m^3$ via nose-only inhalation, 4 hours/day for 60 days (Archibong et al., 2008; Ramesh et al., 2008). Rats in the control group were subjected to the nose-only restraint, but were not exposed to the CB carrier. Blood samples were collected at 0, 24, 48, and 72 hours after exposure terminated, and the animals sacrificed for tissue analyses following the last blood sampling. Data were analyzed statistically for benzo[a]pyrene effects on weekly body weights, total plasma testosterone and LH concentrations, testis weights, density of stored spermatozoa, sperm morphological forms and motility, benzo[a]pyrene metabolite concentrations and AHH activity, and morphometric assessments of testicular histologies. Relative to controls, the results indicated 34% reduced testis weight ($p < 0.025$), reduced daily sperm production ($p < 0.025$) and reduced intratesticular testosterone concentrations ($p < 0.025$). Plasma testosterone concentrations were reduced to about one-third of the level in controls on the last day of exposure (day 60) and at 24, 48, and 72 hours later ($p < 0.05$). However, plasma LH concentrations in benzo[a]pyrene exposed rats were elevated throughout the blood sampling time periods compared with controls ($p < 0.05$). In testis, lung, and liver, AHH activity, and benzo[a]pyrene-7,8-dihydrodiol (precursor to the DNA-

reactive BPDE) and benzo[a]pyrene-3,6-dione metabolites were significantly ($p < 0.05$) elevated relative to controls. Progressive motility and mean density of stored spermatozoa were significantly reduced ($p < 0.05$). Weekly body weight gains were not affected by benzo[a]pyrene exposure. These results indicate that 60-day exposure of adult male rats to benzo[a]pyrene:CB aerosols at 75 $\mu g/m^3$ produced decreased testis weight; decreased intratesticular and plasma testosterone concentrations; and decreased sperm production, motility, and density.

4.4. OTHER DURATION OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Neurological Studies

Saunders et al. (2001) administered benzo[a]pyrene (>97% pure in peanut oil) to F344 rats (10/sex/dose group) via a single gavage dose of 0, 12.5, 25, 50, 100, or 200 mg/kg. Separate groups of animals were used for motor activity assessment and functional observational battery. Motor activity (horizontal, vertical, total distance, and stereotypic activity) was measured over 2-hour intervals during the nocturnal phase (12 hours) for 5 consecutive days after treatment (day 1). The functional observational battery, consisting of 29 tests assessing autonomic, neuromuscular, CNS excitability, CNS activity, sensorimotor, and physiological activity, was administered before dosing and at 2, 4, 6, 12, 24, 48, 72, and 96 hours after dosing. Body weight was measured after the functional observational battery.

In both sexes of rat, body weight gain was significantly reduced on day 4 and/or 5 at doses ≥25 mg/kg (Saunders et al., 2001). Body weight gains were comparable to controls in the 25 and 50 mg/kg groups by day 6. Higher doses of benzo[a]pyrene resulted in prolonged reductions in body weight gain, with reductions of 21–26% in both sexes on day 9 after treatment. Weight gain had returned to control levels after 2 weeks postdosing. At doses of 50 mg/kg and higher, all measures of motor activity were significantly depressed in both sexes beginning 2 hours after dosing and persisting through the 12-hour post-dosing measurement. At the 25 mg/kg dose, significant changes in motor activity were not observed until 4–6 hours after treatment. When motor activity was measured over 24-hour intervals, significant depression of motor activity (all measures) was observed at all doses on day 1 post-treatment and at ≥50 mg/kg on day 2. The results of the functional observational battery showed significant effects on neuromuscular endpoints (decreased mobility and grip strength, abnormal gait, loss of righting reflex), autonomic endpoints (increased defecation and urination), and sensorimotor endpoints (decreased response to sound, touch, and pain) at all doses and in both sexes. Effects on most parameters peaked at 6 hours post-dosing, with return to control levels by 72 hours post-dosing. The severity of effects on the FOB tests was greater in males than in females. This study identified a LOAEL of 25 mg/kg benzo[a]pyrene for acute neurotoxicity; the NOAEL is 12.5 mg/kg.

In a study with nearly identical design, Saunders et al. (2002) treated male F344 rats (10/dose) with single gavage doses of 0, 25, 50, 100, or 200 mg/kg benzo[a]pyrene (>97% pure,

in peanut oil). As in the study by Saunders et al. (2001), separate groups of animals were used for motor activity assessment and functional observational battery (using the same endpoints measured at the same time intervals); in addition, a third group of animals was treated at the same doses and used for measurement of benzo[a]pyrene and its metabolites in plasma and brain tissue. At 2, 4, 6, 12, 24, 48, 72, and 96 hours after dosing, rats in the metabolism groups were sacrificed for analysis of benzo[a]pyrene and its metabolites in plasma and brain tissue. Dose- and time-dependent effects on locomotor activity were observed in all treated groups. In measurements conducted over the 12 hours on day 1 after treatment, significant ($p < 0.05$ relative to vehicle controls) reductions in total distance traveled (considered by the authors to represent the most accurate measure of locomotor activity) were observed at doses ≥50 mg/kg beginning at 2–4 hours post dosing and persisting up to the 12-hour time point. When assessed on a daily basis over the 5 posttreatment days, total distance traveled was significantly lower than controls in all treated groups on day 1 and in groups exposed to ≥50 mg/kg on day 2. Significant ($p < 0.05$) dose-dependent effects on neuromuscular, sensorimotor, and autonomic parameters were observed with benzo[a]pyrene treatment. Significant increases in the severity of abnormal gait and impaired sound and tail pinch responses, as well as decreased forelimb grip strength, occurred in all dose groups; the severity of effects peaked at 4 or 6 hours after treatment. Increased severity of landing foot splay was observed at doses ≥50 mg/kg, also peaking in severity at 6 hours after treatment. Effects on this endpoint persisted from 2 to 24 hours post treatment in the two high dose groups. Autonomic effects, consisting of increased frequency of urination and defecation, occurred at doses ≥50 mg/kg; in the two high dose groups, these effects began 4 hours after dosing and persisted through 24 hours. The onset and duration of the effects observed in this study corresponded well with the plasma and brain tissue concentrations of benzo[a]pyrene and its metabolites. In particular, levels of benzo[a]pyrene metabolites in brain tissue peaked between 2 and 6 hours after dosing, and plasma levels peaked at 6 hours after dosing, in all treated groups. By 72 hours after dosing, metabolite levels had returned to baseline. Unmetabolized benzo[a]pyrene was detected in brain tissue only in the two highest dose groups; levels in both brain tissue and plasma peaked between 2 and 6 hours after dosing. Analysis of specific metabolite levels over time showed that the diol metabolites comprised a larger percentage of the total metabolites at earlier time points (up to 12 hours after dosing), while the hydroxyl metabolites predominated at later times. The authors postulated that the observed toxic effects were associated with the production of benzo[a]pyrene diol metabolites and/or related generation of ROS rather than an effect of the parent compound or hydroxyl metabolites. The LOAEL in this study was 25 mg/kg based on suppression of locomotor activity and evidence of impairment in the functional observational battery.

In a follow-up study, Saunders et al. (2006) attempted to correlate neurobehavioral changes with levels of benzo[a]pyrene metabolites, antioxidant enzyme levels, and measures of lipid peroxidation in selected brain regions. Single oral doses of 0, 25, 50, 100, or 200 mg/kg

benzo[a]pyrene (97% pure, in peanut oil) were administered by gavage to groups of 10 male F344 rats. Motor activity, measured as total distance traveled over a 2-hour interval during the nocturnal phase, was assessed at 0, 2, 4, 6, 12, 24, 48, 72, and 96 hours post treatment. Additional groups of animals were exposed to the same doses and sacrificed for collection of blood and specific brain tissues (hippocampus, striatum) at the same time points; benzo[a]pyrene and its metabolites were measured by reverse phase HPLC in blood and brain tissues. In addition, the activities of superoxide dismutase, catalase, glutathione peroxidase, and levels of malondialdehyde in striatum and hippocampus were determined at 6 and 96 hours after treatment. The authors reported that motor activity was significantly ($p < 0.01$) suppressed as early as 2 hours after treatment and remained suppressed through the 48-hour time point in the groups exposed to 50 and 200 mg/kg benzo[a]pyrene; however, the data were not reported, and it was not clear whether the 100 mg/kg dose group was affected. The authors indicated that the maximum suppression occurred at 12 hours, when motor activity was 72% lower than controls; however, the affected dose group(s) was not reported. Data were reported graphically for the 25 mg/kg group only; based on the graph, a significant ($p < 0.05$) suppression of motor activity occurred by 4 hours after treatment and persisted through the 12-hour measurement. Activity had returned to control levels by 72 or 96 hours in all dose groups. As in the study reported by Saunders et al. (2002), the levels of benzo[a]pyrene metabolites in the plasma and brain correlated with the onset and duration of behavioral effects; metabolite concentrations peaked between 2 and 6 hours after treatment, when suppression of motor activity occurred. In addition, the toxification/detoxification ratio of benzo[a]pyrene metabolites (measured as the ratio of 7,8-dihydrodiol 9,10-epoxide to 3[OH]benzo[a]pyrene) in plasma, cortex, cerebellum, hippocampus, and striatum was higher (between 2 and 7) for the first 6 hours, indicating higher levels of the more toxic epoxide metabolite. From 24 to 96 hours after treatment, the ratio was <1, indicating that the hydroxyl form predominated. Measurement of antioxidant enzyme levels in the striatum and hippocampus at 6 and 96 hours after dosing showed significant dose-related decreases in the activities of superoxide dismutase and glutathione peroxidase, but enhanced catalase activity and increased lipid peroxidation products in the striatum and hippocampus. The authors suggested that benzo[a]pyrene-induced acute neurobehavioral effects may be associated with oxidative stress resulting from generation of ROS and inhibition of brain antioxidants.

Evidence of neurotoxicity has also been reported in studies of acute exposure to benzo[a]pyrene administered via parenteral routes. As part of a study of the neurotoxicity of motorcycle exhaust, Liu et al. (2002) administered benzo[a]pyrene dissolved in corn oil via i.p. injection to ICR mice (sex not specified; 4–6/group) at doses of 0, 50, or 100 mg/kg-day for 3 consecutive days. One day after the last treatment, motor nerve conduction velocity was measured in the tails. The mice were then sacrificed for removal of the sciatic nerve, which was assayed for Na^+/K^+-ATPase activity. Although the methods section indicated that benzo[a]pyrene-treated animals were tested for rotarod performance, results of this evaluation

were not reported. Data on motor nerve conduction velocity and Na^+/K^+-ATPase activity were presented graphically. Exposure to benzo[a]pyrene at resulted in significant ($p < 0.05$) depression of motor nerve conduction velocity (about 25 and 40% decrease from control for 50 and 100 mg/kg-day doses, respectively, based on visual inspection data presented graphically), and decreased the Na^+/K^+-ATPase activities of sciatic nerves (about 20 and 30% decreases from control, respectively).

Grova et al. (2007) evaluated the effects on short-term exposure to benzo[a]pyrene on learning, memory, locomotor activity, and motor coordination. Groups of 10 female Balb/c mice were treated with i.p. injections of benzo[a]pyrene (>97% pure, in vegetable oil) at doses of 0, 0.02, 0.2, 2, 20, or 200 mg/kg-day for 10 consecutive days. At the end of exposure, locomotor activity was measured in open field activity, and motor coordination was assessed. Memory and learning were evaluated using the Y maze (measures spontaneous alternation behavior) and the Morris water maze (measures learning as escape latency in repeated trials). Finally, the animals were sacrificed for removal of the brain; expression of the NMDA R1 receptor (involved in cognitive function) subunit gene was measured in eight brain regions (cerebral trunk, cerebellum, mesencephalum, hippocampus, hypothalamus, thalamus, frontal cortex, and temporal cortex) by quantitative real-time reverse transcription PCR assay. In contrast to oral studies of benzo[a]pyrene exposure (Saunders et al., 2006, 2002, 2001), injection of benzo[a]pyrene did not affect locomotor activity at any dose in this study. At the lowest doses (0.02 and 0.2 mg/kg-day), benzo[a]pyrene exposure resulted in reductions in the percentage of spontaneous alternation in the Y maze. However, at the higher doses (≥2 mg/kg-day), there was no difference from controls. The authors attributed this finding to increased activity and arousal at the higher doses, postulated to result from an anxiolytic effect of benzo[a]pyrene. In the 5^{th} trial of the water maze, all benzo[a]pyrene groups showed impairment; the escape latency was significantly higher than controls. In contrast, the higher doses of benzo[a]pyrene resulted in significantly reduced latency during the first trial. No differences from control were observed in the 2^{nd}, 3^{rd}, and 4^{th} trials, or 1 day after the last dose. Benzo[a]pyrene exposure resulted in modulation of NMDA-R1 subunit gene expression; expression was significantly ($p < 0.05$) increased in the cerebellum, mesencephalus, and hippocampus, but decreased in the frontal cortex and cerebral trunk. Effects on gene expression in the cerebellum, frontal cortex, and hippocampus occurred at all doses, while gene expression in the cerebral trunk was affected only at doses of ≥0.2 mg/kg-day, and expression in the mesencephalus occurred only at doses of ≥2.0 mg/kg-day.

In a follow-up study, Grova et al. (2008) evaluated the effects of exposure to benzo[a]pyrene on anxiety-related behaviors (performance in elevated-plus maze and hole-board apparatus). The animals, group sizes, and doses were the same as in Grova et al. (2007), but exposure occurred over 11 days. Behavioral tests were administered 30 minutes after the final dose, and the animals were sacrificed 1 hour after the tests. Benzo[a]pyrene exposure at 20 and 200 mg/kg-day resulted in reduction in anxiety-related behavior as measured by the increased

number of head dippings in the hole-board apparatus. In the elevated-plus maze test, only the highest dose resulted in a significant reduction in anxiety (as measured by higher percentage of open arm entries and time spent in open arms).

4.4.2. Immunological Studies

Immunological effects (e.g., decreased thymus weight, decreased number of B cells in spleen) have been reported in Wistar rats repeatedly exposed to benzo[a]pyrene doses ≥10–15 mg/kg-day in standard oral toxicity studies (Kroese et al., 2001; De Jong et al., 1999). Diminished immune responses elicited by the dermal sensitizer, 2,4-dinitrochlorobenzene (DNCB) have been observed in C56BL/6 mice orally exposed to 13 mg/kg-day benzo[a]pyrene, 3 times/week for 4 weeks (van den Berg et al., 2005). No studies were located examining immune system endpoints following inhalation exposure of animals to benzo[a]pyrene. Results from studies of immune system endpoints in mice following i.p., subcutaneous (s.c.), or intratracheal instillation exposure are consistent with immune suppression at dose levels generally ≥40 mg/kg-day. The available animal studies identify immune suppression as a potential hazard of repeated oral exposure to benzo[a]pyrene at doses ≥10–15 mg/kg-day.

4.4.2.1. *Oral Exposure Immunological Studies*

As discussed in Section 4.2.1.1, dose-related decreases in thymus weight and relative number of B cells in the spleen were observed in male Wistar rats administered gavage doses ≥10 mg/kg-day for 35 days (De Jong et al., 1999). At higher doses (≥30 mg/kg-day), serum IgM and IgA levels were decreased. At the highest dose tested (90 mg/kg-day), the relative cortex surface area of thymus and thymic medullar weight were significantly reduced; NK cell activity in the spleen was also reduced at this dose. No effects on the immune system were observed at 3 mg/kg-day (De Jong et al., 1999). In two additional subchronic gavage studies, thymus weight was decreased in a dose-related manner in male Wistar rats exposed to doses ≥15 mg/kg-day (5 days/week) for at least 5 weeks and in females exposed to doses of ≥30 mg/kg-day (5 days/week) for 90 days (Kroese et al., 2001). No other immune system parameters were assessed in these studies. In an adaptation of the sensitization-specific murine local lymph node assay for use in testing immune function, van den Berg et al. (2005) tested several immunomodulating compounds, including benzo[a]pyrene, for effects on the T-cell-dependent immune response induced by the contact sensitizer, DNCB. Groups of eight male and eight female C56BL/6 mice were given gavage doses of 13 mg/kg-day benzo[a]pyrene (purity not reported) 3 times/week for 4 weeks, followed by sensitization with DNCB (0, 0.33, 0.66, or 1% solutions in acetone:corn oil) applied topically to the backs of both ears for 3 consecutive days. Three days after the last DNCB treatment, the lymph nodes under the application area were excised, weighed, and homogenized for preparation of cell suspensions. The lymph node cell suspensions were tested for cell proliferation capacity via measurement of [^{3}H]-thymidine

incorporation. Releases of the cytokines interferon (IFN)-γ and interleukin (IL)-4 following concanavalin A stimulation were assayed by ELISA. At the highest concentration of the sensitizer, benzo[a]pyrene treatment reduced [^{3}H]-thymidine incorporation into lymphocytes by approximately 30% (based on visual inspection of data presented graphically; $p = 0.008$) compared with untreated controls. In this treatment group, benzo[a]pyrene also reduced the release of IFN-γ (approximately 75% less than controls based on graphical data, not significant) and IL-4 (approximately 60% less than controls based on graphical data, $p < 0.001$). The results indicated that benzo[a]pyrene modulates the immune response elicited by the sensitizer DNCB.

4.4.2.2. *Inhalation Exposure Immunological Studies*

No studies were located that examined immune system endpoints following inhalation exposure of animals to benzo[a]pyrene.

4.4.2.3. *Other Exposure Route Immunological Studies*

A number of studies have shown suppression of both humoral and cell-mediated immune responses in mice following i.p., s.c., or intratracheal administration. Dose-related decreases in spleen and serum IgM levels after challenge by sheep red blood cells (SRBC) were reported in rats (10, 40 mg/kg-day) and mice (5, 20, 40 mg/kg-day) following s.c. injection of benzo[a]pyrene for 14 days (Temple et al. 1993). Reduced spleen cell response to SRBC and lipopolysaccharides were observed in B6C3F$_1$ mice exposed to doses ≥40 mg/kg-day benzo[a]pyrene by i.p. or s.c. injection for 4–14 days (Lyte and Bick, 1985; Dean et al., 1983; Munson and White, 1983) or by intratracheal instillation for 7 days (Schnizlein et al., 1987). B6C3F$_1$ mice exhibited dose-dependent decreased resistance to Streptococcus pneumonia or Herpes simplex type 2 following s.c. injection of 5, 20, or 40 mg/kg benzo[a]pyrene for 14 days (Munson et al., 1985). Galvan et al. (2006) reported that single i.p. injections of mice with 50 mg/kg benzo[a]pyrene caused decreased pro/pre B-lymphocytes and neutrophils in bone marrow, without affecting numbers of immature and mature B-lymphocytes or GR-1+ myeloid cells. Several i.p. injection studies reported immune suppression effects in mice exposed to benzo[a]pyrene *in utero* at doses ranging from 50 to 150 mg/kg; effects included decreased spleen or thymus weights, suppression of antibody forming cells in response to sheep red blood cells, decreased spleen, thymic, or bone marrow cellularity, and disrupted T-cell development (Rodriguez et al. 1999; Holladay and Smith, 1995; Lummus and Henningsen, 1995; Holladay and Smith, 1994; Urso and Johnson 1988; Urso et al., 1988; Urso and Gengozian, 1984, 1982, 1980).

In contrast to the studies that have shown decrements in immune response, benzo[a]pyrene may also induce sensitization responses. Epicutaneous application of benzo[a]pyrene (100 μg benzo[a]pyrene to C3H/HeN mice followed by ear challenge with 20 μg

benzo[a]pyrene 5 days later) produced a contact hypersensitivity (a significant ear swelling) response (Klemme et al., 1987).

4.4.2.4. *Other Exposure Route Developmental Immunotoxicy*

While there are no oral or inhalation studies of benzo[a]pyrene on the developing immune system, several i.p. injection studies indicate that this is an area of concern for both cell-mediated and humoral immune ontogeny. In terms of cell-mediated effects, Urso and Gengozian (1984) reported severe suppression of the mixed lymphocyte response and moderate suppression of the graft-versus-host response in mice exposed *in utero* to 150 mg/kg from GD 11 to 17. Both effects persisted until 18 mo of age. Holladay and Smith (1994) found that mice exposed to 0, 50, 100, or 150 mg/kg from GD 13 to 17 exhibited severe fetal thymic atrophy when examined on GD 18. In the same study, expression data of cell surface markers (e.g. CD4, CD8) indicate that benzo[a]pyrene may inhibit and/or delay thymocyte maturation, possibly contributing to the observed thymic atrophy. Several other studies also show decreased thymocyte numbers and disrupted T cell maturation after *in utero* exposure to benzo[a]pyrene (Rodriguez et al., 1999; Holladay and Smith, 1995; Lummus and Henningsen, 1995; Urso et al., 1992; Urso and Johnson, 1987).

In addition to direct thymus effects, Holladay and Smith (1994) reported a large reduction in total cellularity in the fetal liver, which is the primary hematopoietic organ during gestation and a major source of thymocyte precursors beginning around GD 10-11 in mice (Pennit and Vaddeur, 1989; Landreth and Dodson, 2005). This was accompanied by decreased expression of terminal deoxynucleotidyl transferase (TdT), an intracellular marker known to be present in cortical thymocyte progenitors in the fetal liver (Fine et al., 1990; Silverstone et al., 1976). This data suggests that benzo[a]pyrene also disrupts liver hematopoiesis during gestation and may interfere with prolymphoid seeding of the thymus, possibly contributing to thymic atrophy and cell-mediated immunosuppression. Rodriguez et al. (1999) assessed downstream affects of T cell development by showing that $CD4^+$ T-cells were reduced in the spleen of 1-week old mice following *in utero* benzo[a]pyrene exposure.

There is also some evidence of humoral immune disruption by benzo[a]pyrene during fetal life. In a series of related studies, mice exposed to benzo[a]pyrene during mid (GD 11 to 13) or late (GD 16-18) gestation or both (GD 11 to 17) exhibited severe suppression of the plaque-forming cell response to sheep red blood cells from 1 wk up to 18 mo after birth (Urso and Gengozian, 1984, 1982, 1980). In their analysis of fetal liver cells, Holladay and Smith (1994) reported large decreases in expression of TdT and CD45R cellular markers, both of which are present on pre-B lymphocytes.

4.4.3. Cancer Bioassays (Other Routes of Exposure)

Cancer bioassays following i.p. injection of mice with benzo[a]pyrene have consistently found cancer responses. Newborn mouse bioassays involving postnatal injections of benzo[a]pyrene (generally in the dose range of 0.5–1 μmol/mouse on PNDs 1, 8, and 15) consistently found increases in liver or lung tumors, either increases in incidence of animals with tumors or increased numbers of tumors per animal (LaVoie et al., 1994, 1987; Busby et al., 1989, 1984; Weyand and LaVoie, 1988; Wislocki et al., 1986; Buening et al., 1978; Kapitulnik et al., 1978). Likewise, i.p. injection of pregnant mice with benzo[a]pyrene (100–150 mg/kg) during gestation induced increased incidences of offspring with liver or lung tumors, compared with controls (Urso and Gengozian, 1984; Bulay and Wattenberg, 1971). A/J adult mice given single i.p. injections of benzo[a]pyrene showed a dose-related increase in the number of lung tumors per mouse with doses ranging from about 5 to 200 mg/kg (Mass et al., 1993).

Tumorigenic responses to s.c. administered benzo[a]pyrene have been observed mostly at the site of injection in studies with mice (Nikonova, 1977; Pfeiffer, 1977; Homburger et al., 1972; Roe and Walters, 1967; Grant and Roe, 1963; Steiner, 1955; Rask-Nielson, 1950; Pfeiffer and Allen, 1948; Bryan and Shimkin, 1943; Barry et al., 1935).

Positive cancer responses from other routes of exposure have included: (1) mammary tumors in rats with intramammilary administration (Cavalieri et al., 1991, 1988a, b, c); (2) cervical tumors in mice with intravaginal application (Naslund et al., 1987); (3) injection site sarcomas with intramuscular injection (Sugiyama, 1973); (4) respiratory tract tumors in hamsters with intratracheal instillation (Henry et al., 1973); and (5) tracheal epithelial tumors in rats with intratracheal implantation (Topping et al., 1981, Nettesheim et al., 1977).

4.4.4. Atherogenesis Studies

Cigarette smoking (see Ramos and Moorthy, 2005; Miller and Ramos, 2001; Thirman et al., 1994, for review) and, to a more limited degree, occupational exposure to PAH mixtures (Burstyn et al., 2005) have been identified as risk factors associated with the development of atherosclerotic vascular disease and increased risk for cardiovascular mortality. Based on results from in vivo and in vitro animal studies, reactive metabolites of PAHs, including benzo[a]pyrene, are thought to play a role in the progression of atherosclerosis leading to hardening and thickening of the arteries (see Ramos and Moorthey, 2005; Miller and Ramos, 2001 for review). For example, in vivo exposure of Sprague-Dawley rats to 10 mg/kg benzo[a]pyrene i.p. injections (once/week for 8 weeks) induced aortic wall lesions related to atherosclerosis including loss of endothelial integrity and increase of smooth muscle cell mass (Zhang and Ramos, 1997). The molecular mechanisms responsible for PAH-induced vascular injury and the development of atherosclerosis are not well established, but current hypotheses include roles for cell proliferative responses to injury of endothelial cells from reactive metabolites (including ROS) and genomic alterations in smooth muscle cells from reactive

metabolites leading to transformed vasculature cells and eventual plaque formation (Ramos and Moorthy, 2005).

Although many studies have been conducted in animal systems to study the mechanisms by which PAHs may participate in the initiation and promotion of atherosclerosis, no studies were located that examined relationships between levels of exposure to benzo[a]pyrene (via environmentally relevant routes) and the development of aortic wall lesions related to atherosclerosis, with the exception of a series of experiments involving repeated exposure of Apolipoprotein E knock out (ApoE-/-) mice to oral doses of 5 mg/kg benzo[a]pyrene (Knaapen et al., 2007; Curfs et al., 2005, 2004; Godschalk et al., 2003). ApoE-/- mice develop spontaneous atherosclerosis, which is thought to be due to enhanced oxidative stress from the lack of ApoE (Godschalk et al., 2003).

Treatment of male ApoE-/- mice with gavage doses of 5 mg/kg-day benzo[a]pyrene for 4 days produced increased levels of lipid peroxidation-derived DNA modifications (etheno-DNA adducts) and BPDE-DNA adducts in aorta, compared with unexposed ApoE-/- controls (Godschalk et al., 2003). Repeated exposure of male ApoE-/- mice to 5 mg/kg once a week for 12 or 24 weeks did not cause enhancement of the initiation of plaques in the aortic arch (compared with unexposed ApoE-/- controls), but caused larger plaques with increased plaque layering and number of lipid cores, and increased plaque content of T-lymphocytes, compared with unexposed ApoE-/- controls (Curfs et al., 2004). In another study, gavage exposure of male ApoE-/- mice with 5 mg/kg benzo[a]pyrene or 5 mg/kg benzo[e]pyrene (BeP) once per week for 24 weeks similarly increased plaque size and T-lymphocyte content (Curfs et al., 2005). In addition, exposure to benzo[a]pyrene, and to a lesser extent BeP, was associated with increased transforming growth factor beta (TGFβ1) protein levels in plaque macrophages; TGFβ1 is thought to play a role in the migration of T-lymphocytes. No exposure-related differences were noted in the location or number of plaques, oxidative DNA damage (assessed by immunostaining for 8-hydroxydeoxyguanosine, 8-OHdG), or apoptosis in the plaques (Curfs et al., 2005). As expected, the lungs of benzo[a]pyrene-exposed mice showed several benzo[a]pyrene-DNA adducts, which were not detectable in the lungs of BeP-exposed or control ApoE-/- mice (Curfs et al., 2005). In another study, increased expression of monocyte-chemoattractant protein-1 (MCP-1) was found in aortic tissue from male ApoE-/- mice exposed to 5 mg/kg benzo[a]pyrene once per week by gavage for 2 weeks; this protein is thought to recruit monocytes into atherosclerotic lesions (Knaapen et al., 2007).

In summary, the results of the studies with ApoE-/- mice indicate that repeated oral exposure to 5 mg/kg gavage doses of benzo[a]pyrene enhance the progression of (but do not initiate) atherosclerosis through a general local inflammatory process. The involvement of PAH-DNA adducts was not evident in these studies, as indicated by observations that BeP, which does not cause DNA adducts, elicited similar plaque responses in ApoE-/- mice as benzo[a]pyrene. Although these results demonstrate that repeated oral exposure to 5 mg/kg benzo[a]pyrene can

enhance atherosclerosis in animals, the altered genetic disposition of ApoE-/- mice limits their usefulness in describing human-relevant dose-response relationships for oral exposure to benzo[a]pyrene and atherosclerosis.

4.4.5. Reproductive Studies (Other Routes of Exposure)

Mattison et al. (1980) examined the response to i.p. exposure to a single dose of benzo[a]pyrene in female DBa/2N mice (n=15 per dose group); effects on fertility, primordial oocyte destruction, and response to pregnant mare's serum gonadotropins were evaluated. In the 10 week breeding study, dose groups included the vehicle control (corn oil) and 10, 100, 200 and 500 mg benzo[a]pyrene/kg. Complete infertility was seen in the 200 and 500 mg/day groups, with decreased fertility seen in the 10 and 100 mg/kg dose groups, too. The total number of pups born was 137, 91, 28, 0 and 0 and the mean number of pups per mouse per week was 0.91, 0.61, 0.20, 0.0, 0.0 in the 0, 10, 100, 200 and 500 mg/kg dose groups, respectively ($p < 0.05$ for comparison of 0 to 10 mg/kg groups and 20 to 100 mg/kg groups). In a parallel study using a single i.p. dose administered 21 days before sacrifice, the percent of primordial oocytes destroyed (compared with controls) was 0, 18, 19, 56, 88, 100% for doses of 0, 5, 10, 50, 100, and 200 mg benzo[a]pyrene/kg. The differences at doses $\geq$ 50 mg/kg were statistically significant ($p < 0.05$) compared with controls. The results from these studies were used to calculated an ED_{50} (i.e., dose producing a reduction in fertility or number of oocytes) of 25.5 mg/kg for fertility reduction and 24.5 mg/kg for primordial oocyte destruction. There was no effect of benzo[a]pyrene exposure on ovary weight or response to pregnant mare's serum gonadotropin, indicating that the effect of exposure did not involve ovulation inhibition.

Another acute exposure study examined the effect of benzo[a]pyrene exposure on ovulatory response (as determined by number of corpora lutea) in female C57BL/6N mice (Swartz and Mattison, 1985). Benzo[a]pyrene was given as a control dose (corn oil vehicle), and 1, 5, 50, 100, 500 mg/kg i.p., 20 animlals were included per dose group and 5 were sacrificed at weekly intervals. Ovaries were removed and serial sections were examined for histological changes and counts of corpora lutea. There was a 35% mortality rate in the 500 mg/kg group, but no evidence of treatment-related mortality in the other groups. Mean number of corpora lutera in controls varied between 5.5 and 10.0 for the samples taken at 1, 2, 3, and 4 weeks post-dose administration, with no time-related trend of increasing or decreasing number. The 1 mg/kg dose group exhibited no decrease in number of corpora lutea compared with controls at any time period (mean count varying between 6.2 and 7.2). The number of corpora lutea was decreased ($p < 0.05$) at all doses $\geq$ 5.0 mg/kg at 1 week post-administration (mean 0.0, 2.0, 0.0, 0.0 and 0.0 for 5.0, 10, 50, 100 and 500 mg/kg compared with 6.8 and 10.0 in the control and 1 mg/kg groups, respectively); at 2 weeks decreases were seen at $\geq$ 50 mg/kg and at weeks 3 and 4 a decrease was seen only at doses $\geq$ 100 mg/kg. Thus in addition to the destruction of primordial follicles seen

in Mattison et al. (1980), this study also demonstrated an inhibition of ovulation by benzo[a]pyrene that was time- and dose-related.

Miller et al. (1992) used the protocol described by Swartz and Mattison (1985) to further examine the ovarian effects of acute benzo[a]pyrene exposure. As in the previous experiment, doses of 1, 5, 10, 50, 100 and 500 mg/kg i.p. (with corn oil vehicle control) were administered to female C57BL/6N mice (20 per group; 5 sacrificed at 1, 2, 3, and 4 weeks post-dose administration). In addition to counts of corpora lutea, total ovarian volume and total and individual corpora lutea volume were measured. The results pertaining to dose- and time-dependent descreases in corpora lutea matched the results seen in Swartz and Mattison, 1985), and similar trends were seen in the total ovarian volume and total corpora luteal volume measures. However, volume of individual corpora lutea was increased in the treated animals compared with controls at weeks 2, 3 and 4 post-treatment. The authors note that the recovery seen in the effect on corpora lutea number by 2 weeks post-treatment at the lower doses may reflect an effect specifically on antral follicles, whereas the longer recover period at higher doses indicates an additional effect on growing follicles.

Borman et al. (2000) compared the ovarian effects of benzo[a]pyrene, two other PAHs (9,10-dimethylbenzanthracene and 3-methylcholanthrene in female B6C3F_1 mice and Fischer 344 rats); the ovotoxic 4-vinylcyclohexene (VHC)and its diepoxide metabolite (4-vinylcyclohexene diexpoxide, VHD) were also included to allow calculation of an "ovotoxic index." as positive controls. Doses of 0.0, 0.0075, 0.015, 0.075, 0.15, 0.75, 3.5, 7.5 and 15 mg/kg in the mouse, and an additional dose of 60 mg/kg for the rat were administered i.p. (sesame oil vehicle) daily for 15 days (6-7 animals per treatment group). The size of the ovaries and the number of primordial, primary and secondary (containing an oocyte) follicles was determined was determined after sacrifice (4 hours after the last dose administration). The ovotoxic index was defined as the lowest dose that resulted in a 50% loss (ED_{50}) of primordial follicles. For benzo[a]pyrene in mice, the ED_{50} was 3 mg/kg (0.012 mmol/kg) for primordial follicles, a 50% loss in primary follicles was seen at 7.5 mg/kg (0.03 mmol/kg), but this level of loss of secondary follicles was not seen even at the highest dose used. The ED_{50} for primordial follicle loss was 0.02 mg/kg for dimethylbenzanthracene and 0.045 mg/kg for 3-methylcholanthrene. In rats, the 60 mg/kg (0.24 mmol/kg) dose of benzo[a]pyrene resulted in a 50% loss of primary and secondary follicles, but a much smaller decrease in primordial follicles was seen (approximately 75% of control counts in the 15 and 60 mg/kg groups).

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MOA

benzo[a]pyrene is a complete carcinogen in that it both initiates and promotes tumor formation. Several mechanistic processes have been associated with benzo[a]pyrene carcinogenicity, including oxidative metabolism, which gives rise to reactive intermediates (see Section 3.3), and formation of DNA adducts, both of which can lead to genotoxicity and

mutations in specific cancer-related genes. The ability of benzo[a]pyrene to function as a tumor promoter may be related to cytotoxicity, AhR affinity, and upregulation of genes related to biotransformation, growth, and differentiation.

The following sections discuss mechanistic evidence for possible key events in the MOA for cancer (a topic that is further discussed in Section 4.7.3). Information regarding MOAs for noncancer effects noted above is discussed in Section 4.6.3.

4.5.1. Genotoxicity

The ability of benzo[a]pyrene to cause mutations and other forms of DNA damage in both *in vivo* and *in vitro* studies is well documented (see Tables 4-24, 4-25 and 4-26). With metabolic activation (inclusion of S9) benzo[a]pyrene is consistently mutagenic in the prokaryotic *Salmonella*/Ames and *E. coli* assays (Table 4-24). A rare exception was observed, in which benzo[a]pyrene did not induce mitotic recombination in eukaryotic *S. cerevisiae* regardless of the presence of S9. In mammalian *in vitro* studies, benzo[a]pyrene is consistently mutagenic, clastogenic and induces cell transformation both with and without metabolic activation (Table 4-25). Cytogenetic damage in the form of chromosomal aberrations, micronuclei, sister chromatid exchanges and aneuploidy are commonplace following benzo[a]pyrene exposure as are DNA adduct formation, single strand breaks, and induction of DNA repair and unscheduled DNA synthesis. The in vitro mammalian cell assays were conducted in various test systems, including human cell lines.

In in vivo studies, benzo[a]pyrene consistently tested positive in multiple species and strains and under various test conditions in the following assays: cell transformation, chromosomal aberrations, DNA adducts, DNA strand breaks, micronuclei formation, gene mutations (H-ras, K-ras, p53, *lacZ*, *Hprt*), sister chromatid exchanges, sperm abnormality, and unscheduled synthesis. Negative results were nominally interspersed throughout the in vivo mammalian assays, except for consistently negative results observed for unscheduled DNA synthesis.

In human in vivo studies, exposures were to mixed PAHs through cigarette smoke or occupational exposure. In a subset of these studies, benzo[a]pyrene-specific DNA adducts have been detected, and it has been demonstrated qualitatively that benzo[a]pyrene metabolites damage DNA in exposed humans (see Table 4-26).

Table 4-24. In vitro genotoxicity studies of benzo[a]pyrene in non-mammallian cells

	Result		
	+S9	– S9	Reference
Endpoint/Test System: *prokaryotic cells*			
Forward mutation			
S. typhimurium TM677	+	–	Rastetter et al. (1982)
S. typhimurium TM677	+	ND	Babson et al. (1986)
Reverse mutation			
S. typhimurium TA1537; TA1538	+	–	Ames et al. (1973)
S. typhimurium TA1535	–	–	Ames et al. (1973)
S. typhimurium TA98; TA1538	+	ND	Ames et al. (1975)
S. typhimurium TA1537; TA1538	+	–	Glatt et al. (1975)
S. typhimurium TA 1535	–	–	Glatt et al. (1975)
S. typhimurium TA98; TA100; TA1538	+	ND	McCann et al. (1975)
S. typhimurium TA 1535	–	ND	McCann et al. (1975)
S. typhimurium TA1538	+	ND	Egert and Greim (1976)
S. typhimurium TA1537	+	ND	Oesch et al. (1976)
S. typhimurium TA1538, TA98	+	–	Wood et al. (1976)
S. typhimurium TA98; TA100; TA1537	+	–	Epler et al. (1977)
S. typhimurium TA1535	–	–	Epler et al. (1977)
S. typhimurium TA98; TA100	+	–	Obermeier and Frohberg (1977)
S. typhimurium TA100	+	ND	Tang and Friedman (1977)
S. typhimurium TA98	+	–	Pitts et al. (1978)
S. typhimurium TA100	+	ND	Bruce and Heddle (1979)
S. typhimurium TA98, TA100	+	ND	LaVoie et al. (1979)
S. typhimurium TA1538	+	–	Rosenkranz and Poirier (1979)
S. typhimurium TA98, TA100	+	–	Simmon et al. (1979a)
S. typhimurium TA98	+	ND	Hermann (1981)
S. typhimurium TA98, TA100	+	ND	Alfheim and Randahl (1984)
S. typhimurium TA100	+	ND	Norpoth et al. (1984)
S. typhimurium TA98, TA100, TA 1538	ND	–	Glatt et al. (1985)
S. typhimurium TA97, TA98, TA100	+	–	Sakai et al. (1985)

S. typhimurium TA100	+	–	Carver et al. (1986)
S. typhimurium TA98	+	–	Alzieu et al. (1987)
S. typhimurium TA97, TA98, TA100, TA1537	+	–	Glatt et al. (1987)
S. typhimurium TA97, TA98, TA100	+	ND	Marino (1987)
S. typhimurium TA100	+	ND	Pahlman and Pelkonen (1987)
S. typhimurium TA 98, TA100	+	–	Prasanna et al. (1987)
S. typhimurium TA98	+	ND	Ampy et al. (1988)
S. typhimurium TA98, TA100	+	ND	Bos et al. (1988)
S. typhimurium TA98	+	ND	Lee and Lin (1988)
S. typhimurium TA100	+	ND	Phillipson and Ioannides (1989)
S. typhimurium TA98	+	ND	Antignac et al. (1990)
S. typhimurium TA98	–	ND	Gao et al. (1991)
S. typhimurium TA98	+	ND	Balansky et al. (1994)
S. typhimurium TA100	–	ND	Balansky et al. (1994)
DNA damage			
E. coli/pol A	+	–	Rosenkranz and Poirier (1979)
E. coli/differential killing test	+	–	Tweats (1981)
E. coli WP2-WP100/rec-assay	+	ND	Mamber et al. (1983)
E. coli/SOS chromotest Pq37	+	–	Mersch-Sundermann et al. (1992)
Endpoint/Test System*: Non-mammalian eukaryotes* **Mitotic recombination**			
S. cerevisiae D4-RDII	ND	–	Siebert et al. (1981)
S. cerevisiae D3	–	–	Simmon (1979b)

1
2

Table 4- 25. In vitro genotoxicity studies of benzo[a]pyrene in mammalian cells			
	Result		
Assay/Test System	+S9	– S9	Reference
Forward Mutation			
Human AHH-1 lymphoblastoid cells	ND	+	Danheiser et al. (1989)
Human lymphoblast (AHH-1) cells (*Hprt*)	ND	+	Crespi et al. (1985)
Human lymphoblastoid (AHH-1) cell line	ND	+	Chen et al. (1996)
Human fibroblast (MRC5CV1) cell line (*Hprt*)	–	ND	Hanelt et al. (1997)
Human lymphoblast (TK) cells	ND	+	Barfknecht et al. (1982)
Human lymphoblast (TK6) cells	+	ND	Crespi et al. (1985)
Human embryonic epithelial (EUE) cells	ND	+	Rocchi et al. (1980)
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)
Human HSC172 lung fibroblasts	+	–	Gupta and Goldstein (1981)
Human Q3-wp normal lung keratinocytes	+	ND	Allen-Hofmann and Rheinwald (1984)
Human SCC-13Y lung keratinocytes	ND	+	Allen-Hofmann and Rheinwald (1984)
Chinese hamster ovary (CHO) cells (*aprt*)	+	ND	Yang et al. (1999)
Chinese hamster ovary 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 (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/Oua^{R}	ND	+	Mishra et al. (1978)
DNA damage			
DNA adducts			

Human fibroblast (MRC5CV1) cell line	+	ND	Hanelt et al. (1997)
Human peripheral blood lymphocytes	ND	+	Wienke et al. (1990)
Hamster tracheal cells	ND	+	Roggeband et al. (1994)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Virus transformed Syrian hamster embryo 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)
DNA damage/single strand breaks			
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 (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)
Unscheduled DNA synthesis			
HeLa cells	+	ND	Martin et al. (1978)
Human fibroblasts	+	ND	Agrelo and Amos (1981)
Human fibroblasts	+	–	Robinson and Mitchell (1981)
Human HepG2	[+]		Valentin-Severin et al. (2004)
Hamster Primary embryo cells	ND	+	Casto et al. (1976)
Hamster tracheal cells	ND	+	Roggeband et al. (1994)
Rat Hepatocytes	[+]		Michalopoulos et al. (1978)
Rat tracheal cells	ND	–	Roggeband et al. (1994)
DNA repair			
Human mammary epithelial cells	ND	+	Leadon et al. (1988)
Human skin fibroblasts	ND	+	Milo et al. (1978)
Baby hamster kidney (BHK21/c13) cells	[+]		Feldman et al. (1978)

secondary mouse embryo fibroblasts (C57BL/6) and human lymphocytes	[+]		Shinohara and Cerutti (1977)
Rat/F344 hepatocytes	ND	+	Williams et al. (1982)
Cytogenetic damage			
Chromosomal aberrations			
Human blood cells	ND	+	Salama et al. (2001)
Human WI38 fibroblasts	+	–	Weinstein et al. (1977)
Chinese hamster CHL	+	–	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)
Micronuclei			
Human AHH-1 lymphoblastoid cells	ND	+	Crofton-Sleigh et al. (1993)
Human HepG2 liver cells	ND	+	Wu et al. (2003b)
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)
Sister chromatid exchanges			
Human C-HC-4 and C-HC-20 hepatoma cells	ND	+	Abe et al. (1983a,b)
Human diploid fibroblast (TIG-II) cell line	+	+	Huh et al. (1982)
Human fibroblasts	ND	+	Juhl et al. (1978)
Human blood cells	ND	+	Salama et al. (2001)
Human peripheral blood lymphocytes	ND	+	Rudiger et al. (1976)
Human peripheral blood lymphocytes	ND	+	Craig-Holmes and Shaw (1977)
Human peripheral blood lymphocytes	ND	+	Schoenwald et al. (1977)
Human peripheral blood lymphocytes	ND	+	Wienke et al. (1990)
Human peripheral blood lymphocytes	ND	+	Wienke et al. (1990)

Human peripheral blood lymphocytes	+	–	Tohda et al. (1980)
Human peripheral blood lymphocytes	+	ND	Lo Jacono et al. (1992)
Chinese hamster Don-6 cells	ND	+	Abe et al. (1983a,b)
Chinese hamster V79 lung epithelial cells	+	–	Popescu et al. (1977)
Chinese hamster V79 lung epithelial cells	+	ND	Mane et al. (1990)
Chinese hamster V79 lung epithelial cells	+	ND	Wojciechowski et al. (1981)
Chinese hamster V79 lung epithelial cells	+	ND	Arce et al. (1987)
Chinese hamster V79 lung epithelial cells	ND	+	Kulka et al. (1993)
Chinese hamster ovary (CHO) cells	+	–	de Raat (1979)
Chinese hamster ovary (CHO) cells	+	–	Husgafvel-Pursiainen et al. (1986)
Chinese hamster ovary (CHO) cells	ND	+	Wolff and Takehisa (1977)
Chinese hamster ovary (CHO) cells	ND	+	Pal et al. (1978)
Hamster Chl cells	ND	+	Shimizu et al. (1984)
Rabbit peripheral blood lymphocytes	ND	+	Takehisa and Wolff (1978)
Rat ascites hepatoma AH66-B	ND	+	Abe et al. (1983a,b)
Rat esophageal tumor R1	ND	+	Abe et al. (1983a,b)
Rat hepatocyte (immortalized) cell lines (NRL cl-B, NRL cl-C and ARL)	+	ND	Kulka et al. (1993)
Rat hepatoma (Reuber H4-II-E) cells	ND	+	Dean et al. (1983)
Rat liver cell line ARL18	ND	+	Tong et al. (1981)
Rat pleural mesothelial cells	ND	+	Achard et al. (1987)
Aneuploidy			
Chinese hamster V79-MZ cells	ND	+	Matsuoka et al. (1998)
Cell transformation			
Human BEAS-2B lung cells	+		van Agen et al. (1997)
Human breast epithelial (MCF-10F, MCF-7, T24) cell lines	ND	+	Calaf et al. (1993)
Baby hamster kidney (BHK21/c13) cells	+	ND	Greb et al. (1980)
Golden hamster embyro cells	+	ND	Mager et al. (1977)
Syrian hamster embryo (SHE) cells	ND	+	DiPaolo et al. (1969, 1971)
Syrian hamster embryo cells	ND	+	Dunkel et al. (1981)

Syrian hamster embryo cells	+		LeBoeuf et al. (1996)
Syrian hamster embyro (SHE) cells/focus assay	ND	+	Casto et al. (1977)
Fetal Syrain hamster lung (FSHL) cells	ND	+	Emura et al. (1980, 1987)
Virus infected rat embryo RLV/RE and RAT cells; mouse embryo AKR/Me cells; Syrain hamster embryo cells	+		Heidelberger et al. (1983)
Virus transformed Syrian hamster embryo and mouse C3H10T1/2 cells	ND	+	Arce et al. (1987)
Mouse C3H/10T1/2 embryo fibroblasts	+		Nesnow et al. (2002, 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	+		Heidelberger et al. (1983)
Mouse BALB/c-3T3 cells	ND	+	Dunkel et al. (1981)
Mouse BALB/c-3T3 cells	+		Matthews (1993)
Mouse BALB/c-3T3 clone A31-1-1	ND	+	Little and Vetroys (1988)
Rat embyro cells/SA7 virus transformation	ND	+	DiPaolo and Casto (1976)
Rat/Fischer, embryo cells (leukemia virus transformed)	ND	+	Dunkel et al. (1981)
Rat/Fischer, embryo cells/Oua^{R}	ND	+	Mishra et al. (1978)

Key: [+] = S9 status not given; "+" = positive; "-" = negative; ND = not determined.

1
2

1

Table 4-26. In vivo genotoxicity studies of benzo[a]pyrene

Endpoint	Test System	Test Conditions	Results	Dose	Comment	Reference
Mutations	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)
Mutations	Mouse/strains:T-stock, (SEC×C57BL)F_1, (C3H×101)F_1, (C3H×C57BL)F_1 for females; (101×C3H)F_1 or (C3H×101)F_1 for males; dominant-lethal mutation assay	12-wk old males dosed with benzo[a]pyrene i.p. and mated 3.5-6.5 days post-treatment with 12-wk old females from different stocks; sacrificed on days 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 expt.; fertilized eggs collected from 9-11 hrs after mating and first-cleavage metaphase chromosomes prepared 20 hrs after mating	+	500 mg/kg b.w.	The % of dominant lethal mutations were in the order of T-stock = (C3H×101)F_1 > (SEC×C57BL)F_1> (C3H×C57BL)F_1	Generoso et al. (1979)
Mutations, GC	Mice/strains: Male stocks: (101×C3H)F_1; Female stocks (A): (101xC3H)F1, (B): (C3Hx101)F1, (C): (C3HxC57BL)F1, (D):(SECxC57BL)F1, (E):T-stock females; dominant lethal mutations	In dominant lethal assay (DLA), 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 days post-dosing, or with (#3b) with stocks B, C, D and E females 3.5-6.5 days post-dosing. Control group mated at time corresponding to 1.5-4.5 days post-treatment in	positive for DLA; negative for HT	500 mg/kg b.w.	Dominant lethal effects were observed in early to middle (4.5-5.5 and 6.5-7.5 days post-treatment, respectively) spermatozoa and in preleptotene spermatocytes (32.5-33.5 and 34.5-35.5 days post-treatment). In the HTA, no significant differences observed between treated and control progeny.	Generoso et al. (1982)

		the test groups.				
Mutations, GC	Mice/strains: Male stocks: (101×C3H)F_1; Female stocks (A): (101xC3H)F1, (B): (C3Hx101)F1, (C): (C3HxC57BL)F1, (D):(SECxC57BL)F1, (E):T-stock females; heritable translocations	For heritable translocation assay (HTA), males were mated with stocks B and D females 3.5-7.7 days post-benzo[a]pyrene treatment and male progeny screened for translocation heterozygosity.	–	500 mg/kg b.w.	No significant differences observed between treated and control progeny.	Generoso et al. (1982)
Mutations, GC/ spot test	Mouse/C57BL female × T-strain male; somatic mutation assay	Mice mated for a 5-day period; $10^1/_4$ days 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 (WMVS) and recessive spots (RS).	+	100 and 500 mg/kg b.w.	Induced coat color mosaics represent genetic changes (e.g. point mutations) in somatic cells. WMVS and RS represent melanocyte cell killing and mutagenicity, respectively. Benzo[a]pyrene caused high incidence of RS but did not correlated with WMVS.	Russell (1977)
Mutations	Mouse/*lacZ* transgenic (Muta^{TM} Mouse)	benzo[a]pyrene given orally in corn oil for 5 consecutive days; sacrificed 14 days after last dosing; Eleven organs analyzed for *lacZ* MF	+	125 mg/kg/day	Highest MF observed in colon followed by ileum > forestomach > bone marrow = spleen > glandular stomach > liver = lung>kidney = heart	Hakura et al. (1998)
Mutations	Mouse/C57BL/6J *Dlb-1* congenic; *Dlb-1*locus assay	Animals dosed i) i.p. with vehicle or benzo[a]pyrene 2, 4, or 6 doses at 96 hr intervals; or ii) single dose of benzo[a]pyrene given i.p. or p.o. alone or 96 hours following a single i.p. dosing with 10 µg/kg TCDD	+	40 mg/kg b.w.	benzo[a]pyrene caused a dose-dependent increase in mutant frequency; i.p. route showed higher mutant frequency than p.o. route; induction of mutations were associated with Ah-responsiveness.	Brooks et al. (1999)

Mutations (*Hprt* locus)	Mice/C57BL/6 (*lacz* negative and $XPA^{+/+}$ and $XPA^{-/-}$); T lymphocytes	Gavage in corn oil 3 times/wk for 0, 1, 5, 9, 13 wks; sacrificed 7 wks after last treatment	+	13 mg/kg	Mutation sensitivity: $XPA^{-/-} > XPA^{+/+}$	Bol et al. (1998)
Mutations	Mouse/Cockayne syndrome-deficient *($Csb^{-/-}$);* heterozygous *($Csb^{+/-}$)* and wild type controls *($Csb^{+/+}$); Hprt* mutation frequency assay	$Csb^{-/-}/lacZ^{+/-}$ and $Csb^{+/-}/lacZ^{+/-}$ mice were dosed i.p. with benzo[a]pyrene thrice a wk for 5, 9, or 13 wks; For *Hprt* MF analysis mice were sacrificed 3 wks after last treatment; spleenocytes collected; For *lac Z* MF analysis mice were sacrificed 3 days after last treatment and liver, lung and spleen collected.	+	13 mg/kg	*lac Z* MF detected in all tissues but no differences between WT and $Csb^{-/-}$ mice; *Hprt* mutations significantly higher in $Csb^{-/-}$ mice than control mice. BPDE-dGuo adducts in Hprt gene are preferentially removed in WT mice than $Csb^{-/-}$ mice.	Wijnhoven et al. (2000)
Mutations	Mouse/B6C3F1, forestomach H-*ras*, K-*ras* & *p53* mutations	benzo[a]pyrene given in feed in a 2-year chronic feeding study;	+	5, 25, 100 ppm	68% K-ras (codons 12,13), 10% H-ras (codon 13), 10% p53 mutations; all G-->T transversions	Culp et al. (2000)
Mutations	Mouse/*lacZ/galE* (Muta™ Mouse); Skin painting study	Mice topically treated with a single dose or in five divided doses daily; sacrificed 7 or 21 days after the single or final treatment; DNA from skin, liver and lung analyzed for mutations.	$+^{Sk}_{Li,Lu}$ or –	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; MF for single or multiple-dose regimens were similar.	Dean et al. (1998)
Mutations/ spot test	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 x 2 mg)		Davidson and Dawson (1976)
Mutations (*Hprt* locus)	Mouse, 129/Ola (Wild type); splenic T lymphocytes	Single i.p. injection followed by sacrifice 7 wks post-treatment	+	0, 50, 100, 200, 400 mg/kg	dose-dependent increase in *Hprt* MF	Bol et al. (1998)
Mutations	Mouse, A/J, male	Single i.p. injection followed by sacrifice 28 days post-treatment	+	0, 0.05, 0.5, 5 50 mg/kg	Dose-dependent increase in lung tissue K-ras codon 12 G-->T mutation frequency	Meng et al. (2010)
Mutations/ gene	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	+	600 nmol/mouse	About 90% of papillomas contained Ha-ras mutations, all of them being	Colapietro et al. (1993)

		initiation promoted twice weekly with 5 nmol TPA for 14 wks. One month after stopping TPA application, papillomas collected and DNA from 10 individual papillomas were analyzed for Ha-ras mutations by PCR and direct sequencing.			transversions at codons 12 (20% GGA-->GTA), 13 (50% GGC-->GTC), and 61 (20% CAA--> CTA).	
Mutation/*In vivo-in vitro*	Rat, Wistar	Single dose by gavage; urine and feces collected 0-24, 24-48 and 48-72 hrs post-treatment; urine and extracts of feces tested in *S. typhimurium* TA100 strain with or without S9 mix and β-glucuronidase	+	0, 1, 5, 10, 100 mg/kg	Fecal extracts and urine showed mutagenicity at and above 1 and 10 mg/kg b.w. Benzo[a]pyrene, respectively. Highest mutagenic activity observed for 0-24 hrs post-treatment for feces and 24-48 hrs post-treatment for urine with β-glucuronidase ± S9 mix.	Willems et al (1991)
Mutations, GC /gene	*D. melanogaster*/ sex-linked recessive lethal test	*Basc* males exposed to benzo[a]pyrene were mated with virgin females of *Berlin* K or *mei-9*^{L1}strains;	±	10 mM	Data inconclusive due to low fertility rates of *mei-9*^{L1} females.	Vogel et al. (1983)
Mutations, GC /gene	*D. melanogaster*/ sex-linked recessive lethal test	Adult *Berlin* males treated orally with benzo[a]pyrene	+	5 or 7.5 mM	Low mutagenic activity	Vogel et al. (1983)
Mutations, GC /gene	*D. melanogaster*/Berlin-K and Oregon-K strains; sex-linked recessive lethal test	benzo[a]pyrene dissolved in special fat and injected into the abdomen of flies.	–	2 and 5 mM	Negative at both doses	Zijlstra and Vogel (1984)
Mutations, GC /gene	*D. melanogaster*/ sex-linked recessive lethal test	Male *Berlin K* larvae treated with benzo[a]pyrene for 9-11 days	+	0.1-4 mM	Threefold enhancement in lethals in treated versus controls	Vogel et al. (1983)

Mutations, GC /gene	*D. melanogaster*/Canton-S (WT) males, FM6 (homozygous for an X chromosome) females; sex-linked recessive lethal test	Adult male flies were fed on filters soaked in benzo[a]pyrene for 48 or 72 hrs; Treated and control males mated with FM6 females, males transferred to new groups of females at intervals of 3, 2, 2, and 3 days; four broods obtained; a group of 100 daughters of each male were mated again; scored for % lethal	–	250, 500 ppm	Authors report incomplete dissolution of benzo[a]pyrene in DMSO as a possible cause of negative result.	Valencia and Houtchens (1981)
Mutation/gene	*D. melanogaster;* somatic mutation - eye color mosaicism	50 females and 20 females were mated in a culture bottle for 48 hrs allowing females to oviposit; adults then discarded and the eggs allowed to hatch; larvae fed on benzo[a]pyrene deposited on food surface and the emerging adult males scored for mosaicly colored eye sectors;	+	1, 2, or 3 mM	benzo[a]pyrene was effective as a mutagen; no dose-response observed	Fahmy and Fahmy (1980)
DNA adducts	Human/white blood cells	Workers were exposed for 6-8 hrs/day for at least 4-6 months before blood collection; leukocyte DNA isolated, digested and benzo[a]pyrene tetrols analyzed by HPLC with fluorescent detection (HPLC-FD). Low, medium, and high exposure groups correspond to < 0.15, 0.15 to 4, > 4 mg/m3 of benzo[a]pyrene, respectively.	+	< 0.15, 0.15 to 4, > 4 μg/m^3 of benzo[a]pyrene	PAH exposure, CYP1A1 status and smoking significantly affected DNA adduct levels, i.e. *CYP1A1*(**1/*2* or **2A/*2*a) > *CYP1A1*1/*1*; occupational > environmental exposure; smokers > nonsmokers; adducts increased with dose and duration of smoking	Rojas et al. (2000)
DNA adducts	Human/white blood cells	Coke oven workers were exposed to PAHs and benzo[a]pyrene-WBC DNA analyzed by HPLC-FD for BPDE-DNA adducts	+	0.14 μg/m^3	BPDE-DNA adducts detectable; no significant difference between smokers and nonsmokers; no correlation with air benzo[a]pyrene levels and adduct levels	Mensing et al. (2005)

DNA adducts and Mutations	Mouse/C57BL/6 *lacZ* transgenic	Mice dosed with single i.p. injection of benzo[a]pyrene in DMSO; sacrificed 1, 3, 5, 7, 14, 21, and 28 days post-treatment; spleen, lung, liver, kidney and brain collected, DNA isolated and analyzed for mutations in lacz reporter gene in *E. coli* and adducts by ^{32}P-postlabeling assay.	+	50 mg/kg b.w.	BPDE-dG adduct levels peaked between 5 and 7 days post-treatment, 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 days in lung, spleen, liver and kidney; brain was not significant at any time point.	Boerrigter (1999)
DNA adducts	Mice/ (*Ahr*$^{+/+}$, *Ahr*$^{+/-}$,*Ahr*$^{-/-}$)	Gavage; sacrificed 24 hr post-treatment	+	100 mg/kg b.w.	No induction of CYP in *Ahr*$^{-/-}$, but all alleles positive for adduct formation	Sagredo et al 2006
DNA adducts	Mice/C57BL/6J *Cyp1a1*(+/-) and *Cyp1a1*(-/-)	Single i.p. injection; sacrificed 24 hrs post-treatment; liver DNA analyzed by ^{32}P-postlabeling assay	+	500 mg/kg b.w.	BPDE-DNA adduct levels 4-fold higher in *Cyp1a1*(-/-) mice than *Cyp1a1*(+/-) mice	Uno et al. (2001)
DNA adducts	Mouse/B6C3F1	benzo[a]pyrene fed in diet for 4 (100 ppm) or for 1, 2, 8, 16 and 32 wks (5 ppm); sacrificed and liver, lungs, forestomach, 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)
DNA adducts	Mouse/Balb/c;	Single i.p. injection; sacrificed 12 hrs post-injection; liver and forestomach collected; DNA binding of [^{3}H]benzo[a]pyrene analyzed by scintillation counting.	+	140 μCi/100 g b.w.	Liver DNA had 3-fold higher binding of benzo[a]pyrene than that of forestomach	Gangar et al. (2006)
DNA adducts	Mice/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 pre-treatment had a greater suppressive effect on	Wu et al. (2008)

					adduct formation in BALB relative to CBA mice at low dose but resulted in no significant difference in adduct levels at high dose.	
DNA adducts	Mice/BALB/c, skin;	Four doses of benzo[a]pyrene topically applied to the shaved backs of animals at 0, 6, 30 and 54 hrs; sacrificed 1 day after last treatment; DNA analyzed by ^{32}P-postlabeling assay	+	4 x 1.2 μmol/animal	Five adducts spots detected	Reddy et al . (1984)
DNA adducts	Mice/Swiss, epidermal and dermal skin	Single topical application on shaved backs; sacrificed 1, 3, and 7 days post-treatment; 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 days with a gradual decline in levels	Oueslati et al. (1992)
DNA adducts	Rats/CD, PBLs, lungs and liver	Single i.p. injection; sacrificed 3 days post-treatment; DNA analyzed by Nuclease P1-endhanced ^{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)
DNA adducts	Rats/Sprague-Dawley, liver	Single i.p. injection followed by sacrifice at 4 hours post-treatment; liver DNA isolated and analyzed by ^{32}P-postlabeling assay.	+	100 mg/kg b.w.	Two adduct spots detected	Reddy et al . (1984)
DNA adducts	Rat/Lewis; lung and liver	Animals received a single oral dose of benzo[a]pyrene in tricaprylin; sacrificed 1, 2, 4, 11, and 21 days post-dosing; analyzed liver and lung DNA for BP-DNA adducts by ^{32}P-postlabeling assay and urine for 8-oxodG adducts by HPLC-ECD.	+	10 mg/kg	BPDE-dG levels peaked 2 days after exposure in both tissues, higher in lungs than liver at all time points, decline faster in liver than lung; Increased 8-oxodG levels in urine and decreased levels in liver and lung.	Briedé et al. (2004)

DNA adducts	Rats/F344; ^{32}P-postlabeling assay	benzo[a]pyrene given in the diet for 30, 60, or 90 days; animals sacrificed and liver and lung isolated and DNA extracted and analyzed for adducts.	+	0, 5, 50, 100 mg/kg	Adduct levels linear at low and intermediate doses, nonlinear at high dose;	Ramesh and Knuckels (2006)
DNA adducts	Rats/Wistar; liver and PBL adducts	Single dose by gavage; sacrificed 24 hrs post-dosing; PBL and liver DNA analyzed by ^{32}P-postlabeling for BP-DNA adducts	+	0, 10, 100 mg/kg b.w.	At 100 mg dose total adduct levels in PBL were twofold higher than the levels in liver; adduct profiles differed between PBL and liver	Willems et al (1991)
DNA strand breaks	Rats/Sprague-Dawley; Comet assay	instilled intratracheally with (i) single dose of benzo[a]pyrene in aqueous suspension; sacrificed at 3, 24, 48 hrs post-treatment; alveolar macrophages, lung cells, lymphocytes, hepatocytes collected (ii) dose-response study and sacrificed at 24 hours post-treatment; lungs collected; Controls received normal saline instillation; All cells analyzed by comet assay.	+	Expt#1: 3 mg of benzo[a]pyrene; Expt#2: dose-response study with 0.75, 1.5, 3 mg benzo[a]pyrene	All time points showed significant increase in SSB (Expt#1); A dose-response in SSB observed (Expt#2)	Garry et al. (2003a,b)
DNA strand breaks	Aquatic organisms:Carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and clams (*Spisula sachalinensis*); Comet assay	All organisms acclimatized in tanks for 2 days, 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 post-treatment; 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 conc.	Kim and Hyun (2006)
DNA strand breaks	Rat, Brown Norway	UDS determined after 5 and 18 hrs of a single i.g. dosing	–	62.5 mg/kg	negative at both time points	Mullaart et al. (1989)

Unscheduled DNA synthesis	Rats/F344;	Single i.p. injection of benzo[a]pyrene or DMSO; sacrificed at 2 or 12 hrs post-exposure; liver isolated, hepatocyte cultures were setup and incubated with 10 mCi/ml ^{3}H-thymidine for 4 hrs; washed and autoradiography performed	–	100 mg/kg b.w.	benzo[a]pyrene was negative at both time points	Mirsalis et al. (1982)
Unscheduled DNA synthesis	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 hr post-treatment, skin isolated [^{3}H]thymidine; cultured in ; 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)
Unscheduled DNA synthesis	Rat/Brown Norway; liver	Single intragastric injection; sacrificed at 5 and 18 hours post-injection	–	62.5 mg/kg b.w.	benzo[a]pyrene was negative at both time points	Mullaart et al. (1989)
Unscheduled DNA synthesis	Mouse/(C3Hf x 101)F_1 hybrid, germ cells	i.p. injection of benzo[a]pyrene; [^{3}H]Thymidine injection later	–	0.3 mL	Concentration not specified	Sega (1979)
Unscheduled DNA synthesis	Mouse, early spermatid	i.p. injection	–	250-500 mg/kg b.w.	Reviewed by Sotomayor and Sega (2000)	Sega (1982)
Chromosomal aberrations	Hamster/Chinese bone marrow	Single, i.p. injection of benzo[a]pyrene dissolved in tricapryline; animals sacrificed 24 hours post-exposure	+	25, 50, 100 mg/kg b.w.	benzo[a]pyrene induced CAs at 50 mg/kg/bw only, with negative responses at the low and high dose	Bayer (1979)
Chromosomal aberrations	Mice/C57 (high AHH inducible) and DBA (low AHH inducible) strains; 11-day old embryos; adult bone marrows	Study used 4 matings (female×male): C57×C57; DBA×DBA; C57×DBA; DBA×C57; Pregnant mice treated orally on GD11 with benzo[a]pyrene; sacrificed 15 hrs post-treatment; material liver, bone	+	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	Adler et al. (1989)

		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.			homozygous embryos. No quantitative correlation between BP-induced CAs and AHH inducibility. No differences in bone marrow mitotic index of males of different strains between control and treatment groups.	
Chromosomal aberrations	Mouse/1C3F1 hybrid (101/E1xC31×E1)F_1; CAs in bone marrow	Single dose by gavage; sacrificed 30 hrs of post-dosing; 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)
Chromosomal aberrations	Rats/Wistar; PBLs	Single dose by gavage; sacrificed 6, 24 and 48 hrs post-treatment; blood from abdominal aorta collected, whole blood cultures set up, CAs scored in 100 first-division PBLs per animal	–	0, 10, 100, 200 mg/kg b.w.	No difference between control and treatment groups at any dose or at any sampling time observed	Willems et al (1991)
Micronuclei	Hamster, Chinese, bone marrow	Single, i.p. injection of benzo[a]pyrene dissolved in tricaprylin; animals sacrificed 30 hours post-exposure	–	100, 300, 500 mg/kg b.w.		Bayer (1979)
Micronuclei	Mice/B6C3F1 (hybrid);	I.p. injection; several doses given to calculate LD_{50}	+	232 mg/kg ($LD_{50/7}$); 259 mg/kg ($LD_{50/4}$)	Study conducted to determine the toxicity of benzo[a]pyrene (LD_{50})	Salamone et al. (1981)
Micronuclei	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, 1000, 2000 mg/kg b.w.	significant increase at all doses; no dose-response; double dosing at 500 mg/kg dose gave best response	Shimada et al. (1990)
Micronuclei	Mouse/CD-1 & 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, 500 mg/kg b.w.	maximum response seen at 48 hrs post-treatment	Shimada et al. (1992)
Micronuclei	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, 1000 mg/kg b.w.	maximum response seen at 72 hrs post-treatment	Shimada et al. (1992)

Micronuclei	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 b.w.	All groups significantly higher than controls for MN; Fetal liver more sensitive than any other group	Harper et al. (1989)
Micronuclei	Mouse/Swiss albino; bone marrow	Given orally in corn oil; sacrificed 24 hr post-exposure	+	75 mg/kg b.w.		Koraktar et al. (1993)
Micronuclei	Mouse/Swiss; bone marrow PCE	Given by gavage and sacrificed 36 hrs post-treatment	+	75 mg/kg b.w.		Rao and Nandan (1990)
Micronuclei	Mice/CD-1 and MS/Ae strains	i.p. and p.o. administration	+	62.5, 125, 250, 500 mg/kg	good dose response by both routes, strains; i.p. better than p.o.; MS/Ae strain more sensitive than CD-1 strain	Awogi and Sato (1989)
Micronuclei	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 post-treatment; bone marrow smears prepared, stained with May-Grunwald-Giemsa technique and scored for MN PCEs.	+	0, 25, 50, 60 mg/kg b.w.	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 post-injection	Balansky et al. (1994)
Micronuclei	Mouse, HRA/Skh hairless, keratinocytes	Single topical application	+	0.5, 5, 50, 100, 500 mg/mouse		He and Baker (1991)
Micronuclei	Mouse/HOS:HR-1, hairless; skin micronuclei	Topical application once daily for 3 days; sacrificed 24 hrs after last treatment	+	0.4, 1, 2, 4 mg		Nishikawa et al. (2005)
Micronuclei	Mice/HR-1 hairless, skin (benzo[a]pyrene with slight radiation)		+		Exposure to sunlight simulator to evaluate photogenotoxicity and chemical exposure	Hara et al. (2007)
Micronuclei	Rat, Sprague-Dawley, pulmonary alveolar macrophages	i.t. instillation, once/day for 3 days	+	25 mg/kg b.w.		De Flora et al. (1991)

Micronuclei	Rat, Sprague-Dawley, bone marrow cells	i.t. instillation, once/day for 3 days	–	25 mg/kg b.w.		De Flora et al. (1991)
Micronuclei	Fish (Carp, rainbow trout, clams); Blood and hemolymph		+	0.05, 0.25, 0.5 and 1 ppm		Kim and Hyun (2006)
Sister chromatid exchanges	Hamster/Chinese; 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 b.w.	Significant increase in metaphase SCEs in benzo[a]pyrene-treated animals compared to vehicle-treated controls.	Roszinsky-Kocher et al. (1979)
Sister chromatid exchanges	Hamster/Chinese,	Animals implanted s.c. with bromodeoxyuridine (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 b.w., sacrificed 2 hrs later; bone marrow from femur prepared for SCE assay	+	50 or 100 mg/kg b.w.	SCEs increased with low dose of phorone significantly.	Bayer et al. (1981)
Sister chromatid exchanges	Hamster/Syrian, fetal liver	i.p. injection to pregnant animals on GDs 11, 13 or 15; fetal liver SCEs were analyzed	+	50 and 125 mg/kg b.w.	Produced doubling of SCE frequency	Pereira et al. (1982)
Sister chromatid exchanges	Hamster/Chinese, bone marrow	NA	+	2.5, 25, 40, 50, 75, 100 mg/kd b.w.	Frequency of SCEs increased ≥40 mg/kg b.w.	Bayer (1979)
Sister chromatid exchanges	Mouse/DBA/2 & C57BL/6, bone marrow cells	Two intragastric injections given; mice implanted with BrdU tablets, sacrificed on day 5, SCE estimated	+	10 or 100 mg/kg b.w.	SCEs and BP-DNA adducts in the order of C57Bl/6 (AHH-inducible) < DBA/2 (AHH-noninducible)	Wielgosz et al. (1991)
Sister chromatid exchanges	Mouse/DBA/2 & C57BL/6, spleenic lymphocytes	Two intragastric injections given; mice killed on 5th day and cells cultured for 48 hrs with BrdU.	+	10 or 100 mg/kg b.w.	SCEs and BP-DNA adducts in the order of C57Bl/6 (AHH-inducible) < DBA/2 (AHH-noninducible)	Wielgosz et al. (1991)

Sister chromatid exchanges	Rats/Wistar; PBLs	Single dose by gavage; sacrificed 6, 24 and 48 hrs post-treatement; blood from abdominal aorta collected, whole blood cultures set up, SCEs scored in 50 second-division metaphases in PBLs per animal	+	0, 10, 100, 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)
Cell transformation	Hamsters/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 days post-culture trypsinized; subcultured every 4-6 days thereafter and scored for plating efficiency and transformation.	+	3 mg/100 g b.w.		Quarles et al. (1979)

8-oxodG, 8-oxodeoxyguanosine; AHH, aryl hydrocarbon hydroxylase; benzo[a]pyrene, benzo[a]pyrene; BPDE, benzo[a]pyrene diol epoxide; BrdU, bromodeoxyuridine; CAs, chromosomal aberrations; CSB, Cockayne syndrome; CYP, cytochrome P450; DLA, dominant lethal assay; DMSO, dimethylsulfoxide; ECD, electrochemical detection; FD, fluorescence detection; FM6, First Multiple No. 6 is an X chromosome with a complex of inversions (to suppress cross-over) and visible markers such as yellow body, white eyes and narrow eyes. GC, germline cell; GD, gestational day; HPLC, high performance liquid chromatography; HPRT, hypoxanthine-guanine phosphoribosyl transferase; HTA, heritable translocation assay; i.p., intraperitoneal; i.t., intratracheal; Li, Liver; Lu, Lung; MF, mutation frequency; PBL, peripheral blood lympbocytes; PCE, polychromatic erythrocytes; SCEs, sister chromatid exchanges; SFS, synchronous fluorescence spectrometry; Sk Skin TCDD, 2,3,7,8-tetrachlorodibenzodioxin; TPA, 12-tetradecanoyl-*O*-phorbol acetate; UDS, unscheduled DNA synthesis; USERIA, ultrasensitive enzyme radioimmunoassay; WT, wild-type; XP, xeroderma pigmentosum;

4.5.2. Metabolic pathways

Diol epoxide pathway

benzo[a]pyrene diol epoxide metabolites interact preferentially with the exocyclic amino groups of deoxyguanine and deoxyadenine (Geacintov et al., 1997; Jerina et al., 1991). Adducts may give rise to mutations unless these adducts are removed by DNA repair processes prior to replication. The stereochemical nature of the diol epoxide metabolite (i.e., anti- vs. syn-diol epoxides) affects the number and type of adducts and mutation that occurs (Geacintov et al., 1997). Transversion mutations (e.g., GC→TA or AT→TA) are the most common type of mutation found in mammalian cells following diol epoxide exposure (Bostrom et al., 2002).

Strong evidence for the association between benzo[a]pyrene activation by the diol epoxide pathway and key DNA-reactive and mutational events associated with tumor initiation comes from the following observations: (1) (+)-anti-BPDE is very reactive with guanine residues in DNA (Koreeda et al., 1978; Jeffrey et al., 1976); (2) (+)-anti-BPDE is more potent than benzo[a]pyrene, benzo[a]pyrene phenols, and benzo[a]pyrene diols in mutagenicity assays in bacterial and mammalian cells (Malaveille et al., 1977; Newbold and Brookes, 1976); (3) When administered by ip injection to newborn mice, (+)-anti-BPDE is more potent than benzo[a]pyrene phenols and benzo[a]pyrene diols and much more potent than benzo[a]pyrene itself in lung tumorigenicity assays (Chang et al., 1987; Buening et al., 1978; Kapitulnik et al., 1978); (4) (+)-anti-BPDE treatment resulted in *ras* gene codon 12 G→T point mutations, the activation of the H-ras-1 proto-oncogene and transformation of NIH/3T3 cells (Marshall et al., 1984); (5) (+)-anti-BPDE forms DNA adducts at specific “hotspots” in the p53 tumor suppressor gene that are commonly mutated in lung and other cancer patients (Denissenko et al., 1996; Puisieux et al., 1991); (6) lung tumors from nonsmoking patients who were chronically exposed to smoky coal emissions contained mutated p53 and showed a spectrum of mutations consistent with (+)-anti-BPDE-associated mutations in the K-ras oncogene (DeMarini et al., 2001); (7) elevated blood BPDE-DNA adducts have been observed in coke oven workers and chimney sweeps, occupations associated with increased risks of cancer from PAH-containing complex mixtures (Pavanello et al., 1999); (8) the spectrum of mutation in the K-ras, H-ras, and p53 genes in forestomach tumors of mice fed benzo[a]pyrene in the diet for 2 years was consistent with (+)-anti-BPDE DNA reactions (Culp et al., 2000); (9) K-ras mutations found in lung tumors from A/J mice given single i.p. injections of benzo[a]pyrene showed several guanine mutations at codon 12, which are indicative of (+)-anti-BPDE DNA adduct formation (Ross and Nesnow, 1999; Nesnow et al., 1998a, b, 1996, 1995; Mass et al., 1993); and (10) the major DNA adduct formed in a murine embryonic fibroblast line transfected with human p53 DNA and exposed to 1uM benzo[a]pyrene for 96 hours was (+)-anti-BPDE-DNA. The concomitant spectrum of p53 mutations in the latter study had features similar to those found in human lung cancer:

predominance of G→T mutations, strand bias of transversions, and mutation hot spots at codons 157 to 158 (Liu et al., 2005).

As pointed out by Penning et al. (1999), the association between BPDE-DNA adducts and tumors from benzo[a]pyrene exposure is not entirely specific given that dihydrodiol and diol epoxides of benzo[a]pyrene are less potent tumorigenic agents in mouse skin than the parent material (Slaga et al., 1977; Chouroulinkov et al., 1976) and oxidative damage to DNA has been observed in rats treated with benzo[a]pyrene (Kim and Lee, 1997) and human mammary epithelial cells exposed to benzo[a]pyrene (Leadon et al., 1988). In addition, although BPDE-DNA adduct levels in forestomach tissue were linearly related to the amount of benzo[a]pyrene consumed by mice in a 28-day study (Culp et al., 2000, 1998, 1996a; Culp and Beland, 1994), levels of BPDE-DNA adduct in lung and liver tissue (which did not develop tumors with 2 years of exposure to benzo[a]pyrene in the diet) were similar at 28 days to those in forestomach tumors (Goldstein et al., 1998). These observations suggest that BPDE-adduct levels alone are not the only path to benzo[a]pyrene-induced tumors and provide indirect evidence for the other mutagenic pathways to benzo[a]pyrene tumor initiation.

Radical cation pathway

Radical cation formation involves a one-electron oxidation by CYP or peroxidase enzymes (i.e., horseradish peroxidase, prostaglandin H synthetase) that produces electrophilic radical cation intermediates (Cavalieri and Rogan, 1995, 1992). Radical cations can be further metabolized to phenols and quinones (Cavalieri et al., 1988d, e), or they can form unstable adducts with DNA that ultimately result in depurination (Cavalieri et al., 2005, 1993; Rogan et al., 1993). The predominant depurinating adducts occur at the N-3 and N-7 positions of adenine and the C-8 and N-7 positions of guanine (Cavalieri and Rogan, 1995).

Abasic sites resulting from base depurination undergo error-prone excision repair and can induce mutations such as those found in the H-ras oncogene in mouse skin (Chakravarti et al., 2000). One pathway to the formation of depurinating DNA adducts involves the formation of DNA-reactive radical cations from benzo[a]pyrene via CYP peroxidases (Cavalieri and Rogan, 1995). In mouse skin exposed to 200 nmol benzo[a]pyrene for 4 hours, a mix of stable and depurinating DNA adducts was found: (+)-anti-BPDE DNA and depurinating adducts accounted for 22 and 74% of the identified adducts, respectively (Rogan et al., 1993). When mouse skin was exposed to either the benzo[a]pyrene-7,8-diol or BPDE, only stable BPDE-DNA adducts were found (Rogan et al., 1993). In mouse skin tumors induced by benzo[a]pyrene and promoted by TPA, 7/13 examined tumors had H-ras oncogene mutations attributed to apurinic sites generated by loss of N-7 and C-8 guanine adducts, and 2/13 tumors had H-ras mutations attributed to loss of N-7 adenine adducts (Chakravarti et al., 1995). Results from the Rogan et al. (1993) and Chakravarti (1995) studies provide strong in vivo evidence for the importance of both

the diol epoxide and the radical cation pathways in the activation of benzo[a]pyrene to initiate mouse skin tumors, possibly by inducing mutations in critical genes.

o-Quinone/ROS pathway

The o-Quinone metabolites of PAHs are formed by enzymatic dehydrogenation of dihydrodiols (Bolton et al., 2000; Penning et al., 1999; Harvey, 1996; ATSDR, 1995). DHH enzymes are members of the α-keto reductase gene superfamily. o-Quinone metabolites are potent cytotoxins, are weakly mutagenic, and are capable of producing a broad spectrum of DNA damage. These metabolites can interact directly with DNA as well as result in the production of ROS (i.e., hydroxyl and superoxide radicals) that may produce further cytotoxicity and DNA damage. The DNA damage caused by o-quinones may include the formation of stable adducts (Balu et al., 2004), N-7 depurinating adducts (McCoull et al., 1999), oxidative base damage (i.e., 8-oxo-2'-deoxyguanosine or 8-oxo-dG) (Park et al., 2006a), and strand scission (Flowers et al., 1997). The ROS generated by the o-quinone metabolites of benzo[a]pyrene and other PAHs have been shown to induce mutation in the p53 tumor suppressor gene using an in vitro yeast reporter gene assay (Park et al., 2008; Shen et al., 2006; Yu et al., 2002).

The o-quinone/ROS pathway also can produce depurinated DNA adducts from benzo[a]pyrene metabolites (Jiang et al., 2007; 2005). In this pathway, and in the presence of $NAD(P)^+$, AKR oxidizes benzo[a]pyrene-7,8-diol to a ketol, which subsequently forms benzo[a]pyrene-7-8-dione. This and other PAH o-quinones react with DNA to form unstable, depurinating DNA adducts. In the presence of cellular reducing equivalents, o-quinones can also activate redox cycles which produce DNA-ROS (Penning et al., 1996). DNA damage in *in vitro* systems following exposure to benzo[a]pyrene-7,8-dione or other o-quinone PAH derivatives occurs through the AKR pathway and can involve the formation of stable DNA adducts (Balu et al., 2004), N-7 depurinated DNA adducts (McCoull et al., 1999), DNA damage from ROS (8-oxo-dG) (Park et al., 2006a) and strand scission (Flowers et al., 1997, 1996). Benzo[a]pyrene-7,8-dione and other PAH o-quinones have been shown to induce mutations in the p53 tumor suppressor gene using an in vitro yeast reporter gene assay (Park et al., 2008; Shen et al., 2006; Yu et al., 2002). When the yeast were exposed to varying concentration of benzo[a]pyrene-7,8-dione or (+)-anti-BPDE, levels of 8-oxo-dG or (+)-anti-BPDE-DNA, adducts were linearly related to p53 mutagenic frequencies with similar slopes, suggesting that these two types of DNA lesions were equipotent in producing p53 mutations in this system (Park et al., 2008). When the p53 mutations were sorted into dominant and recessive mutants, the dominant mutations clustered to p53 mutation hotspots observed in human lung cancer tissue (Park, 2008). The combined results provide strong in vitro evidence for the potential for *the o-Quinone/ROS pathway* to produce several DNA-damaging products from benzo[a]pyrene (e.g., benzo[a]pyrene-7,8-dione and ROS) that lead to p53 mutations associated with human lung cancer. In support of the operation of this pathway, and the other bioactivation pathways, in

humans, Jiang et al. (2007) used liquid chromatograpy-mass spectrometry (LC-MS) to provide evidence for the formation of radical cations, diol epoxides, and o-quinones in cultured human lung H358 cells following exposure to 4 μM [^{3}H]-benzo[a]pyrene.

4.5.3. Mechanistic Studies- Mutagenesis and Tumor Initiation

Oncogene/tumor suppressor gene mutations (in vivo)

DeMarini et al. (2001) demonstrated mutations in the p53 tumor suppressor gene and the K-ras oncogene in the lung tumors of nonsmokers, whose tumors were associated with exposure to smoky coal. Lung tumors were obtained from 24 nonsmoking women from China (age 30–63, mean age 48.5 ± 8.8 years) who used smoky coal in their homes without chimneys. Bronchioloalveolar adenocarcinoma and acinar adenocarcinoma were observed in 54 and 46% of the women studied, respectively. The observed mutations in lung tumors were primarily G→T transversions at either K-ras or p53. Mutation hotspots in the lung tumors examined corresponded with hot spots for PAH adducts (codon 154), cigarette smoke associated mutations (codon 249), and both of these events together (codon 273). The mutation spectrum was described as unique and consistent with exposure to PAHs in the absence of cigarette smoke.

Mutations in the K-ras, H-ras, and p53 genes were assessed in forestomach tumors (n = 31) of mice fed benzo[a]pyrene in the diet (0, 5, 25, or 100 ppm) for 2 years (Culp et al., 2000). Forestomach tumors had K-ras mutations (68% of tumors) that were G→T or C transversions in codon 12 or 13. H-ras (codon 13) and p53 mutations characterized as G→T or C transversions were also each found in 10% of forestomach tumors.

K-ras mutations were observed in A/J mouse lung tumors (Nesnow et al., 1998a, b, 1996, 1995; Mass et al., 1993). Benzo[a]pyrene was administered to male A/J mice (20/group) as a single i.p. injection (0, 10, 50, 100, or 200 mg/kg in tricaprylin) and the presence of lung adenomas were evaluated 8 months following injection. The number of lung adenomas/mouse was significantly greater than control ($p < 0.05$) for benzo[a]pyrene doses ≥50 mg/kg. Lung tumor DNA was isolated and DNA sequence analysis of K-ras mutations was performed for 19 separate lung tumors. The DNA sequence analysis demonstrated several guanine mutations at codon 12, including GGT→TGT (56% of tumors), GGT→GTT (25% of tumors), and GGT→GAT (19% of tumors).

H-ras mutations were studied in skin papillomas of SENCAR mice resulting from dermal initiation by benzo[a]pyrene or benzo[a]pyrene-7,8-dihydrodiol (400 nmol) followed by TPA promotion (Chakravarti et al., 2000, 1995). PCR amplification of the H-ras gene and sequencing revealed that codon 13 (GGC to GTC) and codon 61 (CAA to CTA) mutations in papillomas corresponded to the relative levels of depurinating adducts of guanine and adenine, despite the formation of significant amounts of stable DNA adducts.

DNA adducts in target tissues detected following chemical exposure

DNA adducts of benzo[a]pyrene have been measured in target tissues of humans exposed to PAH mixtures and experimental animals exposed to benzo[a]pyrene. Phillips et al. (2002) provided a review of smoking-related DNA adducts detected in human respiratory tract tissues. BPDE-DNA adducts were detected in the lung, parenchyma of cigarette smokers with lung cancer (Godschalk et al., 2002; Bartsch et al., 1999; Alexandrov et al., 1992). DNA was isolated from the normal tissue (i.e., noncancerous) of the lung which was obtained during surgery. A study using lung samples obtained on autopsy revealed that the average level of BPDE-DNA adducts was higher in smokers (4.46 ± 5.76 per 10^8 bases) compared to ex-smokers (4.04 ± 2.37 per 10^8 bases) and nonsmokers (1.76 ± 1.69 per 10^8 bases) (Lodovici et al., 1998).

BPDE-DNA adducts were measured in skin biopsies of eczema patients treated with coal tar preparations (Godschalk et al., 2001). Godschalk et al. (1998a) performed a study examining the level of DNA adducts in biopsies of treated skin and in WBCs in psoriasis patients being treated with coal tar. Urinary 1-OH-Py levels were serially monitored in all subjects. Skin biopsies were taken before and after five treatments, at which point the average number of DNA adduct levels increased from 2.9 to 63.3 per 10^8 nucleotides. Total WBC DNA adducts increased from 0.33 to 0.89 per 10^8 after five treatments, and then doubled to 1.59 per 10^8 when sampled 1 week later. There was an increase in 1-OH-Py levels from 0.75 to 186 μg/L after one treatment and to 266 μg/L after five treatments. One week later, mean 1-OH-Py levels were reduced to 2.4 μg/L. Adduct levels in the skin increased over 20-fold with five treatments, while WBC adduct levels approximately doubled over the same period.

DNA adduct levels were examined in the forestomach of groups of female $B6C3F_1$ mice fed benzo[a]pyrene in the diet at concentrations of 5, 25, or 100 ppm for 28 days (Culp et al., 2000, 1998, 1996a, b; Culp and Beland, 1994). [^{32}P]-postlabeling of forestomach DNA of benzo[a]pyrene-treated mice revealed one major adduct characterized as dG-N^2-BPDE. There was a linear relationship between the amount of benzo[a]pyrene consumed and the concentration of dG-N^2-BPDE in the forestomach of mice. For benzo[a]pyrene, forestomach tumor incidence increased sharply with adduct concentrations between 50 and 140 fmol/mg DNA and in coal-tar fed mice. Tumor incidence increased sharply with dG-N^2-BPDE adduct levels between 20 and 60 fmol/mg DNA. The same levels of adduct were present in lung and liver of benzo[a]pyrene-treated mice, although only the forestomach exhibited benzo[a]pyrene-induced tumors (Goldstein et al., 1998). The presence of adducts in tumor-free tissue suggests that DNA adduct levels alone are not necessarily predictors of tumor outcome.

DNA adducts were identified and quantified in experiments using the A/J mouse lung model which results in lung adenomas in male A/J mice 8 months following a single i.p. injection (Nesnow et al., 1998a, b, 1996, 1995; Ross et al., 1995). Benzo[a]pyrene was administered to male A/J mice (20-25/group) as a single i.p. injection (0, 20, 50, or 100 mg/kg in

tricaprylin) and DNA was isolated from lung tissues at several time points between 1 and 21 days following injection. The primary DNA adduct identified in mouse lung tumors was a benzo[a]pyrene bay region diol epoxide adduct of guanine (7R, 8S, 9S-trihydroxy-10R-[2N-2'-deoxyguanosyl]-7, 8, 9, 10-tetrahydro-benzo[a]pyrene). Two minor adducts were also observed to result from the metabolism of 9-hydroxy-benzo[a]pyrene and trans-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene. Quantitative analysis of DNA adducts by [^{32}P]-postlabeling illustrated the importance of measuring DNA adduct levels over time. Total DNA adducts accumulated rapidly between 3 and 9 days after exposure followed by a gradual decrease. A time-integrated DNA adduct level (TIDAL) was linearly related to the administered dose of benzo[a]pyrene (Ross et al., 1995).

Following i.p injection, benzo[a]pyrene also induced DNA adducts in the lungs, liver, and peripheral blood lymphocytes of rats (Ross et al., 1991, 1990), the liver of Lewis rats (Godschalk et al., 1998b), and lungs of BALB/c mice (Van Schooten et al., 1991). Qian et al. (1998) treated male CD rats with intratracheal instillation of fume condensates of roofing asphalts and evaluated adducts in lung cells and WBCs. Adducts were seen in the lungs but not in WBCs, leading the authors to conclude that WBCs may not be a suitable surrogate for lung cells. The adducts were not characterized, so the benzo[a]pyrene-specificity of the results cannot be evaluated. Formation of DNA adducts from benzo[a]pyrene metabolites also has been observed in the lung and liver of male Sprague-Dawley rats after intratracheal administration of benzo[a]pyrene (De Flora et al., 1991; Weyand and Bevan, 1987).

DNA adducts have been reported in the lung and skin of dermally treated SENCAR mice (Mukhtar et al., 1986), in the epidermis of Swiss mice (Oueslati et al., 1992), and in the skin of an unspecified strain of mice (Ingram et al., 2000). When Talaska et al. (1996) compared the dose-duration-response of benzo[a]pyrene-induced adducts in the skin, lung, and liver of Hsd (ICR) BR mice treated dermally with 10, 25, or 50 nmol benzo[a]pyrene, accumulation of adducts was found to be linear with dose in the skin and lung. In skin painting studies with female SENCAR mice and various PAHs, Melendez-Colon et al. (1999) found that carcinogenic potency correlated with DNA adduct levels in epidermal DNA rather than in the formation of apurinic sites. Alexandrov and Rojas-Moreno (1990) found DNA adducts in epidermal keratinocytes and dermal fibroblasts of Swiss mice treated dermally with benzo[a]pyrene but not in similarly treated Wistar rats. BPDE-DNA adducts were measured in the lung, stomach, and skin of male Lewis rats (15/group) following a single exposure to 10 mg/kg benzo[a]pyrene via the intratracheal, gavage, and dermal routes, respectively (Godschalk et al., 2000).

4.5.3. Tumor Promotion and Progression

benzo[a]pyrene has been shown to promote the growth of previously initiated cells, resulting in the formation of tumors in the skin (see Section 4.2.3.1). The tumor promotion

properties of benzo[a]pyrene may be due to a compensatory response to cytotoxicity or via an AhR-mediated effect on cell growth and differentiation.

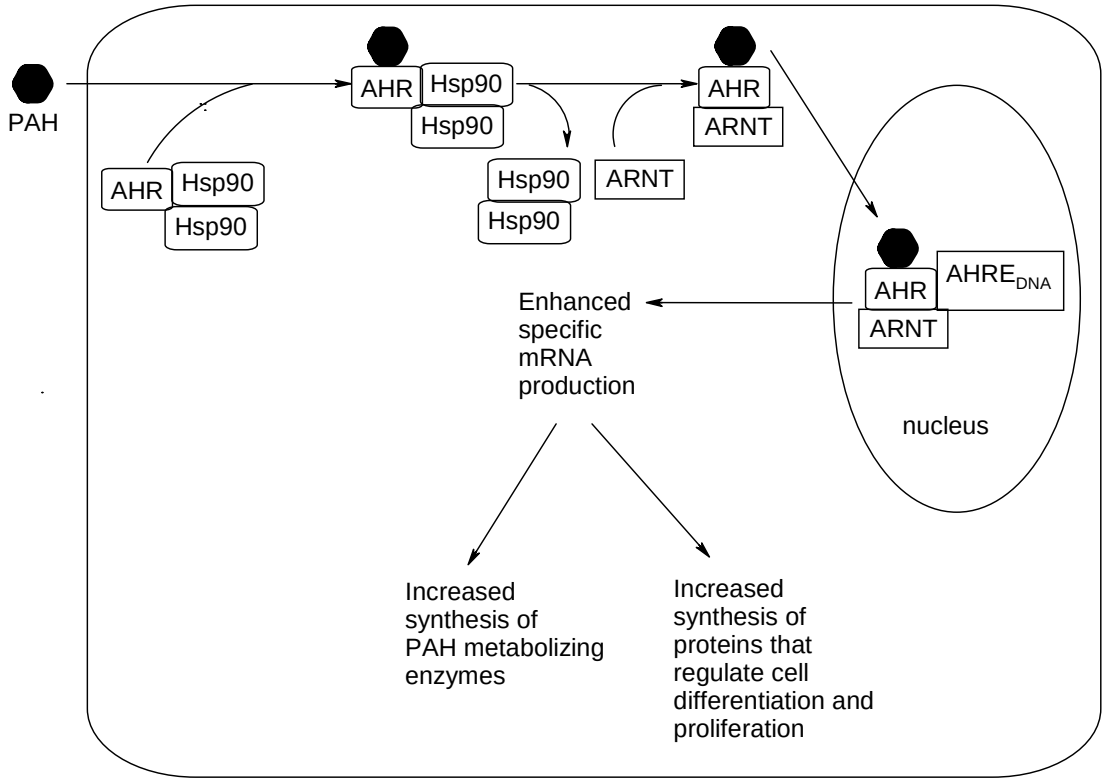
Cytotoxicity and inflammatory response

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

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

AhR-mediated effects

The promotional effects of benzo[a]pyrene may also be related to AhR affinity and the upregulation of genes related to biotransformation (i.e., induction of CYP1A1), growth, and differentiation (Bostrom et al., 2002). Figure 4-1 illustrates the function of the AhR and depicts the genes regulated by this receptor as belonging to two major functional groups (i.e., induction of metabolism or regulation cell differentiation and proliferation). PAHs bind to the cytosolic AhR in complex with heat shock protein 90 (Hsp90). The ligand-bound receptor is then transported to nucleus in complex with the ARNT. The AhR complex interacts with the Ah responsive elements (AHRE) of the DNA to increase the transcription of proteins associated with induction of metabolism and regulation of cell differentiation and proliferation.



AHRE$_{DNA}$ = Ah-responsive elements of DNA; Hsp90 = heat shock protein 90

Source: Okey et al. (1994).

Figure 4-1. Interaction of PAHs with the AhR.

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

90% in AhR knock out mice compared with WT C57Bl6/J mice following a single dermal application of 33 mg/kg benzo[a]pyrene for 24 hours. Ma and Lu (2007) further noted that Ah-nonresponsive mice were at greater risk of toxicity and tumorigenicity in remote organs, distant from the site of exposure (i.e., bone marrow). As an example, Uno et al. (2006) showed that benzo[a]pyrene (125 mg/kg/-day, p.o. for 18 days) caused marked wasting, immunosuppression, and bone marrow hypocellularity in CYP1A1 knock out mice, but not in WT mice.

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

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

Dertinger et al. (2001, 2000) demonstrated that AhR signaling may play a role in cytogenetic damage caused by benzo[a]pyrene. The in vivo formation of micronuclei in

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

Several in vitro studies have suggested that the AhR plays a role in the disruption of cell cycle control, possibly leading to cell proliferation and tumor promotion following exposure to benzo[a]pyrene (Andrysik et al., 2007; Chung et al., 2007; Chen et al., 2003). Chung et al. (2007) showed that benzo[a]pyrene-induced cytotoxicity and apoptosis in mouse hepatoma (Hepa1c1c7) cells occurred through a p53 and caspace-dependent process requiring the AhR. An accumulation of cells in the S-phase of the cell cycle (i.e., DNA synthesis and replication) was also observed, suggesting that this process may be related to cell proliferation. Chen et al. (2003) also demonstrated the importance of the AhR in benzo[a]pyrene-7,8-dihydrodiol- and BPDE-induced apoptosis in human HepG2 cells. Both the dihydrodiol and BPDE affected Bcl2 (a member of a family of apoptosis suppressors) and activated caspase and p38 mitogen-activated protein (MAP) kinases, both enzymes that promote apoptosis. When the experiments were conducted in a cell line that does not contain ARNT (see Figure 4-1), the dihydrodiol was not able to initiate apoptotic event sequences, indicating that activation to BPDE by CYP1A1 was required. BPDE did not induce apoptosis-related events in a p38-defective cell line, illustrating the importance of MAP kinases in this process. In rat liver epithelial cells (WB-F344 cells), in vitro exposure to benzo[a]pyrene resulted in apoptosis, a decrease in cell number, an increase in the percentage of cells in S-phase (comparable to a proliferating population of WB-F334 cells), and increased expression of cell cycle proteins (e.g., cyclin A) (Andrysik et al., 2007). Benzo[a]pyrene-induced apoptosis was attenuated in cells transfected with a dominant-negative mutation of the AhR.

Inhibition of gap junctional intercellular communication

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

Blaha et al. (2002) surveyed the potency of 35 PAHs, including benzo[a]pyrene, to inhibit gap junctional intercellular communication (GJIC). The scrape loading/dye transfer assay was employed using a rat liver epithelial cell line that was incubated in vitro for 15, 30, or 60 minutes with 50 μM benzo[a]pyrene. After incubation, cells were washed, and then a line was

scraped through the cells with a surgical blade. Cells were exposed to the fluorescent dye lucifer yellow for 4 minutes and then fixed with formalin. Spread of the dye from the scrape line into cells remote from the scrape was estimated under a fluorescence microscope. Benzo[a]pyrene reduced spread of the dye after 30 minutes of exposure (approximately 50% of control). Recovery of GJIC was observed 60 minutes after exposure.

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

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Numerous epidemiological studies are available which investigate associations between PAH (including benzo[a]pyrene) dietary intake and cancer incidence; however, no studies were found which evaluate the contribution of benzo[a]pyrene through dietary exposure in humans and noncancer health effects. Several studies in animal models are available evaluating the sensitive noncancer effects following subchronic or chronic exposure to benzo[a]pyrene. The types of effects observed following oral exposure were predominantly effects in the reproductive and immune systems. Additionally, some minor hematological effects and kidney and forestomach effects were observed. Studies that identified NOAELs and LOAELS for noncancer effects in animals repeatedly exposed to benzo[a]pyrene by the oral route are summarized in Table 4-27.

Table 4-27. NOAELS and LOAELs for noncancer effects in animals repeatedly exposed to benzo[a]pyrene by the oral route

Species/sex	Dose	Duration	NOAEL mg/kg-d	LOAEL mg/kg-d	Response at LOAEL	Comments	Reference
Wistar rat/ male and female	0, 3, 10, or 30 mg/kg-d, gavage, 5 d/wk	2 yrs	ND	3	↑ Forestomach hyperplasia, ↑ liver clear cell foci of alteration	Hematological and organ weight variables were not measured at terminal sacrifice. No exposure-related changes in noncancer histology in oral cavity, esophagus, forestomach, jejunum, liver, kidney, skin, mammary gland, or auditory canal (noncancer lesions were only detected in tissues that developed tumors). ↑ Forestomach tumors in males at ≥3 mg/kg-d. ↑ Forestomach, liver, and kidney (males only) tumors at 10 and 30 mg/kg-d.	Kroese et al., 2001
$B6C3F_1$ mouse/female only	Estimated doses: 0, 0.7, 3.3, or 16.5 mg/kg-d in diet	2 yrs	ND 0.7 3.3	0.7 3.3 16.5	↑ Forestomach hyperplasia ↑ Forestomach hyperkeratosis ↑ Esophagus (basal cell hyperplasia)	No exposure-related changes in weight or histology of liver, kidney, or lung weight (other organs not measured). Hematologic variables were not examined. ↑ Forestomach tumors at 3.3 and 16.5 mg/kg-d; ↑ Esophagus and tongue tumors at 16.5 mg/kg-d.	Beland and Culp, 1998; Culp et al., 1998
Wistar rat/ male and female	0, 3, 10, or 30 mg/kg-d, 5 d/wk	90 d	10 3	30 10	↓ Thymus weight, ↑ liver weight, ↑ forestomach hyperplasia, ↑ slight thymic atrophy ↑ Forestomach epithelial cell proliferation index (BrdU incorporation)	No exposure-related changes in hematological variables or histology of lung, spleen, or lymph node.	Kroese et al., 2001
F344 rat/ male and female	0, 5, 50, or 100 mg/kg-d in diet	90 d	ND 5 50	5 50 100	↑ renal tubular casts in males ↓ RBCs and hematocrit in males ↓ RBCs and hematocrit in females, ↓ hemoglobin in both sexes, ↑liver:body weight ratio in males	No exposure-related changes in other organ weights measured (stomach, testes, ovaries), or in histology of stomach, liver, testes or ovaries (other tissues were not examined).	Knuckles et al., 2001

Table 4-27. NOAELS and LOAELs for noncancer effects in animals repeatedly exposed to benzo[a]pyrene by the oral route

Species/sex	Dose	Duration	NOAEL	LOAEL	Response at LOAEL	Comments	Reference
			mg/kg-d				
SD male rats	0, 1 or 5 mg/kg-day by gavage	90 d	1	5	↓ testicular testosterone	Testosterone levels measured in animals sacrificed after 30 d, were not statistically different than controls	Zheng et al., 2010
SD female rats	0, 2.5 or 5 mg/kg-day, gavage[1]	60 d	ND 2.5	2.5 5	↓ ovary weight ↓ estrogen and primordial follicles; altered estrous cyclicity		Xu et al., 2010
C57BL/6 male mice	0, 1, or 10 mg/kg-day exposure to F0 generation	42 d	ND 1	1 10	↓ epididymal sperm count in F0 and F1 generations ↓ sperm motility in F0 mice		Mohamed et al., 2010
Wistar rat/male only	0, 3, 10, 30, or 90 mg/kg-d, gavage, 5 d/wk	35 d	3 10 30	10 30 90	↓ RBCs, hemoglobin, and hematocrit, ↓ thymus weight, ↓ percent B cells in spleen ↑ Forestomach hyperplasia, ↓serum IgM and IgA ↑ Liver oval cell hyperplasia	No exposure-related histological changes in adrenals, brain, bone marrow, colon, caecum, jejunum, heart, kidney, lung, lymph nodes, esophagus, pituitary, spleen, stomach, testis, or thymus.	De Jong et al., 1999
Wistar rat/ male and female	0, 1.5, 5, 15, or 50 mg/kg-d, gavage, 5 d/wk	35 d	5 15	15 50	↓ Thymus weight, ↑ forestomach hyperplasia ↑ Liver weight	No exposure-related changes in hematological variables, weights of kidney, spleen, lung, adrenals or ovaries, or histology of liver, kidney, spleen, thymus, lung, or mammary gland.	Kroese et al., 2001
CD-1 mouse/ F0 female; F1 male and female	0,10, 40, or 160 mg/kg-d, gavage	GDs 7–16 of F0 pregnancy	40 10 ND ND	160 40 10 10	↓ Number of F0 females with viable litters ↓ F1 body weight at PND 20 ↓ F1 body weight at PND 42 ↓ F1 male and F1female fertility index	Beginning at 6–7 wks of age, each F1 male mouse (20–45/group) was exposed to 10 untreated females over a period of 25 d. Beginning at 6 wks of age, each F1 female mouse (20–55/group) was cohabitated with an untreated male for a period of 6 mo.	MacKenzie and Angevine, 1981

Table 4-27. NOAELS and LOAELs for noncancer effects in animals repeatedly exposed to benzo[a]pyrene by the oral route

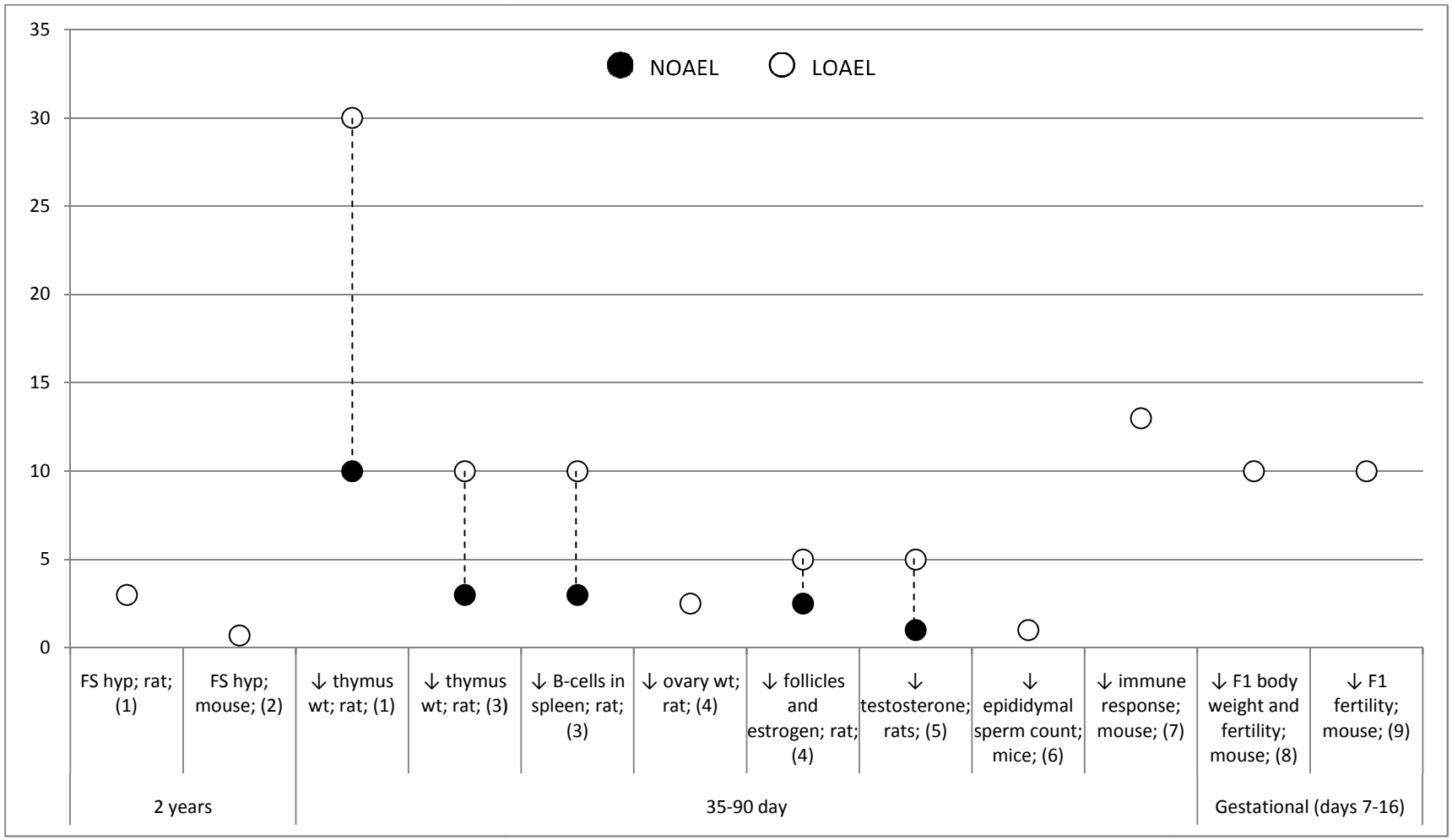
Species/sex	Dose	Duration	NOAEL	LOAEL	Response at LOAEL	Comments	Reference
			mg/kg-d				
NMRI mouse/ F0 female; F1 female	0 or 10 mg/kg-d	GDs 7–16 of F0 pregnancy	ND	10	↓ F1 female fertility (↓ number of F2 litters and F2 litter size; ↓ ovary weight, and ↓ numbers of small, medium, or large follicles and corpora lutea)	Exposed F0 females showed no gross signs of toxicity and no effects on fertility. One F1 female from each litter was continuously bred with an untreated male for 6 mo.	Kristensen et al., 1995

ND = not determined

1 Time weighted average dose ; animals treated by gavage, every other day to 0, 5, or 10 mg/kg-day

1

2



(1) Kroese et al., 2001; (2) Beland and Culp, 1998; Culp et al., 1998; (3) De Jong et al., 1999; (4) Xu et al., 2010; (5) Zheng et al., 2010; (6) Mohamed et al., 2010; (7) van den Berg et al., 2005; (8) MacKenzie and Angevine, 1981; (9) Kristensen et al., 1995; ↑= increased; ↓= decreased; FS = forestomach; hyp = hyperplasia; wt = weight.

Figure 4-2. NOAELs and LOAELs for selected noncancer effects from repeated oral exposure to benzo[a]pyrene.

The two oral chronic-duration studies identify forestomach hyperplasia in rats (gavage exposure) and mice (dietary exposure) as a sensitive effect—a LOAEL of 3 mg/kg-day for forestomach hyperplasia in male and female Wistar rats (Kroese et al., 2001) and a LOAEL of 0.7 mg/kg-day for forestomach hyperplasia in female $B6C3F_1$ mice (Beland and Culp, 1998; Culp et al., 1998). In both rats and mice, an increasing incidence of animals with forestomach tumors with increasing dose was also observed.

Several immune related effects have been observed in animals treated subchronicly with benzo[a]pyrene, including decreased thymus weight, decreased % of B cells in the spleen, decreased RBCs, and decreased serum immunoglobulins. LOAELs for decreased thymus weights were 10 and 15 mg/kg-day in two different studies of Wistar rats exposed by gavage for 35 days (Kroese et al., 2001; De Jong et al., 1999) and 30 mg/kg-day for Wistar rats exposed to benzo[a]pyrene in the diet for 90 days (Kroese et al., 2001). Thymus weights were not measured in the the available chronic studies. Decreased thymus weight was accompanied by a decreased percentage of B cells in spleen and decreased serum IgM and IgA in one 35-day Wistar rat study (De Jong et al., 1999) and increased incidence of slight thymic atrophy in the 90-day Wistar rat study (Kroese et al., 2001). Thymus atrophy, but no histological thymus lesions, was noted in the other 35-day study with Wistar rats (Kroese et al., 2001). Other support for immune effects as a potential effect from repeated oral exposure to benzo[a]pyrene is shown by decreased immune responses in lymph nodes to the dermal sensitizer, DNCB, in C56BL/6 mice given 13 mg/kg-day (LOAEL) 3 times/week for 4 weeks (van den Berg et al., 2005; see Section 4.4.2).

Effects on RBC counts were also observed across the rat subchronic duration studies (Table 4-25). LOAELs for decreased RBCs were 10 mg/kg-day in Wistar rats exposed by gavage for 35 days (De Jong et al., 1999) and 50 mg/kg-day in male F344 rats exposed in the diet for 90 days (Knuckles et al., 2001), but no significant exposure-related changes in RBC counts were observed in Wistar rats in another 35 day study at doses up to 50 mg/kg-day (Kroese et al., 2001) or at 30 mg/kg-day in Wistar rats exposed in the diet for 90 days (Kroese et al., 2001; see Table 4-26). When observed, the magnitudes of the decreases in RBC, hemoglobin, or hematocrit were generally small: about 18% at 90 mg/kg-day and <10% at lower doses in Wistar rats (De Jong et al., 1999) and about 10% in F344 rats (Knuckles et al., 2001). Hematologic variables were not measured at the terminal sacrifices in the chronic duration studies in rats (Kroese et al., 2001) or mice (Beland and Culp, 1998; Culp et al., 1998).

Kidney effects characterized as increased incidence of renal tubular casts in male F344 rats were observed in a study by Knuckles et al 2001). The most sensitive effect observed in this study was an increase in abnormal tubular casts in the kidney in males at 5 mg/kg-day (40%), 50 mg/kg-day (80%) and 100 mg/kg-day (100%), compared to 10% in the controls. In females,

only 10% showed significant kidney tubular changes at the two high dose levels compared to zero incidence in controls.

Reproductive and developmental effects following gestational exposure to benzo[a]pyrene have been observed in animal models. Decreased male reproductive endpoints including decreased testicular testosterone, decreased epididymal sperm count, and decreased sperm motility have been observed in rodents treated subchronically (Mohamed et al., 2010; Zheng et al., 2010). In addition, female reproductive endpoints including decreased ovary weight, decreased estrogen, decreased primordial follicles, and estrus cyclicity have been observed in female rats treated for 60 days (Xu et al., 2010). Impaired reproductive performance in F1 mouse offspring (male and female) has been observed following exposure of F0 mice to oral doses as low as 10 mg/kg-day during GDs 7–16 (Kristensen et al., 1995; MacKenzie and Angevine, 1981). Effects observed included decreased ovary weight in F1 females and reduced fertility as reflected by decreased mean number of F2 litters. F1 females had statistically significantly lower median numbers of offspring, number of litters, and litter sizes and a statistically significantly greater median number of days between litters as compared with the controls (Kristensen et al., 1995). Another study of gestationally treated dams (GD 7-16) identified statistically significant decrements in fertility, pup weight, and reproductive organ weights and histology (MacKenzie and Angevine, 1981). These mouse developmental/ reproductive toxicity studies observed effects at the lowest dose tested (10 mg/kg-day). Reductions in motor activity, decreased grip strength, and decreased response to sound, touch, and pain were observed in F344 rats following administration of single gavage doses of ≥25 mg/kg (Saunders et al., 2006, 2002, 2001; see Section 4.4.1), but similar evaluations of neurological endpoints following repeated oral exposure of animals to benzo[a]pyrene were not located.

Studies with ApoE-/- mice, which spontaneously develop atherosclerosis, show that repeated oral exposure to 5 mg/kg gavage doses of benzo[a]pyrene enhances the progression of atherosclerosis through a general local inflammatory process (Knaapen et al., 2007; Curfs et al., 2005, 2004; Godschalk et al., 2003; see Section 4.4.4); however, available data are inadequate to assess oral exposure dose-response relationships for benzo[a]pyrene-induced atherosclerosis in normal test animals.

4.6.2. Inhalation

Several epidemiological studies have associated increased occupational exposure of benzo[a]pyrene with cardiac endpoints, specifically ischemic heart disease (Friesen et al., 2010; Burstyn et al., 2005). Other studies have reported potential prenatal effects, birth outcomes, and decreased fertility associated with increased exposure to benzo[a]pyrene. Decreased head

circumference, decreased birth weight, and decreased postnatal weight have been reported (Tang et al., 2006; Perera et al., 2005a,b;) in addition to increased risk of early fetal death (Wu et al., 2010). Furthermore, elevated levels of benzo[a]pyrene in follicular fluid have been associated with reduced fertility (Neal et al., 2008).

In addition to epidemiological studies, several repeated-exposure inhalation toxicity studies in animals exist for benzo[a]pyrene (Archibong et al., 2002, 2008; Ramesh et al., 2008; Inyang et al., 2003; Wormley et al., 2004). A lifetime-exposure carcinogenicity study of Syrian golden hamsters exposed to benzo[a]pyrene condensed onto NaCl aerosols at nominal concentrations of 2, 10, or 50 mg/m^3 is available (Thyssen et al., 1981); however, noncancer effects were not evaluated.

Although no standard developmental toxicity studies are available for benzo[a]pyrene via the inhalation route, decreased fetal survival and number of pups per litter were observed following exposure of pregnant F344 rats to aerosols of benzo[a]pyrene and CB at concentrations ≥25 μg/m^3 on GDs 11–20 (Archibong et al., 2002). Decreased levels of plasma progesterone, estradiol, and prolactin were observed on GD 17 in dams exposed to 75 μg/m^3, but not in those exposed to 25 μg/m^3 (Archibong et al., 2002). Other rat studies from the same laboratory have associated inhalation exposures to 100 μg/m^3 benzo[a]pyrene:CB aerosols during gestation with changes in electrophysiological variables in the hippocampus (Wormley et al., 2004). Duration-dependent effects on male reproductive endpoints including increased luteinizing hormone, decreased circulating and intratesticular concentrations of testosterone, decreased testis weight, and decreased sperm motility have also been observed following exposure of adult male F344 rats to benzo[a]pyrene:CB aerosols at 75 μg/m^3 for 10 or 60 days (Archibong et al., 2008; Ramesh et al., 2008; Inyang et al., 2003).

Table 4-28. NOAELS and LOAELs for noncancer effects in animals repeatedly exposed to benzo[a]pyrene by the inhalation route

Species/sex	Dose	Duration	NOAEL	LOAEL	Response at LOAEL	Comments	Reference
			µg/m^3				
F344 rats	0, 25, 75, 100 µg/m^3 4hr/d	GD 11-20	ND 25	25 75	↓ pups/litter, litter survival (%) ↑ resorptions ↓ plasma progesterone, estradiol, and prolactin	carbon black used as carrier particle	Archibong et al., 2002; Wu et al 2003
F344 rats	0, 75 µg/m^3 4hr/d	60 d	ND	75	↑ luteinizing hormone ↓ testosterone ↓ decreased testis weight ↓ sperm motility	carbon black used as carrier particle in treatment group; controls not exposed to carbon black	Archibong et al., 2008
F344 rats	0, 100 µg/m^3 4hr/d	GD	ND	100	↓ pups/litter electrophysiological changes in the hippocampus		Wormley et al., 2004

1

4.6.3. Dermal

Though numerous chronic cancer bioassays exist for benzo[a]pyrene by the dermal route, noncancer effects were not reported in these studies, nor are studies available evaluating noncancer effects in humans exposed dermally to benzo[a]pyrene.

4.6.4. Mode-of-Action Information

4.6.4.1. *Forestomach Lesions from Oral Exposure*

The development of forestomach hyperplasia in mice and rats from subchronic or chronic oral exposures (by gavage and diet) to benzo[a]pyrene is reasonably expected to involve a cell proliferative response to cytotoxicity from reactive benzo[a]pyrene metabolic intermediates, based on the extensive findings from research on the bioactivation of benzo[a]pyrene and carcinogenicity (see reviews on the bioactivation of benzo[a]pyrene by Xu et al., 2009; Jiang et al., 2007, 2005; Xue and Warshawsky, 2005; Penning et al., 1999; Harvey 1996; Cavalieri and Rogan, 1995). Reactive intermediates that can react with cellular macromolecules and potentially lead to cytotoxicity include BPDE, benzo[a]pyrene radical cations, benzo[a]pyrene o-quinones, and ROS. Reactive benzo[a]pyrene metabolites are also well known to reduce the viability and survival of cultured cells involving mechanisms related to stimulation of apoptosis (Andrysik et al., 2007; Chung et al., 2007; Nwagbara et al., 2007; Chen et al., 2003; Jyonouchi et al., 1999; Flowers-Geary et al., 1996, 1993). Molecular details of cell proliferative responses to cytotoxicity or apoptosis from benzo[a]pyrene metabolites are poorly understood, but Burdick et al. (2006, 2003) provided evidence that benzo[a]pyrene o-quinones could inhibit apoptosis and increase cell proliferation in a model human mammary epithelial cell system (MCF10A) via activation of the epidermal growth factor receptor (EGFR) by ROS. The relationship of these findings to development of benzo[a]pyrene-induced forestomach hyperplasia is unknown.

4.6.4.2. *Immune System Effects*

Decreased thymus weight, decreased number of B cells in spleen, and immune suppression have been observed following oral, i.p., s.c., or intratracheal instillation exposure to benzo[a]pyrene. DeJong et al. (1999) and Kroese et al. (2001) found decreased thymus weight due to benzo[a]pyrene at oral doses ≥10 mg/kg body weight. In addition, several studies report thymus effects at higher doses and/or by other routes of exposure (e.g. Rodriguez et al., 1999; Holladay and Smith, 1994). Reduced thymus size or weight have been noted to be among the first indicators of immunotoxicity (Schuurman et al. 1992; Luster et al. 1988), and correlate well with adverse histopathologic effects and the presence of lesions in the thymic cortex (Germolec et al. 2004a, 2004b; Wachsmuth 1983).

Interpretation of decreased thymus weight as an adverse effect is supported by general immunology literature as well as chemical-specific data. The thymic cortex is known to be a major site of thymocyte proliferation and selection for maturation, and impairment can lead to cell-mediated immune suppression (Kuper 2002, 1992; De Waal et al. 1997). Reduced thymus weight is often attributed to decreased thymocyte proliferation or increased thymocyte apoptosis in the thymic cortex (Kamath et al., 1997; Vandebriel et al., 1999). DeJong et al. (1999) reported a decrease in estimated thymic cortex weight at 10, 30, and 90 mg/kg benzo[a]pyrene, and reduced medulla weight at 90 mg/kg, but did not calculate the ratio between the two. This suggests that both parts of the thymus were affected by benzo[a]pyrene, but that the cortex may be more sensitive. De Jong also used immunohistochemistry data to show that cell proliferation was not affected, suggesting that thymic atrophy may be due to increased rates of thymocyte apoptosis. However, a study using the murine LLNA showed that proliferation activity decreased after a single 13 mg/ml oral dose of benzo[a]pyrene (van den Berg et al., 2005).

In addition to thymus effects, decreased B cell percentages in the spleen were observed at 10, 30, and 90 mg/kg-day benzo[a]pyrene in a dose response pattern. The absolute number of B cells, however, was not significantly lower than control animals until 90 mg-kg/day. There is also supportive evidence of humoral immune suppression at higher doses, as well as evidence of overall toxicity of the bone marrow. This theory is supported by the decrease in IgA and IgM (incating T-cell dependant effects), and the dose-related toxicity of RBCs observed by Dejong et al. (1999).

The MOA by which benzo[a]pyrene produces immune system effects is not understood, but several in vitro studies have been conducted to investigate potential contributing mechanisms. Benzo[a]pyrene induced myelotoxicity in human cord blood cells (Carfi et al., 2007) and mouse bone marrow cultures (Legraverend et al., 1983), suppressed mouse B cell lymphopoiesis (Hardin et al., 1992), and inhibited mitogen-induced proliferative responses of mouse spleen cell cultures (Lee and Urso, 2007). Benzo[a]pyrene inhibition of the proliferative responses of spleen cells to a mitogen was diminished by the presence of the AhR antagonist and CYP inhibitor, α-NF, indicating the potential importance of benzo[a]pyrene metabolites in the immune suppression effect (Lee and Urso, 2007). Similarly, the CYP1A1 inhibitor 1-(1-propynyl)pyrene blocked B-cell growth inhibition by benzo[a]pyrene, but not through the metabolite BPDE (Allan et al. 2006).

Carfi et al. (2007) described a series of in vitro assays designed to assess cytotoxicity, myelotoxicity, cytokine release, and mitogen responsiveness in rat, mouse, and human cells (i.e., peripheral lymphocytes and cord blood cells, and spleen cells from rats and mice only). The cytotoxicity half maximal inhibitory concentration (IC_{50}) value for benzo[a]pyrene was >200 μM in human, rat, and mouse cells. Benzo[a]pyrene produced myelotoxicity as evaluated by a dose-related decrease in colony scoring for the colony forming unit-granulocyte macrophage (CFU-GM) assay using human cord blood cells. Benzo[a]pyrene reduced the release of specific

cytokines from HL following phytohemagglutinin (PHA), gamma-interferon (γ-INF), and lipopolysaccharide (LPS) tumor necrosis factor (TNF-α) stimulation. Mitogen responsiveness in rat and mouse spleen cells following stimulation with LPS or PHA was decreased by exposure to benzo[a]pyrene. T-lymphocyte proliferation induced by anti-CD3 antibody was also inhibited by benzo[a]pyrene in HL, but was not affected in mouse spleen cells at the highest concentration (160 μM).

Myelotoxicity was also observed in mouse bone marrow cultures exposed to benzo[a]pyrene as evidenced by decreased cell survival (Legraverend et al., 1983). The findings in bone marrow cultures from Ah-responsive (C57BL/6) and Ah-nonresponsive (DBA/2) mice suggest that AhR affinity may play a role in benzo[a]pyrene-induced myelotoxicity. Benzo[a]pyrene is more toxic to bone marrow cells from C57BL/6 mice in vitro compared to cells cultured from DBA/2 mice.

Hardin et al. (1992) treated cultured bone marrow cells from DBA/2 and C57BL/6 mice with benzo[a]pyrene at concentrations between 10^{-4} and 10^{-8} M. Benzo[a]pyrene suppressed B cell lymphopoiesis in a dose-dependent manner even at the lowest concentration used. Bone marrow cells from Ah-nonresponsive DBA/2 mice were less sensitive to the immunosuppressive action of benzo[a]pyrene, compared with Ah-responsive C57BL/6 mice. The AhR antagonist and CYP450 inhibitor α-NF prevented benzo[a]pyrene-induced inhibition of B cell lymphopoiesis from C57BL/6 mice in a concentration-dependent fashion. Benzo[a]pyrene also induced apoptosis in cultured bone marrow cells.

Spleen cell cultures derived from C3H/HeJ and CBY/D2 mice were exposed to benzo[a]pyrene and assessed for T-lymphocyte proliferation in response to mitogenic or antigenic stimulation (Lee and Urso, 2007). Benzo[a]pyrene (10 μM) produced an 80% decrease in the allogeneic mixed lymphocyte response (MLR) assay, which is a measure of the proliferative response to antigenic stimulation. Benzo[a]pyrene (0.1, 1, and 10 μM) also produced a dose-dependent inhibition of the proliferative response to the mitogen Concanavalin A (Con A). This inhibition did not occur in spleen cells treated with the AhR antagonist and CYP450 inhibitor, α-NF. BPDE-DNA adducts were detected in CH3 spleen cells cultured with 10 μM benzo[a]pyrene in the presence of Con A.

4.6.4.3. *Developmental and Reproductive Toxicity Effects*

Developmental and reproductive toxicity effects have been associated with oral and inhalation exposure to benzo[a]pyrene. Impaired fertility, with associated lesions in ovarian (decreased follicles) and testicular (atrophic seminiferous tubules) tissues, has been observed in male and female F1 offspring following exposure of F0 female mice to 10 mg/kg-day benzo[a]pyrene on GDs 7–16; a decrease in the number of F0 females with viable litters was observed at a higher dose level of 160 mg/kg-day (Kristensen et al., 1995; MacKenzie and Angevine, 1981). Inhalation exposure of pregnant rats to benzo[a]pyrene:CB aerosols during

gestation has also been associated with decreased fetal survival and number of pups per litter (Archibong et al., 2002), decreased levels of plasma progesterone, estradiol, and prolactin (Archibong et al., 2002), changes in electrophysiological variables in the hippocampus (Wormley et al., 2004) and decreased cortical neuron activity (McCallister et al., 2008). Inhalation exposure of adult male rats to benzo[a]pyrene:CB aerosols (75 $\mu g/m^3$) for 10 or 60 days caused decreased circulating and intratesticular concentrations of testosterone, decreased testis weight, and decreased sperm motility (Archibong et al., 2008; Ramesh et al., 2008; Inyang et al., 2003).). Acute i.p. exposure studies in adult female DBa/2N or C57BL/6N mice demonstrated a 50% destruction of primordial follicles with a single dose of 25 mg/kg (Mattison et al., 1980) and a 15-day exposure of 3 mg/kg-day (Boorman et al., 2000). Other reproductive-related effects in these studies include decreased fertility (number of pups) (Mattison et al., 1980) and ovulatory inhibition, as indicated by decreased number of corpora lutea, one week after a dose of 5 mg/kg and 3-4 weeks after a dose of 100 mg/kg (Miller et al., 1992; Swartz and Mattison, 1985).

In vivo studies have suggested that the mechanism of decreased fertility in females and decreased fetal survival may result from changes in circulating hormones (i.e., decreased progesterone, estradiol-17β, and prolactin levels) responsible for maintaining the uterine environment in a state that can support embryonic and fetal development (Archibong et al., 2002, see Section 4.3.2). Several in vitro studies have demonstrated low affinity binding of benzo[a]pyrene to the estrogen receptor and alteration of estrogen-dependent gene expression (Liu et al., 2006; Van Lipzig et al., 2005; Vondracek et al., 2002; Fertuck et al., 2001; Charles et al., 2000); however, the role of these changes in benzo[a]pyrene-induced reproductive toxicity is unknown. Fertuck et al. (2001) showed in vitro effects of benzo[a]pyrene on estrogen-receptor-mediated gene expression, but did not demonstrate estrogen-mediated uterotrophic effects (increased uterine weight or lactoferrin mRNA expression) in ovarectomized C57BL/J6 or DBA/2 mice following in vivo administration of benzo[a]pyrene (0.1–10 mg/kg-day, p.o., for 3 consecutive days).

The mechanism(s) by which benzo[a]pyrene or its metabolites impair the development of follicles in the ovary have been the focus of study for more than 30 years (Mattison and Thorgeirsson, 1977). AHH is found in the ovary, and inhibition of AHH activity reduces the level of oocyte destruction seen with benzo[a]pyrene exposures in mice (Mattison and Thorgeirsson, 1979). AHH activation is not sufficient to explain the variation across strains in oocyte destruction, however (Mattison and Nightingale, 1980). Studies using intraovarian injection of metabolites of benzo[a]pyrene indicate that the epoxide metabolite (+)-(7R,8S)-diol-(9S,10R)-epoxide-2 is most strongly correlated with the oocyte counts in exposed mice (Takizawa et al.,1984).

Most of the loss of oocytes that occurs in utero and throughout the reproductive lifespan in mice and in women occurs through programmed cell death (apoptosis), which is regulated by

the protein Bax. Activation of *Bax* leads to increased oocyte death, and PAHs (including benzo[a]pyrene) have been demonstrated to activate *Bax* gene transcription in mice (Matikainen et al., 2002; 2001). However, an in vitro study of mouse ovarian cells obtained from 4-day old pups did not show any evidence of increased markers of apoptosis with treatments of benzo[a]pyrene concentrations of up to 1000 ng/ml for 6 and for 24 hours (Tuttle et al., 2009).

Other mechanisms may be more relevant for the ovulatory inhibition effects seen with benzo[a]pyrene exposures. In an intro experiment, Neal et al. (2007) demonstrated a dose-dependent decrease in FSH-stimulated rat follicle growth, with 158, 99, 75, 38, 30 and 38% change in follicle area for benzo[a]pyrene exposure concentrations of 0, 1.5, 5.0, 15, 45 and 135 ng /ml ($p < 0.05$ for all differences compared with controls). The authors noted that the lowest dose at which this effect was seen, 1.5 ng/ml, was similar to the mean concentration of benzo[a]pyrene seen in follicular fluid samples from women who smoked.

Several in vitro studies have investigated the possible mechanisms for impaired spermatogenesis by benzo[a]pyrene including enhancement of apoptosis of spermatogonia (Revel et al., 2001), inhibition of spermatid meiosis (Georgellis et al., 1990), Sertoli cell cytotoxicity (Raychoudhury and Kubinski, 2003), and altered androgen hormone regulation (Inyang et al., 2003; Vinggaard et al., 2000).

Revel et al. (2001) reported dose-related increases in apoptosis of spermatogonia harvested from the vas deferens of male BALB/c mice administered benzo[a]pyrene doses ranging from 0.5 to 50 mg/kg via s.c. injection for 5 weeks. The competitive AhR inhibitor, resveratrol (50 mg/kg, s.c.) given simultaneously with 5 mg/kg benzo[a]pyrene s.c. for 5 weeks, suppressed both BPDE DNA adduct formation and apoptosis, suggesting a role for the AhR in this benzo[a]pyrene-induced male reproductive toxicity (Revel et al., 2001).

Georgellis et al. (1990) reported that concentrations of 0.1 μM benzo[a]pyrene incubated in vitro with seminiferous tubule segments from Sprague-Dawley rats with microsome preparations from the whole rat testes inhibited meiotic division of the spermatids and was highly cytotoxic.

Raychoudhury and Kubinski (2003) isolated Sertoli cells from CD rats and incubated the cells in culture with benzo[a]pyrene. Benzo[a]pyrene was cytotoxic to these cells at 50 and 100 μg/mL. Treatment of the cells for 24 hours with 10 μg/mL benzo[a]pyrene induced cell killing through an apoptotic response (as measured by fluorescence labeling of apoptotic DNA fragments).

Inyang et al. (2003) demonstrated that benzo[a]pyrene inhalation altered circulating levels or cellular responsiveness to androgenic hormones such as testosterone (see Section 4.3.2). Vinggaard et al., 2000) showed that benzo[a]pyrene antagonized the human androgen receptor (hAR) in a sensitive reporter gene assay based on CHO cells transiently cotransfected with a hAR vector and an MMTV-LUC vector (antiandrogen IC_{50} of 3.9 μM).

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans." This conclusion is based on evidence of carcinogenicity in humans following exposure to different PAH mixtures containing benzo[a]pyrene, extensive and consistent evidence of carcinogenicity in laboratory animals exposed to benzo[a]pyrene via all routes of administration, and strong evidence that the biological processes leading to benzo[a]pyrene carcinogenesis in laboratory animals are also present in humans. Bioactivation of benzo[a]pyrene leads to the formation of DNA-reactive metabolites which can produce mutations in key genes, such as the p53 tumor suppressor gene and the ras oncogene, leading to tumor formation (see Section 4.7.3.).

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

There is a large body of evidence for human carcinogenicity for several PAH mixtures containing benzo[a]pyrene, such as soot, coal tars, coal-tar pitch, mineral oils, and shale oils (IARC, 2010; Baan et al., 2009; Straif et al., 2005). There is also evidence of carcinogenicity in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as aluminum production, chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, and paving and roofing with coal tar pitch (IARC, 2010; Baan et al., 2009; Straif et al., 2005). Increased cancer risks have been reported among other occupations involving exposure to PAH mixtures such as carbon black and diesel exhaust (Bosetti et al., 2007; Straif et al., 2005). Benzo[a]pyrene is also a notable constituent of tobacco smoke (IARC 2004). An increasing number of studies report exposure biomarkers such as benzo[a]pyrene- or PAH-DNA adducts in white blood cells, and several cohort studies (summarized in Section 4.1) demonstrate a positive exposure-response relationship with cumulative PAH exposure using benzo[a]pyrene—or a proxy such as BSM that can be converted to benzo[a]pyrene—as an indicator substance. Because benzo[a]pyrene is only one of many PAHs that could contribute to these observed increases in cancer, the epidemiologic studies provide credible but limited support for a causative role of benzo[a]pyrene in human cancer.

In laboratory animals (i.e., rats, mice, and hamsters), exposure to benzo[a]pyrene via the oral, inhalation, and dermal routes have been associated with carcinogenic responses both systemically and at the site of administration. Chronic oral exposure to benzo[a]pyrene was associated with forestomach and liver tumors in male and female Wistar rats (Kroese et al., 2001), forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981), and forestomach, esophagus, tongue, and larynx tumors in female $B6C3F_1$ mice (Beland and Culp, 1998; Culp et al., 1998). Auditory canal tumors were also observed in male and female Wistar rats (Kroese et al., 2001). Repeated or short-term oral exposure to benzo[a]pyrene was associated with forestomach tumors in more than 10 additional bioassays with several strains of

mice (see Table 4-4 in Section 4.2.1.2). Chronic inhalation exposure to benzo[a]pyrene was associated with tumors in the larynx and pharynx of male Syrian golden hamsters exposed to benzo[a]pyrene:NaCl aerosols (Thyssen et al., 1981). Intratracheal instillation of benzo[a]pyrene was associated with respiratory tract tumors in more than 10 additional studies with hamsters (see Section 4.2.2.2 for references). Chronic dermal application of benzo[a]pyrene (2–3 times/week) has been associated with mouse skin tumors in 12 bioassays (see Section 4.2.3.2 for references). Skin tumors in rats, rabbits, and guinea pigs have also been associated with repeated application of benzo[a]pyrene to skin in the absence of exogenous promoters (WHO, 1998; ATSDR, 1995; IARC, 1983, 1973). When followed by repeated exposure to a potent tumor promoter, acute dermal exposure to benzo[a]pyrene induced skin tumors in numerous studies of mice, indicating that benzo[a]pyrene is a strong tumor-initiating agent in the mouse skin model (see Section 4.2.3.1 for references).

Carcinogenic responses in animals exposed to benzo[a]pyrene by other routes of administration include: (1) liver or lung tumors in newborn mice given acute postnatal i.p. injections; (2) increased lung tumor multiplicity in A/J adult mice given single i.p. injections; (3) injection site tumors in mice following s.c. injection; (4) injection site sarcomas in mice following intramuscular injection; (5) mammary tumors in rats with intramammilary administration; (6) cervical tumors in mice with intravaginal application; and (7) tracheal tumors in rats with intratracheal implantation (see Section 4.4.3 for references).

Benzo[a]pyrene is classified as an alternant PAH, or a compound composed solely of fused benzene rings. Nonalternant PAHs contain both benzene and five carbon rings. Among alternant PAHs, important structural features related to enhanced mutagenicity and carcinogenicity include the presence of at least four rings (Bostrom et al., 2002). The carcinogenic activity of PAH compounds is influenced by specific structural features. Recently, this knowledge has been exploited in an effort to derive quantitative structure activity relationship (QSAR) methods to evaluate the relationship between specific PAH structural features and mechanistic events related to carcinogenesis (Bruce et al., 2008; Vijayalakshmi et al., 2008). Alternant PAHs having four or more benzene rings exhibit greater carcinogenic potency than PAHs with two or three benzene rings (Bostrom et al., 2002). The carcinogenic activity of PAHs is also related to the specific arrangement of the benzene rings. As a general rule, PAHs with at least four rings and a classic bay- or fjord-region (formed entirely by benzene rings) may be characterized as containing structural alerts for carcinogenesis. However, this structural characterization is likely to be overly simplistic and other features may be important to carcinogenesis.

As discussed in Section 4.5.1.1, several lines of evidence related to tumor initiation following mutagenicity are available for benzo[a]pyrene including: (1) in vivo detection of cancer-relevant oncogene/tumor suppressor gene mutations in target tissue; (2) in vivo detection of DNA adducts in target tissue; (3) in vivo DNA adducts, gene mutations, cytogenetic damage,

and other measures of primary DNA damage in non-target tissues; and (4) in vitro DNA adduct formation, mutations, cytogenetic damage, and primary DNA damage in cells from target and nontarget tissues.

4.7.3. Mode-of-Action Information

4.7.3.1. *Hypothesized MOA*

The carcinogenicity of benzo[a]pyrene, the most studied and best characterized PAH, is well documented (Xu et al., 2009; Jiang et al., 2007, 2005; Xue and Warshawsky, 2005; Ramesh et al., 2004; Bostrom et al., 2002; Penning et al., 1999; WHO, 1998; Harvey, 1996; ATSDR, 1995; Cavalieri and Rogan, 1995; U.S. EPA, 1991b). EPA has concluded that benzo[a]pyrene induces carcinogenicity via a mutagenic mode of action. Mutagenicity is a well-established cause of carcinogenicity. This hypothesized mode of action is presumed to apply to all tumor types and is relevant for all routes of exposure. The principal key events associated with the mode of action for benzo[a]pyrene include: (1) bioactivation of benzo[a]pyrene to reactive metabolites (2) direct DNA damage by the reactive metabolites, including the formation of DNA adducts (3) formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes and (4) clonal expansion of mutated cells. These events are depicted in Figure 4-3.

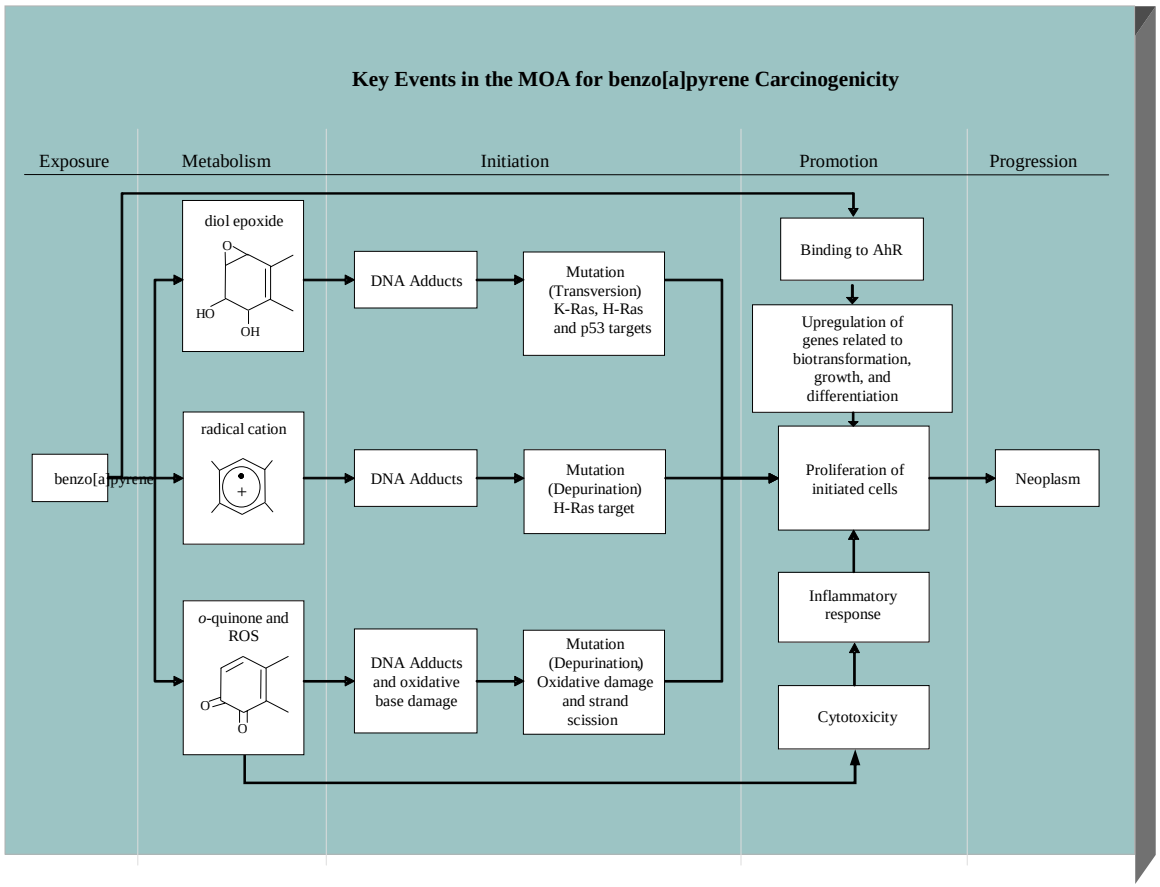


Figure 4-3. Proposed principal pathways and key events in the benzo[a]pyrene carcinogenic MOA.

4.7.3.2. *Experimental Support for the Hypothesized MOA*

Strength, consistency, specificity of association. There is an extensive database of in vitro and in vivo studies demonstrating the genotoxicity and mutagenicity of benzo[a]pyrene following metabolic activation (see Tables 4-24, 4-25 and 4-26). In vitro studies overwhelmingly support the formation of DNA adducts, mutagenesis in bacteria, yeast and mammalian cells, several measures of cytogenetic damage (CA, SCE, MN), and DNA damage. In vivo systems in animal models are predominantly positive for somatic mutations following benzo[a]pyrene exposure. Additionally, some evidence exists that benzo[a]pyrene can induce mutations in germ cells.

Benzo[a]pyrene is thought to be converted into reactive intermediates via three principal metabolic pathways: (1) activation to a reactive diol epoxide via CYP1A1/1B1 and epoxide hydrolase; (2) activation to a reactive radical cation via CYP peroxidases and (3) activation to a reactive and redox active o-quinone metabolite via AKR1A1 and AKR1C1-1C4 (Xu et al., 2009; Jiang et al., 2007, 2005; Xue and Warshawsky, 2005; Penning et al., 1999; Harvey 1996; Cavalieri and Rogan, 1995). All three of these pathways (discussed in detail in Sections 3.3. and 4.5.2) lead to DNA damage including DNA adducts, depurination, and/or oxidative damage to DNA. DNA damage, if not correctly repaired prior to replication, can subsequently give rise to mutations.

Benzo[a]pyrene-DNA adducts, biomarkers of exposure and of effect, have been extensively demonstrated with in vitro cell systems, in vivo animals studies, and in human target tissues, including skin and lung (see Section 4.1.2.). Specifically, elevated BPDE-DNA adducts have been observed in coke oven workers and chimney sweepers, occupations associated with increased risks of cancer from complex PAH-containing mixtures (Pavanello et al., 1999). BPDE-DNA adducts were also found to be elevated in the lungs of cigarette smokers with lung cancer (Godschalk et al., 2002; Phillips et al., 2002; Bartsch et al., 1999; Alexandrov et al., 1992). Multiple epidemiological studies have indicated that PAH exposed individuals who are homozygous for a CYP1A1 polymorphism which increases the inducability of this enzyme (thus increasing the production of reactive diol epoxide metabolites) have increased levels of benzo[a]pyrene-DNA adducts (Bartsch et al., 2006; Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). In addition, this population of individuals also has a greater risk of certain tumors, including those of the lung.

Mutations in the K-ras, H-ras, and p53 genes were assessed in forestomach tumors of mice fed benzo[a]pyrene in the diet for 2 years (Culp et al., 2000). Forestomach tumors had K-ras mutations (68% of tumors) that were G→T or C transversions in codon 12 or 13. H-ras (codon 13) and p53 mutations characterized as G→T or C transversions were also found.

K-ras mutations were observed in the A/J mouse lung tumor model following IP treatment with benzo[a]pyrene, and were observed to be qualitatively different than K-ras mutations in spontaneous lung tumors from control animals (Nesnow et al., 1996). Lung tumor DNA was isolated and DNA sequence analysis of K-ras mutations was performed for 19 separate lung tumors in the benzo[a]pyrene treated group and the control group. The DNA sequence analysis demonstrated several guanine mutations at codon 12, which were different than the spectrum of mutations found in untreated animals. Specifically, the frequency of GGT→TGT transversion mutations were significantly higher in the benzo[a]pyrene treated animals compared to controls (56% vs. 0%) whereas GGT→GAT transition mutations were the predominant mutation in lung tumors from control animals (58% vs. 19% in benzo[a]pyrene group).

Some human data exist which correlate the frequency of PAH-DNA adducts with gene mutations in highly PAH-exposed populations. In a study of iron foundry workers (a high PAH exposure population which has been demonstrated to have an increased risk of lung cancer [Bosetti et al., 2007]), biological samples from workers were analyzed for DNA adducts and somatic gene mutations at the hprt locus (Perera et al.1994, 1993). A strong correlation between PAH-DNA adduct levels and incidence of hprt mutations was observed in individuals with detectable levels of adducts, indicating that somatic mutations were increased in parallel with PAH-DNA adducts in workers exposed to PAHs.

Data in humans from a study by Marini et al. (2001) indicate that the types of mutations commonly found in response to benzo[a]pyrene exposure in in vitro and animal models are similar to the spectrum of mutations in critical tumor suppressor genes and/or oncogenes in populations highly exposed to PAHs. DeMarini et al. (2001) demonstrated mutations in the p53 tumor suppressor gene and the K-ras oncogene in lung tumors obtained from 24 nonsmoking women from China, whose tumors were associated with exposure in their homes to smoky coal from the use of stoves with no chimneys. The observed mutations in lung tumors were primarily G→T transversions at either K-ras or p53. Mutation hotspots in the lung tumors corresponded with hot spots for PAH mutations (codon 154, codon 249, and codon 273).

Dose-response concordance and temporal relationship. The metabolism of benzo[a]pyrene to reactive metabolites is a necessary event which precedes mutagenesis. Mutation assays of benzo[a]pyrene in *salmonella typhimurium* are overwhelmingly positive with the inclusion of S9 metabolic liver fractions, but are negative without the addition of the S9 metabolic enzymes (see Table 4-24).

In mice, a dose-response and temporal relationship has been demonstrated between the formation of BPDE-DNA adducts and skin and forestomach tumors. In a study using mice treated dermally with benzo[a]pyrene once or twice per week for 15 weeks, a linear dose-response of benzo[a]pyrene-induced adducts in the skin, lung, and liver was observed (Talaska et al. 1996). Another study examined the dose-response relationship and the time course of

benzo[a]pyrene-induced skin damage, DNA adduct formation, and tumor formation in female mice. Mice were treated dermally with 0, 16, 32, or 64 μg of benzo[a]pyrene once per week for 29 weeks (Albert et al., 1991). Indices of skin damage and levels of BPDE-DNA adducts in skin reached plateau levels in exposed groups by 2–4 weeks of exposure. With increasing dose level, levels of BPDE-DNA adducts (fmol/μg DNA) initially increased in a linear manner and began to plateau at doses ≥32 μg/week. Tumors began appearing after 12–14 weeks of exposure for the mid- and high-dose groups and at 18 weeks for the low-dose group. At study termination (35 weeks after start of exposure), the mean number of tumors per mouse was approximately one per mouse in the low- and mid-dose groups and eight per mouse in the high-dose group. The time-course data indicate that benzo[a]pyrene-induced increases in BPDE-DNA adducts preceded the appearance of skin tumors, consistent with the formation of DNA adducts as a precursor event in benzo[a]pyrene induced skin tumors.

Culp et al. (1996a) compared dose-response relationships for BPDE-DNA adducts and tumors in female $B6C3F_1$ mice exposed to benzo[a]pyrene in the diet at 0, 18.5, 90, or 350 μg/day for 28 days (to examine adducts) or 2 years (to examine tumors). The benzo[a]pyrene dose-tumor response data showed a sharp increase in forestomach tumor incidence between the 18.5 μg/day group (6% incidence) and the 90 μg/day group (78% incidence). The BPDE-DNA adduct levels in forestomach showed a relatively linear dose-response throughout the benzo[a]pyrene dose range tested. The appearance of increased levels of BPDE-DNA adducts in the target tissue at 28 days is temporally consistent with the contribution of these adducts to the initiation of forestomach tumors. Furthermore, about 60% of the examined tumors had mutations in the K-ras oncogene at codons 12 and 13, which were G→T or G→C transversions indicative of BPDE reactions with DNA (Culp et al., 1996a).

Biological plausibility and coherence. A mutagenic MOA for benzo[a]pyrene is supported by a large body of research over time with consistent evidence of benzo[a]pyrene activation to reactive metabolites leading to DNA-damage and mutational events associated with tumor initiation. Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The formation of DNA adducts and oncogene/tumor suppressor mutations in organs that also displayed an increase in tumor incidence in rats and mice indicates coherence of these effects. Benzo[a]pyrene has been shown to be mutagenic in vivo and in vitro, across species and tissue types.

4.7.3.2. Other Possible MOAs

In addition to mutagenicity, other MOAs which contribute to the carcinogenicity of benzo[a]pyrene are possible, but are not as well studied. The tumor promotion properties of benzo[a]pyrene may involve cell proliferative responses to cytotoxicity or apoptosis from benzo[a]pyrene metabolites, AhR-mediated effects on cell growth and differentiation, or anti-

apoptotic signals elicited by metabolites (Burdick et al., 2006, 2003; Chen et al., 2003; Nebert et al., 1993). Results from some studies indicate that exposure to benzo[a]pyrene or its metabolites increases the production of inflammatory cytokines, such as IL-1, which may contribute to tumor promotion (N'Diaye et al., 2006; Tamaki et al., 2004; Garçon et al., 2001a, b). Benzo[a]pyrene also has been shown to inhibit GJIC, a characteristic associated with well-known tumor promoters such as TPA (Sharovskaya et al., 2006; Blaha et al., 2002). In summary, though there are limited data which support other processes that may contribute to the carcinogenicity of benzo[a]pyrene (inflammation, cytotoxicity, anti-apoptotic signaling, etc.); the available evidence indicates that the primary mode of action for benzo[a]pyrene involves DNA reactivity and mutagenicity leading to carcinogenesis.

4.7.3.4. *Conclusions About the Hypothesized Mode of Action*

The MOA of mutagenicity of benzo[a]pyrene through reactive metabolites is extensively supported by a large body of research. Mutations from DNA reactive benzo[a]pyrene metabolites occur as early events in the carcinogenic process and are not believed to be acquired following cytoxicity or regenerative proliferation. Several lines of evidence relating to mutagenicity and tumor initiation are available for benzo[a]pyrene including: in vitro evidence of DNA adducts, mutations, cytogenetic damage, and primary DNA damage; in vivo DNA adducts, gene mutations, cytogenetic damage, and other measures of primary DNA damage; detection of DNA adducts in target tissue in vivo; and detection of cancer-relevant oncogene/tumor suppressor gene mutations in target tissue in vivo. Taken together, these data provide support for a mutagenic MOA for benzo[a]pyrene-induced cancer.

Support for the hypothesized MOA in test animals

Benzo[a]pyrene induces gene mutations in a variety of in vivo and in vitro systems and produces tumors in all animal species tested and all routes of exposure. Strong, consistent evidence indicates that the postulated key events: the metabolism benzo[a]pyrene to a DNA-reactive intermediates, the formation of DNA adducts, and the occurrence of subsequent mutations in oncogenes and tumor suppressor genes occur in animal models.

Relevance of the hypothesized MOA to humans

Mutagenicity is a well-established cause of carcinogenicity. A substantial database of information on benzo[a]pyrene indicates that the postulated key events: the metabolism of benzo[a]pyrene to a DNA-reactive intermediates, the formation of DNA adducts, and the formation of subsequent mutations in oncogenes and tumor suppressor genes all occur in human tissues. The following lines of evidence from human studies provide support that the hypothesized mutagenic MOA is relevant to humans: the activation of benzo[a]pyrene to DNA reactive metabolites occurs in humans in qualitatively and quantitatively similar manner

compared to animals; DNA adducts specific to benzo[a]pyrene have been found in a wide variety of human tissues and are elevated in populations exposed to high levels PAHs; increased DNA mutations have been strongly associated with increasing levels of PAH-DNA adducts in workers occupationally exposed to PAHs; and the spectra of benzo[a]pyrene induced mutations in p53 tumor suppressor genes and ras oncogenes observed in controlled in vitro cell systems and in vivo animal studies are similar to the spectra of mutations in tumors from PAH-exposed humans.

Populations or lifestages particularly susceptible to the hypothesized MOA

The mutagenic mode of action is considered relevant to all populations and lifestages. The current understanding of biology of cancer indicates that mutagenic chemicals, such as benzo[a]pyrene, are expected to exhibit a greater effect in early life versus later life exposure (U.S. EPA, 2005b; Vesselinovitch et al., 1979). Although the developing fetus and infants may have lower levels of some bioactivating enzymes than adults (e.g., CYP1A1/1B1), infants or children are expected to be more susceptible to benzo[a]pyrene-induced cancer at certain tissue sites. Newborn or infant mice developed liver and lung tumors more readily than young adult mice following acute i.p. exposures to benzo[a]pyrene (Vesselinovitch et al., 1975; see Section 4.8.1). These results indicate that exposure to benzo[a]pyrene during early life stages presents additional risk for cancer, compared with exposure during adulthood. The *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action. Given the weight of the available evidence, benzo[a]pyrene acts through a mutagenic mode of carcinogenic action and the ADAFs should be applied.

Population variability in metabolism and detoxification of benzo[a]pyrene, in addition to DNA repair capability, may affect cancer risk. Polymorphic variations in the human population in CYP1A1, CYP1B1, and other CYPs have been implicated as determinants of increased individual lung cancer risk in some studies (Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). The Phase II cytosolic GST, mediated by variants of the GSTM1 and GSTT1 genes, prevents the formation of BPDE-DNA adducts. Some evidence suggests that humans lacking a functional GST gene have higher BPDE-DNA adduct levels and thus are at greater risk for cancer (Vineis et al., 2007a; Pavanello et al., 2004; Perera and Weinstein, 2000; Alexandrov et al., 2002). In addition, acquired deficiencies or inherited gene polymorphisms that affect the efficiency or fidelity of DNA repair may also influence individual susceptibility to cancer from environmental mutagens (Matullo et al., 2003; Shen et al., 2003; Cheng et al., 2000; Perera and Weinstein, 2000; Wei et al., 2000; Amos et al., 1999). In general, however, available support for the role of single polymorphisms in significantly modulating human PAH cancer risk is relatively weak or inconsistent. Combinations of metabolic polymorphisms, on the other hand,

may be critical determinants of cumulative DNA-damaging dose, and thus susceptibility to cancer from benzo[a]pyrene exposure.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

Increased childhood susceptibility to benzo[a]pyrene is supported by several lines of evidence including epidemiological studies reporting associations between adverse birth outcomes and developmental effects and internal biomarkers of exposure to benzo[a]pyrene, presumably via exposure to complex PAH mixtures (Tang et al. 2008, 2006; Perera et al., 2005a,b). The occurrence of BPDE-DNA in maternal and umbilical cord blood in conjunction with exposure to ETS was associated with reduced birth weight and head circumference in pregnant women living in the vicinity of fires from the 09/11/2001 disaster site in New York City (Perera et al., 2005a). In other studies, elevated levels of BPDE-DNA adducts in umbilical cord blood were associated with: (1) reduced birth weights or reduced head circumference in the offspring of 529 Dominican or African-American nonsmoking women (Perera et al., 2005b); and (2) decreased body weight at 18, 24, and 30 months and deficits in several areas of development as assessed by the Gesell Developmental Schedules at 24 months in the offspring of nonsmoking Chinese women living in the vicinity of a coal-fired power plant (Tang et al., 2008, 2006).

Developmental neurotoxicity

Studies in humans and experimental animals indicate that exposure to PAHs in general, and benzo[a]pyrene in particular, may impact neurological development at relatively low exposure levels. Observational studies in humans have suggested associations between gestational exposure to PAHs and later measures of neurodevelopment (Perera et al., 2009; Tang et al., 2008). An observational study of a Chinese population living in close proximity to a coal fired power plant found increased levels of benzo[a]pyrene-DNA adducts in cord blood were associated with decreased developmental quotients in offspring (Tang et al., 2008). In addition, a study of pregnant women living or working near the World Trade Center site in NYC found high PAH exposure during pregnancy was associated with a reduction in verbal and full scale IQ of offspring at 5 years of age (Perera et al., 2009).

A study in pregnant rats exposed by inhalation showed a dose related increase in benzo[a]pyrene metabolites in the cerebral cortex and hippocampus of pups, indicating the fetal brain is exposed to benzo[a]pyrene and/or its metabolites following maternal inhalation exposure (Wu et al., 2003). Another study which treated pregnant rat dams to benzo[a]pyrene by inhalation found a decrease in long term potentiation (LTP) in the hippocampus of gestationally treated pups compared with controls, indicating a possible effect on learning and memory in benzo[a]pyrene exposed animals, though functional tests were not conducted (Wormley et al., 2004). Another study by the same group treated rat dams by gavage with low levels of

benzo[a]pyrene (300 µg/kg) on GD 14-17 and observed benzo[a]pyrene metabolites in the brains of pups and diminished cortical neuronal activity to sensory input in exposed offspring compared with controls (McCallister et al., 2008). In addition, a study of mice exposed to benzo[a]pyrene lactationally observed statistically significant differences in performance in several neuromotor and behavioral tests which indicated decreased righting reflex and disinhibition behavior (Bouayed et al., 2009).

Reproductive effects

Epidemiological studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (El Nemr et al., 1998; Neal et al., 2005) and that prenatal exposure to cigarette smoking is associated with reduced fertility of women later in life (Weinberg et al., 1989). A case-control study in a Chinese population has also indicated that women with elevated levels of benzo[a]pyrene-DNA adducts in maternal blood were four times more likely to have experienced a missed abortion (Wu et al., 2010).

Oral multigenerational studies of benzo[a]pyrene exposure in mice demonstrate effects on fertility and the development of reproductive organs in male and female offspring exposed to benzo[a]pyrene during development at levels in which no overt toxicity or depression in fertility is seen in the parental animals (Mackenzie and Angevine 1981; Kristensen et al., 1995).

MacKenzie and Angevine (1981) exposed groups of mice to benzo[a]pyrene on GDs 7–16 and reproductive outcomes of the offspring were investigated. Fertility of the F1 generation was decreased in a dose dependant manner. At maturity, the fertility of these animals was tested. The F1 male and F1 female fertility indices were significantly decreased in each exposure group. These reductions in fertility indices were associated with decreased testes and ovary weight in the F1 animals. Male offspring showed histological damage of the seminiferous tubules and female offspring had hypoplastic ovaries with few follicles and corpora lutea. Similar results were reported in a study in which female mice were exposed by gavage to benzo[a]pyrene on GDs 7–16 (Kristensen et al., 1995). F1 females had decreased mean ovary weight and reduced fertility. At necropsy, the F1 females had reduced ovary weights with decreased numbers of small, medium, or large follicles and corpora lutea. Inhalation exposure of pregnant female rats to benzo[a]pyrene:CB aerosols during gestation has also been associated with decreased fetal survival and number of pups per litter associated with decreased levels of plasma progesterone, estradiol, and prolactin (Archibong et al., 2002).

These reductions in fertility observed in animal models are supported by a large database of animal studies in adult animals indicating that benzo[a]pyrene is ovotoxic with effects including decreased ovary weight, decreased primordial follicles, and reduced fertility (Mattison et al., 1980; Swartz and Mattison 1985; Miller et al., 1992; Borman et al, 2000).

Developmental Immune effects

The severity and persistence of immune effects observed during in utero studies suggests that immunotoxicity may be greater during gestation than adulthood (Dietert and Pieperbrink, 2006; Holladay and Smialowicz, 2000). Urso and Gengozian (1982) provide experimental support demonstrating immunosuppression from benzo[a]pyrene exposure during gestation was greater than for mice exposed after birth to a 25-fold higher dose. There is also substantial general literature indicating that disruption of the immune system during certain critical periods of development (e.g., initiation of hematopoiesis; migration of stem cells; expansion of progenitor cells) may have significant and lasting impacts on lifetime immune function (e.g. Burns-Naas et al., 2008; Dietert, 2008; Landreth et al., 2002; Dietert et al., 2000), as well as more specific studies showing increased dose sensitivity and disease persistence from developmental versus adult chemical exposure (reviewed in Luebke et al., 2006).

Thymus toxicity is a sensitive and specific effect of benzo[a]pyrene and has been observed in both prenatal and adult exposure studies. The thymus serves as a major site of thymocyte proliferation and selection for maturation, and impairment can lead to cell-mediated immune suppression (Kuper 2002, 1992; De Waal et al., 1997). The thymus is believed to be critical for T lymphocyte production during early life and not in adulthood (Hakim et al., 2005; Schonland et al., 2003; Petrie et al., 2002; Mackall et al., 1995). Therefore, the decreases in thymus weight observed in studies of adult animals exposed to benzo[a]pyrene suggest that immunosuppression may be a heightened concern for individuals developmentally exposed to benzo[a]pyrene.

Cancer

As mentioned above in section 4.7.3.4, investigations in young animals exposed to benzo[a]pyrene provide evidence that early life exposure may present increased risk of cancer. Comparisons of cancer responses in newborn (1 day old), infant (15 days old), and young adult (42 days old) mice indicate that exposure to benzo[a]pyrene during early life stages can present additional risk for cancer, compared with exposure during young adulthood (Vesselinovitch et al., 1975), but studies designed to compare risks of cancer from early-life (including gestational and pre-weaning) plus chronic adulthood exposures with risks from chronic adulthood exposure alone were not located. Following i.p. injection of single doses of 75 or 150 mg/kg benzo[a]pyrene to newborn (1 day old), infant (15 days old), or young adult (42 days old), newborn and infant mice more readily developed tumors than young adult mice in the liver and lung, the most predominant tissue sites of cancer development under these exposure conditions (Vesselinovitch et al., 1975). The benzo[a]pyrene-exposed groups also displayed increased incidences of stomach and lymphoreticular tumors, but the data indicated that these tumors developed more readily with exposure at 42 or 15 days, compared with exposure on PND 1 .

4.8.2. Possible Gender Differences

Cheng et al. (2007) had conducted a study in which they found that lung tumor tissue from nonsmoking females contained higher benzo[a]pyrene-DNA adduct levels than that from nonsmoking males. The adduct levels were associated with CYP1A1 protein levels in the same tissues. Female lung cancer tissue contains higher levels of DHH activity than male lung cancer tissue. DHH is an enzyme that can divert benzo[a]pyrene-7,8-dihydrodiol into the quinone pathway, thus preventing BPDE and DNA adduct formation. It is highly expressed in liver, but only weakly in lung. Cheng et al. (2007) decided to investigate benzo[a]pyrene-DNA adduct formation in several lung cancer cell lines to elucidate the roles of CYP1A1 and DHH in this process. They found that DNA adduct levels were increased in cell lines that contain elevated CYP1A1 and DHH isoform 1 activities. When DHH1 activity was blocked in these cells, DNA adduct levels were increased. Benzo[a]pyrene-DNA adduct levels in 120 lung tumor samples were associated with the protein levels of CYP1A1, but not DHH1. Comparing tumor tissues from both genders lung cancer patients they observed that a significantly higher percentage of female lung tumors had measurable CYP1A1 levels, but were negative for DHH1, compared with male tumors. The authors suggested that a gender difference in DHH1 activity was in part responsible for the increased lung tumor incidence in females.

Chang et al. (2007) conducted a study also based in the increased incidence of lung cancer in human females, but focused on the benzo[a]pyrene interaction with estrogen that result in elevated COX-2 expression. COX-2 (aka PHS-2) can activate the procarcinogen benzo[a]pyrene-7,8-dihydrodiol to BPDE. Human bronchial epithelial cells were treated with benzo[a]pyrene and/or 17β-estradiol. The combined, but not the individual treatments induced COX-2 expression. The authors considered their findings as mechanistic evidence towards understanding the gender difference in susceptibility towards benzo[a]pyrene.

Taioli et al. (2007) reviewed the evidence for a connection between MPO polymorphism and lung cancer (a more detailed overview of this study is given in Section 4.8.3.3). MPO converts benzo[a]pyrene metabolites into highly reactive epoxides and a known polymorphism in its promoter region is said to afford some protection from lung cancer. Several genetic variants of MPO are known, most of which result in deficiency of the enzyme. The MPO-*G/G* genotype (WT) is said to be associated, among others, with acute promyelocytic leukemia, aerodigestive tract cancer, coronary artery disease, early-onset multiple sclerosis, and an increased incidence of Alzheimer disease. A multi-study analysis was conducted after several epidemiologic studies had suggested an association between this gene polymorphism and lung cancer incidence. The data were stratified for ethnicity, age, gender, and smoking status but neither age nor gender showed any association for MPO polymorphism and lung cancer risk. The authors hypothesized that age- and gender-related associations with MPO genotype and lung cancer incidence, as had been observed in other studies, may be related to age- and gender-dependent smoking habits rather than to the gene polymorphism itself.

Mammary epithelium and tissues from the female genital tract have the ability to activate benzo[a]pyrene. Morris and Seifter (1992) made a strong case for benzo[a]pyrene as a potential causative agent in breast cancer, not only based on the anti-estrogenic action of benzo[a]pyrene but also on its tendency to accumulate in adipose and hence breast tissue. Jeffy et al. (2002) also pointed out that PAHs are risk factors for breast cancer. Because the populations in the studies presented in Section 4.1.4 were predominantly, if not exclusively males, data for breast or cervical cancers in relation to benzo[a]pyrene exposure are not available.

Gender differences in the response to benzo[a]pyrene have been demonstrated in numerous animal studies (Knuckles et al., 2001; Kroese et al., 2001; Ramesh et al., 2001a, 2000; Hood et al., 2000; Rodriguez et al., 1997; Weyand et al., 1994; Turusov et al., 1990; Weyand and Bevan, 1987). The differences ranged from variations in feed intake, with or without effects on body weight, to differences in disposition, all the way to different susceptibility towards benzo[a]pyrene-induced cancers. In some studies, females and males were dosed differently; in general, females were more resistant to benzo[a]pyrene toxicity than males. None of the studies presented cogent explanations for the observed differences.

In the 2-year bioassay by Kroese et al. (2001), female rats had fewer tumors of the forestomach and auditory canal, but more tumors of the liver, compared with males. There was a rather striking negative dose response for pituitary tumors in females (eight to zero tumors from control to highest dose), but not in males. Brune et al. (1981) used both sexes of animals in their study, but did not report their findings for the sexes separately, allowing the conclusion that no obvious sex differences were observed.

Soyka (1980) found that prenatal treatment of mice with benzo[a]pyrene affected response to an enzyme-inducing challenge with 3-MC in a gender-specific way when the animals were 3 months old. Female offspring of benzo[a]pyrene-treated mice had significantly elevated hepatic microsomal aminopyrine demethylase activity, while CYP450 levels were significantly lower in male offspring.

Sharma et al. (1997) specifically attempted to resolve the gender difference in cancer susceptibility of CD-1 mice. They focused on glutathione S-transferase π (GSTP) because it detoxifies BPDE. They noted that constitutive expression of GSTP in the liver of the male CD-1 mouse was higher than in the female and that GSTP activity was much more inducible by the antioxidant butylhydroxyanisole in the female than in the male mouse. They reported that female mice were more susceptible to the carcinogenic effect of benzo[a]pyrene than males, but only females could be partially protected from benzo[a]pyrene-induced carcinogenesis by the co-administration of butylhydroxyanisole. This is an indication that Phase II enzymes may play a role in the gender difference towards benzo[a]pyrene toxicity.

Martin et al. (2004) used a transgenic mouse model to address the question of gender differences. They used p53 heterozygous Tg.AC (v-Ha-ras) mice, a strain possessing a carcinogen-inducible ras oncogene, but only one functional p53 tumor suppressor gene. The

authors emphasized that these two mutations have been observed frequently in human tumors. Female and male animals received 20 mg/kg benzo[a]pyrene by gavage in corn oil twice weekly for 10 weeks. Eighteen weeks after termination of dosing, tissues were collected for histologic processing. There were evident differences in gender response (percent of total, female control/male control:female treated/male treated): mortality (60/60:70/40), thymus hyperplasia (9/30:27/10), bladder papilloma (0/0:0/13), hepatocyte vacuolization (54/92:86/55), hematopoietic cell proliferation in spleen (57/8:82/27), lymph node hyperplasia (0/0:0/33), and malignant lymphoma (0/0:7/36). Neoplasia of the forestomach did not show a gender difference. The focus of this study was evaluation of feed modifications in carcinogenesis studies (some of the animals were given N-acetyl cysteine in the feed); the authors did not speculate on the reasons for the gender differences. Wei et al. (2000) (see Section 4.1.4) observed that female lung cancer patients displayed less DNA repair capacity than males.

benzo[a]pyrene has also been described as an anti-androgen (see Sections 4.4.1.3 and 4.5.3) in animal studies. The AhR mediates anti-estrogenic effects of its ligands (see Section 4.6.3.2). The findings in animals—anti-estrogenic and potential protection from breast cancer—are at odds with postulates made for humans that benzo[a]pyrene may advance the development of breast cancer (Jeffy et al., 2002; Morris and Seifter, 1992). Li et al. (1999) reported that 41% of the samples of noncancerous breast tissue from breast cancer patients contained benzo[a]pyrene-like DNA adducts, while no such adducts were detected in tissues obtained from breast reduction surgery patients. Gender-specific expression of glycine N-methyltransferase (GNMT) has been shown for mice (Section 4.5.2) and might help to explain gender-related effects, including cancer formation, in this species. Applicability to human populations, however, has not been addressed.

4.8.3. Genetic Polymorphisms

The metabolic formation and subsequent binding to critical positions in DNA of ultimate carcinogenic forms of PAH are recognized as key mechanistic events in tumorigenesis. Increased PAH exposure concentrations are associated with increased levels of DNA adducts and other biomarkers in both target and surrogate tissues. Observed biomarker levels vary considerably even among persons with apparently comparable exposures (Garte et al., 2007; Perera and Weinstein, 2000). While laboratory variation and uncertain exposure estimates contribute to the differences, inter-individual variation in PAH metabolism (activation and detoxification) may be especially important. In particular, heritable metabolic gene (single nucleotide polymorphisms [SNPs]) appear in some cases to influence individual susceptibility to specific types of cancer, in addition to factors such as ethnicity, age, gender, nutrition, hormonal and immune status, and preexisting health impairment.

In humans, benzo[a]pyrene is metabolized to the highly DNA-reactive BPDE by microsomal CYP Phase I enzymes, mediated primarily by the CYP1A1 and CYP1B1 genes.

Polymorphic variations in the human population that result in high inducibility of CYP1A1, CYP1B1, and other CYPs have been implicated as determinants of increased lung cancer risk in some studies (Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). The Phase II cytosolic GST, mediated by variants of the GSTM1 and GSTT1 genes, prevent the formation of BPDE-DNA adducts. Some evidence suggests that humans lacking a functional GST gene have higher PAH(BPDE)-DNA adduct levels and thus be at greater risk for cancer (Vineis et al., 2007a; Pavanello et al., 2004; Alexandrov et al., 2002; Perera and Weinstein, 2000). Similarly, acquired deficiencies or inherited gene polymorphisms that affect the efficiency or fidelity of DNA repair may also influence individual susceptibility to cancer (Matullo et al., 2003; Shen et al., 2003; Cheng et al., 2000; Perera and Weinstein, 2000; Wei et al., 2000; Amos et al., 1999). In general, however, support for the role of SNP in significantly modulating PAH cancer risk is relatively weak (i.e., small increases in cancer risk) or inconsistent, possibly due in part to small study size and the use of DNA-adduct detection methods with low specificity (i.e., bulky DNA-adducts) and sensitivity. Combinations of metabolic polymorphisms, on the other hand, are receiving increased attention as critical determinants of cumulative DNA-damaging dose, and thus individual cancer risk.

Following a report from Japan that the GSTM1 null genotype combined with a mutated CYP1A1 genotype was associated with increased lung cancer risk, Rojas and coworkers (2000) measured specific BPDE-DNA adducts in leukocytes (HPLC with fluorometric detection) to evaluate the impact of CYP1A1, GSTM1, and GSTT1 genotype combinations. The human subjects were 89 PAH-exposed coke oven workers (smokers and nonsmokers) and 44 power plant workers (all smokers) not occupationally exposed to PAH. Increased adduct levels were significantly correlated with CYP1A1 polymorphism, occupational PAH exposure, and smoking. Combinations of genotypes were observed to have a significant impact on BPDE-DNA adducts, ranging from the absence of adducts in subjects with the active CYP1A1/GSTM1 genotypes to the highest BPDE-DNA adduct level in the most susceptible combination of mutated CYP1A1 with null GSTM1 genotype. The results provide mechanistic support for distinguishing high-susceptibility benzo[a]pyrene-exposed subgroups, and for understanding their association with increased cancer rates.

Pavanello et al. (2005) studied associations between xeroderma pigmentosum (XP)-linked gene polymorphisms, the GSTM1 polymorphism, and bulky BPDE-type DNA adduct formation in peripheral lymphocytes from 67 highly PAH-exposed male Polish coke oven workers. The four XP genotypes studied impart low NER capacity, while the GSTM1 active or null genotypes are associated with effective or ineffective removal of biologically active benzo[a]pyrene metabolites via GSH conjugation. Workers were questioned for smoking habits, charbroiled meat consumption, and other factors that might have affected their PAH exposure. PAH exposure was assessed via urinary 1-OH-pyrene levels. There was a statistically significant difference in the number of DNA adducts between the GSTM1 active and null carriers (3.37 ±

2.20 vs. 6.73 ± 6.61 adducts per 10^8 nucleotides, respectively). For the DNA repair gene polymorphisms, there was an increase in DNA adduct numbers from homozygous WT carriers to low-DNA-repair homozygous variant carriers. This difference was statistically significant for homozygous carriers of the XPC-PAT and XPA-A23G variants, but not for the homozygous XPD-Lys751Gln and XPD-Asp312Asn variants. Individuals with a combination of DNA repair-unfavorable XPC or XPA genotypes and the GSTM1-null variant generally fell into the highest tertile of DNA adduct numbers. Smoking status and diet did not influence urinary 1-OH-Py or BPDE-DNA adduct levels. These results support the conclusion that certain gene polymorphism combinations affecting DNA repair or detoxification capacities may increase health risks resulting from PAH exposure.

Porter et al. (2005) also evaluated the influence of XPA gene variants involved in NER on BPDE-induced cytotoxicity. SV40-transformed human skin fibroblasts from an XP patient with a nonsense XPA mutation were stably transfected with the WT XPA gene or either of two rare XPA variants; the transfected genes could be overinduced with ponasterone A. The WT XPA and both variants had greatly improved survival compared to XPA-free cells. Survival was even more improved by ponasterone A induction in the variant, but not the WT cells. These findings indicate that the polymorphic XPAs show greater ability to repair BPDE-induced DNA damage, and thus may offer some protection from benzo[a]pyrene-induced genotoxicity, while the nonsense mutation is likely to increase genotoxic risk.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

There are limited data establishing associations between increased risk for noncancer health effects in humans and exposure to benzo[a]pyrene. Several epidemiology studies have reported associations between adverse birth outcomes including reduced birth weight, postnatal body weight, and head circumference and internal biomarkers of exposure to benzo[a]pyrene (BPDE-DNA adducts) via exposure to complex PAH mixtures (Tang et al., 2008, 2006; Perera et al., 2005a, b). However, extrapolations from these studies are complicated by the concomitant exposure to multiple PAHs and other components in the mixture. Thus, studies in humans were not selected to serve as the basis of the RfD.

The subchronic and chronic oral exposure animal database includes a 2-year gavage cancer bioassay with male and female Wistar rats (Kroese et al. 2001), a 2-year dietary cancer bioassay with female B6C3F_1 mice (Beland and Culp, 1998; Culp et al., 1998), a 90-day gavage study with male and female Wistar rats (Kroese et al., 2001), a 90-day dietary study with male and female F344 rats (Knuckles et al., 2001), and a 35-day study in male Wistar rats evaluating immune endpoints (De Jong et al., 1999). Also available are five reproductive/developmental toxicity studies in rodents examining reproductive endpoints in male Sprague-Dawley rats (Zheng et al., 2010) and C57BL/6 mice (Mohamed et al., 2010), in offspring of treated CD-1 and NMRI female mice (Kristensen et al., 1995; MacKenzie and Angevine, 1981), and in female Sprague-Dawley rats (Xu et al., 2010).

Kroese et al. (2001) exposed Wistar rats to benzo[a]pyrene in soybean oil by gavage at doses of 0, 3, 10, or 30 mg/kg-day, 5 days/week, for 2 years. This study was primarily designed as a cancer bioassay and did not evaluate other endpoints. An increase in the incidence of animals with forestomach hyperplasia, compared with the control incidence, occurred at the low and mid-dose but not the high-dose level; an dose-related, increased incidence of forestomach tumors was observed at doses $\geq$ 3 mg/kg-day. An increased incidence of hepatic clear cell foci of cellular alteration was also observed at the 3 mg/kg-day, but not at the 10 or 30 mg/kg-day. At the two highest exposure levels, elevated incidences of liver tumors were observed.

Female B6C3F_1 mice were administered benzo[a]pyrene in the diet at average daily doses of 0, 0.7, 3.3, and 16.5 mg/kg-day for 2 years (Beland and Culp, 1998; Culp et al., 1998). An increase in the incidence of mice with forestomach hyperplasia, compared with the control incidence, occurred at the lowest exposure level (23/47 at 0.7 mg/kg-day versus 13/48 in controls). Similar to the rat bioassay (Kroese et al., 2001), forestomach hyperplasia was observed with increasing incidence of animals with forestomach tumors (squamous cell

papillomas or carcinomas) with increasing dose. No other dose-related effects were reported in this cancer bioassay.

A 90 day study also reported by Kroese et al. (2001) treated animals by gavage 5 days/week with 0, 3, 10, or 30 mg/kg benzo[a]pyrene in corn oil. The most sensitive effects observed included increased liver weight and decreased thymus weight. Increases in liver weight greater than 10% of controls were observed at 10 and 30 mg/kg-day in males only, and were statistically significant. However, there were no statistically significant elevations in liver enzymes screened in serum (ALT, AST, LDH, and GGT). The biological significance of increased liver weight in males in the absence of elevated liver enzymes in the serum is unclear. A decrease in thymus weight was observed in both sexes at 30 mg/kg-day, at 17 and 33% in females and males, respectively, compared with controls. At 10 mg/kg-day, thymus weight in males was decreased by 15% (not statistically significant). An increase in the incidence of thymus atrophy was also observed in the 30 mg/kg-day males that showed a reduction of thymus weight. Incidences for thymus atrophy (categorized in severity as slight) for the control through high-dose groups were 0/10, 0/10, 0/10, and 3/10 for females and 0/10, 2/10, 1/10, and 6/10 for males. The thymus is an organ involved in the maturation of immune cells especially early in development. A change in thymus weight in the adult animal may be accompanied by alterations of the immune system in functional assays, but the significance of thymus weight changes alone is unknown.

Knuckles et al. (2001) exposed male and female F344 rats (6-8 per group) to benzo[a]pyrene at doses of 0, 5, 50, or 100 mg/kg-day in the diet for 90 days. Statistically significant decreases in RBC counts and hematocrit level (decreases as much as 10 and 12%, respectively) were observed in males at doses ≥50 mg/kg-day and in females at 100 mg/kg-day. The effect observed in this study at the lowest dose was an increase in abnormal tubular casts in the kidney in males in which increases were observed at 5 mg/kg-day (40%), 50 mg/kg-day (80%) and 100 mg/kg-day (100%), compared to 10% in the controls. In females, only 10% showed significant kidney tubular changes at the two high dose levels compared to zero incidence in controls. The incidences for kidney lesions were not provided; instead the data are reported graphically as rounded percent incidences. Several reporting gaps in Knuckles et al (2001) make interpretation of the results difficult. Specifically, the authors do not provide statistical analysis of the renal endpoint nor do they provide the incidence data which would allow for independent statistical analysis. The study author was contacted, but additional clarification of the study data was not provided. Therefore, due to reporting gaps and resulting reduced confidence, this study was not considered further in selecting the principal study.

De Jong et al. (1999) treated male Wistar rats (eight/dose group) with benzo[a]pyrene by gavage 5 days/week for 35 days at doses of 0, 3, 10, 30, and 90 mg/kg-day. Hematological and immunological changes were reported. Small, but statistically significant, dose-related decreases in RBC count (5%) and associated measures (hemoglobin, and hematocrit) were observed at

≥10 mg/kg-day. In addition, a dose-related and statistically significant decrease in the relative number of B cells (13%) in the spleen was observed at ≥10 mg/kg-day compared to controls. Dose-related decreases in thymus weight were statistically significant at ≥10 mg/kg-day. Decreases in heart weight at 3 mg/kg-day and in kidney weight at 3 and 30 mg/kg-day were also observed, but these changes did not show dose-dependent responses. At doses above 10 mg/kg-day, significant decreases were observed in absolute number of cells harvested in the spleen, in the number of B cells in the spleen, and in NK cell activity in the spleen, as well as a decrease in serum IgM and IgA in rats.

Zheng et al., (2010) treated male Sprague-Dawley rats (8/group) to 0, 1, or 5 mg/kg-day benzo[a]pyrene by daily corn oil gavage for a duration of 30 or 90 days. Testicular testosterone was statistically significantly decreased in the high dose group (approximately 15%) following 90 days of exposure. The low dose group also appeared to have a similar average depression of testosterone levels; however, the change did not reach statistical significance.

Mohamed et al. (2010) investigated multi-generational effects in male mice following exposure of six-week old C57BL/6 mice (10/group) to 0 (corn oil), 1, or 10 mg/kg-day benzo[a]pyrene for 6 weeks by daily corn oil gavage. Following final treatment, male mice were mated with two untreated female mice to produce an F1 generation; F1 and F2 males were also mated with untreated female mice. The mice of the F1, F2, and F3 generations were not exposed to benzo[a]pyrene. Statistically significant reductions of approximately 50% were observed in epididymal sperm counts of F0 and F1 generations treated with the low dose of benzo[a]pyrene. For F0 and F1 generations of the high dose group, epididymal sperm counts were reduced approximately 70%. Means and variances were not reported but were presented graphically. This study indicates that exposure to benzo[a]pyrene may have transgenerational effects on sperm count. However, due to incomplete reporting, this study was not considered further for selection as the principal study but was considered to be supportive of low dose male reproductive effects following benzo[a]pyrene exposure.

MacKenzie and Angevine (1981) exposed groups of 30–60 female CD-1 mice to 0, 10, 40, or 160 mg/kg-day benzo[a]pyrene on GDs 7–16. Crossover mating studies were then conducted in which F1 offspring were mated continuously with untreated mice to determine effects on fertility. Benzo[a]pyrene did not appear to be overtly toxic to mothers or offspring. However, statistically significant decreased pup weight was observed at all dose levels at day 42. At the lowest dose tested, pup weight was decreased 6% compared to control. At maturity, the fertility of these animals was tested. The F1 male and F1 female fertility indices (i.e., percent of mated animals that were pregnant) were significantly decreased in each exposure group as follows (control through high-dose groups): F1 males: 80.4, 52.0, 4.7, and 0.0; F1 females 100, 65.7, 0.0, and 0.0. After six months on the breeding study, 34% of the gestationally treated females in the 10 mg/kg-day dose group failed to produce any litters, and the the F1 females in this dose group that did litter produced statistically significantly smaller litter sizes (19%

reduction in mean litter size). These reductions in fertility indices were associated with decreased testes and ovary weight in the F1 animals. Testes weight was decreased 40 and 88% at 10 and 40 mg/kg-day, respectively, and was associated with histologic evidence of injury to the seminiferous tubules. In female F1 animals, severe reductions in ovarian tissues were observed at all dose levels such that ovary weight measurements were difficult to obtain. Examination of available tissue in these females revealed hypoplastic ovaries with few follicles and corpora lutea (10 mg/kg-day) or with no evidence of folliculogenesis at the higher dose (40 mg/kg-day).

Similar results were reported in a study in which groups of nine NMRI F0 female mice were exposed by gavage to 0 or 10 mg/kg-day benzo[a]pyrene on GDs 7–16 (Kristensen et al., 1995). F1 females were continuously bred with an untreated male for 6 months. F1 females had decreased mean ovary weight (30% decreased, compared with controls) and reduced fertility as reflected by decreased mean number of F2 litters (three compared with eight for control F1 females). F1 females had statistically significantly lower median numbers of offspring, number of litters, and litter sizes and a statistically significantly greater median number of days between litters as compared with the controls. At necropsy, the F1 females had statistically significantly reduced ovary weight with histologic examination revealing decreased numbers of small, medium, or large follicles and corpora lutea.

Xu et al., (2010) treated female Sprague-Dawley rats (6/group) to 0, 5, or 10 mg/kg-day benzo[a]pyrene by corn oil gavage every other day for a duration of 60 days. This resulted in time weighted average doses of 0, 2.5, and 5 mg/kg-day over the study period of 60 days. Absolute ovary weight was statistically significantly reduced in the both the low and high benzo[a]pyrene dose groups (11 and 15%, respectively; see Table 5-1). Animals in the high dose group also had statistically significantly depressed levels of estradiol (by approximately 25%) and decreased numbers of primordial follicles (by approximately 20%) compared to controls. Statistically significantly altered estrus cyclicity was also evident in the high dose of benzo[a]pyrene.

Table 5-1. Means ± SD for ovary weight in female SD-rats

	Dose (mg/kg-d)[a]		
	0	**2.5**	**5**

Ovary weight (g)	0.160 ± 0.0146	0.143 ± 0.0098[b]	0.136 ± 0.0098[b]
Body weight (g)	261.67 ± 12.0	249.17 ± 11.2	247.25 ± 11.2

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

Source: Xu et al. (2010).

5.1.2. Methods of Analysis

A number of noncancer effects observed following chronic or subchronic administration of benzo[a]pyrene were modeled with U.S. EPA's Benchmark Dose (BMD) Modeling Software (BMDS) where data were amenable. These endpoints included increased liver weight, decreased thymus weight, decreased percent of splenic B-cells, increased forestomach hyperplasia, and decreased ovary weight (Xu et al., 2010; Kroese et al., 2001; De Jong et al., 1999). Zheng et al. (2010) did not provide enough information (i.e., incidences or means and variances) to allow for BMD modeling. Data from other studies could not be modeled due study design utilizing only one dose (Kristensen et al., 1995) or a highly elevated magnitude of response at the lowest dose (MacKenzie and Angevine 1981) in which extrapolation down to a suitable benchmark response would be unsupported by the available models.

In accordance with U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), the BMD and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 1 standard deviation (SD) from the control mean for continous data or a BMR of 10% extra risk for dichotomous data in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across endpoints and assessments. A summary of modeling results for each endpoint is listed below in Table 5-2. Further details including the output and graph for the best fit model can be found in Appendix B. In general, model fit was assessed by a chi-square goodness-of-fit test (i.e., models with $p < 0.1$ failed to meet the goodness-of-fit criterion) and the Akaike Information Criterion (AIC) value (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint). Of the models exhibiting adequate fit, the model yielding the lowest AIC value was selected as the best-fit model. (U.S. EPA, 2000b).

For the forestomach hyperplasia endpoint, all data sets provided adequate descriptions of the dose-response relationship from chronic oral exposure to benzo[a]pyrene, but at the highest dose level for the rats (Kroese et al., 2001), the incidence of forestomach hyperplasia was not increased relative to controls. It is possible that the forestomach hyperplasia observed following benzo[a]pyrene exposure may be a precursor to the development of forestomach tumors, but specific data supporting this conclusion are unavailable. Regardless, the male and female data

sets in rats (Kroese et al., 2001) were modeled without the data from the highest dose group due to the nonmontonic increase in response to increasing dose (Kroese et al., 2001).

Points of departure (PODs) for endpoints that were not amenable to BMD modeling were identified using a NOAEL/LOAEL approach (Zheng et al., 2010; Kristensen et al., 1995; MacKenzie and Angevine, 1981). A LOAEL of 5 mg/kg-day was identified for Zheng et al. (2010) for significantly descreased testicular testosterone. A LOAEL of 10 mg/kg-day was identified as the POD for Mackenzie and Angevine for decreased postnatal body weight and decreased fertility of male and female mice treated during gestation. A POD based on the LOAEL of 10 mg/kg/day was established from Kristensen et al. (1995) based on decreased fertility and decreased ovary weight in female mice treated during gestation.

Table 5-2. Summary of BMDs and BMDLs for modeled noncancer effects following oral exposure								
Endpoint/data	**Exposure duration**	**BMR**	**Fitted model**	**Goodness-of-fit *p*-value**	**AIC**	**BMD (mg/kg-d)**	**BMDL (mg/kg-d)**	**Reference**
Increased Liver Weight in Male Wistar Mice	90 d	10%	**Linear (1° polynomial), Power**	**0.58**	**49.51**	**8.11**	**5.8**	Kroese et al., 2001
			Polynomial (2°)	0.74	50.53	4.53	2.29	
			Hill	0.82	50.48	4.1	1.24	
Decreased Thymus Weight in Male Wistar Mice	90 d	1 SD	**Linear , Polynomial (2°), Power (nonconstant variance)**	**0.23**	**380.71**	**16.40**	**11.30**	Kroese et al., 2001
			Hill (nonconstant variance)	NA				
Decreased Thymus Weight in Female Wistar rats	90 d	1 SD	**Linear**	**0.81**	**349.12**	**10.52**	**7.64**	Kroese et al., 2001
			Hill	NA				
			Polynomial (2°)	0.77	350.80	13.29	7.77	
			Power	NA				
Decreased Thymus Weight in Male Wistar Mice	35 d	1 SD	**Linear , Polynomial (2°)**	**0.52**	**381.41**	**14.41**	**11.58**	De Jong et al., 1999
			Hill	0.42	382.91	11.15	6.19	
			Power	NA				
Decreased Splenic B-cells in Male Wistar rats	35 d	1 SD	**Linear, Polynomial (2°), Power (constant variance)**	**0.21**	**145.28**	**15.58**	**12.43**	De Jong et al., 1999
			Hill (constant variance)	0.18	146.18	10.24	5.31	
Increased Forestomach Hyperplasia[a] in Male Wistar Rats	2 yrs	10%	**Log-logistic**	**0.13**	**112.27**	**5.31**	**2.39**	Kroese et al., 2001
			Gamma, Multistage, Weibull	0.12	112.37	5.63	2.67	
			Logistic	0.09	112.93	7.25	4.35	
			LogProbit	0.06	113.88	8.36	4.52	
			Probit	0.10	112.87	7.09	4.13	
Increased Forestomach Hyperplasia[a] in Female	2 yrs	10%	**Log-logistic**	**0.32**	**117.04**	**2.15**	**1.35**	Kroese et al., 2001
			Logistic	0.06	120.02	4.23	3.28	

Wistar Rats			LogProbit	0.02	121.13	3.91	2.57	
			Gamma, Multistage, Weibull	0.24	117.42	2.40	1.59	
			Probit	0.06	119.74	3.99	3.06	
Increased Forestomach Hyperplasia in Female $B6C3F_1$ mice	2 yrs	10%	**Log-logistic**	**0.21**	**193.3**	**0.329**	**0.115**	Beland and Culp, 1998
			Logistic	0.06	194.7	0.757	0.545	
			LogProbit	0.29	192.1	0.670	0.448	
			Gamma, Multistage, Weibull	0.42	191.3	0.421	0.295	
			Probit	0.03	196.6	0.946	0.711	
Decreased Ovary Weight in Female Sprague-Dawley Rats	60 d	1 SD	**Linear , Polynomial (1°)**	**0.39**	**-138.67**	**2.3**	**1.5**	Xu et al., 2010
			Power	NA				

[a] The best fit of each model considered is summarized. For continuous models (linear, polynomial, power, Hill), if an adequate required including a variance model, only the results including modeled variance are summarized and the use of nonconstant variance is indicated; otherwise constant variance was assumed. Details in Appendix B.
[b]Data for the high-dose group were excluded from the modeled dataset due to a decreased incidence judged to be due to competing effects masking the response.
NA = not applicable, model failed

Several candidate principal studies (Kroese et al., 2001; De Jong et al., 1999; Zheng et al., 2010; Kristensen et al., 1995; MacKenzie and Angevine, 1981) reported liver, thymus, immune, and reproductive effects at higher doses relative to the ovary and forestomach effects (reported by Kroese et al., 2001; Beland and Culp, 1998) and were considered less sensitive measures of benzo[a]pyrene effects. Forestomach hyperplasia was not selected as the critical effect, even though it was observed at lower doses compared with other effects, based on the consideration that the reproductive and fertility effects, observed in animals and supported by human data, appear to better characterize noncancer low dose effects of BaP. Specifically, the Xu et al., (2010) study was chosen as the principal study and decreased ovarian weight as the critical effect for the derivation of the RfD. This study identified biologically and statistically significant decreases in ovary weight, estrogen, and primordial follicles, and altered estrus cycling in treated animals. These reductions in female reproductive parameters are supported by a large database of animal studies indicating that benzo[a]pyrene is ovotoxic with effects including decreased ovary weight, decreased primordial follicles, and reduced fertility (Mattison et al., 1980; MacKenzie and Angevine 1981; Swartz and Mattison 1985; Miller et al., 1992; Kristensen et al., 1995; Borman et al, 2000). Additionally, studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (El Nemr et al., 1998; Neal et al., 2005). Specific associations have also been made between infertility and increased levels of benzo[a]pyrene in follicular fluid in women undergoing in vitro fertilization (Neal et al., 2008).

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

Of the endpoints discussed in section 5.1.1., decreased ovary weight in female rats ($BMDL_{1SD}$ of 1.5 mg/kg-day) reported by Xu et al. (2010) was selected to serve as the critical effect for the RfD. A total UF of 3000 was applied to the POD of 1.5 mg/kg-day to account for several areas of uncertainty.

An UF_A of 10 was applied to account for toxicokinetic and toxicodynamic differences associated with extrapolation from animals to humans. The available data do not provide quantitative information on the difference in susceptibility to benzo[a]pyrene between rats and humans.

An UF_H of 10 was applied to account for variability in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to quantitatively estimate variability in human susceptibility to benzo(a)pyrene.

An UF_S of 10 was applied for the extrapolation of subchronic-to-chronic exposure duration. The 60-day study by Xu et al. (2010) falls well short of a lifetime duration. Therefore, it is unknown whether effects would be more severe or would be observed at lower doses with a longer exposure duration.

An UF_L of 1 was applied for LOAEL-to-NOAEL extrapolation because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 1 SD change from the control mean in ovary weight was selected under an assumption that it represents a minimal biologically significant response level.

An UF_D of 3 was applied to account for deficiencies in the benzo[a]pyrene toxicity database. Limited observational studies in humans have suggested associations between biomarkers of internal dose of benzo[a]pyrene and adverse birth outcomes (including reduced birth weight, postnatal body weight, head circumference, and neurodevelopment) and decreased fertility (Edwards et al., 2010; Neal et al., 2008; Tang et al., 2008, 2006; Perera et al., 2009; 2005a, b). However, the likely contribution of multiple exposure routes in these studies make extrapolation to exposure concentrations uncertain. Several animal studies exist for benzo[a]pyrene to inform noncancer effects, including subchronic oral toxicity studies in rats and mice, and two developmental studies and several reproductive studies in mice and rats. The lack of a standard multigenerational study (specifically, one which includes exposure from pre-mating to lactation) is a data gap, especially considering benzo[a]pyrene has been shown to affect fertility in adult male and female animals by multiple routes of exposure (Mohamed et al., 2010; MacKenzie and Angevine 1981; Kristensen et al., 1995; Archibong et al., 2008; Borman et al., 2000; Swartz and Mattison 1985). In addition, the lack of a study examining functional neurological endpoints following in utero exposure is also a data gap considering the available epidemiological evidence showing the association of in utero PAH exposure and indicators of decreased neurological development (Edwards et al., 2010, Perera et al., 2009, Tang et al., 2008). Therefore, an UF of 3 was applied to the POD for the lack of a standard multigenerational reproductive toxicity study and a neurodevelopmental study.

The RfD for benzo[a]pyrene was calculated as follows:

$$\begin{aligned} \text{RfD} &= BMDL_{1SD} \div UF \\ &= 1.5 \text{ mg/kg-day} \div 3000 \\ &= 0.0005 \text{ mg/kg-day} \end{aligned}$$

5.1.4. Previous RfD Assessment

No RfD was derived in the previous IRIS assessment.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

The only chronic inhalation study available for benzo[a]pyrene was designed as a cancer bioassay and did not report noncancer endpoints (Thyssen et al.1981). However, several repeat

dose reproductive and developmental toxicity studies are available in which effects on fetal survival and the male reproductive system have been observed.

Reproductive system variables were adversely impacted in male F344 rats (10/group) exposed to benzo[a]pyrene aerosols at 75 μg/m^3 for 60 days (Archibong et al., 2008; Ramesh et al., 2008). The testes from exposed rats weighed 34% less than those from unexposed controls. Treatment with benzo[a]pyrene was also associated with reductions (compared with control values) in total tubular volume and weight (~20%) and tubular length (~40%); total weight and volume of interstitium per paired testis (12%); and percentage of progressively motile stored spermatozoa, stored sperm density, and percentage of morphologically normal sperm (~70–80%). Daily sperm production and levels of circulating and intratesticular testosterone were also decreased in treated rats (by about 50, 70, and 80% respectively), compared with controls. Luteinizing hormone levels were 50–60% higher in treated rats than controls 72 hours after exposure.

In a developmental study, timed-pregnant F344 rats (10/group) were exposed to benzo[a]pyrene on GDs 11–21 as a carbon black aerosol at 100 μg/m^3 to assess neurological endpoints in offspring during PNDs 60–70 (Wormley et al., 2004). Pups of the F1 generation were weaned on PND 30 and tested for long-term potentiation electrophysiological responses in the hippocampus during PNDs 60–70. Although the number of implantation sites in treated rats was within 1% of unexposed controls, the percentage of pups born relative to recorded implantation sites in each dam (the birth index) was reduced by 65% in treated rats compared with unexposed controls. In addition, protein levels of NMDA receptor subunit 1 were down-regulated (by 18% on PND 10 and 67% on PND 30) in the hippocampus of benzo[a]pyrene-exposed F1 pups, and the magnitude of the long-term potentiation response across the perforant path-granular cells in the hippocampus of F1 rats was consistently weaker than the response observed for the controls (about 25% weaker), suggesting that exposure to benzo[a]pyrene via the inhalation route attenuates the capacity for long-term potentiation in the F1 generation. However, no functional tests to assess neurotoxicity were conducted in this study.

In another developmental toxicity study, timed-pregnant F344 rats (10/group) were exposed to benzo[a]pyrene aerosols at concentrations of 25, 75, or 100 μg/m^3 on GDs 11–20 and evaluated for post-implantation fetal survival and hormone levels associated with pregnancy (Archibong et al., 2002). The total number of implantation sites in treated rats was within 5% of the values obtained for sham-exposed and unexposed controls. However, dose-dependent trends were observed for decreased numbers of pups per litter and percent fetal survival per litter with increasing benzo[a]pyrene concentrations. The number of pups/litter was decreased by approximately14, 50, and 65% at 25, 75, and 100 μg/m^3, respectively, compared with sham-exposed and unexposed controls. Percent survival/was similarly reduced by about 20, 60, and 65%, respectively, at the same exposure concentrations. In addition, biologically significant decreases in pup weights (presented as g/litter) were observed at concentrations ≥75 μg/m^3 (14

and 16% decreases at 75 and 100 μg/m^3, respectively). Levels of plasma progesterone, estradiol-17β, and prolactin on GD 17 were decreased by about 12, 14, and 35%, respectively, at 25 μg/m^3 and 17, 60, and 70%, respectively, at 75 μg/m^3 compared with respective controls.

The study by Archibong et al. (2002) was selected as the principal study as it observed biologically significant effects at the lowest dose tested by the inhalation route. This study indicates that the developing fetus is a sensitive target following inhalation exposure to benzo[a]pyrene. A LOAEL of 25 μg/m^3 was identified based on exposure to benzo[a]pyrene on GDs 11–20 that caused biologically significant reductions in fetal survival and body weight decreases in the surviving pups (see Table 5-3). The observed decrease in pup weight and fetal survival were selected as critical effects as they are the most sensitive noncancer effects observed following inhalation exposure to benzo[a]pyrene. Though only a few studies exist which evaluate benzo[a]pyrene by the inhalation route, additional support for this endpoint can be found from oral studies of benzo[a]pyrene. A developmental/reproductive study conducted via the oral route in mice observed decreased survival of litters, decreased pup weight, and decreased reproductive organ weight following in utero exposure to benzo[a]pyrene on GD 7-16 (MacKenzie and Angevine, 1981).

5.2.2. Methods of Analysis- Adjustment to a Human Equivalent Concentration (HEC)

By definition, the RfC is intended to apply to continuous lifetime exposures for humans (U.S. EPA, 1994). EPA recommends that adjusted continuous exposures be used for inhalation developmental toxicity studies as well as for studies of longer durations (U.S. EPA, 2002). The LOAEL of 25 μg/m^3 based on decreased pup weight and fetal survival reported in the developmental study by Archibong et al. (2002) was selected to serve as the POD.

Table 5-3. Pregnancy outcomes in female F344 rats treated with benzo[a]pyrene on GDs 11–21 by inhalation

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

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

Source: Archibong et al. (2002).

Data for decreased pup survival were not amenable to BMD modeling due to the pattern of variability (heterogeneous variances) in the data set. Therefore, the LOAEL from this study was used as the POD. The LOAEL from this study is based on a 4 hour exposure of pregnant rats to 25 μg/m^3 benzo[a]pyrene on GDs 11-20. This concentration was adjusted to account for the discontinuous daily exposure as follows:

POD_{ADJ} = POD x hours exposed per day/24 hours
= LOAEL x (4 hr/24 hr)
= 25 μg/m^3 x 4/24
= 4.2 μg/m^3

The human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by a dosimetric adjustment factor (DAF), which, in this case, was the regional deposited dose ratio ($RDDR_{ER}$) for extrarespiratory (i.e. systemic) effects as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). The observed developmental effects are considered systemic in nature (i.e., extrarespiratory) and the current normalizing factor for extrarespiratory effects of particles is body weight. In the case of benzo[a]pyrene, the $RDDR_{ER}$ was calculated as follows:

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

where:
BW = body weight (kg)
V_E = ventilation rate (L/min)
F_{TOT} = total fractional deposition

The total fractional deposition (F_{TOT}) includes particle deposition in the nasal-pharyngeal region, the tracheobronchial region, and the pulmonary region. F_{TOT} for both animals and humans was calculated using the Multi-Path Particle Dosimetry model, a computational model that can be used for estimating human and rat airway particle deposition and clearance (MPPD; Version 2.0 © 2006, publicly available through the Hamner Institute). The F_{TOT} was calculated based on the average particle size of 1.7 ± 0.085 (mass median aerodynamic diameter ± geometric standard deviation) as reported in the description of particle generation methods in Ramesh et al. (2000). For the model runs, the Yeh-Schum 5-lobe model was used for the human and the asymmetric multiple path model was used for the rat (see Appendix C for MPPD model output). Both models were run under nasal breathing scenarios with the inhalability adjustment selected. A geometric standard deviation (GSD) of 1 was used as the default by the model because the reported GSD of 0.085 ≤ 1.05.

The human parameters used in the model for calculating F_{TOT} and in the subsequent calculation of the POD_{HEC} were as follows: human - BW, 70 kg; V_E, 13.8 L/min; breathing frequency, 16 per minute; tidal volume, 860 mL; FRC (functional residual capacity), 3300 mL; and URT (upper respiratory tract) volume, 50 mL. Although the most sensitive population in the principal study is the developing fetus, the adult rat dams were exposed. Thus, adult human parameters were used in the calculation of the HEC to extrapolate from a pregnant rat to a pregnant human. The parameters used for the rat were BW, 0.25 kg (based on the approximate weight of a 100 day-old, female timed-pregnant Sprague-Dawley rat); V_E, 0.18 L/min; breathing frequency, 102 per minute; tidal volume, 1.8 mL; FRC (functional residual capacity), 4 mL; and URT (upper respiratory tract) volume, 4.42 mL. All other parameters were set to the default value (see Appendix C).

Under these conditions, the MPPD model calculated F_{TOT} values of 0.621 for the human and 0.181 for the rat. Using the above equation, the $RDDR_{ER}$ was calculated to be 1.06. From this, the POD_{HEC} was calculated as follows:

$$POD_{HEC} = POD_{ADJ} \times RDDR_{ER}$$
$$POD_{HEC} = 4.2\ \mu g/m^3 \times 1.1$$
$$POD_{HEC} = 4.6\ \mu g/m^3$$

5.2.3. RfC Derivation- Including Application of Uncertainty Factors (UFs)

The critical effect for the derivation of the RfC was identified as decreased fetal survival and decreased pup weight associated with inhalation exposure to pregnant rats on GDs 11–20. The LOAEL for decreased fetal survival was adjusted to a continuous human equivalent concentration and used as the POD for the derivation of the RfC. A total UF of 1000 was applied to the POD_{HEC} to account for four main areas of uncertainty:

A UF_A of 3 was applied to account for uncertainties in extrapolating from rats to humans. Application of a UF of 10 encompasses two areas of uncertainty: toxicokinetic and toxicodynamic uncertainties. In this assessment, the toxicokinetic component is mostly addressed by the determination of a HEC as described in the RfC methodology (U.S. EPA, 1994b). Therefore, a UF of 3 was applied to account for the remaining toxicodynamic uncertainties in the extrapolation from rats and humans.

A UF_H of 10 was applied to account for variability in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to quantitatively estimate variability in human susceptibility to benzo(a)pyrene.

A UF_L of 10 was applied to account for the use of a LOAEL. A NOAEL was not identified for decreased fetal survival observed by Archibong et al (2002). At the lowest dose, benzo[a]pyrene treated dams gave birth to 15% fewer pups compared to dams treated with

vehicle alone (carbon black particles). Due to the lack of a NOAEL and the inability to model the data set for decreased fetal survival, a UF of 10 was applied to extrapolate to a NOAEL.

A UF_S of 1 was applied to account for extrapolation from subchronic to chronic exposure because developmental toxicity resulting from a narrow period of exposure was used as the critical effect. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991a).

A UF_D of 3 was applied to account for deficiencies in the benzo[a]pyrene toxicity database. One developmental study exists for benzo[a]pyrene by the inhalation route. The developmental study by Archibong et al., 2002, which was used as the basis for the RfC, observed decreased fetal survival and decreased litter size following gestational treatment on GDs 11-20. Limited observational studies in humans have suggested associations between biomarkers of internal doses of benzo[a]pyrene and adverse birth outcomes (including reduced birth weight and postnatal weight, decreased head circumference, and impaired neurodevelopment) and decreased fertility (Neal et al., 2008; Tang et al., 2008, 2006; Perera et al., 2005a, b). A multigenerational reproductive study examining these types of effects in animals does not exist for the inhalation route. However, oral multigenerational studies indicate that effects on fertility would be expected in male and female offspring exposed to benzo[a]pyrene during development (Mackenzie and Angevine 1981; Kristensen et al., 1995). In addition, the lack of a study examining functional neurological endpoints following in utero exposure is also a data gap considering the available epidemiological evidence showing the association of in utero PAH exposure and indicators of decreased neurological development (Edwards et al., 2010, Perera et al., 2009, Tang et al., 2008). Therefore, a UF of 3 was applied to the POD for the lack of a multigenerational reproductive toxicity study and a neurodevelopmental study.

The RfC for benzo[a]pyrene was calculated as follows:

$$\begin{aligned} \text{RfC} &= LOAEL_{ADJ[HEC]} \div UF \\ &= 4.6\ \mu g/m^3\text{-day} \div 1000 \\ &= 4.6 \times 10^{-3}\ \mu g/m^3\text{-day or } 5 \times 10^{-6}\ mg/m^3 \end{aligned}$$

5.2.4. Previous RfC Assessment

An RfC was not derived in the previous IRIS assessment.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

The following discussion identifies uncertainties associated with the RfD and RfC for benzo[a]pyrene. To derive the RfD, the UF approach (U.S. EPA, 2000, 1994b) was applied to a POD based on decreased ovary weight in female rats exposed to benzo[a]pyrene. To derive the RfC, this same approach was applied to a POD from a developmental study for the effect of decreased fetal survival. Uncertainty factors were applied to the POD to account for extrapolating from an animal bioassay to human exposure, the likely existence of a diverse population of varying susceptibilities, and for database deficiencies. These extrapolations are carried out with default approaches given the lack of data to inform individual steps.

The database for benzo[a]pyrene contains limited human data. The observation of effects associated with benzo[a]pyrene exposure in humans is complicated by several factors including the existence of benzo[a]pyrene in the environment as one component of complex mixtures of PAHs, exposure to benzo[a]pyrene by multiple routes of exposure, and the difficulty in obtaining accurate exposure information. Data on the effects of benzo[a]pyrene alone are derived from a large database of studies in animal models. The database for oral benzo[a]pyrene exposure includes two chronic bioassays in rats and mice, two developmental studies in mice, and several subchronic studies in rats.

Although the database is adequate for RfD derivation, there is uncertainty associated with the database, because a NOAEL was not identified in the reproductive and developmental oral toxicity studies, comprehensive two-generation reproductive/developmental toxicity studies are not available, and immune system endpoints affected in the subchronic-duration studies were not evaluated in the chronic-duration toxicity studies. Additionally, the only available chronic studies of oral exposure to benzo[a]pyrene focused primarily on neoplastic effects. These studies identify forestomach hyperplasia as one of the more sensitive histological effects following repeated oral exposure to benzo[a]pyrene. However, data from chronic cancer bioassays for benzo[a]pyrene show no increase in this endpoint at the high dose in rats. An increased incidence of forestomach tumors is observed at similar doses; suggesting that this effect may be pre-neoplastic in nature.

The only chronic inhalation study of benzo[a]pyrene was designed as a lifetime carcinogenicity study and did not examine noncancer endpoints (Thyssen et al., 1981). However subchronic and short term inhalation studies are available which examine developmental and reproductive endpoints in rats. Developmental studies by the inhalation route identified biologically significant reductions in the number of pups/litter and percent fetal survival and possible neurodevelopmental effects (e.g., diminished electrophysiological responses to stumuli in the hippocampus) following gestational exposures. Additionally, a 60 day oral study in male rats reported male reproductive effects (e.g., decreased testes weight and sperm production and motility), but provides limited information to characterize dose-response relationships with

chronic exposure scenarios. One area of uncertainty pertains to the lack of information regarding fertility in animals exposed gestationally to benzo[a]pyrene, especially in light of developmental studies by the oral route indicating reduced fertility in the F1 generation and decreased reproductive organ weights. The database also lacks a multigenerational reproductive study via the inhalation route. Areas of uncertainty include the lack of chronic inhalation studies focusing on noncancer effects, limited data on dose-response relationships for impaired male or female fertility with gestational exposure or across several generations, and limited data on immune system endpoints with chronic exposure to benzo[a]pyrene.

The toxicokinetic and toxicodynamic differences for benzo[a]pyrene between the animal species in which the POD was derived and humans are unknown. PBPK models can be useful for the evaluation of interspecies toxicokinetics; however, the benzo[a]pyrene database lacks an adequate model that would inform potential differences. There is some evidence from the oral toxicity data that mice may be more susceptible than rats to some benzo[a]pyrene effects (such as ovotocity [Borman et al., 2000]), though the underlying mechanistic basis of this apparent difference in not understood. Most importantly, it is unknown which animal species may be more comparable to humans.

5.4. CANCER ASSESSMENT

As discussed in Section 4.7, benzo[a]pyrene is "carcinogenic to humans" based on evidence of carcinogenicity in humans exposed to different PAH mixtures containing benzo[a]pyrene, extensive and consistent evidence of carcinogenicity in laboratory animals exposed to benzo[a]pyrene via several routes of administration, and extensive and consistent evidence that the mode of action of carcinogenesis in laboratory animals also occurs in humans exposed to PAH mixtures containing benzo[a]pyrene.

5.4.1. Oral Exposure—Oral Slope Factor

5.4.1.1. *Choice of Study/Data—with Rationale and Justification—Oral Exposure*

Numerous cancer bioassays exist which identify tumors, primarily of the alimentary tract, following oral exposure in rodents (see Table 4-8 for references). These studies provide support for the carcinogenic hazard for benzo[a]pyrene, however, are not suitable for dose-response analysis due to limitations in study design, methods, and/or reporting. Specifically, several of these studies 1) lack a vehicle control group 2) use only one benzo[a]pyrene dose group or 3) use a single one-time exposure to benzo[a]pyrene (Benjamin et al., 1988; Robinson et al., 1987; El Bayoumy, 1985; Wattenberg, 1974; Roe et al., 1970; Biancifiori et al. 1967; Chouroulinkov et al., 1967; Field and Roe, 1970; Berenblum and Haran 1955). Of the controlled, multiple dose-group, repeat-dosing studies that remain, most treated animals for less than a year, which is less

optimal for extrapolating to a lifetime exposure (Weyand et al., 1995; Triolo et al., 1977; Fedorenko et al., 1967; Neal and Rigdon 1967).

Three 2-year oral bioassays remain which associate lifeftime benzo[a]pyrene exposure with forestomach, liver, oral cavity, jejunum, kidney, auditory canal (Zymbal's gland) tumors, and skin or mammary gland tumors in male and female Wistar rats (Kroese et al., 2001); forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981); and forestomach, esophagus, tongue, and larynx tumors in female $B6C3F_1$ mice (male mice were not tested; Beland and Culp, 1998; Culp et al., 1998). Brune et al. (1981) dosed rats (32/sex/group) with several concentration of benzo[a]pyrene dissolved a 1.5% caffeine solution, sometimes as infrequently as once every 9^{th} day, for up to two years and observed increased forestomach tumors. This study was not selected for quantitation due to the non-standard treatment protocol. The rat bioassay by Kroese et al. (2001) and the mouse bioassay by Beland and Culp (1998) were conducted in accordance to Good Laboratory Practice (GLP) principles as established by OECD. These studies included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for two years or until death, and included detailed reporting of methods and results (including individual animal data).

Therefore, the Kroese et al. (2001) and Beland and Culp s(1998) tudies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene.

5.4.1.2. *Dose-response Data—Oral Exposure*

Details of the rat (Kroese et al., 2001) and female mouse (Beland and Culp, 1998) study designs are provided in Section 4.2.1.2. Dose-related, statistically significant increasing trends in tumors were noted at the following sites:

- Squamous cell carcinomas or papillomas of the forestomach or oral cavity in male and female rats;
- Squamous cell carcinomas or papillomas of the forestomach, tongue, larynx, or esophagus in female mice;
- Auditory canal carcinomas in male and female rats;
- Kidney urothelial carcinomas in male rats;
- Jejunum adenocarcinomas in female and male rats;
- Hepatocellular adenomas or carcinomas in male and female rats;
- Squamous cell carcinomas or basal cell tumors of the skin or mammary gland in male rats.

These tumors were generally observed earlier during the study with increasing exposure levels, and showed statistically significantly increasing trends in incidence with increasing

exposure level (Cochran-Armitage trend test, $p \leq 0.001$). These data are summarized in Tables 5-4 (male and female rats) and 5-5 (female mice). As recommended by the NTP (McConnell et al., 1986), etiologically similar tumor types, i.e., benign and malignant tumors of the same cell type, were combined for these tabulations when it was judged that the benign tumors could progress to the malignant form, as outlined in the *Cancer Guidelines* (U.S. EPA, 2005a). In addition, when one tumor type occurred across several functionally related tissues, as with squamous cell tumors in the tongue, esophagus, larynx and forestomach, or adenocarcinomas of the jejunum or duodenum, these incidences were also aggregated as counts of tumor-bearing animals.

In the rat study (Kroese et al., 2001), the oral cavity and auditory canal were examined histologically only if a lesion or tumor was observed grossly at necropsy. Consequently, dose-response analysis for these sites was not straightforward. Use of the number of tissues examined histologically as the number at risk would tend to overestimate the incidence, because the unexamined animals were much less likely to have a tumor. On the other hand, use of all animals in a group as the number at risk would tend to underestimate if any of the unexamined animals had tumors which could only be detected microscopically. The oral cavity squamous cell tumors were combined with those in the forestomach because both are part of the alimentary tract, recognizing that there was some potential for underestimating this cancer risk.

The auditory canal tumors from the rat study were not considered for dose-response separately or combined with another site. First, very few tissues were examined in the control and lower dose groups (see Table 4-4). Also, the tumors were not clearly related to any other site or incidence type, as they were described as a mixture of squamous and sebaceous cells derived from pilosebaceous units. The tumors found were observed mainly in the high dose groups and were highly coincident with the oral cavity and forestomach tumors. That is, only one mid-dose male with an auditory canal tumor did not also have a forestomach or oral cavity squamous cell tumor. No low-dose male or female rats were found with auditory canal tumors. While the investigators did not suggest that these tumors were metastases from other sites (in which the auditory canal tumors could be reflections of other tumor types), it is difficult to conclude that they are independent on a purely statistical basis without sufficient low-dose data. Therefore dose-response analysis was not pursued for this site.

Table 5-4. Incidence data for tumors in Wistar rats exposed to benzo[a]pyrene by gavage, 5 days/week for 104 weeks

Tumor site/type[a]	Administered dose (mg/kg-d)			
	0	3	10	30
	HED (mg/kg-d)[b]			
Male rats	**0**	**0.54**	**1.81**	**5.17**
Forestomach or oral cavity: squamous cell papilloma or carcinoma	0/51	8/51	45/49	52/52
Hepatocellular adenoma or carcinoma	0/51	4/50	38/49	49/50
Jejunum/duodenum: adenocarcinoma	0/50	0/48	1/48	9/39
Kidney: urothelial carcinoma	0/52	0/52	0/52	3/52
Skin or mammary gland:				
Basal cell adenoma or carcinoma	2/52	1/50	1/49	13/40
Squamous cell carcinoma	0/51	1/50	1/49	6/40
Female rats	**0**	**0.49**	**1.62**	**4.85**
Forestomach or oral cavity: squamous cell papilloma or carcinoma	1/52	6/50	30/47	50/51
Hepatocellular adenoma or carcinoma	0/52	1/50	39/47	51/51
Jejunum/duodenum: adenocarcinoma	0/50	0/46	0/45	4/42

[a]For each tissue site, the numerator of the tumor incidence value is the number of animals bearing the specified tumors. The denominators are the number of animals examined histologically, minus the number of animals who died before the earlier of the first occurrence of the tumor type in each group or Week 52.
[b]HEDs for continuous exposure were calculated using the animal to human scaling factor for each dose group × the administered dose × 5 d/7 d. Scaling factors used the form (TWA body weight/70)$^{0.25}$, with the U.S. EPA (1988) reference body weight for humans (70 kg), and the TWA body weight for each dose group. See Table D-4 for more information.

Source: Kroese et al. (2001).

Table 5-5. Incidence data for tumors in female B6C3F$_1$ mice exposed to benzo[a]pyrene in the diet for 104 weeks

Tumor site/type[a]	Administered dose (mg/kg-d)[b]			
	0	0.7	3.3	16.5
	HED (mg/kg-d)[c]			
	0	0.10	0.48	2.32
Forestomach, esophagus, tongue, larynx: squamous cell papilloma or carcinoma	1/48	3/48	38/46	46/47

[a] The numerator of the tumor incidence value is the number of animals bearing any of the listed tumors (see Table 4-6 in Section 4.2.1.2). The denominators are the number of tissues examined histologically, minus the number of animals who died before the earlier of the first occurrence of the tumor type in each group or Week 52.
[b]Administered doses were calculated using TWA body weight for mice and reported food intakes.
[c]HEDs were calculated using the animal to human scaling factor for each dose group × the administered dose. Scaling factors were calculated using U.S. EPA (1988) reference body weights for humans (70 kg), and the TWA body weight for each dose group: (TWA body weight/70)$^{0.25}$ × dose = HED. See Table D-5 for more information.

Source: Beland and Culp (1998).

5.4.1.3. *Dose Adjustments and Extrapolation Method(s)—Oral Exposure*

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The dose response is assumed to be linear in the low dose range, when evidence supports a mutagenic mode of action because of DNA reactivity, or if another mode of action that is anticipated to be linear is applicable. In this assessment, EPA concluded that benzo[a]pyrene causes cancer via a mutagenic MOA (as discussed in Section 4.7.3). Thus, a linear approach to low-dose extrapolation was used.

The high-dose groups of both the rat and mouse studies were dead or moribund by week 79 for female mice, week 72 for female rats, and week 76 for male rats. Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of the high-dose group in each study, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. EPA has generally used a model which incorporates the time at which death-with-tumor occurred as well as the dose; the multistage-Weibull model is multistage in dose and Weibull in time, and has the form:

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

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

Two general characteristics of the observed tumor types were considered prior to modeling; allowance for different, although unidentified modes of action, and allowance for relative severity of tumor types. First, etiologically different tumor types were not combined across sites prior to modeling (that is, overall counts of tumor-bearing animals were not tabulated), in order to allow for the possibility that different tumor types could have different dose-response relationships due to different underlying mechanisms or factors, such as latency. Consequently, all of the tumor types listed separately in Tables 5-4 and 5-5 were also modeled separately.

Additionally, the multistage-Weibull model can address relative severity of tumor types by distinguishing between tumors as being either fatal or incidental to the death of an animal, in order to adjust partially for competing risks. Incidental tumors are those tumors thought not to

have caused the death of an animal, while fatal tumors are thought to have resulted in animal death. Cause of death information for most early animals deaths was provided by the investigators of both of the bioassays. In the rat study, tumors of the forestomach or liver were the principal cause of death for most animals dying or sacrificed (due to moribundity) before the end of the study, while tumors of the forestomach were the most common cause of early deaths in the mouse study.

Adjustments for approximating human equivalent slope factors applicable for continuous exposure were applied prior to dose-response modeling. First, continuous daily exposure for the gavage study in rats (Kroese et al, 2001) was estimated by multiplying each administered dose by (5 days)/(7 days) = 0.71, under the assumption of equal cumulative exposure yielding equivalent outcomes. Dosing was continuous in the mouse diet study (Beland and Culp, 1998), so no continuous adjustment was necessary. Next, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), an adjustment for cross-species scaling was applied to address toxicological equivalence across species. Following EPA's cross-species scaling methodology, the time-weighted daily average doses were converted to human equivalent doses on the basis of (body weight)$^{3/4}$ (U.S. EPA, 1992). This was accomplished by multiplying administered doses by (animal body weight(kg)/70 kg)$^{0.25}$ (U.S.EPA, 1992), where the animal body weights were time-weighted averages from each group (see Tables D-4, D-5), and the U.S. EPA (1988) reference body weight for humans is 70 kg. It was not necessary to adjust the administered doses for lifetime equivalent exposure prior to modeling for the groups terminated early, because the multistage-Weibull model characterizes the tumor incidence as a function of time, from which it provides an extrapolation to lifetime exposure.

The multistage-Weibull model was applied to the datasets. Specific n-stage Weibull models were selected for each tumor dataset based on the values of the log-likelihoods according to the strategy used by EPA (U.S. EPA, 2002). If twice the difference in log-likelihoods was less than a χ^2 with degrees of freedom equal to the difference in the number of stages included in the models being compared, the models were considered comparable and the most parsimonious model (i.e., the lowest-stage model) was selected. This method generally led to the same conclusion as selecting the model fit with the lowest AIC. If a model with one more stage fitted the low-dose data better than the most parsimonious model, then the model with one higher stage was selected.

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

5.4.1.4. *Oral Slope Factor Derivation*

The PODs estimated for each tumor site are summarized in Table 5-5. Details of the model selection process are provided in Table D-6. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination and are listed in Table 5-6.

Table 5-6. Human equivalent PODs and oral slope factors derived from multistage-Weibull modeling of tumor incidence data at multiple tissue sites in Wistar rats and B6C3F$_1$ mice exposed to benzo[a]pyrene orally for 2 years

Species	Sex	Tumor	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	Slope Factor[a] $(mg/kg\text{-}d)^{-1}$	
Rats	Male	Forestomach, oral cavity: squamous cell tumors	0.453	0.281	0.4	
		Hepatocellular adenomas or carcinomas	0.651	0.449	0.2	
		Jejunum/duodenum adenocarcinomas	3.03	2.38	0.04	0.5[b]
		Kidney: urothelial carcinomas	4.65	2.50	0.04	
		Skin, mammary:				
		Basal cell tumors	2.86	2.35	0.04	
		Squamous cell tumors	2.64	1.77	0.06	
	Female	Forestomach, oral cavity: squamous cell tumors	0.539	0.328	0.3	
		Hepatocellular adenomas or carcinomas	0.575	0.507	0.2	0.3[b]
		Jejunum/duodenum adenocarcinomas	3.43	1.95	0.05	
Mice	Female	Forestomach, esophagus, tongue, larynx: squamous cell tumors	0.127	0.071	1	

[a]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix D for details of modeling results.
[b] Estimates of risk of incurring at least one of the tumor types listed.

Oral slope factors derived from rat bioassay data varied by gender and tumor site (Table 5-6). Values ranged from 0.04 per mg/kg-day, based on kidney tumors in males, to 0.4 per mg/kg-day, based on alimentary tract tumors in males. Slope factors based on liver tumors in male and female rats (approximately 0.2 per mg/kg-day) were only slightly lower than slope factors based on alimentary tract tumors. The oral slope factor for female mice was highest, at 1 per mg/kg-day for alimentary tract tumors (Table 5-6), approximately fourfold higher than the oral slope factor derived from the alimentary tract tumors in male rats.

Although the time-to-tumor modeling helps account for competing risks associated with decreased survival times and other tumors, considering the tumor sites individually still does not convey the total amount of risk potentially arising from the sensitivity of multiple sites—that is, the risk of developing any combination of the increased tumor types, not just the risk of developing all simultaneously. One approach suggested in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used this approach until the National Resource Council (NRC) document *Science and Judgment* (NRC, 1994) made a case that this approach would tend to underestimate overall risk when tumor types occur in a statistically independent manner. In addition,

application of one model to a composite data set does not accommodate biologically relevant information that may vary across sites or may only be available for a subset of sites. For instance, the time courses of the multiple tumor types evaluated varied, as is suggested by the variation in estimates of *z* (see Table 5-6), from 1.5 (e.g., male rat skin or mammary gland basal cell tumors), indicating relatively little effect of age on tumor incidence, to 3.7 (e.g., male mouse alimentary tract tumors), indicating a more rapidly increasing response with increasing age (in addition to exposure level). The result of fitting a model with parameters which can reflect underlying mechanisms, such as *z* in the multistage-Weibull model, would be difficult to interpret with composite data (i.e., counts of tumor-bearing animals). A simpler model, such as the multistage model, could be used for the composite data but relevant biological information would then be ignored.

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

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

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

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

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

$$95\%\ UCL = MLE + 1.645 \times s.d.,$$

rearranged to:

$$s.d. = (UCL - MLE) / 1.645,$$

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

The resulting composite slope factor for all tumor types for male rats was 0.5 per mg/kg-d, about 25% higher than the slope factor based on the most sensitive tumor site, oral cavity and forestomach, while for female rats the composite slope factor was equivalent to that for the most sensitive site (Table 5-6; see Table D-7 for details of the composite slope factor estimates).

The risk estimates from rats and mice spanned a nearly five-fold range. As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result was used to derive the oral slope factor. The recommended slope factor for assessing human cancer risk associated with chronic oral exposure to benzo[a]pyrene is **1 per mg/kg-day,** based on the alimentary tract tumor response in female $B6C3F_1$ mice.

5.4.2. Inhalation Exposure—Inhalation Unit Risk

5.4.2.1. *Choice of Study/Data—with Rationale and Justification—Inhalation Exposure*

Inhalation exposure to benzo[a]pyrene was associated with nasal adenocarcinomas and squamous cell tumors in the larynx, pharynx, trachea, esophagus, and forestomach, of male Syrian golden hamsters exposed to benzo[a]pyrene:NaCl aerosols at concentrations of 10 or 50 mg/m^3 until natural death (up to 133 weeks) for 3–4.5 hours/day, 5-7 days/week (Thyssen et al., 1981). Supportive evidence for the carcinogenicity of inhaled benzo[a]pyrene comes from 10 additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation (see Section 4.2.2.2 for references). However, the use of intratracheal dosing alters the deposition, clearance, and retention of substances and therefore studies utilizing this exposure technique are not as useful for the quantitative extrapolation of cancer risk from the inhalation of benzo[a]pyrene in the environment (Driscoll et al., 2000).

The Thyssen et al. (1981) bioassay represents the only lifetime inhalation cancer bioassay available for describing dose-response relationships for cancer from inhaled benzo[a]pyrene. Limitations of the study include the following: (1) only male animals were included; (2) particle analysis of aerosols was not reported [i.e., MMAD and geometric SD were not reported], and (3) benzo[a]pyrene exposure occurred through the inhalation of hygroscopic particles [benzo[a]pyrene was adsorbed onto NaCl aerosols] which may have a different deposition than

benzo[a]pyrene adsorbed onto non-hygroscopic particles in the environment. Strengths of the study include exposure to hamsters for life, histological tumor examination of organs, use of multiple exposure groups, including approximately 30 male hamsters per group, and the availability of individual animal pathology reports with time of death and tumor detection data. Although the study has a few limitations, the strengths of the study support use of the data to derive an inhalation unit risk for benzo[a]pyrene.

5.4.2.2. *Dose-response Data—Inhalation Exposure*

Survival was decreased relative to control only in the high-dose exposure group; mean survival times in the 0, 2, and 10 mg/m^3 concentration groups were 96.4, 95.2, and 96.4 weeks, respectively, and 59.5 weeks in the 50 mg/m^3 group animals. Overall, tumors occurred earlier in the highest benzo[a]pyrene exposure group than in the mid-exposure group. Increased incidences of benign and malignant tumors of the larynx, trachea, pharynx, esophagus and forestomach were seen with increasing exposure concentration. Benign tumors—papillomas, polyps and papillary polyps—were considered by the study authors as early stages of the squamous cell carcinomas in these tissues.

Nasal cavity tumors were also observed in the mid- and high-dose groups. Consideration of early mortality (using the poly-3 approach; Bailer and Portier, 1988) suggested that an increasing dose-response was consistent with these data. However trend testing was not statistically significant (p=0.08), and the site was not considered further for unit risk derivation. Table E-1 in Appendix E summarizes the individual animal tumor data, noting the presence or absence of a tumor in these tissues, whether or not the tissue was available for examination by the pathologist, and the time of death. A summary of the incidence of these tumors is provided in Table 5-7.

Table 5-7. Incidence of tumors in male hamsters exposed by inhalation to benzo[a]pyrene for life

Average continuous benzo[a]pyrene concentration[a] (mg/m^3)	Number of hamsters in group[b]	Larynx	Pharynx	Trachea	Esophagus	Forestomach	Any Tumor[c]	Nasal Cavity Tumors
Control	27	0	0	0	0	0	0	0
0.25	27	0	0	0	0	0	0	0
1.01	26	11	9	2	0	1	18	4
4.29	34	12	18	3	2	1	18	1

[a]Calculated from air monitoring data.
[b]Number of animals examined histologically, minus the number of animals who died before the earlier of the first occurrence of the tumor type in each group or Week 52.
[c] Includes any animal with squamous cell carcinoma of the larynx, pharynx, trachea, esophagus, or forestomach.

Source: Thyssen et al. (1981) and a reanalysis of this data by Clement Associates (1990). See Appendix E for more detailed incidence data.

5.4.2.3. *Dose Adjustments and Extrapolation Method(s)—Inhalation Exposure*

A toxicokinetic model to assist in cross species scaling of benzo[a]pyrene inhalation exposure was not available. In addition, default dosimetry adjustments utilized in the benzo[a]pyrene RfC calculation could not be applied because aerosol particle distribution data were not available for the hamster inhalation bioassay by Thyssen et al. (1981). The carrier particle used in Thyssen et al. (1981) was sodium chloride, a soluble hygroscopic particle, and the approaches presented in the RfC methodology guidelines (US EPA 1994b) were developed for insoluble and nonhygroscopic particles.

The availability of the raw chamber air monitoring data and individual times on study allowed the calculation of time-weighted average (TWA) continuous exposure rates for each hamster. Group averages of individual TWA continuous exposure concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3, respectively, for the 0, 2, 10, and 50 mg/m^3 study concentrations.

A time-to-tumor dose-response model was fit to the time-weighted average exposure concentrations and the individual animal occurrence data for tumors in the larynx, pharynx, trachea, esophagus, and forestomach (Table E-1 in Appendix E) using the computer software program multistage-Weibull (U.S. EPA, 2010) as described in Section 5.4.1.3. The investigators did not determine cause of death for any of the animals. Since in the available oral bioassays the investigators considered these same tumors to be fatal at least some of the time, bounding estimates for the Thyssen et al. data were developed by treating the tumors alternately as either all incidental or all fatal. In either case, therefore, an estimate of t_0 (the time between a tumor first becoming observable and causing death) could not be estimated.

Because benzo[a]pyrene is expected to cause cancer via a mutagenic MOA, a linear approach to low dose extrapolation from the $BMCL_{10}$ was used (U.S. EPA, 2005a).

5.4.2.4. *Inhalation Unit Risk Derivation*

Modeling results are provided in Appendix E. The BMC (0.28 mg/m^3) and BMCL (0.20 mg/m^3) associated with an extra risk of 10% were calculated based on the occurrence of upper respiratory and upper digestive tract tumors in male hamsters exposed to aerosols of benzo[a]pyrene for 104 weeks using the multistage-Weibull model Using linear extrapolation from the $BMCL_{10}$ of 0.20 mg/m^3, an inhalation unit risk of 0.5 per mg/m^3 or **5×10^{-4} per $\mu g/m^3$** was calculated.

5.4.3. Dermal Exposure—Dermal Slope Factor

5.4.3.1. *Choice of Study/Data—with Rationale and Justification—Dermal Exposure*

Skin cancer in humans has been documented to result from occupational exposure to complex mixtures of PAHs including benzo[a]pyrene such as coal tar, coal tar pitches, non-refined mineral oils, shale oils and soot (IARC, 2010; Baan et al., 2009; Boffetta et al., 1997;

WHO, 1998; ATSDR, 1995), but no studies of human exposures to benzo[a]pyrene alone are known to exist. In animal models, numerous dose response studies have demonstrated an increased incidence of skin tumors with increasing dermal exposure to benzo[a]pyrene, in all species tested (mice, rabbits, rats, and guinea pigs), though most benzo[a]pyrene chronic dermal bioassays which provide quantitative information have been conducted in mice. In addition, mice appear to be the most sensitive laboratory model of carcinogenesis following dermal benzo[a]pyrene exposure. Therefore, this analysis focuses on chronic carcinogenicity bioassays in several strains of mice demonstrating increasing incidence of benign and malignant skin tumors, and earlier occurrence of tumors with increasing exposure, following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime. These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and included histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984; 1983; Habs et al., 1980; 1984; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960; 1959). These data sets are described in greater detail in Section 4.2.3.2.

Because of the availability of the lifetime studies listed above, other carcinogenicity studies were not considered for this assessment. The other studies included: 1) early "skin painting" studies of benzo[a]pyrene carcinogenicity in mouse skin which did not report sufficient information to estimate the doses applied (e.g., Wynder and Hoffman 1959; Wynder et al, 1957); 2) initiation-promotion studies utilizing acute dosing of benzo[a]pyrene followed by repeated exposure to a potent tumor promoter (sometimes benzo[a]pyrene at a lower dose than the initiation step), because they are not as relevant for calculating risks from constant benzo[a]pyrene exposure alone; 3) bioassays with one benzo[a]pyrene dose level or with only dose levels inducing 90–100% incidence of mice with tumors, because they provide relatively little information about the shape of the dose-response relationship (e.g., Wilson and Holland, 1988); 4) studies with shorter exposure and observation periods (i.e., less than one year; Levin et al., 1977; Nesnow et al, 1983; Albert et al., 1991; Emmett et al, 1981; Higginbotham et al., 1993) which are less relevant for characterizing lifetime risk; and 5) studies involving vehicles expected to interact with or enhance benzo[a]pyrene carcinogenicity (e.g., Bingham and Falk, 1969) which precludes assessment of carcinogenic risks of benzo[a]pyrene alone.

5.4.3.2. *Dose-response Data—Dermal Exposure*

Several studies were considered for dose-response modeling for derivation of the dermal slope factor for benzo[a]pyrene, reflecting a relatively large database. Study designs and the extent of data reported varied across the studies, with no individual animal data available. All of the studies identified in the previous section (Sivak et al., 1997; Grimmer et al., 1984; 1983; Habs et al., 1980; 1984; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960; 1959) were considered further in order to evaluate overall consistency of the available database. These data sets are presented in Tables 5-8 through 5-12, and are grouped by study

characteristics such as mouse strain and vehicle in order to facilitate qualitative comparisons where possible.

Each data set was examined for study design and both strengths and limitations that could potentially impact the dose-response evaluation, including the potential of early mortality to impact the number at risk for developing tumors, and the length of exposure. Although nearly all studies reported lifetime exposures, this term most often indicated that exposure continued until natural death, not a scheduled sacrifice at 104 weeks.

The studies by Poel (1959, 1960) were conducted in male mice and used toluene as the vehicle (see Tables 4-11, 4-12, respectively). In addition to a control group, the 1959 study included nine dose groups of one mouse strain (C57L) and the 1960 study included seven dose groups of 3 other mouse strains. Both studies demonstrated high mortality and tumor incidence at higher exposure levels. As noted in Table 4-11, all C57L mice in dose groups with >3.8 μg/application died by Week 44 of the study (Poel, 1959). Therefore, these five dose groups were omitted prior to dose-response modeling because of the relatively large uncertainty in characterizing cancer risk in relation to lifetime exposure. Four dose groups in addition to control remained. Among these groups mice survived and were exposed until weeks 83–103. According to the lifespan ranges provided, at least one mouse in each dose group died before the first appearance of tumor, but insufficient information was available to determine how many; consequently the incidence denominators were not adjusted. The dose-response data are summarized in Table 5-7.

For the Poel (1960) studies, all tumors in the highest three dose groups for each of the three mouse strains had occurred by Week 40 (see Table 4-12). While these observations support concern for cancer risk, as noted above such results are relatively uncertain for estimating lifetime cancer risk. In addition, there was no information indicating duration of exposure for the mice without tumors; although exposure was for lifetime, it might have been as short as for the mice with tumors. Overall, these datasets did not provide sufficient information to estimate the extent of exposure associated with the observed tumor incidence. Consequently the experiments reported by Poel (1960) were not used for dose-response modeling.

The studies listed in Table 5-9 all used acetone as the vehicle and either Swiss or NMRI, female mice (Roe et al., 1970; Schmidt et al., 1973; Schmähl et al., 1977; Habs et al., 1980, 1984). Roe et al. (1970) applied benzo[a]pyrene dermally for 93 weeks or until natural death; with the exception of the highest dose group, each group still had approximately 20 animals at 86 weeks (Table 4-14). The tumors were first observed in the lowest and highest dose groups during the interval of weeks 29-43. Mice that died before week 29 were likely not at risk of tumor development. However because tumor incidence and mortality were reported in 100-day intervals, mice that had not been on study long enough to develop tumors were not easily identifiable. Incidence denominators reflect the number of animals alive at Week 29, and thus

may tend to lead to underestimates of tumor risk if the number of animals at risk have been overestimated.

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

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

Habs et al. (1980) reported age-standardized skin tumor incidence rates, indicating earlier mortality in the two highest dose groups (2.8 and 4.6 μg/application). These rates were used to estimate the number at risk in the dose-response modeling, by dividing the number of mice with tumors by the age-standardized rates (see Table 5-9). Exposure lasted longer than 104 weeks in the two lower exposure groups, at about 120 and 112 weeks, and until about 88 weeks in the highest exposure group. Incidence in the two lower exposure groups may be higher than if the exposure had lasted just 104 weeks. There was mortality in the first 52 weeks of exposure, about 10−15% in the three exposure groups, but because there was no information concerning when tumors first appeared it is not possible to determine how much the early mortality may have impacted the number of mice at risk in each group.

Habs et al. (1984) reported mean survival times (with 95% confidence intervals) for each dose group. The confidence intervals supported the judgment that the control and lower dose groups were treated for 104 weeks. The higher dose group (4 μg/application) was probably treated for less than 104 weeks, because the upper 95% confidence limit for the mean survival was approximately 79 weeks (Table 4-20). However, since it was not possible to estimate a more realistic duration for this group, an estimate of 104 weeks was used.

Grimmer et al. (1983 and 1984), studied female CFLP mice, using acetone:DMSO (1:3) as the vehicle (see Table 5-10). Mean or median latency times were reported (as well as measures of variability), but no information concerning overall length of exposure or survival was included in the results. The total of tumor-bearing mice and the reported percentages of mice with any skin tumors was reported and varied at most one animal from the number of

animals initially placed on study. The decreasing latency and variability and increasing tumor incidence with increasing benzo[a]pyrene exposure suggests that exposure probably did not last for 104 weeks in at least the high dose group, but the available information did not provide duration of exposure. The data reported were modeled under the assumption that at least some animals in each group were treated and survived until Week 104.

The study listed in Table 5-11, Sivak et al. (1997) exposed male C3H /HeJ mice dermally to benzo[a]pyrene in cyclohexanone/acetone (1:1) for 24 months, and reported mean survival times for each group (see Table 4-21). All high dose mice died before the final sacrifice. From the information provided it is apparent that the animals in the control and lower two dose groups survived until study termination at Week 104. The study authors did not report how long treatment in the highest dose group lasted, but estimation of the figure from the publication suggest that exposure duration was 74 weeks. The tumor incidences and estimated duration of exposure for each dose group are presented in Table 5-11.

Table 5-8. Skin tumor incidence, benign or malignant, in C57L male mice dermally exposed to benzo[a]pyrene

Study	Mouse strain	Dose (µg)[a]	Average daily dose (µg/d)	First appearance of tumor (weeks)	Length of Exposure (weeks)	Lifetime Average Daily Dose[b]	Skin tumor incidence (all types)
Poel (1959)	C57L	0 (toluene)	0	—	92	0.00	0/33 (0%)
		0.15	0.06	42	98	0.05	5/55 (9%)
		0.38	0.16	24	103	0.16	11/55 (20%)
		0.75	0.32	36	94	0.24	7/56 (13%)
		3.8	1.63	21–25	82	0.80	41/49 (84%)

[a]Doses were applied to interscapular skin 3 times/wk for up to 103 weeks or until time of appearance of a grossly detected skin tumor. See Table 4-11 for data of five highest dose groups (19-752 µg**)** in which all mice died by Week 44. These groups were not considered for dose-response modeling.
[b]See Section 5.4.3.3. for discussion of extrapolation to lifetime average daily doses.

1

2

Table 5-9. Skin tumor incidence, benign or malignant in female Swiss or NMRI mice dermally exposed to benzo[a]pyrene

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

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

Table 5-10. Skin tumor incidence, benign or malignant, in female CFLP mice dermally exposed to benzo[a]pyrene

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

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

Table 5-11. Skin tumor incidence, benign or malignant, in male C3H /HeJ mice dermally exposed to benzo[a]pyrene(Sivak et al., 1997)

Dose (µg)[a]	Average daily dose (µg/d)	First appearance of tumor (weeks)	Length of exposure (weeks)[b]	Lifetime average daily dose (µg/d)	Skin tumor incidence (all types)
0 (1:1 cyclohexanone/acetone)	0	—	104	0.0	0/30 (0%)
0.05	0.01	—	104	0.01	0/30 (0%)
0.5	0.14	NR	104	0.14	5/30 (17%)
5.0	1.4	~43	*74*	0.51	27/30 (90%)

[a]Indicated doses were applied twice/week to shaved dorsal skin.
[b]Assumed exposure period is indicated in italics.
NR=not reported.

5.4.3.3. *Dose Adjustments and Extrapolation Method(s)—Dermal Exposure*

As with the oral and inhalation benzo[a]pyrene carcinogenicity data (see sections 5.4.1.3 and 5.4.2.3), benzo[a]pyrene's dermal exposure carcinogenicity data were generally characterized by earlier occurrence of tumors and increased mortality with increasing exposure level. However, individual animal data were not available for any of the identified studies. Therefore, time to tumor modeling was not possible. Each of the dermal data sets was modeled using the multistage model, incorporating adjustments for early mortality, when data were available.

First, for all studies, administered doses were converted to average daily doses using the equation:

Average dose/day = (μg/application) × (number of exposures/week ÷ 7 days/week).

Next, lifetime equivalent doses were estimated for study groups that were reported to end before 104 weeks by multiplying the relevant average daily doses by $(L_e/104)^3$, where L_e is the length of exposure, based on observations that tumor incidence tends to increase with age (Doll, 1971). Note that exposure periods less than 52 weeks would lead to a relatively large adjustment [i.e., $(52/104)^3 = 0.125$, or an eightfold lower dose than administered], reflecting considerable uncertainty in lifetime equivalent dose estimates generated from relatively short studies.

The multistage-cancer model in the EPA BMDS (version 2.1) was fit to each data set in Tables 5-8 through 5-11. The multistage model with the most parsimonious fit (fewest parameters yielding an adequate fit) was selected to calculate the potential POD from each data set (see Appendix F for details). Because the multistage model is preferred for cancer modeling, the conventional α-level of 0.05 was used to judge goodness-of-fit. If there was no adequate fit using the multistage-cancer model, then other dichotomous models were considered. If there was still no adequate fit, high doses were dropped incrementally and the multistage-cancer model was considered before attempting other models. BMDs and BMDLs associated with an extra risk of 10% were calculated. Because benzo[a]pyrene is expected to cause cancer via a mutagenic MOA, a linear approach to low dose extrapolation from the PODs (i.e., $BMDL_{10}$) was used (U.S. EPA, 2005a) for candidate dermal slope factors.

5.4.3.4. *Dermal Slope Factor Derivation*

Adequate model fits were found using the multistage model for all but one of the mouse skin tumor incidence data sets in Tables 5-8 to 5-11, as described in Appendix F. In one case, the data from Grimmer et al. (1984) could not be adequately fit by the multistage model initially, and the other dichotomous models available in BMDS were considered. Due to the supralinear shape of the dose-response data, only the log-logistic and dichotomous Hill models provided adequate fits. Also due to the supralinear dose-response shape, the point of departure for slope factor derivation was identified near the lowest response of ~70%, in order to avoid excessive extrapolation of the fitted model.

Dermal slope factors, calculated in units of risk per (μg/day) using linear extrapolation from the $BMDL_{10}$ values, ranged from 0.25 to 1.8 per μg/day, a roughly 7-fold range (see Table 5-12). A number of differences among studies contribute to this range, including solvent choice, sex and strain of mice studied, dose ranges and the level of detail reported. Mouse strains were not repeated across sexes among these studies, so it cannot be established whether male mice are

generally more or less sensitive than female mice to benzo[a]pyrene dermal carcinogenicity, or whether Swiss or NRMI mice are more or less sensitive than other strains. In addition, different solvents were used in the various studies with varying strain and sex combinations tested. For example, toluene was used in one male study only and all of the female studies used acetone. Thus, any possible impact of the solvents used is not clear. The estimates derived from the two studies in males were at the higher end of the range of slope factors derived, but the available information is too limited to conclude that males are more sensitive than females. Also, as noted earlier, incomplete mortality information in several of the female mouse studies (Schmidt et al., 1973; Schmähl et al., 1977; Grimmer et al., 1983, 1984; and Habs et al., 1980, 1984) suggests that the derived dermal slope factors may underestimate cancer risk.

The $BMDL_{10}$ of 0.066 µg/animal-day, based on the tumor response in C3H/HeJ male mice (Sivak et al., 1997), is recommended for developing a human dermal slope factor because it is the lowest POD among studies with lower doses where intercurrent mortality was less likely to impact the number at risk and represented a chronic duration of exposure.

Table 5-12. PODs derived from skin tumor incidence data in mice exposed to benzo[a]pyrene by the dermal route of exposure[a]

Reference	Mouse strain	Solvent	BMD_{10} (µg/animal-d)	$BMDL_{10}$ (µg/animal-d)
Male mice				
Sivak et al., 1997	C3H/HeJ	Acetone/ cyclohexanone	0.12	0.066
Poel, 1959	C57L	Toluene	0.12	0.077
Female mice				
Habs et al., 1984	NMRI	Acetone	0.078	0.056
Grimmer et al., 1984	CFLP	Acetone/DMSO	1.07[b]	0.48[b]
Schmahl et al., 1977	NMRI	Acetone	0.23	0.15
Schmidt et al., 1973	Swiss	Acetone	0.28	0.22
Grimmer et al., 1983	CFLP	Acetone/DMSO	0.24	0.21
Habs et al., 1980	NMRI	Acetone	0.29	0.22
Schmidt et al., 1973	NMRI	Acetone	0.33	0.29
Roe et al., 1970	Swiss	Acetone	0.69	0.39

[a]See Appendix F for details of modeling results.
[b]BMR=70% for this dataset, in order to avoid excessive extrapolation via the fitted model.

5.4.3.5. *Dermal Slope Factor Cross Species Scaling*

Different methodologies have been established for interspecies scaling of points of departure (PODs) used to derive oral slope factors and inhalation unit risks. Cross-species adjustment of oral doses is based on allometric scaling using the three-fourths power of body weight. This adjustment accounts for more rapid distribution, metabolism, and clearance in small animals (US EPA 2005). Cross-species extrapolation of inhalation exposures is based on standard dosimetry models that consider factors such as solubility, reactivity, and persistence

(US EPA 1994). However, no established methodology exists to adjust for interspecies differences in dermal toxicity at the point of contact. Because there is no established methodology for cross-species extrapolation of dermal toxicity, several alternative approaches were evaluated (see Appendix H). Among the alternative described in Appendix H, cross-species adjustment based on allometric scaling using body weight to the 3/4 power was selected. Under this approach, rodents and humans exposed to the same daily dose of a carcinogen, adjusted for $BW^{3/4}$, would be expected to have equal lifetime risks of cancer.

The POD_M derived from the mouse study by Sivak et al., (1997) is adjusted to a human equivalent dose (HED) as follows:

$$POD_{HED}(\mu g/day) = POD_M\ (\mu g/day) \times (BW_H / BW_M)^{3/4}$$

$$POD_{HED}\ (\mu g/day) = 0.066\ \mu g/day \times (70\ kg / 0.035\ kg)^{3/4}$$
$$= 19.7\ \mu g/day$$

The resulting POD_{HED} is used to calculate the dermal slope factor for benzo[a]pyrene:

$$DSF = 0.1/ POD_{HED}$$

$$DSF = 0.1/(19.7\ \mu g/day) = \mathbf{0.005\ (\mu g/day)^{-1}}$$

Note that the DSF should only be used with lifetime human exposures < 20 µg/day, the human equivalent of the bioassay POD, because above this level the dose-response relationship may not be proportional to mass of the compound applied.

Several assumptions are made in the use of this scaling method. First, it is assumed that the toxicokinetic processes in the skin will scale with interspecies differences in whole body toxicokinetics. Secondly, it is assumed that the risk at low doses of benzo[a]pyrene is linear; however, one study indicates that at high doses of benzo[a]pyrene, carcinogenic potency is related to mass applied per unit skin and not to total mass (Davies 1967). However, this may be due to promotional effects, such as inflammation, that are observed at high doses of benzo[a]pyrene.

This slope factor has been developed for a local effect and it is not intended to estimate systemic risk of cancer following dermal absorption of benzo[a]pyrene into the systemic circulation. Although some information suggests that benzo[a]pyrene metabolites can enter systemic circulation following dermal exposure (Godschalk et al 1998), lifetime skin cancer bioassays which have included pathological examination of other organs, have not found

elevated incidences of tumors at distal sites (Poel 1959; Roe et al., 1970; Schmidt et al., 1973; Schmahl et al., 1977; Habs et al., 1980; Higginbotham et al.,1993). In addition, benzo[a]pyrene tends to bind to targets within the skin rather than enter the plasma receptor fluid (a surrogate measure of systemic absorption) in in vitro human skin experiments. These data are consistent with benzo[a]pyrene's metabolism to reactive metabolites within the viable layers of the skin (Wester et al., 1990). Some studies indicate that the fraction of benzo[a]pyrene left within the viable layers of the skin is a large portion of the applied dose (Moody et al., 2007; 1995). Taken together, these data support the conclusion that the risk of skin cancer following dermal exposure likely outweighs cancer risks at distal organs.

5.4.4. Application of Age-Dependent Adjustment Factors

Based on sufficient support in laboratory animals and relevance to humans (see Section 4.7.3) benzo[a]pyrene is determined to be carcinogenic by a mutagenic MOA. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* ("*Supplemental Guidance*") (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic MOA are assumed to have increased risk for cancer. The oral slope factor of 1.4 per mg/kg-day, inhalation unit risk of 0.5 per mg/m^3, and dermal slope factor of 0.0051 per µg/day for benzo[a]pyrene, calculated from data applicable to adult exposures, do not reflect presumed early life susceptibility to this chemical. Though some chemical specific data exist for benzo[a]pyrene which demonstrate increased early life susceptibility to cancer (Vesselinovitch et al. 1984), these data were not considered sufficient to develop separate risk estimates for childhood exposure, as they used acute, i.p. exposures (U.S. EPA, 2005b). In the absence of adequate chemical-specific data to evaluate differences in age-specific susceptibility, the Supplemental Guidance (U.S. EPA, 2005b) recommends that age-dependent adjustment factors (ADAFs) be applied in estimating cancer risk.

The *Supplemental Guidance* (U.S. EPA, 2005b) establishes ADAFs for three specific age groups. These ADAFs and their corresponding age groupings are: 10 for individuals exposed <2 years, 3 for exposed individuals 2 to <16 years, and 1 for exposed individuals ≥16 years. The 10- and 3-fold adjustments are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years age) exposures to benzo[a]pyrene To illustrate the use of the ADAFs established in the *Supplemental Guidance* (U.S. EPA, 2005b), sample calculations are presented for three exposure duration scenarios, including full lifetime, assuming a constant benzo[a]pyrene exposure of 0.001 mg/kg-day (Table 5-13).

Calculations for the application of ADAFs to oral exposures are presented in Table 5-13; calculations for exposures by the inhalation and dermal routes follow the same procedure (Table 5-13 and 5-14). Exposure duration scenarios include full lifetime exposure (assuming a 70-year lifespan), and two 30-year exposures at ages 0–30 and ages 20–50. Table 5-13 lists the four factors (ADAFs, cancer risk estimate, assumed exposure, and duration adjustment) that are

needed to calculate the partial cancer risk based on the early age-specific group. The cancer risk for each age group is the product of the four factors in columns 2–5. Therefore, the cancer risk following daily benzo[a]pyrene oral exposure in the age group 0 to <2 years is the product of the values in columns 2–5 or $10 \times 1 \times 0.001 \times 2/70 = 4 \times 10^{-4}$. The cancer risk for specific exposure duration scenarios that are listed in the last column are added together to get the total risk. Thus, a 70-year (lifetime) risk estimate for continuous exposure to 0.001 mg/kg-day benzo[a]pyrene is 2×10^{-3}, which is adjusted for early-life susceptibility and assumes a 70-year lifetime and constant exposure across age groups.

Table 5.13. Application of ADAFs to benzo[a]pyrene cancer risk following a lifetime (70-year) oral exposure

Age Group	ADAF	Unit risk (per mg/kg-day)	Exposure Concentration (mg/kg-day)	Duration adjustment	Cancer Risk for Specific Exposure Duration Scenarios
0–<2 yrs	10	1	0.001	2 yrs/70 yrs	0.0003
2–<16 yrs	3	1	0.001	14 yrs/70 yrs	0.0006
≥16 yrs	1	1	0.001	54 yrs/70 yrs	0.0007
				Total Risk	0.002

In calculating the cancer risk for a 30-year constant exposure to benzo[a]pyrene at an exposure level of 0.001 mg/kg-day from ages 0–30, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks for the three age groups would be 3×10^{-4}, 6×10^{-4}, and 2×10^{-4}, which would result in a total risk estimate of 1×10^{-3}.

In calculating the cancer risk for a 30-year constant exposure to benzo[a]pyrene at an exposure level of 0.001 mg/kg-day from ages 20–50, the duration adjustments would be 0/70, 0/70, and 30/70. The partial risks for the three groups are 0, 0, and 4×10^{-4}, which would result in a total risk estimate of 4×10^{-4}.

Consistent with the approaches for the oral route of exposure, the ADAFs should also be applied when assessing cancer risks for subpopulations with early life exposures to benzo[a]pyrene via the inhalation and dermal routes are presented in Tables 5-14 and 5-15.

Table 5-14. Application of ADAFs to benzo[a]pyrene cancer risk following a lifetime (70-year) inhalation exposure

Age Group	ADAF	Unit risk (per µg/m^3)	Exposure Concentration (µg/m^3)	Duration adjustment	Cancer Risk for Specific Exposure Duration Scenarios

0–<2 yrs	10	5×10^{-4}	1	2 yrs/70 yrs	0.0001
2–<16 yrs	3	5×10^{-4}	1	14 yrs/70 yrs	0.0003
≥16 yrs	1	5×10^{-4}	1	54 yrs/70 yrs	0.0004
				Total Risk	0.0008

Table 5-15. Application of ADAFs to benzo[a]pyrene cancer risk following a lifetime (70-year) dermal exposure

Age Group	ADAF	Unit risk (per µg/day)	Exposure Concentration (µg/day)	Duration adjustment	Cancer Risk for Specific Exposure Duration Scenarios
0–<2 yrs	10	0.005	0.001	2 yrs/70 yrs	1×10^{-6}
2–<16 yrs	3	0.005	0.001	14 yrs/70 yrs	3×10^{-6}
≥16 yrs	1	0.005	0.001	54 yrs/70 yrs	4×10^{-6}
				Total Risk	8×10^{-5}

5.4.5. Uncertainties in Cancer Risk Values

5.4.5.1. *Oral Slope Factor*

Uncertainty in the recommended oral slope factor is reflected in the range of slope factors among tumors sites and species; the lowest and highest slope factors listed in Table 5-6 show about a 35-fold difference. While the highest risk estimates were derived from the incidence data for forestomach tumors in both rats and mice, the oral slope factor based on the mouse forestomach data was about threefold higher than the oral slope factor based on male rat data (Table 5-6). These comparisons show that the selection of target organ, animal species, and dosimetric extrapolation can impact the oral cancer risk estimate. However, all of the activation pathways implicated in benzo[a]pyrene carcinogenicity have been observed in human tissues and associations have been made between the spectra of mutations in tumor tissues from benzo[a]pyrene-exposed animals and humans exposed to complex PAH mixtures containing benzo[a]pyrene (see Section 4.7.3).

5.4.5.2. *Inhalation Unit Risk*

Only one animal cancer bioassay by the inhalation route is available which describes the dose-response relationship for respiratory tract tumors with chronic inhalation exposure to benzo[a]pyrene (Thyssen et al., 1981). Although corroborative information on dose-response relationships in other animal species is lacking, the findings for upper respiratory tract tumors are consistent with findings in other hamster studies with intratracheal administration of benzo[a]pyrene. This study is adequate for dose-response analysis and derivation of an inhalation unit risk estimate, but some associated uncertainty includes the inability to apply U.S.

EPA (1994b) dosimetry approaches to extrapolate inhaled doses from animals to humans, due to the use of a soluble hygroscopic carrier particle (NaCl) for the delivery of benzo[a]pyrene. One likely consequence of the use of hygroscopic carrier particles would be the growth of benzo[a]pyrene-NaCl particles in the humid environment of the respiratory tract resulting in increased particle diameter and resulting changes in particle deposition, specifically, increased impaction in the upper respiratory tract (Xu and Yu 1985; Ferron 1994; Asgharian 2004; Varghese and Gangamma 2009). Exposure to benzo[a]pyrene in the environment predominantly occurs via non-soluble, non-hygroscopic particles. The potential impact of differences in carrier particle on the magnitude of the inhalation unit risk is unknown.

5.4.5.3 *Dermal Slope Factor*

Uncertainty in the recommended dermal slope factor is partly reflected in the range of slope factors derived from the modeled mouse skin tumor data sets: the lowest and highest dermal slope factors listed in Table 5-12 show a 7-fold difference (0.25-1.8 ug/day) in magnitude. There is some indication that the recommended dermal slope factor may underestimate cancer risk, due to inadequate data to take the observed decreasing tumor latency with increasing exposure level into account. Reliance on studies with the lowest exposure levels where early mortality due to benzo[a]pyrene exposure was low and exposures continued for approximately 104 weeks may minimize this source of uncertainty.

Human dermal exposure to benzo[a]pyrene in the environment likely occurs predominantly through soil contact. The available mouse dermal bioassays of benzo[a]pyrene relied on delivery of benzo[a]pyrene to the skin in a solvent solution (typically acetone or toluene). The use of a volatile solvent likely results in a larger dose of benzo[a]pyrene available for uptake into the skin (compared to soil). Reliance on these studies may overestimate the risk of skin tumors from benzo[a]pyrene contact through soil; however, cancer bioassays delivering benzo[a]pyrene through a soil matrix are not available.

There is uncertainty in extrapolating from the intermittent exposures in the mouse assays to daily exposure scenarios. This assessment makes the assumption that risk is proportional to total cumulative exposure. The extent to which this assumption under- or overestimates risk is unknown.

The available data were not useful to determine which animal species may be the best surrogate for human dermal response to benzo[a]pyrene. In extrapolation of the animal dermal information to humans the inherent assumption is that equal area of skin from a mouse or human would have equal probability of developing a tumor upon benzo[a]pyrene exposure. Qualitatively, the toxicokinetics and toxicodynamics in mouse and human skin appear to be similar (Knafla et al., 2010; Bickers et al., 1984). Specifically, all of the activation pathways implicated in benzo[a]pyrene carcinogenicity have been observed in mouse and human skin and associations have been made between the spectra of mutations in tumor tissues from

benzo[a]pyrene-exposed animals and humans exposed to complex PAH mixtures containing benzo[a]pyrene (see Section 4.7.3).

This dermal slope factor for benzo[a]pyrene is based on skin cancer and it is not developed to represent systemic cancer risk from dermal exposure. It is unclear whether dermal exposure to benzo[a]pyrene would result in elevated risk of systemic tumors. Some studies in humans suggest that although the skin may be responsible for a "first pass" metabolic effect, benzo[a]pyrene-specific adduct levels have been detected in WBC following dermal exposure to benzo[a]pyrene, indicating that dermally applied benzo[a]pyrene enters systemic circulation (Godschalk et al., 1998). Although none of the lifetime dermal bioassays in mice, which included macroscopic examination of internal organs, reported an elevation of systemic tumors in benzo[a]pyrene-treated mice compared to controls (Poel 1959; Roe et al., 1970; Schmidt et al., 1973; Schmahl et al., 1977; Habs et al., 1980; Higginbotham et al.,1993), most of these studies attempted to remove animals with grossly observed tumors of the skin from the study before the death of the animal, possibly minimizing the development of more distant tumors with longer latency. The risk of benzo[a]pyrene-induced point of contact tumors in the skin likely competes with systemic risk of tumors. Currently, the potential contribution of dermally absorbed benzo[a]pyrene to systemic cancer risk is unclear.

5.5.5. Previous Cancer Assessment

The previous cancer assessment for benzo[a]pyrene was posted on the IRIS database in 1987. At that time, benzo[a]pyrene was classified as a probable human carcinogen (Group B2) based on inadequate data in humans and sufficient data in animals via several routes of exposure. An oral slope factor was derived from the geometric mean of four slope factor estimates based on studies in Sprague-Dawley rats (Brune et al., 1981) and CFW-Swiss mice (Neal and Rigdon, 1967). Brune et al. (1981) administered 0.15 mg/kg benzo[a]pyrene in the diet every 9th day or 5 days/week in a 1.5% caffeine solution until rats were moribund or dead. A single slope factor estimate of 11.7 per mg/kg-day, based on a linearized multistage model applied to the combined incidence of forestomach, esophageal, and laryngeal tumors, was derived. In the Neal and Rigdon (1967) bioassay, mice administered benzo[a]pyrene in the diet at concentrations ranging from 1 to 250 ppm for up to 197 days developed significantly increased incidences of forestomach tumors. This study utilized mixed sex dose groups with mice from 3 weeks to 6 months old at the start of dosing. This study did not include concurrent controls. This necessitated the use of historical controls (from SWR/J mice) for the incidence of forestomach tumors from a study by (Rabstein et al., 1973). Three modeling procedures were used to derive risk estimates from these data. For one risk estimate, Clement Associates (1990) fit a two-stage response model, based on exposure-dependent changes in both transition rates and growth rates of preneoplastic cells, to derive a value of 5.9 per mg/kg-day. In a U.S. EPA report (1991b), a value of 9.0 per mg/kg-day, derived by linear extrapolation from the 10% response point to the

background of an empirically fitted dose-response curve, was identified. Finally, using a Weibull-type model to reflect less-than-lifetime exposure to benzo[a]pyrene, the same U.S. EPA report (1991b) derived an upper-bound slope factor estimate of 4.5 per mg/kg-day. Since the variance for the four slope factor estimates was low, and in order to consider all of the available data, the geometric mean of these four estimates, 7.3 per mg/kg-day, was recommended as the oral slope factor.

An inhalation unit risk and dermal slope factor were not previously available on IRIS.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

benzo[a]pyrene is a five-ring nonsubstituted PAH, which is produced through natural and anthropogenic processes involving the incomplete combustion or pyrrolysis of carbon-containing materials. Benzo[a]pyrene exists in the environment in complex mixtures, which may consist of numerous PAHs, including heterocyclic and nonheterocyclic forms as well as aza arenes and nitro-substituted PAHs. The magnitude of human exposure to benzo[a]pyrene and other PAHs depends on several factors related to lifestyle (e.g., diet, tobacco smoking), occupation, and living conditions (e.g., urban versus rural setting, domestic heating, and cooking methods).

There are limited data establishing associations between increased risk for noncancer health effects in humans and exposure to benzo[a]pyrene. Several epidemiology studies have reported associations between adverse birth outcomes including reduced birth weight, postnatal body weight, and head circumference with internal biomarkers of exposure to benzo[a]pyrene (BPDE-DNA adducts) via exposure to complex PAH mixtures (Tang et al., 2008, 2006; Perera et al., 2005a, b). However, extrapolations from these studies are complicated by the concomitant exposure to multiple PAHs and other components in the mixture.

There is evidence of human carcinogenicity for several PAH mixtures containing benzo[a]pyrene, such as soot, coal tars, coal-tar pitch, mineral oils, and shale oils (IARC, 2010; Baan et al., 2009; Straif et al., 2005). There is also evidence of carcinogenicity in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as aluminum production, chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, and paving and roofing with coal tar pitch (IARC, 2010; Baan et al., 2009; Straif et al., 2005). Benzo[a]pyrene is also a notable constituent of tobacco smoke (IARC 2004). An increasing number of studies report exposure biomarkers such as benzo[a]pyrene- or PAH-DNA adducts, and several cohort studies demonstrate a positive exposure-response relationship with cumulative PAH exposure using benzo[a]pyrene as an indicator substance. Because benzo[a]pyrene is only one of many PAHs that could contribute to these observed increases in cancer, the epidemiologic studies provide credible but limited support for a causative role of benzo[a]pyrene in human cancer. Studies in multiple species of laboratory animals indicate that benzo[a]pyrene is carcinogenic by all routes of exposure.

6.2. DOSE RESPONSE

6.2.1. RfD

Limited human data are available to inform noncancer health effects following chronic oral exposure to benzo[a]pyrene. Animal studies reporting effects of benzo[a]pyrene include

several chronic cancer bioassays (which report limited noncancer endpoints; Kroese et al. 2001; Beland and Culp, 1998; Culp et al., 1998), several subchronic studies (Knuckles et al., 2001; De Jong et al., 1999), developmental toxicity studies (Kristensen et al., 1995; MacKenzie and Angevine, 1981) and several reproductive toxicity studies (Mohamed et al., 2010; Xu et al., 2010; Zheng et al., 2010).

In consideration of the available studies reporting low-dose effects of chronic and subchronic oral exposure to benzo(a)pyrene in animals, the Xu et al., (2010) study was chosen as the principal study. This study identified biologically and statistically significant decrements in ovary weight, number of primordial follicles, estrogen levels, and estrus cyclicity. These reductions in female reproductive endpoints observed in rats are supported by a large database of animal studies indicating that benzo[a]pyrene, administered by multiple routes of exposure, is ovotoxic with effects including decreased ovary weight, decreased primordial follicles, and reduced fertility (Mattison et al., 1980; MacKenzie and Angevine, 1981; Swartz and Mattison 1985; Miller et al., 1992; Kristensen et al.,1995; Borman et al, 2000; Archibong et al., 2002). Additionally, studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (El Nemr et al., 1998; Neal et al., 2005). Specific associations have also been made between infertility and increased levels of benzo[a]pyrene in follicular fluid in women undergoing in vitro fertilization (Neal et al., 2008).

The RfD of 0.0005 mg/kg-day (0.5 µg/kg-day) was derived using a $BMDL_{1SD}$ of 1.5 mg/kg-day for reduced ovary weight in SD rats exposed to benzo[a]pyrene via gavage for 60 days (Xu et al., 2010). To derive the RfD, this POD was divided by a total UF of 3000 (factors of 10 for animal-to-human extrapolation, human interindividual variability in susceptibility, and subchronic to chronic extrapolation, and 3 for database deficiencies). The default animal-to-human extrapolation and human variability factors were applied because of the lack of quantitative information to assess toxicokinetic or toxicodynamic differences between animals and humans and the range of susceptibilities in human populations. A subchronic to chronic extrapolation factor was applied because the POD was chosen from a study with a less than lifetime exposure duration. In addition, a database uncertainty factor of 3 was applied to account for deficiencies in the benzo[a]pyrene toxicity database, primarily the lack of a standard multigenerational reproductive study and the lack of a neurodevelopmental study.

The overall confidence in the RfD is low-to-medium. Confidence in the principal study (Xu et al., 2010) is medium. The design, conduct, and reporting of this subchronic toxicity study were adequate; however, the number of dose groups and number of animals per group were low. Confidence in the database is low-to-medium primarily due to the lack of a multigeneration reproductive toxicity study (with exposure from pre-mating to sexual maturity) and the lack of a neurodevelopmental study. Reflecting medium confidence in the principal study and low-to-medium confidence in the database, confidence in the RfD is low-to-medium.

6.2.2. RfC

The only chronic inhalation study available for benzo[a]pyrene was designed as a cancer bioassay and did not report noncancer endpoints (Thyssen et al.1981). However, several repeated dose reproductive and developmental toxicity studies are available in which effects on fetal survival and the male reproductive system have been observed following inhalation exposure.

Archibong et al. (2002) was selected as the principal study as it observed biologically significant effects in F344 rats at the lowest dose tested by the inhalation route. This study indicates that the developing fetus is a sensitive target following inhalation exposure to benzo[a]pyrene. Exposure to benzo[a]pyrene at 25 µg/m^3 on GDs 11–20 caused biologically significant reductions in fetal survival and body weight decreases in the surviving pups. The observed decrease in pup weight and fetal survival were selected as critical effects as they are the most sensitive noncancer effects observed following inhalation exposure to benzo[a]pyrene. Additional support for this endpoint can be found from an oral study of benzo[a]pyrene in mice which observed decreased survival of litters, decreased pup weight, and decreased reproductive organ weight following in utero exposure to benzo[a]pyrene on GD 7-16 (MacKenzie and Angevine, 1981). Though only a few studies exist that evaluate benzo[a]pyrene by the inhalation route, the oral studies support the reproductive and developmental effects observed in the available inhalation studies.

The RfC of 5 x 10^{-6} mg/m^3-day was derived using a $LOAEL_{ADJ[HEC]}$ of 4.6 µg/m^3-day for decrease in pup weight and fetal survival in F344 rats exposed to benzo[a]pyrene aerosols on GDs 11-20 (Archibong et al., 2002). To derive the RfC, the POD was divided by a total UF of 1000 (factors of 3 for animal-to-human extrapolation, 10 for human interindividual variability in susceptibility and LOAEL-to-NOAEL extrapolation, and 3 for database deficiencies). The default animal-to-human extrapolation and human variability factors were applied because of the lack of quantitative information to assess toxicodynamic differences between animals and humans, whereas the toxicokinetic component is addressed by the determination of a HEC as described in the RfC methodology (U.S. EPA, 1994b). The default human variability factor was applied because of the lack of information regarding the range of susceptibilities in human populations. A LOAEL-to-NOAEL extrapolation factor was applied because a NOAEL was not identified for decreased fetal survival observed by Archibong et al (2002). In addition, a database uncertainty factor of 3 was applied to account for deficiencies in the benzo[a]pyrene toxicity database, primarily the lack of a standard multigenerational reproductive study.

The overall confidence in the RfC is low-to-medium. Confidence in the principal study (Archibong et al., 2002) is medium. The conduct, and reporting of this developmental dietary study were adequate, however, a NOAEL was not identified. Confidence in the database is low-to-medium due to the lack of a multigeneration reproductive toxicity study, the lack of studies on

immune endpoints, and the lack of information regarding subchronic and chronic inhalation exposure. Reflecting medium confidence in the principal study and low-to-medium confidence in the database, confidence in the RfC is low-to-medium.

6.2.3. Cancer

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans" based on evidence of carcinogenicity in humans exposed to different PAH mixtures containing benzo[a]pyrene, extensive and consistent evidence of carcinogenicity in laboratory animals exposed to benzo[a]pyrene via several routes of administration, and strong evidence that mechanisms of carcinogenesis in laboratory animals also occur in humans exposed to PAH mixtures containing benzo[a]pyrene. Strong evidence links the metabolism of benzo[a]pyrene to DNA-reactive agents with key mutational events in genes that can lead to tumor initiation. Specifically, the metabolic activation of benzo[a]pyrene occurs in human tissues, and associations have been made between spectra of mutations in the p53 tumor suppressor gene or ras oncogenes induced by benzo[a]pyrene metabolites and the spectra of mutations in these genes in tumor tissue from benzo[a]pyrene-exposed animals and humans.

Several lines of evidence relating to mutagenicity and tumor initiation are available for benzo[a]pyrene including: in vitro evidence of DNA adducts, mutations, cytogenetic damage, and primary DNA damage; in vivo DNA adducts, gene mutations, cytogenetic damage, and other measures of primary DNA damage; detection of DNA adducts in target tissue in vivo; and detection of cancer-relevant oncogene/tumor suppressor gene mutations in target tissue in vivo. Taken together, these data provide support for a mutagenic MOA for benzo[a]pyrene-induced cancer. Because benzo[a]pyrene is expected to cause cancer via a mutagenic MOA, a linear approach to low-dose extrapolation was used in the derivation of the cancer risk estimates.

In the absence of appropriate benzo[a]pyrene-specific data to adjust cancer risk values for early life exposure, ADAFs combined with age-specific exposure estimates should be applied to the cancer risk values (oral slope factor, inhalation unit risk, and dermal slope factor) when assessing cancer risks for individuals exposed during early life periods, as per U.S. EPA (2005b) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*.

6.2.3.1. Cancer—Oral

Lifetime oral exposure to benzo[a]pyrene has been associated with forestomach, liver, oral cavity, jejunum or duodenum, and auditory canal tumors in male and female Wistar rats (Kroese et al., 2001), with forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981), and with forestomach, esophagus, tongue, and larynx tumors in female B6C3F1 mice (male mice were not tested); (Beland and Culp, 1998; Culp et al., 1998). Less-than-lifetime oral exposure to benzo[a]pyrene is also associated with forestomach tumors in more than 10

additional bioassays with several strains of mice (see Section 4.2.1.2.). Both the rat bioassay by Kroese et al. (2001) and the mouse bioassay by Beland and Culp (1998) included histological examinations for tumors, three exposure levels and controls, and 50 animals per dose group. The chronic studies by Kroese et al. and Beland and Culp studies were therefore selected for dose-response analysis.

EPA used the multistage-Weibull model in the derivation of the oral slope factor because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups in the rat bioassay. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination (slope factor = $0.1/BMDL_{10}$). The oral slope factor of **1 per mg/kg-day** is based on the tumor response in the alimentary tract (forestomach, esophagus, tongue, larynx) of female $B6C3F_1$ mice exposed to benzo[a]pyrene in the diet for 2 years (Beland and Culp, 1998). The slope factor was derived by linear extrapolation from a human equivalent $BMDL_{10}$ of 0.07 mg/kg-day for forestomach, esophagus, tongue, and larynx papillomas or carcinomas. The recommended slope factor was selected as the factor with the highest value among a range of slope factors derived from tumor responses at several sites in the 2-year male and female Wistar rat bioassay by Kroese et al. (2001) and the 2-year female $B6C3F_1$ mouse bioassay by Beland and Culp (1998).

6.2.3.2. Cancer—Inhalation

Inhalation exposure to benzo[a]pyrene was associated with squamous cell neoplasia in the larynx, pharynx, trachea, esophagus, and forestomach, of male Syrian golden hamsters exposed to benzo[a]pyrene condensed onto NaCl particles (Thyssen et al., 1981). Supportive evidence for the carcinogenicity of inhaled benzo[a]pyrene comes from 10 additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation (see Section 4.2.2.2 for references). The Thyssen et al. (1981) bioassay represents the best available data for describing dose-response relationships for cancer from inhaled benzo[a]pyrene.

A time-to-tumor dose-response model was fit to the time-weighted average exposure concentrations and the individual animal occurrence data for tumors in the larynx, pharynx, trachea, esophagus, and forestomach. The inhalation unit risk of **5×10^{-4} per µg/m^3** was calculated by linear extrapolation (slope factor = $0.1/BMDL_{10}$) from a $BMDL_{10}$ of 0.20 mg/m^3 for the occurrence of upper respiratory and upper digestive tract tumors in male hamsters chronically exposed by inhalation to benzo[a]pyrene (Thyssen et al., 1981).

6.2.3.3. Cancer—Dermal

Skin cancer in humans has been documented to result from occupational exposure to complex mixtures of PAHs including benzo[a]pyrene such as coal tar, coal tar pitches, non-refined mineral oils, shale oils and soot (Boffetta et al., 1997; WHO, 1998; ATSDR, 1995). No

human studies of exposure to benzo[a]pyrene alone are known to exist. In animal models, numerous dose response studies have demonstrated the increased incidence of skin tumors with increasing dermal exposure of benzo[a]pyrene, in all species tested (mice, rabbits, rats, and guinea pigs), though most benzo[a]pyrene chronic dermal bioassays have been conducted in mice. This analysis focuses on chronic carcinogenicity bioassays in several strains of mice demonstrating increasing incidence of benign and malignant skin tumors following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime.

As with the oral and inhalation benzo[a]pyrene carcinogenicity data (see Sections 5.4.1.3 and 5.4.2.3), benzo[a]pyrene's dermal exposure carcinogenicity data were generally characterized by earlier occurrence of tumors with increasing exposure and increased mortality with increasing exposure level. Each of the dermal data sets was modeled using the multistage model, incorporating adjustments for early mortality, when data were available, prior to modeling.

The POD of 0.0066 µg/day, based on the tumor response in C3H/HeJ male mice (Sivak et al., 1997), is recommended for developing a human dermal slope factor because it is the highest POD among studies with low observed tumor response to benzo[a]pyrene (20%). Following the modeling, this POD from the Sivak et al. (1997) dataset in male C3H/HeJ mice was adjusted by allometric scaling. The dermal slope factor of **0.005 per µg/day** was calculated by linear extrapolation (slope factor = $0.1/BMDL_{10\text{-}HED}$) from the human equivalent POD (19.7 µg/day) for the occurrence of skin tumors in male mice chronically exposed dermally to benzo[a]pyrene (Sivak et al., 1997).

This dermal slope factor has been calculated based on the risk of skin tumors in mice following dermal exposure to benzo[a]pyrene. As this slope factor has been developed for a local effect, it is not intended to estimate systemic risk of cancer following dermal absorption of benzo[a]pyrene into the systemic circulation.

7. REFERENCES

Agarwal, R; Medrano, EE; Khan, IU; et al. (1991) Metabolism of benzo[a]pyrene by human melanocytes in culture. Carcinogenesis 12(10):1963–1966.

Aklillu, E; Ovrebo, S; Botnen, IV; et al. (2005) Characterization of common CYP1B1 variants with different capacity for benzo[a]pyrene-7,8-dihydrodiol epoxide formation from benzo[a]pyrene. Cancer Res 65(12):5105–5111.

Albert, RE; Miller, ML; Cody, T; et al. (1991) Benzo[a]pyrene-induced skin damage and tumor promotion in the mouse. Carcinogenesis 12(7):1273–1280.

Alexandrie, A-K; Warholm, M; Carstensen, U; et al. (2000) CYP1A1 and GSTM1 polymorphisms affect urinary 1-hyroxypyrene levels after PAH exposure. Carcinogenesis 21(4):699–676.

Alexandrie, AK; Nyberg, F; Warholm, M; et al. (2004) Influence of CYP1A1, GSTM1, GSTT1, and NQO1 genotypes and cumulative smoking dose on lung cancer risk in a Swedish population. Cancer Epidemiol Biomarkers Prev 13(6):908–914.

Alexandrov, K; Rojas-Moreno, M. (1990) In vivo DNA adduct formation by benzo(a)pyrene in mouse and rat epidermal and dermal fibroblasts after topical application of an initiating dose of benzo(a)pyrene. Arch Geschwulstforsch 60(5):329–340.

Alexandrov, K; Rojas, M; Goldberg, M; et al. (1990) A new sensitive fluorometric assay for the metabolism of (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by human hair follicles. Carcinogenesis 11:2157–2161.

Alexandrov, K; Rojas, M; Geneste, O; et al. (1992) An improved fluorometric assay for dosimetry of benzo(a)pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res 52(22):6248–6253.

Alexandrov, K; Cascorbi, I; Rojas, M; et al. (2002) CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. Carcinogenesis 23(12):1969–1977.

Amos, CI; Xu, W; Spitz, MR. (1999) Is there a genetic basis for lung cancer susceptibility? Recent Results Cancer Res 151:3–12.

Anderson, KE; Kadlubar, FF; Kulldorff, M; et al. (2005) Dietary intake of heterocyclic amines and benzo(a)pyrene: associations with pancreatic cancer. Cancer Epidemiol Biomarkers Prev 14(9):2261–2265.

Andersson, K; Levin, JO; Nilsson, CA. (1983) Sampling and analysis of particulate and gaseous polycyclic aromatic hydrocarbons from coal tar sources in the working environment. Chemosphere 12(2):197–207.

Andrysik, Z; Vondracek, J; Machala, M; et al. (2007) The aryl hydrocarbon receptor-dependent deregulation of cell cycle control induced by polycyclic aromatic hydrocarbons in rat liver epithelial cells. Mutat Res 615(1-2):87–97.

Archibong, AE; Inyang, F; Ramesh, A; et al. (2002) Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene. Reprod Toxicol 16(6):801–808.

Archibong, AE; Ramesh, A; Niaz, MS; et al. (2008) Effects of benzo(a)pyrene on intra-testicular function in F-344 rats. Int J Environ Res Public Health 5(1):32–40.

Ariese, F; Verkaik, M; Hoornweg, GP; et al. (1994) Trace analysis of 3-hydroxy benzo[a]pyrene in urine for the biomonitoring of human exposure to polycyclic aromatic hydrocarbons. J Anal Toxicol 18(4):195–204.

Arlt, VM; Frei, E; Schmeiser, HH. (2007) ECNIS-sponsored workshop on biomarkers of exposure and cancer risk: DNA damage and DNA adduct detection and 6th GUM-^{32}P-postlabelling workshop, German Cancer Research Center, Heidelberg, Germany, 29-30 September 2006. Mutagenesis 22(1):83–88.

Armstrong, B; Tremblay, C; Baris, D; et al. (1994) Lung cancer mortality and polynuclear aromatic hydrocarbons: a case-cohort study of aluminum production workers in Arvida, Quebec, Canada. Am J Epidemiol 139(3):250–262.

Armstrong, B; Hutchinson, E; Unwin, J; et al. (2004) Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. Environ Health Perspect 112(9):970–978.

Arnould, JP; Pfohl-Leszkowicz, A; Bach, V; et al. (1999) Biological monitoring exposure of workers from plant producing carbon electrodes: quantification of benzo[a]pyrene DNA-adducts in leukocytes, by a ^{32}P-postlabelling method and an immunoassay. Hum Exp Toxicol 18(5):314–321.

Arnould, JP; Kubiak, R; Belowski, J; et al. (2000) Detection of benzo[a]pyrene-DNA adducts in leukocytes of coke oven workers. Pathol Biol (Paris) 48(6):548–553.

Assennato, G; Ferri, GM; Foa, V; et al. (1993a) Correlation between PAH airborne concentration and PAH-DNA adducts levels in coke-oven workers. Int Arch Occup Environ Health 65(1 Suppl):S143–S145.

Assennato, G; Ferri, GM; Tockman, MS; et al. (1993b) Biomarkers of carcinogen exposure and cancer risk in a coke plant. Environ Health Perspect 99:237–239.

ATSDR (Agency for Toxic Substances and Disease Registry). (1995) Toxicological profile for polycyclic aromatic hydrocarbons. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Autrup, H; Seremet, T. (1986) Excretion of benzo[a]pyrene-Gua adduct in the urine of benzo[a]pyrene-treated rats. Chem Biol Interact 60(2):217–226.

Autrup, H; Harris, CC; Stoner, GD; et al. (1978) Metabolism of [3H]benzo[a]pyrene by cultured human bronchus and cultured human pulmonary alveolar macrophages. Lab Invest 38(3):217–224.

Autrup, H; Wefald, FC; Jeffrey, AM; et al. (1980) Metabolism of benzo[a]pyrene by cultured tracheobronchial tissues from mice, rats, hamsters, bovines and humans. Int J Cancer 25(2):293–300.

Balu, N; Padgett, WT; Lambert, GR; et al. (2004) Identification and characterization of novel stable deoxyguanosine and deoxyadenosine adducts of benzo[a]pyrene-7,8-quinone from reactions at physiological pH. Chem Res Toxicol 17(6):827–838.

Bao, H; Vepakomma, M; Sarkar, MA. (2002) Benzo(a)pyrene exposure induces CYP1A1 activity and expression in human endometrial cells. J Steroid Biochem Mol Biol 81(1):37–45.

Barry, G; Cook, JW; Haslewood, AD; et al. (1935) The production of cancer by pure hydrocarbons-Part III. Proc R Soc London 117:318–351.

Bartsch, H; Rojas, M; Nair, U; et al. (1999) Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. Cancer Detect Prev 23(6):445–453.

Becher, G; Bjorseth, A. (1983) Determination of exposure to polycyclic aromatic hydrocarbons by analysis of human urine. Cancer Lett 17(3):301–311.

Beland, F; Culp, S. (1998) Chronic bioassay of two composite samples from selected manufactured gas plant waste sites. Jefferson, AK: Research, NCfT; Technical Report 6722.02 (unpublished).

Benjamin, H; Storkson, J; Tallas, PG; et al. (1988) Reduction of benzo[a]pyrene-induced forestomach neoplasms in mice given nitrite and dietary soy sauce. Food Chem Toxicol 26(8):671–678.

Bentsen-Farmen, RK; Botnen, I; Eilertsen, E; et al. (1999a) The effect of CYP1A1 induction on the formation of benzo(a)pyrene adducts in liver and lung DNA and plasma albumin in rats exposed to benzo(a)pyrene: Adduct quantitation by immunoassay and an HPLC method. Biomarkers 4(1):37–47.

Bentsen-Farmen, RK; Botnen, IV; Noto, H; et al. (1999b) Detection of polycyclic aromatic hydrocarbon metabolites by high-pressure liquid chromatography after purification on immunoaffinity columns in urine from occupationally exposed workers. Int Arch Occup Environ Health 72(3):161–168.

Berenblum, I; Haran, N. (1955) The influence of croton oil and of polyethylene glycol-400 on carcinogenesis in the forestomach of the mouse. Cancer Res 15(8):510–516.

Bevan, DR; Sadler, VM. (1992) Quinol diglucuronides are predominant conjugated metabolites found in bile of rats following intratracheal instillation of benzo[a]pyrene. Carcinogenesis 13(3):403–407.

Bevan, DR; Ulman, MR. (1991) Examination of factors that may influence disposition of benzo[a]pyrene in vivo: vehicles and asbestos. Cancer Lett 57(2):173–179.

Bevan, DR; Weyand, EH. (1988) Compartmental analysis of the disposition of benzo[a]pyrene in rats. Carcinogenesis 9(11):2027–2032.

Biancifiori, C; Caschera, F; Giornelli-Santulli, F; et al. (1967) The action of oestrone and four chemical carcinogens in intact and ovariectomized BALB/c/Cb/Se mice. Br J Cancer 21:452–459.

Bingham, E; Falk, HL. (1969) Environmental carcinogens. The modifying effect of cocarcinogens on the threshold response. Arch Environ Health 19(6):779–783.

Bjørseth, A; Bjorseth, O; Fjeldstad, PE. (1978a) Polycyclic aromatic hydrocarbons in the work atmosphere. I. Determination in an aluminum reduction plant. Scand J Work Environ Health 4(3):212–223.

Bjørseth, A; Bjorseth, O; Fjeldstad, PE. (1978b) Polycyclic aromatic hydrocarbons in the work atmosphere. II. Determination in a coke plant. Scand J Work Environ Health 4(3):224–236.

Blaha, L; Kapplova, P; Vondracek, J; et al. (2002) Inhibition of gap-junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons. Toxicol Sci 65(1):43–51.

Boerrigter, ME. (1999) Treatment of lacZ plasmid-based transgenic mice with benzo[a]pyrene: measurement of DNA adduct levels, mutant frequencies, and mutant spectra. Environ Mol Mutagen 34(2-3):140–147.

Boffetta, P; Jourenkova, N; Gustavsson, P. (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 8(3):444–472.

Bol, SA; van Steeg, H; Jansen, JG; et al. (1998) Elevated frequencies of benzo(a)pyrene-induced Hprt mutations in internal tissue of XPA-deficient mice. Cancer Res 58(13):2850–2856.

Bolton, JL; Trush, MA; Penning, TM; et al. (2000) Role of quinones in toxicology. Chem Res Toxicol 13(3):135–160.

Bompart, G; Clamens, S. (1990) In vitro and in vivo benzo(a)pyrene metabolism in rat liver and lung microsomes: effect of sodium selenite. J Toxicol Clin Exp 10(1):3–14.

Bond, JA; Harkema, JR; Russell, VI. (1988) Regional distribution of xenobiotic metabolizing enzymes in respiratory airways of dogs. Drug Metab Dispos 16(1):116–124.

Borman, SM; Christian, PJ; Sipes, IG; Hoyer, PB. (2000) Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol App Pharmacol 167;191-198.

Bosetti, C; Boffetta, P; La Vecchia, C. (2007) Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. Ann Oncol 18:431–446.

Bostrom, CE; Gerde, P; Hanberg, A; et al. (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect 110(Supp 3):451–488.

Bowman, ED; Rothman, N; Hackl, C; et al. (1997) Interindividual variation in the levels of certain urinary polycyclic aromatic hydrocarbon metabolites following medicinal exposure to coal tar ointment. Biomarkers 2:321–327.

Brooks, RA; Gooderham, NJ; Edwards, RJ; et al. (1999) The mutagenicity of benzo[a]pyrene in mouse small intestine. Carcinogenesis 20(1):109–114.

Bruce, ED; Austenrieth, RL; Burghardt, RC; et al. (2008) Using quantitative structure-activity relationships (QSAR) to predict toxic endpoints for polycyclic aromatic hydrocarbons (PAH). J Toxicol Environ Health Part A 71:1073–1084.

Brune, H; Deutsch-Wenzel, RP; Habs, M; et al. (1981) Investigation of the tumorigenic response to benzo(a)pyrene in aqueous caffeine solution applied orally to Sprague-Dawley rats. J Cancer Res Clin Oncol 102(2):153–157.

Bryan, WR; Shimkin, MB. (1943) Quantitative analysis of dose-response data obtained with three carcinogenic hydrocarbons in strain C3H male mice. J Natl Cancer Inst 3:503–531.

Buckley, TJ; Waldman, JM; Dhara, R; et al. (1995) An assessment of a urinary biomarker for total human environmental exposure to benzo[a]pyrene. Int Arch Occup Environ Health 67(4):257–266.

Buening, MK; Wislocki, PG; Levin, W; et al. (1978) Tumorigenicity of the optical enantiomers of the diastereomeric benzo[a]pyrene 7,8-diol-9,10-epoxides in newborn mice: exceptional activity of (+)-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. Proc Natl Acad Sci USA 75(11):5358–5361.

Bulay, OM; Wattenberg, LW. (1971) Carcinogenic effects of polycyclic hydrocarbon carcinogen administration to mice during pregnancy on the progeny. J Natl Cancer Inst 46(2):397–402.

Burdick, AD; Davis, JW; Liu, KJ; et al. (2003) Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. Cancer Res 63(22):7825–7833.

Burdick, AD; Ivnitski-Steele, ID; Lauer, FT; et al. (2006) PYK2 mediates anti-apoptotic AKT signaling in response to benzo[a]pyrene diol epoxide in mammary epithelial cells. Carcinogenesis 27(11):2331–2340.

Burns-Naas, LA; Hastings, KL; Ladics, GS; et al. (2008) What's so special about the developing immune system? Int J Toxicol 27(2):223-254.

Burstyn, I; Kromhout, H; Partanen, T; et al. (2005) Polycyclic aromatic hydrocarbons and fatal ischemic heart disease. Epidemiology 16(6):744–750.

Burstyn, I; Kromhout, H; Johansen, C; et al. (2007) Bladder cancer incidence and exposure to polycyclic aromatic hydrocarbons among asphalt pavers. Occup Environ Med 64(8):520–526.

Busby, WF. Jr.; Goldman, ME; Newberne, PM; et al. (1984) Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5(10):1311-1316.

Busby, WF, Jr.; Stevens, EK; Martin, CN; et al. (1989) Comparative lung tumorigenicity of parent and mononitro-polynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. Toxicol Appl Pharmacol 99(3):555–563.

Byczkowski, JZ; Gessner, T. (1989) Effects of inhibition of NADPH: cytochrome P-450 reductase on benzo(a)pyrene metabolism in mouse liver microsomes. Int J Biochem 21(5):525–529.

Byczkowski, JZ; Kulkarni, AP. (1990) Lipid peroxidation-coupled co-oxygenation of benzo(a)pyrene and benzo(a)pyrene-7,8-dihydrodiol in human term placental microsomes. Placenta 11(1):17–26.

Byczkowski, JZ; Kulkarni, AP. (1992) Vanadium redox cycling, lipid peroxidation and co-oxygenation of benzo(a)pyrene-7,8-dihydrodiol. Biochim Biophys Acta 1125(2):134–141.

Carfi, M; Gennari, A; Malerba, I; et al. (2007) In vitro tests to evaluate immunotoxicity: a preliminary study. Toxicology 229(1-2):11–22.

Cavalieri, EL; Rogan, EG. (1992) The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation. Pharmacol Ther 55(2):183–199.

Cavalieri, EL; Rogan, EG. (1995) Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. Xenobiotica 25(7):677–688.

Cavalieri, E; Rogan, E; Toth, B; et al. (1981) Carcinogenicity of the environmental pollutants cyclopenteno-[cd]pyrene and cyclopentano[cd]pyrene in mouse skin. Carcinogenesis 2(4):277–281.

Cavalieri, E; Rogan, E; Cremonesi, P; et al. (1988a) Tumorigenicity of 6-halogenated derivatives of benzo[a]pyrene in mouse skin and rat mammary gland. J Cancer Res Clin Oncol 114:10–15.

Cavalieri, E; Rogan, E; Sinha, D. (1988b) Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. J Cancer Res Clin Oncol 114:3–9.

Cavalieri, E; Rogan, E; Higginbotham, S; et al. (1988c) Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of fluorinated derivatives of benzo[a]pyrene and 3-methylcholanthrene. J Cancer Res Clin Oncol 114(1):16–22.

Cavalieri, EL; Devanesan, PD; Rogan, EG. (1988d) Radical cations in the horseradish peroxidase and prostaglandin H synthase mediated metabolism and binding of benzo[a]pyrene to deoxyribonucleic acid. Biochem Pharmacol 37(11):2183–2187.

Cavalieri, EL; Rogan, EG; Cremonesi, P; et al. (1988e) Radical cations as precursors in the metabolic formation of quinones from benzo[a]pyrene and 6-fluorobenzo[a]pyrene. Fluoro substitution as a probe for one-electron oxidation in aromatic substrates. Biochem Pharmacol 37(11):2173–2182.

Cavalieri, EL; Rogan, EG; Devanesan, PD; et al. (1990) Binding of benzo[a]pyrene to DNA by cytochrome P-450 catalyzed one-electron oxidation in rat liver microsomes and nuclei. Biochemistry 29(20):4820–4827.

Cavalieri, EL; Higginbotham, S; RamaKrishna, NV; et al. (1991) Comparative dose-response tumorigenicity studies of dibenzo[alpha,l]pyrene versus 7,12-dimethylbenz[alpha]anthracene, benzo[alpha]pyrene and two dibenzo[alpha,l]pyrene dihydrodiols in mouse skin and rat mammary gland. Carcinogenesis 12(10):1939–1944.

Cavalieri, EL; Rogan, EG; Ramakrishna, NVS; et al. (1993) Mechanisms of benzo[a]pyrene and 7,12-dimethylbenz[a]-anthracene activation: qualitative aspects of the stable and depurination DNA adducts obtained from radical cations and diol epoxides. In: Garrigues, P; Lamotte, M; eds. Polycyclic aromatic compounds. Synthesis, properties, analytical measurements, occurrence and biological effects. Langhorne, PA: Gordon and Breach Science Publishers; pp. 725–732.

Cavalieri, EL; Rogan, EG; Li, KM; et al. (2005) Identification and quantification of the depurinating DNA adducts formed in mouse skin treated with dibenzo[a,l]pyrene (DB[a,l]P) or its metabolites and in rat mammary gland treated with DB[a,l]P. Chem Res Toxicol 18(6):976–983.

Cavret, S; Laurent, C; Feidt, C; et al. (2003) Intestinal absorption of 14C from 14C-phenanthrene, 14C-benzo[a]pyrene and 14C-tetrachlorodibenzo-para-dioxin: approaches with the Caco-2 cell line and with portal absorption measurements in growing pigs. Reprod, Nutr, Dev 43(2):145–154.

Cenni, A; Sciarra, G; Sartorelli, P; et al. (1993) Environmental and biological monitoring of polycyclic aromatic hydrocarbons (PAHs) in coke plants and other workplaces. Med Lav 84(5):379–386.

Chakravarti, D; Pelling, JC; Cavalieri, EL; et al. (1995) Relating aromatic hydrocarbon-induced DNA adducts and c-H-ras mutations in mouse skin papillomas: the role of apurinic sites. Proc Natl Acad Sci U S A 92(22):10422–10426.

Chakravarti, D; Mailander, PC; Cavalieri, EL; et al. (2000) Evidence that error-prone DNA repair converts dibenzo[a,l]pyrene-induced depurinating lesions into mutations: formation, clonal proliferation and regression of initiated cells carrying H-ras oncogene mutations in early preneoplasia. Mutat Res 456(1-2):17–32.

Chanda, S; Erexson, G; Frost, D; et al. (2007) 26-Week dermal oncogenicity study evaluating pure trans-capsaicin in Tg.AC hemizygous mice. Int J Toxicol 26:123-133.

Chang, RL; Wood, AW; Conney, AH; et al. (1987) Role of diaxial versus diequatorial hydroxyl groups in the tumorigenic activity of a benzo[a]pyrene bay-region diol epoxide. Proc Natl Acad Sci U S A 84(23):8633–8636.

Chang, LW; Chang, YC; Ho, CC; et al. (2007) Increase of carcinogenic risk via enhancement of cyclooxygenase-2 expression and hydroxyestradiol accumulation in human lung cells as a result of interaction between benzo[a]pyrene and 17-beta estradiol. Carcinogenesis 28(7):1606–1612.

Charles, GD; Bartels, MJ; Zacharewski, TR; et al. (2000) Activity of benzo[a]pyrene and its hydroxylated metabolites in an estrogen receptor-alpha reporter gene assay. Toxicol Sci 55(2):320–326.

Chen, S; Nguyen, N; Tamura, K; et al. (2003) The role of the Ah receptor and p38 in benzo[a]pyrene-7,8-dihydrodiol and benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide-induced apoptosis. J Biol Chem 278(21):19526–19533.

Cheng, L; Spitz, MR; Hong, WK; et al. (2000) Reduced expression levels of nucleotide excision repair genes in lung cancer: a case-control analysis. Carcinogenesis 21(8):1527–1530.

Cheng, YW; Tsai, HJ; Wu, JY; et al. (2007) A possible role for dihydrodiol dehydrogenase in the formation of benzo[a]pyrene-DNA adducts in lung cancer cells and tumor tissues. Environ Mol Mutagen 48(1):14–21.

Chiazze, L, Jr.; Watkins, DK; Amsel, J. (1991) Asphalt and risk of cancer in man. Br J Ind Med 48(8):538–542.

Chipman, JK; Bhave, NA; Hirom, PC; et al. (1982) Metabolism and excretion of benzo[a]pyrene in the rabbit. Xenobiotica 12(6):397–404.

Choi, JY; Lee, KM; Cho, SH; et al. (2003) CYP2E1 and NQO1 genotypes, smoking and bladder cancer. Pharmacogenetics 13(6):349–355.

Chouroulinkov, I; Gentil, A; Guerin, M. (1967) Study of the carcinogenic activity of 9,10-dimethyl-benzanthracene and of 3,4-benzopyrene given orally. Bull Cancer 54(1):67–78.

Chouroulinkov, I; Gentil, A; Grover, PL; et al. (1976) Tumour-initiating activities on mouse skin of dihydrodiols derived from benzo[a]pyrene. Br J Cancer 34(5):523–532.

Chung, JY; Kim, JY; Kim, WR; et al. (2007) Abundance of aryl hydrocarbon receptor potentiates benzo[a]pyrene-induced apoptosis in Hepa1c1c7 cells via CYP1A1 activation. Toxicology 235(1-2):62–72.

Clement Associates. (1990) Development of a dose-response model for inhaled B[a]P. Fairfax, VA: Clement Associates.

Cogliano, VJ; Baan, RA; Straif, K; et al. (2008) Use of mechanistic data in IARC evaluations. Environ Mol Mutagen 49(2):100–109.

Cohen, GM; Haws, SM; Moore, BP; et al. (1976) Benzo(a)pyren-3-yl hydrogen sulphate, a major ethyl acetate-extractable metabolite of benzo(a)pyrene in human, hamster and rat lung cultures. Biochem Pharmacol 25(23):2561–2570.

Conney, AH; Chang, RL; Jerina, DM; et al. (1994) Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. Drug Metab Rev 26(1-2):125–163.

Cooper, AR; Moley, KH. (2008) Maternal tobacco use and its preimplantation effects on fertility: more reasons to stop smoking. Semin Reprod Med. 26:204-212.

Creasia, DA; Poggenburg, JK, Jr.; Nettesheim, P. (1976) Elution of benzo[alpha]pyrene from carbon particles in the respiratory tract of mice. J Toxicol Environ Health 1(6):967–975.

Cresteil, T. (1998) Onset of xenobiotic metabolism in children: toxicological implications. Food Addit Contam 15(Suppl):45–51.

Crofton-Sleigh, C; Doherty, A; Ellard, S; et al. (1993) Micronucleus assays using cytochalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase. Mutagenesis 8(4):363–372.

Culp, SJ; Beland, FA. (1994) Comparison of DNA adduct formation in mice fed coal tar or benzo[a]pyrene. Carcinogenesis 15(2):247–252.

Culp, SJ; Gaylor, D; Sheldon, W; et al. (1996a) DNA adduct measurements in relation to small intestine and forestomach tumor incidence during the chronic feeding of coal tar or benzo(a)pyrene to mice. Polycyclic Aromat Compd 11:161–168.

Culp, SJ; Gaylor, DW; Sheldon, WG; et al. (1996b) Relationship between DNA adduct levels and tumor incidence in mice fed coal tar or benzo[a]pyrene for two years. Proc Am Assoc Cancer Res 37:101.

Culp, SJ; Gaylor, DW; Sheldon, WG; et al. (1998) A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis 19(1):117–124.

Culp, SJ; Warbritton, AR; Smith, BA; et al. (2000) DNA adduct measurements, cell proliferation and tumor mutation induction in relation to tumor formation in B6C3F1 mice fed coal tar or benzo[a]pyrene. Carcinogenesis 21(7):1433–1440.

Curfs, DM; Lutgens, E; Gijbels, MJ; et al. (2004) Chronic exposure to the carcinogenic compound benzo[a]pyrene induces larger and phenotypically different atherosclerotic plaques in ApoE-knockout mice. Am J Pathol 164(1):101–108.

Curfs, DM; Knaapen, AM; Pachen, DM; et al. (2005) Polycyclic aromatic hydrocarbons induce an inflammatory atherosclerotic plaque phenotype irrespective of their DNA binding properties. FASEB J 19(10):1290–1292.

Dahl, AR; Coslett, DS; Bond, JA; et al. (1985) Metabolism of benzo[a]pyrene on the nasal mucosa of Syrian hamsters: comparison to metabolism by other extrahepatic tissues and possible role of nasally produced metabolites in carcinogenesis. J Natl Cancer Inst 75(1):135–139.

Davidson, GE; Dawson, GW. (1976) Chemically induced presumed somatic mutations in the mouse. Mutat Res 38(2):151–154.

Davidson, GE; Dawson, GW. (1977) The induction of somatic mutations in mouse embryos by benzo(a)pyrene. Arch Toxicol 38(1-2):99–103.

Davies RF. (1969) Tumorigenicity of very fresh tobacco smoke condensate to mouse skin. Br J Cancer. 1969 23(4):858-60.

Dean, JH; Luster, MI; Boorman, GA; et al. (1983) Selective immunosuppression resulting from exposure to the carcinogenic congener of benzopyrene in B6C3F1 mice. Clin Exp Immunol 52(1):199–206.

Dean, SW; Coates, A; Brooks, TM; et al. (1998) Benzo[a]pyrene site of contact mutagenicity in skin of Muta mouse. Mutagenesis 13(5):515–518.

De Flora, S; D'Agostini, F; Izzotti, A; et al. (1991) Prevention by N-acetylcysteine of benzo[a]pyrene clastogenicity and DNA adducts in rats. Mutat Res 250(1-2):87–93.

De Jong, WH; Kroese, ED; Vos, JG; et al. (1999) Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci 50:214–220.

DeMarini, DM; Landi, S; Tian, D; et al. (2001) Lung tumor KRAS and TP53 mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. Cancer Res 61(18):6679–6681.

Denissenko, MF; Pao, A; Tang, M; et al. (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science 274:430–432.

Dennison, JE. (2005) Alkene and aromatic compounds. In: Reddy, MB; Yang, RSH; Clewell, HJ; et al.; eds. Physiologically based pharmacokinetic modeling. Hoboken, NJ: John Wiley & Son, Inc.; pp. 103–104.

Dertinger, SD; Lantum, HB; Silverstone, AE; et al. (2000) Effect of 3'-methoxy-4'-nitroflavone on benzo[a]pyrene toxicity. Aryl hydrocarbon receptor-dependent and -independent mechanisms. Biochem Pharmacol 60(2):189–196.

Dertinger, SD; Nazarenko, DA; Silverstone, AE; et al. (2001) Aryl hydrocarbon receptor signaling plays a significant role in mediating benzo[a]pyrene- and cigarette smoke condensate-induced cytogenetic damage in vivo. Carcinogenesis 22(1):171–177.

Deutsch, J; Vatsis, KP; Coon, MJ; et al. (1979) Catalytic activity and stereoselectivity of purified forms of rabbit liver microsomal cytochrome P-450 in the oxygenation of the (-) and (+) enantiomers of trans-7,8-dihydroxy-7,8-dihydrobenzo[alpha]pyrene. Mol Pharmacol 16(3):1011–1018.

De Waal, EJ; Schuurman, HJ; Van Loveren, H; et al. (1997) Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, bis(tri-n-butyltin) oxide and cyclosporine on thymus histophysiology. Crit Rev Toxicol 27(4):381-430.

Diamond, L; Kruszewski, F; Aden, DP; et al. (1980) Metabolic activation of benzo[a]pyrene by a human hepatoma cell line. Carcinogenesis 1(10):871–875.

Dietert, RR. (2008) Developmental immunotoxicology (DIT): Windows of vulnerability, immune dysfunction and safety assessment. J Immunotoxicol 5(4):401-412.

Dietert, RR; Etzel, RA; Chen, D; et al. (2000) Workshop to identify critical windows of exposure for children's health: Immune and respiratory systems work group summary. Environ Health Perspect 108(Suppl 3):483-490.Divi, RL; Beland, FA; Fu, PP; et al. (2002) Highly sensitive chemiluminescence immunoassay for benzo[a]pyrene-DNA adducts: validation by comparison with other methods, and use in human biomonitoring. Carcinogenesis 23(12):2043–2049.

Doll, R. (1971) The age distribution of cancer: implication for models of carcinogenesis. J R Statistical Society A134: 133-166.

Donovan, PJ; Smith, GT; Nardone, R. (2004) The mutagenic effects of 7,12-dimethylbenz[a]anthacene, 3-methylcholanthrene and benzo[a]pyrene to the developing Syrian hamster fetus measured by an in vivo/in vitro mutation assay. Mutation Research 554(1–2):111–120.

Dreij, K; Sundberg, K; Johansson, AS; et al. (2002) Catalytic activities of human alpha class glutathione transferases toward carcinogenic dibenzo[a,l]pyrene diol epoxides. Chem Res Toxicol 15(6):825–831.

Eaton, DL; Bammler, TK. (1999) Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Sci 49(2):156–164.

Edwards, SC; Jedrychowski, W; Butscher, M; et al. (2010). Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children's intelligence at 5 years of age in a prospective cohort study in Poland. Environ Health Perspect. 118(9):1326-31.

El-Bayoumy, K. (1985) Effects of organoselenium compounds on induction of mouse forestomach tumors by benzo(a)pyrene. Cancer Res 45:3631–3635.

El-Bayoumy, K; Hecht, SS; Hoffmann, D. (1982) Comparative tumor initiating activity on mouse skin of 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 3-nitroperylene, 1-nitropyrene and their parent hydrocarbons. Cancer Lett 16(3):333–337.

Emmett, EA; Bingham, EM; Barkley, W. (1981) A carcinogenic bioassay of certain roofing materials. Am J Ind Med 2(1):59–64.

Ewing, P; Blomgren, B; Ryrfeldt, A; et al. (2006) Increasing exposure levels cause an abrupt change in the absorption and metabolism of acutely inhaled benzo(a)pyrene in the isolated, ventilated, and perfused lung of the rat. Toxicol Sci 91(2):332–340.

Fang, JL; Lazarus, P. (2004) Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(-) in liver microsomes from subjects with the UGT1A1*28 variant. Cancer Epidemiol Biomarkers Prev 13(1):102–109.

Fedorenko, Z; Yansheva, N. (1967) Experimental reproduction of tumors of the antral part of the stomach in mice by administration of various dose of 3,4-benzpyrene. Hyg Sanit 32(5):168–173.

Feron, VJ; Kruysse, A. (1978) Effects of exposure to furfural vapour in hamsters simultaneously treated with benzo[alpha] pyrene or diethylnitrosamine. Toxicology 11(2):127–144.

Feron, VJ; de Jong, D; Emmelot, P. (1973) Letter: Dose-response correlation for the induction of respiratory-tract tumours in Syrian golden hamsters by intratracheal instillations of benzo(a)pyrene. Eur J Cancer 9(5):387–390.

Fertuck, KC; Matthews, JB; Zacharewski, TR. (2001) Hydroxylated benzo[a]pyrene metabolites are responsible for in vitro estrogen receptor-mediated gene expression induced by benzo[a]pyrene, but do not elicit uterotrophic effects in vivo. Toxicol Sci 59(2):231–240.

Field, E; Roe, F. (1965) Tumor promotion in the forestomach epithelium of mice by oral administration of citrus oils. J Natl Cancer Inst 35(5):775–784.

Fine, JS; Silverstone, AE; Gasiewicz, TA. (1990) Impairment of prothymocyte activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Immunol 144(4):1169-1176.

Flowers, L; Bleczinski, WF; Burczynski, ME; et al. (1996) Disposition and biological activity of benzo[a]pyrene-7,8-dione. A genotoxic metabolite generated by dihydrodiol dehydrogenase. Biochemistry 35(42):13664–13672.

Flowers, L; Ohnishi, ST; Penning, TM. (1997) DNA strand scission by polycyclic aromatic hydrocarbon o-quinones: role of reactive oxygen species, Cu(II)/Cu(I) redox cycling, and o-semiquinone anion radicals. Biochemistry 36(28):8640–8648.

Flowers-Geary, L; Harvey, RG; Penning, TM. (1993) Cytotoxicity of polycyclic aromatic hydrocarbon o-quinones in rat and human hepatoma cells. Chem Res Toxicol 6(3):252–260.

Flowers-Geary, L; Bleczinki, W; Harvey, RG; et al. (1996) Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon ortho-quinones produced by dihydrodiol dehydrogenase. Chem Biol Interact 99(1-3):55–72.

Fontana, RJ; Lown, KS; Paine, MF; et al. (1999) Effects of a chargrilled meat diet on expression of CYP3A, CYP1A, and P-glycoprotein levels in healthy volunteers. Gastroenterology 117(1):89–98.

Forster, K; Preuss, R; Rossbach, B; et al. (2008) 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. Occup Environ Med 65(4):224–229.

Foth, H; Kahl, R; Kahl, GF. (1988) Pharmacokinetics of low doses of benzo[a]pyrene in the rat. Food Chem Toxicol 26(1):45–51.

Fowler, P; Whitwell, J; Jeffrey, L; et al. (2010) Cadmium chloride, benzo[a]pyrene and cyclophosphamide tested in the in vitro mammalian cell micronucleus test (MNvit) in the human lymphoblastoid cell line TK6 at Covance laboratories, Harrogate UK in support of OECD draft Test Guideline 487. Mutat Res.

Frederick, CB; Bush, ML; Lomax, LG; et al. (1998) Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharmacol 152(1):211–231.

Friesen, MC; Demers, PA; Spinelli, JJ; et al. (2010) Chronic and acute effects of coal tar pitch exposure and cardiopulmonary mortality among aluminum smelter workers. Am J Epidemiol. 172(7):790-9.

Fu, PP; Harvey, RG; Beland, FA. (1978) Molecular orbital theoretical prediction of the isomeric products formed from reactions of arene oxides and related metabolites of polycyclic aromatic hydrocarbons. Tetrahedron 34(7):866.

Galvan, N; Page, TJ; Czuprynski, CJ; et al. (2006) Benzo(a)pyrene and 7,12-dimethylbenz(a)anthrecene differentially affect bone marrow cells of the lymphoid and myeloid lineages. Toxicol Appl Pharmacol 213(2):105–116.

Gangar, SC; Sandhir, R; Rai, DV; et al. (2006) Preventive effects of Azadirachta indica on benzo(a)pyrene-DNA adduct formation in murine forestomach and hepatic tissues. Phytother Res 20:889-895.

Garcon, G; Gosset, P; Garry, S; et al. (2001a) Pulmonary induction of proinflammatory mediators following the rat exposure to benzo(a)pyrene-coated onto Fe2O3 particles. Toxicol Lett 121(2):107–117.

Garçon, G; Zerimech, F; Hannothiaux, M; et al. (2001b) Antioxidant defense disruption by polycyclic aromatic hydrocarbons-coated onto Fe(2)O(3) particles in human lung cells (A549). Toxicology 166(3):129–137.

Garte, S; Taioli, E; Raimondi, S; et al. (2007) Effects of metabolic genotypes on intermediary biomarkers in subjects exposed to PAHS: results from the EXPAH study. Mutat Res 620(1–2):7–15.

Geacintov, NE; Cosman, M; Hingerty, BE; et al. (1997) NMR solution structures of stereoisometric covalent polycyclic aromatic carcinogen-DNA adduct: principles, patterns, and diversity. Chem Res Toxicol 10(2):111–146.

Gelboin, HV. (1980) Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. Physiol Rev 60(4):1107–1166.

Georgellis, A; Toppari, J; Veromaa, T; et al. (1990) Inhibition of meiotic divisions of rat spermatocytes in vitro by polycyclic aromatic hydrocarbons. Mutat Res 231(2):125–135.

Gerde, P; Scholander, P. (1989) An experimental study of the penetration of polycyclic aromatic hydrocarbons through a model of the bronchial lining layer. Environ Res 48(2):287–295.

Gerde, P; Muggenburg, BA; Hoover, MD; et al. (1993a) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. I. The alveolar region. Toxicol Appl Pharmacol 121(2):313–318.

Gerde, P; Muggenburg, BA; Sabourin, PJ; et al. (1993b) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. II. The conducting airways. Toxicol Appl Pharmacol 121(2):319–327.

Gerde, P; Muggenburg, BA; Henderson, RF. (1993c) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. III. Mechanisms of the dosimetry. Toxicol Appl Pharmacol 121(2):328–334.

Gerde, P; Muggenburg, BA; Lundborg, M; et al. (2001) The rapid alveolar absorption of diesel soot-adsorbed benzo[a]pyrene: bioavailability, metabolism and dosimetry of an inhaled particle-borne carcinogen. Carcinogenesis 22(5):741–749.

Germolec, DR; Kashon, M; Nyska, A; et al. (2004) The accuracy of extended histopathology to detect immunotoxic chemicals. Toxicol Sci 82(2):504-514.

Germolec, DR; Nyska, A; Kashon, M; et al. (2004) Extended histopathology in immunotoxicity testing: Interlaboratory validation studies. Toxicol Sci 78(1):107-115.

Gibbs, GW. (1985) Mortality of aluminum reduction plant workers, 1950 through 1977. J Occup Med 27(10):761–770.

Ginsberg, GL; Atherholt, TB. (1989) Transport of DNA-adducting metabolites in mouse serum following benzo[a]pyrene administration. Carcinogenesis 10(4):673–679.

Godschalk, RW; Ostertag, JU; Moonen, EJ; et al. (1998a) Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. Cancer Epidemiol Biomarkers Prev 7(9):767–773.

Godschalk, RW; Maas, LM; Kleinjans, JC; et al. (1998b) Influences of DNA isolation and RNA contamination on carcinogen-DNA adduct analysis by 32P-postlabeling. Environ Mol Mutagen 32(4):344–350.

Godschalk, RW; Moonen, EJ; Schilderman, PA; et al. (2000) Exposure-route-dependent DNA adduct formation by polycyclic aromatic hydrocarbons. Carcinogenesis 21(1):87–92.

Godschalk, RW; Ostertag, JU; Zandsteeg, AM; et al. (2001) Impact of GSTM1 on aromatic-DNA adducts and p53 accumulation in human skin and lymphocytes. Pharmacogenetics 11(6):537–543.

Godschalk, R; Nair, J; Van Schooten, FJ; et al. (2002) Comparison of multiple DNA adduct types in tumor adjacent human lung tissue: effect of cigarette smoking. Carcinogenesis 23(12):2081–2086.

Godschalk, R; Curfs, D; Bartsch, H; et al. (2003) Benzo[a]pyrene enhances lipid peroxidation induced DNA damage in aorta of apolipoprotein E knockout mice. Free Radic Res 37(12):1299–1305.

Gold, LS; Zeiger, E. (1997) Handbook of carcinogenicity potency and genotoxicity databases (CPDB). Boca Raton, FL: CRC Press.

Goldstein, LS; Weyand, EH; Safe, S; et al. (1998) Tumors and DNA adducts in mice exposed to benzo[a]pyrene and coal tars: implications for risk assessment. Environ Health Perspect 106 (Suppl 6):1325–1330.

Goshman, LM and Heidelberger, C. (1967). Binding of tritium-labeled polycyclic hydrocarbons to dna of mouse skin. Cancer Research 27: 1678-1688.

Gozukara, EM; Belvedere, G; Robinson, RC; et al. (1981) The effect of epoxide hydratase on benzo[a]pyrene diol epoxide hydrolysis and binding to DNA and mixed-function oxidase proteins. Mol Pharmacol 19(1):153–161.

Granberg, AL; Brunstrom, B; Brandt, I. (2000) Cytochrome P450-dependent binding of 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (B[a]P) in murine heart, lung, and liver endothelial cells. Arch Toxicol 74(10):593–601.

Grant, G; Roe, FJ. (1963) The effect of phenanthrene on tumour induction by 3,4-benzopyrene administered to newly born mice. Br J Cancer 17:261–265.

Grimmer, G; Brune, H; Deutsch-Wenzel, R; et al. (1983) On the contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of automobile exhaust condensate evaluated by local application onto mouse skin. Cancer Lett 21(1):105–113.

Grimmer, G; Brune, H; Deutsch-Wenzel, R; et al. (1984) Contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of gasoline engine exhaust condensate evaluated by implantation into the lungs of rats. J Natl Cancer Inst 72(3):733–739.

Grimmer, G; Dettbarn, G; Jacob, J. (1993) Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols). Int Arch Occup Environ Health 65(3):189–199.

Grimmer, G; Dettbam, G; Naujack, K; et al. (1994) Relationship between inhaled PAH and urinary excretion of phenanthrene, pyrene and benzo(a)pyrene metabolites in coke plant workers. Polycyclic Aromat Compd 5:269–277.

Gross, P; Tolker, E; Babyak, MA; et al. (1965) Experimental lung cancer in hamsters. Repetitive intratracheal applications of two carcinogenic hydrocarbons. Arch Environ Health 11:59–65.

Grova, N; Valley, A; Turner, JD; et al. (2007) Modulation of behavior and NMDA-R1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. Neurotoxicology 28(3):630–636.

Grova, N; Schroeder, H; Farinelle, S; et al. (2008) Sub-acute administration of benzo[a]pyrene (B[a]P) reduces anxiety-related behaviour in adult mice and modulates regional expression of N-methyl-D-aspartate (NMDA) receptors genes in relevant brain regions. Chemosphere 73(1 Suppl):S295–302.

Grover, PL. (1986) Pathways involved in the metabolism and activation of polycyclic hydrocarbons. Xenobiotica 16(10-11):915–931.

Gu, J; Horikawa, Y; Chen, M; et al. (2008) Benzo(a)pyrene diol epoxide-induced chromosome 9p21 aberrations are associated with increased risk of bladder cancer. Cancer Epidemiol Biomarkers Prev 17(9):2445–2450.

Guillemette, C. (2003) Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J 3(3):136–158.

Gündel, J; Schaller, KH; Angerer, J. (2000) Occupational exposure to polycyclic aromatic hydrocarbons in a fireproof stone producing plant: biological monitoring of 1-hydroxypyrene, 1-, 2-, 3- and 4-hydroxyphenanthrene, 3-hydroxybenz(a)anthracene and 3-hydroxybenzo(a)pyrene. Int Arch Occup Environ Health 73(4):270–274.

Gunter, MJ; Divi, RL; Kulldorff, M; et al. (2007) Leukocyte polycyclic aromatic hydrocarbon-DNA adduct formation and colorectal adenoma. Carcinogenesis 28(7):1426–1429.

Gyorffy, E; Anna, L; Kovacs, K; et al. (2008) Correlation between biomarkers of human exposure to genotoxins with focus on carcinogen-DNA adducts. Mutagenesis 23(1):1–18.

Habs, M; Schmahl, D; Misfeld, J. (1980) Local carcinogenicity os some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. Arch Geschwulstforsch 50(3):266–274.

Habs, M; Jahn, SA; Schmahl, D. (1984) Carcinogenic activity of condensate from coloquint seeds (Citrullus colocynthis) after chronic epicutaneous administration to mice. J Cancer Res Clin Oncol 108(1):154–156.

Hakim, FT; Memon, SA; Cepeda, R; et al. (2005) Age-dependent incidence, time course, and consequences of thymic renewal in adults. J Clin Invest 115(4):930-939.

Hakura, A; Tsutsui, Y; Sonoda, J; et al. (1998) Comparison between in vivo mutagenicity and carcinogenicity in multiple organs by benzo[a]pyrene in the lacZ transgenic mouse (Muta Mouse). Mutat Res 398(1-2):123–130.

Hakura, A; Shimada, H; Nakajima, M; et al. (2005) Salmonella/human S9 mutagenicity test: a collaborative study with 58 compounds. Mutagenesis 20(3):217–228.

Hall, M; Grover, PL. (1988) Stereoselective aspects of the metabolic activation of benzo[a]pyrene by human skin in vitro. Chem Biol Interact 64(3):281–296.

Hammond, EC; Selikoff, IJ; Lawther, PL; et al. (1976) Inhalation of benzpyrene and cancer in man. Ann N Y Acad Sci 271:116–124.

Hanelt, S; Helbig, R; Hartmann, A; et al. (1997) A comparative investigation of DNA adducts, DNA strand breaks and gene mutations induced by benzo[a]pyrene and (+/-)-anti-benzo[a]pyrene-7,8-diol 9,10-oxide in cultured human cells. Mutat Res 390:179-188.

Hansen, ES. (1989) Cancer incidence in an occupational cohort exposed to bitumen fumes. Scand J Work Environ Health 15(2):101–105.

Hansen, ES. (1991) Mortality of mastic asphalt workers. Scand J Work Environ Health 17(1):20–24.

Hara, T; Nishikawa, T; Sui, H; et al. (2007) In vivo photochemical skin micronucleus test using a sunlight simulator: detection of 8-methoxypsoralen and benzo[a]pyrene in hairless mice. Mutat Res 631:1-8.

Hardin, JA; Hinoshita, F; Sherr, DH. (1992) Mechanisms by which benzo[a]pyrene, an environmental carcinogen, suppresses B cell lymphopoiesis. Toxicol Appl Pharmacol 117(2):155–164.

Harvey, RG. (1996) Mechanisms of carcinogenesis of polycyclic aromatic compounds. Polycyclic Aromat Compd 9:1–23.

Hayes, JD; Pulford, DJ. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30(6):445–600.

Hayes, JD; Strange, RC. (2000) Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 61(3):154–166.

Hecht, SS. (2002) Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis 23(6):907–922.

Hecht, SS. (2004) Carcinogen derived biomarkers: applications in studies of human exposure to secondhand tobacco smoke. Tob Control 13 (Suppl 1):48–56.

Hecht, SS; Grabowski, W; Groth, K. (1979) Analysis of faeces for benzo[a]pyrene after consumption of charcoal-broiled beef by rats and humans. Food Cosmet Toxicol 17(3):223–227.

Hecht, SS; Chen, M; Yagi, H; et al. (2003) r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. Cancer Epidemiol Biomarkers Prev 12(12):1501–1508.

Henry, MC; Port, CD; Bates, RR; et al. (1973) Respiratory tract tumors in hamsters induced by benzo(a)pyrene. Cancer Res 33(7):1585–1592.

Herbert, R; Marcus, M; Wolff, MS; et al. (1990) A pilot study of detection of DNA adducts in white blood cells of roofers by 32P-postlabelling. IARC Sci Publ (104):205–214.

Herrold, KM; Dunham, LJ. (1962) Induction of carcinoma and papilloma of the Syrian hamster by intratracheal instillation of benzo[a]-pyrene. J Natl Cancer Inst 28:467–491.

Higginbotham, S; RamaKrishna, NV; Johansson, SL; et al. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[a,l]pyrene versus 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene at low doses in mouse skin. Carcinogenesis 14(5):875–878.

Himmelstein, MW; Boogaard, PJ; Cadet, J; et al. (2009). Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage. Crit Rev Toxicol. 39(8):679-94.

Hirom, PC; Chipman, JK; Millburn, P; et al. (1983) Enterohepatic circulation of the aromatic hydrocarbons benzo[a]pyrene and naphthalene. In: Extrahepatic drug metabolism and chemical carcinogenesis. Amsterdam: Elsvier Science Publishers; pp. 275–281.

Hoffmann, D; Rathkamp, G; Nesnow, S; et al. (1972) Fluoranthenes: quantitative determination in cigarette smoke, formation by pyrolysis, and tumor-initiating activity. J Natl Cancer Inst 49(4):1165–1175.

Holladay, SD; Smith, BJ. (1994) Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: a cytometric evaluation. J Toxicol Environ Health 42(3):259–273.

Holladay, SD; Smith, BJ. (1995) Benzo[a]pyrene-induced alterations in total immune cell number and cell-surface antigen expression in the thymus, spleen and bone marrow of b6c3f1 mice. Vet Hum Toxicol 37(2):99-104.

Holladay, SD; Smialowicz, RJ. (2000) Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure. Environ Health Perspect 108(Suppl 3):463-473.

Homburger, F; Hsueh, SS; Kerr, CS; et al. (1972) Inherited susceptibility of inbred strains of Syrian hamsters to induction of subcutaneous sarcomas and mammary and gastrointestinal carcinomas by subcutaneous and gastric administration of polynuclear hydrocarbons. Cancer Res 32(2):360–366.

Horton, AW; Bingham EL; Graf Burton, MJ; et al. (1965) Carcinogenesis of the skin. III. The contribution of elemental sulfur and of organic sulfur compounds. Cancer Res 25:17591763.

Howard, P; Meylan, W. (1997) Handbook of physical properties of organic chemicals. Boca Raton, FL: Lewis Publishers, Inc.

Hu, Z; Wells, PG. (2004) Human interindividual variation in lymphocyte UDP-glucuronosyltransferases as a determinant of in vitro benzo[a]pyrene covalent binding and cytotoxicity. Toxicol Sci 78(1):32–40.

IARC (International Agency for Research on Cancer). (1973) IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 3. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. Lyon, France: International Agency for Research on Cancer

IARC (International Agency for Research on Cancer). (1983) IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 32. Polynuclear aromatic hydrocarbons, Part 1, chemical environmental and experimental data. Lyon, France: International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer). (1984) Polynuclear aromatic compounds. Part 3, Industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 34. Lyon, France: International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer). (1985) Polynuclear aromatic compounds. Part 4, bitumens coal-tars and derived products shale-oils and soots. IARC International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 35. Lyon, France: International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer). (1987a) IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluation of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. Supplement 7. Lyon, France: International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer). (1987b) Tobacco smoking. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 30. Lyon, France: International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer). (1998) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: preliminary classification. Available online at http://monographs.iarc.fr/ENG/Meetings/92-pahs.pdf .

Ingram, AJ; Phillips, JC; Davies, S. (2000) DNA adducts produced by oils, oil fractions and polycyclic aromatic hydrocarbons in relation to repair processes and skin carcinogenesis. J Appl Toxicol 20(3):165–174.

Inyang, F; Ramesh, A; Kopsombut, P; et al. (2003) Disruption of testicular steroidogenesis and epididymal function by inhaled benzo(a)pyrene. Reprod Toxicol 17(5):527–537.

Izzotti, A; Balansky, R; Scatolini, L; et al. (1991) [Research on adducts of benzo(a)pyrene and DNA in different cells using synchronous fluorescence spectrophotometry]. Bollettino - Societa Italiana Biologia Sperimentale 67(9):869–874.

Izzotti, A; Bagnasco, M; D'Agostini, F; et al. (1992) Evaluation of BPDE-DNA adducts in lung cells. Perspectives in human biomonitoring and chemoprevention of cancer. Mutat Res 27(2):191.

Jarabek, AM; Pottenger, LH; Andrews, LS; et al. (2009); Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Crit Rev Toxicol. 39(8):659-78.

Jeffrey, AM; Jennette, KW; Blobstein, SH; et al. (1976) Letter: Benzo[a]pyrene-nucleic acid derivative found in vivo: structure of a benzo[a]pyrenetetrahydrodiol epoxide-guanosine adduct. J Am Chem Soc 98(18):5714–5715.

Jeffy, BD; Chirnomas, RB; Romagnolo, DF. (2002) Epigenetics of breast cancer: polycyclic aromatic hydrocarbons as risk factors. Environ Mol Mutagen 39(2–3):235–244.

Jerina, DM; Chadha, A; Cheh, AM; et al. (1991) Covalent bonding of bay-region diol epoxides to nucleic acids. Adv Exp Med Biol 283:533–553.

Jiang, H; Shen, YM; Quinn, AM; et al. (2005) Competing roles of cytochrome P450 1A1/1B1 and aldo-keto reductase 1A1 in the metabolic activation of (+/-)-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene in human bronchoalveolar cell extracts. Chem Res Toxicol 18(2):365–374.

Jiang, H; Gelhaus, SL; Mangal, D; et al. (2007) Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. Chem Res Toxicol 20(9):1331–1341.

Johnson, D; Bruice, TC. (1975) Nucleophilic catalysis of the aromatization of an arene oxide. The reaction of trimethylamine with 4-carbo-tert-butoxybenzene. J Am Chem Soc 97:6901–6903.

Johnson, M; Pullen, L; Cogliano, J. (1997) An approach for calculating risk from dermal exposure to benzo[a]pyrene based on point of contact effects. Unpublished review draft report.

Joseph, P; Jaiswal, AK. (1998) NAD(P)H:quinone oxidoreductase 1 reduces the mutagenicity of DNA caused by NADPH:P450 reductase-activated metabolites of benzo(a)pyrene quinones. Br J Cancer 77(5):709–719.

Jyonouchi, H; Sun, S; Iijima, K; et al. (1999) Effects of anti-7,8-dihydroxy-9,10-epoxy- 7,8,9,10-tetrahydrobenzo[a]pyrene on human small airway epithelial cells and the protective effects of myo-inositol. Carcinogenesis 20(1):139–145.

Kalina, I; Brezani, P; Gajdosova, D; et al. (1998) Cytogenetic monitoring in coke oven workers. Mutat Res 417(1):9–17.

Kamath, AB; Xu, H; Nagarkatti, PS; et al. (1997) Evidence for the induction of apoptosis in thymocytes by 2,3,7,8-tetrachlorodibenzo-p-dioxin in vivo. Toxicol Appl Pharmacol 142(2):367-377.

Kapitulnik, J; Wislocki, PG; Levin, W; et al. (1978) Tumorigenicity studies with diol-epoxides of benzo[a]pyrene which indicate that (+/-)-trans-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydr obenzo[a]pyrene is an ultimate carcinogen in newborn mice. Cancer Res 38:354-358.

Kao, J; Hall, J; Shugart, LR; et al. (1984) An in vitro approach to studying cutaneous metabolism and disposition of topically applied xenobiotics. Toxicol Appl Pharmacol 75(2):289–298.

Kao, J; Patterson, FK; Hall, J. (1985) Skin penetration and metabolism of topically applied chemicals in six mammalian species, including man: an in vitro study with benzo[a]pyrene and testosterone. Toxicol Appl Pharmacol 81(3 Pt 1):502–516.

Kao, J; Hall, J; Helman, G. (1988) In vitro percutaneous absorption in mouse skin: influence of skin appendages. Toxicol Appl Pharmacol 94(1):93–103.

Kapitulnik, J; Wislocki, PG; Levin, W; et al. (1978) Tumorigenicity studies with diol-epoxides of benzo(a)pyrene which indicate that (+/-)-trans-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydr obenzo(a)pyrene is an ultimate carcinogen in newborn mice. Cancer Res 38(2):354–358.

Kawamura, Y; Kamata, E; Ogawa, Y; et al. (1988) The effect of various foods on the intestinal absorption of benzo-a-pyrene in rats. J Food Hyg Soc Jpn 29(1):21–25.

Keller, GM; Christou, M; Pottenger, LH; et al. (1987) Product inhibition of benzo[a]pyrene metabolism in uninduced rat liver microsomes: effect of diol epoxide formation. Chem Biol Interact 61(2):159–175.

Ketkar, M; Reznik, G; Schneider, P; et al. (1978) Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo(a)pyrene in bovine serum albumin in Syrian hamsters. Cancer Lett 4(4):235–239.

Kiefer, F; Cumpelik, O; Wiebel, FJ. (1988) Metabolism and cytotoxicity of benzo(a)pyrene in the human lung tumour cell line NCI-H322. Xenobiotica 18(6):747–755.

Kim, KB; Lee, BM. (1997) Oxidative stress to DNA, protein, and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene. Cancer Lett 113(1-2):205–212.

Kim, HS; Kwack, SJ; Lee, BM. (2000) Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene-quinones in the blood of rats treated with benzo[a]pyrene. Chem Biol Interact 127(2):139–150.

Kim, IY; Hyun, CK. (2006) Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms. Ecotoxicol Environ Saf 64:288-297.

Kleiner, HE; Vulimiri, SV; Hatten, WB; et al. (2004) Role of cytochrome p4501 family members in the metabolic activation of polycyclic aromatic hydrocarbons in mouse epidermis. Chem Res Toxicol 17(12):1667–1674.

Klemme, JC; Mukhtar, H; Elmets, CA. (1987) Induction of contact hypersensitivity to dimethylbenz(a)anthracene and benzo(a)pyrene in C3H/HeN mice. Cancer Res 47(22):6074–6078.

Knaapen, AM; Curfs, DM; Pachen, DM; et al. (2007) The environmental carcinogen benzo[a]pyrene induces expression of monocyte-chemoattractant protein-1 in vascular tissue: a possible role in atherogenesis. Mutat Res 621(31):41.

Knafla, A; Phillips, KA; Brecher, RW; et al. (2006) Development of a dermal cancer slope factor for benzo[a]pyrene. Reg Toxicol Pharmacol 45:159–168.

Knox, EG. (2005) Childhood cancers and atmospheric carcinogens. J Epidemiol Community Health 59(2):101–105.

Knuckles, ME; Inyang, F; Ramesh, A. (2001) Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. Toxicol Sci 61(2):382–388.

Kobayashi, N. (1975) Production of respiratory tract tumors in hamsters by benzo(a)pyrene. Gann 66(3):311–315.

Kondraganti, SR; Fernandez-Salguero, P; Gonzalez, FJ; et al. (2003) Polycyclic aromatic hydrocarbon-inducible DNA adducts: evidence by 32P-postlabeling and use of knockout mice for Ah receptor-independent mechanisms of metabolic activation in vivo. Int J Cancer 103(1):5–11.

Koreeda, M; Moore, PD; Wislocki, PG; et al. (1978) Binding of benzo[a]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity. Science 199(4330):778–781.

Kriek, E; Van Schooten, FJ; Hillebrand, MJ; et al. (1993) DNA adducts as a measure of lung cancer risk in humans exposed to polycyclic aromatic hydrocarbons. Environ Health Perspect 99:71–75.

Kristensen, P; Eilertsen, E; Einarsdottir, E; et al. (1995) Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. Environ Health Perspect 103(6):588–590.

Kroese, ED; Muller, JJA; Mohn, GR; et al. (2001) Tumorigenic effects in Wistar rats orally administered benzo(a)pyrene for two years: Implications for human cancer risks associated with oral exposure to polycyclic aromatic hydrocarbons. Bilthoven, Netherlands: RIVM 658603.

Kunstler, K. (1983) Failure to induce tumors by intratracheal instillation of automobile exhaust condensate and fractions thereof in Syrian golden hamsters. Cancer Lett 18(1):105–108.

Kuper, C; Beems, R; Bruijntjes, J; et al. (1992) Normal development, growth, and aging of the thymus. In: Mohr, U; Dungworth, DL; Capen, CC; eds. Pathobiology of the aging rat, Vol. 1. Washington, DC: ILSI Press; pp. 25-48.

Kuper, CF; De Heer, E; Van Loveren, H; et al. (2002) Immune system. In: Hascheck-Hock, WM; Rousseaux, CG; Wallig, MA; eds. Handbook of Toxicologic Pathology, Vol. 2. New York: Academic Press; pp. 585-644.

LaGoy, PK; Quirk, TC. (1994) Establishing generic remediation goals for the polycyclic aromatic hydrocarbons: critical issues. Environ Health Perspect 102:348–352.

Landreth, K; Dodson, S. (2005) Development of the rodent immune system. In: Holladay, SD; ed. Developmental Immunotoxicology. Boca Raton, FL: CRC Press; pp. 3-19.

Landreth, KS. (2002) Critical windows in development of the rodent immune system. Human and Experimental Toxicology 21(9-10):493-498.

LaVoie, EJ; Amin, S; Hecht, SS; et al. (1982) Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. Carcinogenesis 3(1):49–52.

LaVoie, EJ; Braley, J; Rice, JE; et al. (1987) Tumorigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. Cancer Lett 34(1):15–20.

LaVoie, EJ; Cai, ZW; Meschter, CL; et al. (1994) Tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn CD-1 mice. Carcinogenesis 15(10):2131–2135.

Lazarova, M; Labaj, J; Eckl, P; et al. (2006) Comparative evaluation of DNA damage by genotoxicants in primary rat cells applying the comet assay. Toxicol Lett 164(1):54–62.

Leadon, SA; Stampfer, MR; Bartley, J. (1988) Production of oxidative DNA damage during the metabolic activation of benzo[a]pyrene in human mammary epithelial cells correlates with cell killing. Proc Natl Acad Sci U S A 85:4365–4368.

Lee, ME; Urso, P. (2007) Suppression of T lymphocyte proliferation to antigenic and mitogenic stimuli by Benzo(alpha)pyrene and 2-aminofluorene metabolites. Immunopharmacol Immunotoxicol 29(3-4):425–438.

Legraverend, C; Harrison, DE; Ruscetti, FW; et al. (1983) Bone marrow toxicity induced by oral benzo[a]pyrene: protection resides at the level of the intestine and liver. Toxicol Appl Pharmacol 70(3):390–401.

Le Marchand, L; Sivaraman, L; Pierce, L; et al. (1998) Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. Cancer Res 58(21):4858–4863.

Leng, SG; Zheng, YX; Pan, ZF; et al. (2004) [A study on the inherited susceptibility of chromosomal damage in peripheral blood lymphocytes among coke oven workers]. Zhonghua Yu Fang Yi Xue Za Zhi 38(2):94–98.

Leung, HW; Henderson, RF; Bond, JA; et al. (1988) Studies on the ability of rat lung and liver microsomes to facilitate transfer and metabolism of benzo[a]pyrene from diesel particles. Toxicology 51(1):1–9.

Levin, W; Wood, AW; Yagi, H; et al. (1976) Carcinogenicity of benzo[a]pyrene 4,5-, 7,8-, and 9,10-oxides on mouse skin. Proc Natl Acad Sci USA 73(1):243–247.

Levin, W; Wood, AW; Wislocki, PG; et al. (1977) Carcinogenicity of benzo-ring derivatives of benzo(a)pyrene on mouse skin. Cancer Res 37(9):3356–3361.

Levin, W; Wood, A; Chang, R; et al. (1982) Oxidative metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens. Drug Metab Rev 13(4):555–580.

Lewtas, J; Walsh, D; Williams, R; et al. (1997) Air pollution exposure-DNA adduct dosimetry in humans and rodents: evidence for non-linearity at high doses. Mutat Res 378(1-2):51–63.

Li, D; Zhang, W; Sahin, AA; et al. (1999) DNA adducts in normal tissue adjacent to breast cancer: a review. Cancer Detect Prev 23(6):454–462.

Li, D; Firozi, PF; Wang, LE; et al. (2001) Sensitivity to DNA damage induced by benzo(a)pyrene diol epoxide and risk of lung cancer: a case-control analysis. Cancer Res 61(4):1445–1450.

Li, D; Day, RS; Bondy, ML; et al. (2007) Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev 16(4):655–661.

Li, P; Li, X; Stagnitti, F; (2009) Studies on the sources of benzo[a]pyrene in grain and aboveground tissues of rice plants. J Hazard Mater. 162(1):463-8.
Liao, KH; Dobrev, ID; Dennison, JE, Jr.; et al. (2002) Application of biologically based computer modeling to simple or complex mixtures. Environ Health Perspect 110 (Suppl 6):957–963.

Likhachev, AJ; Beniashvili, DS; Bykov, VJ; et al. (1992) Relevance of quantitation of benzo(a)pyrene metabolites in animal excretes to evaluate individual human cancer risk. Prog Clin Biol Res 374:435–452.

Lin, P; Hsueh, YM; Ko, JL; et al. (2003) Analysis of NQO1, GSTP1, and MnSOD genetic polymorphisms on lung cancer risk in Taiwan. Lung Cancer 40(2):123–129.

Liu, SH; Wang, JH; Chuu, JJ; et al. (2002) Alterations of motor nerve functions in animals exposed to motorcycle exhaust. J Toxicol Environ Health A 65:803–812.

Liu, Z; Muehlbauer, KR; Schmeiser, HH; et al. (2005) p53 mutations in benzo(a)pyrene-exposed human p53 knock-in murine fibroblasts correlate with p53 mutations in human lung tumors. Cancer Res 65(7):2583–2587.

Liu, S; Abdelrahim, M; Khan, S; et al. (2006) Aryl hydrocarbon receptor agonists directly activate estrogen receptor alpha in MCF-7 breast cancer cells. Biol Chem 387(9):1209–1213.

Lodovici, M; Akpan, V; Giovannini, L; et al. (1998) Benzo[a]pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. Chem Biol Interact 116(3):199–212.

Luebke, RW; Chen, DH; Dietert, R; et al. (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. J Toxicol Environ Health B Crit Rev 9(1):1-26.

Lummus, ZL; Henningsen, G. (1995) Modulation of t-cell ontogeny by transplacental benzo(a)pyrene. Int J Immunopharmacol 17(4):339-350.

Luster, MI; Munson, AE; Thomas, PT; et al. (1988) Development of a testing battery to assess chemical-induced immunotoxicity: National toxicology program's guidelines for immunotoxicity evaluation in mice. Fundam Appl Toxicol 10(1):2-19.

Lyte, M; Bick, PH. (1985) Differential immunotoxic effects of the environmental chemical benzo[a]pyrene in young and aged mice. Mech Ageing Dev 30(3):333–341.

Lyte, M; Blanton, RH; Myers, MJ; et al. (1987) Effect of in vivo administration of the carcinogen benzo(a)pyrene on interleukin-2 and interleukin-3 production. Int J Immunopharmacol 9(3):307–312.

Ma, Q; Lu, AY. (2007) CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies. Drug Metab Dispos 35(7):1009–1016.

Mackall, CL; Fleisher, TA; Brown, MR; et al. (1995) Age, thymopoiesis, and cd4+ t-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 332(3):143-149.

MacKenzie, KM; Angevine, DM. (1981) Infertility in mice exposed in utero to benzo(a)pyrene. Biol Reprod 24(1):183–191.

MacNicoll, AD; Grover, PL; Sims, P. (1980) The metabolism of a series of polycyclic hydrocarbons by mouse skin maintained in short-term organ culture. Chem Biol Interact 29(2):169–188.

Malaveille, C; Kuroki, T; Sims, P; et al. (1977) Mutagenicity of isomeric diol-epoxides of benzo[a]pyrene and benz[a]anthracene in S. typhimurium TA98 and TA100 and in V79 Chinese hamster cells. Mutat Res 44(3):313–326.

Mallet, WG; Mosebrook, DR; Trush, MA. (1991) Activation of (+-)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to diolepoxides by human polymorphonuclear leukocytes or myeloperoxidase. Carcinogenesis 12(3):521–524.

Mancini,R; Romano, G; Sgambato, A; et al. (1999). Polycyclic aromatic hydrocarbon-DNA adducts in cervical smears of smokers and nonsmokers. Gynecol Oncol. 75:68-71.

Marnett, LJ. (1990) Prostaglandin synthase-mediated metabolism of carcinogens and a potential role for peroxyl radicals as reactive intermediates. Environ Health Perspect 88:5–12.

Marshall, CJ; Vousden, KH; Phillips, DH. (1984) Activation of c-Ha-ras-1 proto-oncogene by in vitro modification with a chemical carcinogen, benzo(a)pyrene diol-epoxide. Nature 310(5978):586–589.

Martin, FL; Cole, KJ; Williams, JA; et al. (2000) Activation of genotoxins to DNA-damaging species in exfoliated breast milk cells. Mutat Res 470(2):115–124.

Martin, FL; Cole, KJ; Muir, GH; et al. (2002) Primary cultures of prostate cells and their ability to activate carcinogens. Prostate Cancer Prostatic Dis 5(2):96–104.

Martin, KR; Jokinen, MP; Honeycutt, HP; et al. (2004) Tumor profile of novel p53 heterozygous Tg.AC (v-Ha-ras) bitransgenic mice treated with benzo(a)pyrene and fed dietary N-acetyl-L-cysteine (NAC). Toxicol Sci 81(2):293–301.

Mass, MJ; Jeffers, AJ; Ross, JA; et al. (1993) Ki-ras oncogene mutations in tumors and DNA adducts formed by benz[j]aceanthrylene and benzo[a]pyrene in the lungs of strain A/J mice. Mol Carcinog 8(3):186–192.

Matkainen,T; Perez, GI; Jurisicova, A; et al. (2001) Aromatic hydrocarbon receptor-driven *BAX* gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nature Genetics 28:355-360.

Matkainen,T; Moriyama, T; Morita, Y; et al. (2002) Ligand activation of the aromatic hydrocarbon receptor transcription factor drives Bax-dependent apoptosis in developing fetal ovarian germ cells. Endocrinology 143;615-620.

Matsuoka, A; Matsuura, K; Sakamoto, H; et al. (1998) Spindle disturbances induced by benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in a Chinese hamster cell line (V79-MZ) and the stability of the numerical chromosome aberrations that follow. Mutat Res 419(1-3):1–12.

Matsuoka, A; Matsuura, K; Sakamoto, H; et al. (1999) A proposal for a simple way to distinguish aneugens from clastogens in the in vitro micronucleus test. Mutagenesis 14(4):385–389.

Mattison, DR; Thorgeirsson, SS. (1977) Genetic differences in mouse ovarian metabolism of benzo[a]pyrene and oocyte toxicity. Biochem Pharmacol 26:909-912.

Mattison, DR; Thorgeirsson, SS. (1979). Ovarian aryl hydrocarbon hydroxylase activity and primordial ooctye toxicity of polcyclic aromatic hydrocarbons in mice. Cancer Res 39;3471-3475.

Mattison, DR; Nightingale, MR. (1980) The biochemical and genetic characteristics of murine ovarian aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity and its relationship to primordial oocyte destruction by polycyclic aromatic hydrocarbons. Toxic App Pharmacol 56:399-408.

Mattison, DR; White, NB; Nightingale, MR (1980). The effect of benzo(a)pyrene on fertility, primordial oocyte number, and ovarian response to pregnant mare's serum gonadotropin. Ped Pharmacol 1:143-151.

Matullo, G; Peluso, M; Polidoro, S; et al. (2003) Combination of DNA repair gene single nucleotide polymorphisms and increased levels of DNA adducts in a population-based study. Cancer Epidemiol Biomarkers Prev 12(7):674–677.

McCabe, DP; Flynn, EJ. (1990) Deposition of low dose benzo(a)pyrene into fetal tissue: influence of protein binding. Teratology 41(1):85–95.

McCallister MM; Maguire M; Ramesh A; et al., (2008) Prenatal exposure to benzo(a)pyrene impairs later-life cortical neuronal function. Neurotoxicology 29(5):846-54.

McCarver, DG; Hines, RN. (2002) The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 300(2):361–366.

McCoull, KD; Rindgen, D; Blair, IA; et al. (1999) Synthesis and characterization of polycyclic aromatic hydrocarbon o-quinone depurinating N7-guanine adducts. Chem Res Toxicol 12(3):237–246.

Melendez-Colon, VJ; Luch, A; Seidel, A; et al. (1999) Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. Carcinogenesis 20(10):1885–1891.

Melikian, AA; Bagheri, K; Hecht, SS; et al. (1989) Metabolism of benzo[a]pyrene and 7 beta,8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[a pyrene in lung and liver of newborn mice. Chem Biol Interact 69(2-3):245–257.

Melikian, AA; Sun, P; Prokopczyk, B; et al. (1999) Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Lett 146(2):127–134.

Meng, F; Knapp, GW; Green, T; et al. (2010) K-Ras mutant fraction in A/J mouse lung increases as a function of benzo[a]pyrene dose. Environ Mol Mutagen. 51(2):146-55.

Mensing, T; Marczynski, B; Engelhardt, B; et al. (2005) DNA adduct formation of benzo[a]pyrene in white blood cells of workers exposed to polycyclic aromatic hydrocarbons. Int J Hyg Environ Health 208:173-178.

Merk, HF; Mukhtar, H; Kaufmann, I; et al. (1987) Human hair follicle benzo[a]pyrene and benzo[a]pyrene 7,8-diol metabolism: effect of exposure to a coal tar-containing shampoo. J Invest Dermatol 88(1):71–76.

Merlo, F; Bolognesi, C; Peluso, M; et al. (1997) Airborne levels of polycyclic aromatic hydrocarbons: 32P-postlabeling DNA adducts and micronuclei in white blood cells from traffic police workers and urban residents. J Environ Pathol Toxicol Oncol 16(2-3):157–162.

Midgette, AS; Baron, JA. (1990) Cigarette smoking and the risk of natural menopause. Epidemiology; 1:474-479.

Miller, MM; Plowchalk, DR; Weitzman, GA et al. (1992) The effect of benzo(a)pyrene on murine ovarian and corpora lutea volumes. Am J OBstet Gynecol 166:1535-1541.

Miller, KP; Ramos, KS. (2001) Impact of cellular metabolism on the biological effects of benzo[a]pyrene and related hydrocarbons. Drug Metab Rev 33(1):1–35.

Mirvish, SS; Ghadirian, P; Wallcave, L; et al. (1981) Effect of diet on fecal excretion and gastrointestinal tract distribution of unmetabolized benzo(a)pyrene and 3- methylcholanthrene when these compounds are administered orally to hamsters. Cancer Res 41(6):2289–2293.

Mohamed, el-SA; Song, WH; Oh, SA; et al.(2010) The transgenerational impact of benzo(a)pyrene on murine male fertility. Hum Reprod. 2010 25(10):2427-33.

Mohr, U. (1971) Diethylnitrosamine-induced carcinogenesis. Diaplacental effect. Fortschr Med 89(6):251-253 [German].

Moir, D. (1999) Physiological modeling of benzo(a)pyrene pharmacokinetics in the rat. In: Salem, H; Katz, SA; eds. Toxicity assessment alternatives: methods, issues, opportunities. Totowa, NJ: Humana Press Inc.; pp. 79–95.

Moir, D; Viau, A; Chu, I; et al. (1998) Pharmacokinetics of benzo[a]pyrene in the rat. J Toxicol Environ Health A 53(7):507–530.

Monteith, DK; Novotny, A; Michalopoulos, G; et al. (1987) Metabolism of benzo[a]pyrene in primary cultures of human hepatocytes: dose-response over a four-log range. Carcinogenesis 8(7):983–988.

Moore, BP; Hicks, RM; Knowles, MA; et al. (1982) Metabolism and binding of benzo(a)pyrene and 2-acetylaminofluorene by short-term organ cultures of human and rat bladder. Cancer Res 42(2):642–648.

Moore, LE; Wiencke, JK; Bates, MN; et al. (2004) Investigation of genetic polymorphisms and smoking in a bladder cancer case-control study in Argentina. Cancer Lett 211(2):199–207.

Moorthy, B; Miller, KP; Jiang, W; et al. (2003) Role of cytochrome P4501B1 in benzo[a]pyrene bioactivation to DNA-binding metabolites in mouse vascular smooth muscle cells: evidence from 32P-postlabeling for formation of 3-hydroxybenzo[a]pyrene and benzo[a]pyrene-3,6-quinone as major proximate genotoxic intermediates. J Pharmacol Exp Ther 305(1):394–401.

Morris, JJ; Seifter, E. (1992) The role of aromatic hydrocarbons in the genesis of breast cancer. Med Hypotheses 38(3):177–184.

Morse, MA; Carlson, GP. (1985) Distribution and macromolecular binding of benzo[a]pyrene in SENCAR and BALB/c mice following topical and oral administration. J Toxicol Environ Health 16(2):263–276.

Motykiewicz, G; Michalska, J; Pendzich, J; et al. (1998) A molecular epidemiology study in women from Upper Silesia, Poland. Toxicol Lett 96–97:195–202.

Mukhtar, H; Asokan, P; Das, M; et al. (1986) Benzo(a)pyrene diol epoxide-I-DNA adduct formation in the epidermis and lung of SENCAR mice following topical application of crude coal tar. Cancer Lett 33(3):287–294.

Munson, AE; White, KLJ. (1983) Effects of subchronic fourteen day exposure to benzopyrene in B6C3F1 male and female mice on specific immunological parameters. American Petroleum Institute. Submitted under TSCA Section FYI; NTIS No. OTSAX-1182-0220.

Munson, AE; White, KL; Lysy, HH. (1985) Effects of subchronic fourteen day exposure to benzopyrene in B6C3F1 female mice on host resistance. Submitted under TSCA Section FYI; EPA Document No. FYI-AX-0485-0220, Fiche No. OTS0000220-2.

Naslund, I; Rubio, CA; Auer, GU. (1987) Nuclear DNA changes during pathogenesis of squamous carcinoma of the cervix in 3,4-benzopyrene-treated mice. Anal Quant Cytol Histol 9(5):411–418.

N'Diaye, M; Le, FE; Lagadic-Gossmann, D; et al. (2006) Aryl hydrocarbon receptor- and calcium-dependent induction of the chemokine CCL1 by the environmental contaminant benzo[a]pyrene. J Biol Chem 281(29):19906–19915.

Neal, J; Rigdon, H. (1967) Gastric tumors in mice fed benzo[a]pyrene: a quantitative study. Tex Rep Biol Med 25(4):553–557.

Neal, MS; Zhu, J; Holloway, AC; Foster, WG. (2007) Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay. Human Repro 22;961-967.

Neal, MS; Zhu, J; Foster, WG. (2008) Quantification of benzo[a]pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers. Repro Toxicol 2008:100-106.

Nebert, DW; Puga, A; Vasiliou, V. (1993) Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. Ann N Y Acad Sci 685:624–640.

Nesnow, S; Triplett, LL; Slaga, TJ. (1983) Mouse skin tumor initiation-promotion and complete carcinogenesis bioassays: mechanisms and biological activities of emission samples. Environ Health Perspect 47:255–268.

Nesnow, S; Gold, A; Sangaiah, R; et al. (1984) Mouse skin tumor-initiating activity of benz[e]aceanthrylene and benz[l]aceanthrylene in SENCAR mice. Cancer Lett 22(3):263–268.

Nesnow, S; Mass, MJ; Ross, JA; et al. (1998a) Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatic hydrocarbons. Environ Health Perspect 106 Suppl 6:1337–1346.

Nesnow, S; Ross, JA; Mass, MJ; et al. (1998b) Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. Exp Lung Res 24(4):395–405.

Nesnow, S; Ross, JA; Stoner, GD; et al. (1995) Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. Toxicology 105(2-3):403–413.

Nesnow, S; Ross, JA; Stoner, GD; et al. (1996) Tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice: linkage to DNA adducts and mutations in oncogenes. Polycyclic Aromat Compd 10:259–266.

Nettesheim, P; Griesemer, RA; Martin, DH; et al. (1977) Induction of preneoplastic and neoplastic lesions in grafted rat tracheas continuously exposed to benzo(a)pyrene. Cancer Res 37(5):1272–1278.

Neubert, D; Tapken, S. (1988) Transfer of benzo(a)pyrene into mouse embryos and fetuses. Arch Toxicol 62(2-3):236–239.

Newbold, RF; Brookes, P. (1976) Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature 261(5555):52–54.

Ng, KM; Chu, I; Bronaugh, RL; et al. (1992) Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: comparison of in vitro and in vivo results in the hairless guinea pig. Toxicol Appl Pharmacol 115(2):216–223.

Nikonova, TV. (1977) [Transplacental effect of benz(a)pyrene and pyrene]. Biulleten Eksperimentalnoi Biologii i Meditsiny 84(7):88–91.

Nikoyan, A; De, MM; Sari-Minodier, I; et al. (2007) Evaluation of a battery of Salmonella typhimurium tester strains for biomonitoring of mutagenic polycyclic aromatic hydrocarbons, nitroarenes and aromatic amines. Mutation Research 626(1-2):88–101.

Niwa, Y ; Terashima, T; Sumi, H. (2003). Topical application of the immunosuppressant tacrolimus accelerates carcinogenesis in mouse skin. Br J Dermatol. 149(5):960-7.

Nordling, MM; Nygren, J; Bergman, J; et al. (2002) Toxicological characterization of a novel in vivo benzo[a]pyrene metabolite, 7-oxo-benz[d]anthracene-3,4-dicarboxylic acid anhydride. Chem Res Toxicol 15(10):1274–1280.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NTP (National Toxicology Program). (2005) 11th Report on carcinogens. Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

Nwagbara, O; Darling-Reed, SF; Tucker, A; et al. (2007) Induction of cell death, DNA strand breaks, and cell cycle arrest in DU145 human prostate carcinoma cell line by benzo[a]pyrene and benzo[a]pyrene-7,8-diol-9,10-epoxide. Int J Environ Res Public Health 4(1):10–14.

Obana, H; Hori, S; Kashimoto, T; et al. (1981) Polycyclic aromatic hydrocarbons in human fat and liver. Bull Environ Contam Toxicol 27(1):23–27.

Oesch, F. (1980) Microsomal epoxide hydrolase. In: Enzymatic basis of detoxification. Academic Press; pp. 277–290.

Okey, AB; Riddick, DS; Harper, PA. (1994) Molecular biology of the aromatic hydrocarbon (dioxin) receptor. Trends Pharmacol Sci 15(7):226–232.

O'Neil, MJ; Smith, A; Heckelman, PE; et al. (2001) The Merck index: An encyclopedia of chemicals, drugs, and biologicals. 13th ed. Whitehouse Station, NJ: Merck & Co., Inc. .

O'Neill, IK; Povey, AC; Bingham, S; et al. (1990) Systematic modulation by human diet levels of dietary fibre and beef on metabolism and disposition of benzo[a]pyrene in the gastrointestinal tract of Fischer F344 rats. Carcinogenesis 11(4):609–616.

O'Neill, IK; Goldberg, MT; el Ghissassi, F; et al. (1991) Dietary fibre, fat and beef modulation of colonic nuclear aberrations and microcapsule-trapped gastrointestinal metabolites of benzo[a]pyrene-treated C57/B6 mice consuming human diets. Carcinogenesis 12(2):175–180.

Oueslati, R; Alexandrov, K; Chouikha, M; et al. (1992) Formation and persistence of DNA adducts in epidermal and dermal mouse skin exposed to benzo(a)pyrene in vivo. In Vivo 6(2):231–235.

Paleologo, M; Van Schooten, FJ; Pavanello, S; et al. (1992) Detection of benzo[a]pyrene-diol-epoxide-DNA adducts in white blood cells of psoriatic patients treated with coal tar. Mutat Res 281(1):11–16.

Pan, G; Hanaoka, T; Yamano, Y; et al. (1998) A study of multiple biomarkers in coke oven workers--a cross-sectional study in China. Carcinogenesis 19(11):1963–1968.

Park, SJ; Zhao, H; Spitz, MR; et al. (2003) An association between NQO1 genetic polymorphism and risk of bladder cancer. Mutat Res 536(1–2):131–137.

Park, JH; Troxel, AB; Harvey, RG; et al. (2006a) Polycyclic aromatic hydrocarbon (PAH) o-quinones produced by the aldo-keto-reductases (AKRs) generate abasic sites, oxidized pyrimidines, and 8-oxo-dGuo via reactive oxygen species. Chem Res Toxicol 19(5):719–728.

Park, JH; Gelhaus, S; Vedantam, S; et al. (2008) The pattern of p53 mutations caused by PAH o-quinones is driven by 8-oxo-dGuo formation while the spectrum of mutations is determined by biological selection for dominance. Chem Res Toxicol 21(5):1039–1049.

Parman, T; Wells, PG. (2002) Embryonic prostaglandin H synthase-2 (PHS-2) expression and benzo[a]pyrene teratogenicity in PHS-2 knockout mice. FASEB J 16(9):1001–1009.

Partanen, T; Boffetta, P. (1994) Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. Am J Ind Med 26(6):721–740.

Pastorelli, R; Guanci, M; Cerri, A; et al. (1998) Impact of inherited polymorphisms in glutathione S-transferase M1, microsomal epoxide hydrolase, cytochrome P450 enzymes on DNA, and blood protein adducts of benzo(a)pyrene-diolepoxide. Cancer Epidemiol Biomarkers Prev 7(8):703–709.

Pataki, J; Huggins, C. (1969) Molecular site of substituents of benz(a)anthracene related to carcinogenicity. Cancer Res 29(3):506–509.

Pavanello, S; Favretto, D; Brugnone, F; et al. (1999) HPLC/fluorescence determination of anti-BPDE-DNA adducts in mononuclear white blood cells from PAH-exposed humans. Carcinogenesis 20(3):431–435.

Pavanello, S; Siwinska, E; Mielzynska, D; et al. (2004) GSTM1 null genotype as a risk factor for anti-BPDE-DNA adduct formation in mononuclear white blood cells of coke-oven workers. Mutat Res 558(1-2):53–62.

Pavanello, S; Pulliero, A; Siwinska, E; et al. (2005) Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B[a]PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. Carcinogenesis 26(1):169–175.

Pavanello, S; Pulliero, A; Saia, BO; et al. (2006) Determinants of anti-benzo[a]pyrene diol epoxide-DNA adduct formation in lymphomonocytes of the general population. Mutat Res 611(1-2):54–63.

Peluso, M; Merlo, F; Munnia, A; et al. (1998) 32P-postlabeling detection of aromatic adducts in the white blood cell DNA of nonsmoking police officers. Cancer Epidemiol Biomarkers Prev 7(1):3–11.

Penning, TM; Ohnishi, ST; Ohnishi, T; et al. (1996) Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. Chem Res Toxicol 9(1):84–92.

Penning, TM; Burczynski, ME; Hung, CF; et al. (1999) Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. Chem Res Toxicol 12(1):1–18.

Pereira, MA; McMillan, L; Kaur, P; et al. (1982) Effect of benzo[a]pyrene on sister-chromatid exchange in fetal hamster liver exposed in utero. Mutat Res 105:343-347.

Perera, FP; Hemminki, K; Young, TL; et al. (1988) Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. Cancer Res 48(8):2288–2291.

Perera, FP; Tang, DL; O'Neill, JP; et al. (1993) HPRT and glycophorin A mutations in foundry workers: relationship to PAH exposure and to PAH-DNA adducts. Carcinogenesis 14(5):969–973.

Perera, FP; Dickey, C; Santella, R; et al. (1994) Carcinogen-DNA adducts and gene mutation in foundry workers with low-level exposure to polycyclic aromatic hydrocarbons. Carcinogenesis 15(12):2905–2910.

Perera, FP; Weinstein, IB. (2000) Molecular epidemiology: recent advances and future directions. Carcinogenesis 21(3):517–524.

Perera, FP; Tang, D; Rauh, V; et al. (2005a) Relationships among polycyclic aromatic hydrocarbon-DNA adducts, proximity to the World Trade Center, and effects on fetal growth. Environ Health Perspect 113(8):1062–1067.

Perera, FP; Rauh, V; Whyatt, RM; et al. (2005b) A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures. Neurotoxicology 26(4):573–587.

Perera FP, Li Z, Whyatt R, Hoepner L, et al., (2009) Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics. 124(2):e195-202.

Persson, E; Larsson, P; Tjalve, H. (2002) Cellular activation and neuronal transport of intranasally instilled benzo(a)pyrene in the olfactory system of rats. Toxicol Lett 133(2–3):211–219.

Petrie, HT. (2002) Role of thymic organ structure and stromal composition in steady-state postnatal t-cell production. Immunol Rev 189:8-19.

Petridou-Fischer, J; Whaley, SL; Dahl, AR. (1988) In vivo metabolism of nasally instilled benzo[a]pyrene in dogs and monkeys. Toxicology 48(1):31–40.

Petry, T; Schmid, P; Schlatter, C. (1996) The use of toxic equivalency factors in assessing occupational and environmental health risk associated with exposure to airborne mixtures of polycyclic aromatic hydrocarbons (PAHs). Chemosphere 32(4):639–648.

Pfeiffer, EH. (1977) Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons in mice. IARC Sci Publ 16:69–77.

Pfeiffer, CA; Allen, E. (1948) Attempts to produce cancer in Rhesus monkeys with carcinogenic hydrocarbons and estrogens. Cancer Res 8(3):97–127.

Phillips, DH. (2002) Smoking-related DNA and protein adducts in human tissues. Carcinogenesis 23(12):1979–2004.

Poel, WE. (1959) Effect of carcinogenic dosage and duration of exposure on skin-tumor induction in mice. J Natl Cancer Inst 22(1):19–43.

Poel, WE. (1960) Skin as a test site for the bioassay of carcinogens and carcinogen precursors. J Natl Cancer Inst Monogr 10:611–631.

Poirier, MC; Santella, RM; Weston, A. (2000) Carcinogen macromolecular adducts and their measurement. Carcinogenesis 21(3):353–359.

Porter, PC; Mellon, I; States, JC. (2005) XP-A cells complemented with Arg228Gln and Val234Leu polymorphic XPA alleles repair BPDE-induced DNA damage better than cells complemented with the wild type allele. DNA Repair (Amst) 4(3):341–349.

Potter, D; Booth, ED; Brandt, HC; et al. (1999) Studies on the dermal and systemic bioavailability of polycyclic aromatic compounds in high viscosity oil products. Arch Toxicol 73(3):129–140.

Puisieux, A; Lim, S; Groopman, J; et al. (1991) Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res 51(22):6185–6189.

Qian, HW; Ong, T; Nath, J; et al. (1998) Induction of DNA adducts in vivo in rat lung cells by fume condensates of roofing asphalt. Teratogenesis, Carcinogenesis, and Mutagenesis 18(3):131–140.

Rahman, A; Barrowman, JA; Rahimtula, A. (1986) The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. Can J Physiol Pharmacol 64(9):1214–1218.

Ramesh, A; Inyang, F; Hood, DB; et al. (2000) Aryl hydrocarbon hydroxylase activity in F-344 rats subchronically exposed to benzo(a)pyrene and fluoranthene through diet. J Biochem Mol Toxicol 14(3):155–161.

Ramesh, A; Greenwood, M; Inyang, F; et al. (2001a) Toxicokinetics of inhaled benzo[a]pyrene: plasma and lung bioavailability. Inhal Toxicol 13(6):533–555.

Ramesh, A; Inyang, F; Hood, DB; et al. (2001b) Metabolism, bioavailability, and toxicokinetics of benzo(alpha)pyrene in F-344 rats following oral administration. Exp Toxicol Pathol 53(4):275–290.

Ramesh, A; Greenwood, M; Inyang, F; et al. (2003) Aryl hydrocarbon hydroxylase (AHH) and benzo(a)pyrene metabolite in F-344 rats subchronically exposed to inhaled benzo[a]pyrene. Toxicologist 72(S-1):325.

Ramesh, A; Walker, SA; Hood, DB; et al. (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol 23(5):301–333.

Ramesh, A; Knuckles, ME. (2006) Dose-dependent benzo(a)pyrene [B(a)P]-DNA adduct levels and persistence in F-344 rats following subchronic dietary exposure to B(a)P. Cancer Lett 240:268-278.

Ramesh, A; Inyang, F; Lunstra, DD; et al. (2008) Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene. Exp Toxicol Pathol 60(4–5):269–280.

Ramos, KS; Moorthy, B. (2005) Bioactivation of polycyclic aromatic hydrocarbon carcinogens within the vascular wall: Implications for human atherogenesis. Drug Metab Rev 37(4):595–610.

Rask-Nielson, R. (1950) The susceptibility of the thymus, lung, subcutaneous and mammary tissues in strain street mice to direct application of small doses of four different carcinogenesis hydrocarbons. Br J Cancer 4:108–116.

Raveh, D; Slaga, TJ; Huberman, E. (1982) Cell-mediated mutagenesis and tumor-initiating activity of the ubiquitous polycyclic hydrocarbon, cyclopenta[c,d]pyrene. Carcinogenesis 3(7):763–766.

Raychoudhury, SS; Kubinski, D. (2003) Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. Environ Health Perspect 111(1):33–38.

Reardon, KF; Liao, KH; Yang, RS. (2005) Development of a hybrid reaction network physiologically based pharmacokinetic model of benzo(a)pyrene and its metabolites. Toxicol Sci 84(1-S):177.

Reddy, MV; Gupta, RC; Randerath, E; et al. (1984) 32P-postlabeling test for covalent DNA binding of chemicals in vivo: application to a variety of aromatic carcinogens and methylating agents. Carcinogenesis 5:231-243.

Renzik-Schuller, H; Mohr, U. (1974) Investigations on the carcinogenic burden by air pollution in man. IX. Early pathological alterations of the bronchial epithelium in Syrian golden hamsters after intratracheal instillation of benzo (a) pyrene. 1. Morphological studies from semi-thin sections. Zentralbl Bakteriol [Orig B] 159(5–6):493–502.

Revel, A; Raanani, H; Younglai, E; et al. (2001) Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. Reprod Toxicol 15(5):479–486.

Rice, JE; Makowski, GS; Hosted, TJ, Jr.; et al. (1985) Methylene-bridged bay region chrysene and phenanthrene derivatives and their keto-analogs: mutagenicity in Salmonella typhimurium and tumor-initiating activity on mouse skin. Cancer Lett 27(2):199–206.

Rigdon, RH; Neal, J. (1965) Effects of feeding benzo(a)pyrene on fertility, embryos, and young mice. J Natl Cancer Inst 34:297–305.

Rigdon, RH; Neal, J. (1966) Gastric carcinomas and pulmonary adenomas in mice fed benzo (a) pyrene. Tex Rep Biol Med 24(2):195–207.

Rigdon, RH; Neal, J. (1969) Relationship of leukemia to lung and stomach tumors in mice fed benzo (a) pyrene. Proc Soc Exp Biol Med 130(1):146–148.

Rigdon, RH; Rennels, EG. (1964) Effect of feeding benzpyrene on reproduction in the rat. Experientia 20(4):224–226.

Robertson, IG; Guthenberg, C; Mannervik, B; et al. (1986) Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7 beta,8 alpha-dihydroxy-9 alpha,10 alpha-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Cancer Res 46(5):2220–2224.

Robinson, DJ; de Bruijn, HS; van der Veen, N; et al. (1998). Fluorescence photobleaching of ala-induced protoporphyrin ix during photodynamic therapy of normal hairless mouse skin: the effect of light dose and lrradiance and the resulting biological effect. Photochemistry and Photobiology 67(1): 140-1 49.

Robinson, M; Laurie, RD; Bull, RJ; et al. (1987) Carcinogenic effects in A/J mice of particulate of a coal tar paint used in potable water systems. Cancer Lett 34(1):49–54.

Rodriguez, LV; Dunsford, HA; Steinberg, M; et al. (1997) Carcinogenicity of benzo[a]pyrene and manufactured gas plant residues in infant mice. Carcinogenesis 18(1):127–135.

Rodriguez, JW; Kirlin, WG; Wirsiy, YG; et al. (1999) Maternal exposure to benzo[a]pyrene alters development of t lymphocytes in offspring. Immunopharmacol Immunotoxicol 21(2):379-396.
Roe, FJ; Waters, MA. (1967) Induction of hepatoma in mice by carcinogens of the polycyclic hydrocarbon type. Nature 214(5085):299–300.

Roe, FJ; Peto, R; Kearns, F; et al. (1970) The mechanism of carcinogenesis by the neutral fraction of cigarette smoke condensate. Br J Cancer 24(4):788–806.

Rogan, EG; Devanesan, PD; RamaKrishna, NV; et al. (1993) Identification and quantitation of benzo[a]pyrene-DNA adducts formed in mouse skin. Chem Res Toxicol 6(3):356–363.

Rogan, EG; RamaKrishna, NV; Higginbotham, S; et al. (1990) Identification and quantitation of 7-(benzo[a]pyren-6-yl)guanine in the urine and feces of rats treated with benzo[a]pyrene. Chem Res Toxicol 3(5):441–444.

Rojas, M; Alexandrov, K; Cascorbi, I; et al. (1998) High benzo[a]pyrene diol-epoxide DNA adduct levels in lung and blood cells from individuals with combined CYP1A1 MspI/Msp-GSTM1*0/*0 genotypes. Pharmacogenetics 8(2):109–118.

Rojas, M; Cascorbi, I; Alexandrov, K; et al. (2000) Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 21(1):35–41.

Rojas, M; Godschalk, R; Alexandrov, K; et al. (2001) Myeloperoxidase--463A variant reduces benzo[a]pyrene diol epoxide DNA adducts in skin of coal tar treated patients. Carcinogenesis 22(7):1015–1018.

Romundstad, P; Haldorsen, T; Andersen, A. (2000a) Lung and bladder cancer among workers in a Norwegian aluminium reduction plant. Occup Environ Med 57(7):495–499.

Romundstad, P; Andersen, A; Haldorsen, T. (2000b) Cancer incidence among workers in six Norwegian aluminum plants. Scand J Work Environ Health 26(6):461–469.

Ross, JA; Nesnow, S. (1999) Polycyclic aromatic hydrocarbons: correlations between DNA adducts and ras oncogene mutations. Mutat Res 424(1–2):155–166.

Ross, J; Nelson, G; Kligerman, A; et al. (1990) Formation and persistence of novel benzo(a)pyrene adducts in rat lung, liver, and peripheral blood lymphocyte DNA. Cancer Res 50(16):5088–5094.

Ross, J; Nelson, G; Erexson, G; et al. (1991) DNA adducts in rat lung, liver and peripheral blood lymphocytes produced by i.p. administration of benzo[a]pyrene metabolites and derivatives. Carcinogenesis 12(10):1953–1955.

Ross, JA; Nelson, GB; Wilson, KH; et al. (1995) Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. Cancer Res 55(5):1039–1044.

Rossella, F; Campo, L; Pavanello, S; et al. (2009) Urinary polycyclic aromatic hydrocarbons and monohydroxy metabolites as biomarkers of exposure in coke-oven workers. Occup Environ Med Epub ahead of print 15 Feb 2009

Roth, RA; Vinegar, A. (1990) Action by the lungs on circulating xenobiotic agents, with a case study of physiologically based pharmacokinetic modeling of benzo(a)pyrene disposition. Pharmacol Ther 48(2):143–155.

Russell, LB. (1977) Validation of the in vivo somatic mutation method in the mouse as a prescreen for germinal point mutations. Arch Toxicol 38(1-2):75–85.

Saffiotti, U; Cefis, F; Kolb, LH. (1968) A method for the experimental induction of bronchogenic carcinoma. Cancer Res 28(1):104–124.

Saffiotti, U; Montesano, R; Sellakumar, AR; et al. (1972) Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo-(a)pyrene and ferric oxide. J Natl Cancer Inst 49(4):1199–1204.

Sagredo, C; Ovrebo, S; Haugen, A; et al. (2006) Quantitative analysis of benzo[a]pyrene biotransformation and adduct formation in Ahr knockout mice. Toxicol Lett 167(3):173–182.

Salama, SA; Sierra-Torres, CH; Oh, HY; et al. (2001) Variant metabolizing gene alleles determine the genotoxicity of benzo[a]pyrene. Environ Mol Mutagen 37:17-26.

Salmon, AG; Brown, JP; Collins, JF; et al. (2003) Evaluation of alternate dose metrics for benzo(a)pyrene inhalation cancer potency using a nasal-PBPK hamster model. Toxicologist 72:85.

Santella, RM; Nunes, MG; Blaskovic, R; et al. (1994) Quantitation of polycyclic aromatic hydrocarbons, 1-hydroxypyrene, and mutagenicity in urine of coal tar-treated psoriasis patients and untreated volunteers. Cancer Epidemiol Biomarkers Prev 3(2):137–140.

Saunders, CR; Shockley, DC; Knuckles, ME. (2001) Behavioral effects induced by acute exposure to benzo[a]pyrene in F-344 rats. Neurotox Res 3:557–579.

Saunders, CR; Ramesh, A; Shickley, DC. (2002) Modulation of neurotoxic behavior of F-344 rats by temporal disposition of benzo[a]pyrene. Toxicol Lett 129:33–45.

Saunders, CR; Das, SK; Ramesh, A; et al. (2006) Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. J Appl Toxicol 26(5):427–438.

Scherer, G; Frank, S; Riedel, K; et al. (2000) Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. Cancer Epidemiol Biomarkers Prev 9(4):373–380.

Schlede, E; Kuntzman, R; Haber, S; et al. (1970) Effect of enzyme induction on the metabolism and tissue distribution of benzo(alpha)pyrene. Cancer Res 30(12):2893–2897.

Schmahl, D; Schmidt, KG; Habs, M. (1977) Syncarcinogenic action of polycyclic hydrocarbons in automobile exhaust gas condensates. IARC Sci Publ (16):53–59.

Schmidt, KG; Schmael, D; Misfield, J. (1973) Investigations on the carcinogenic burden in man. VI. Experimental investigation to determine a dose-response relationship and to estimate a threshold dose of benzo[a]pyrene in the skin of two different mouse strains. Zbl Bakt Hyg I Abs B 158:62–68.

Schnizlein, CT; Munson, AE; Rhoades, RA. (1987) Immunomodulation of local and systemic immunity after subchronic pulmonary exposure of mice to benzo(a)pyrene. Int J Immunopharmacol 9(1):99–106.

Schoket, B; Doty, WA; Vincze, I; et al. (1993) Increased sensitivity for determination of polycyclic aromatic hydrocarbon-DNA adducts in human DNA samples by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA). Cancer Epidemiol Biomarkers Prev 2(4):349–353.

Schonland, SO; Zimmer, JK; Lopez-Benitez, CM; et al. (2003) Homeostatic control of t-cell generation in neonates. Blood 102(4):1428-1434.

Schuurman, HJ; Van Loveren, H; Rozing, J; et al. (1992) Chemicals trophic for the thymus: Risk for immunodeficiency and autoimmunity. Int J Immunopharmacol 14(3):369-375.

Schwarz, D; Kisselev, P; Cascorbi, I; et al. (2001) Differential metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-dihydrodiol by human CYP1A1 variants. Carcinogenesis 22(3):453–459.

Sega, GA. (1979) Unscheduled DNA synthesis (DNA repair) in the germ cells of male mice--its role in the study of mammalian mutagenesis. Genetics 92:s49-58.

Sharma, R; Haque, AK; Awasthi, S; et al. (1997) Differential carcinogenicity of benzo[a]pyrene in male and female CD-1 mouse lung. J Toxicol Environ Health 52(1):45–62.

Sharovskaya, J; Kobliakova, I; Solomatina, N; et al. (2006) Effect of some carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons on gap junction intercellular communication in hepatoma cell cultures. Eur J Cell Biol 85(5):387–397.

Shen, H; Spitz, MR; Qiao, Y; et al. (2003) Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. Int J Cancer 107(1):84–88.

Shen, YM; Troxel, AB; Vedantam, S; et al. (2006) Comparison of p53 mutations induced by PAH o-quinones with those caused by anti-benzo[a]pyrene diol epoxide in vitro: role of reactive oxygen and biological selection. Chem Res Toxicol 19(11):1441–1450.

Shendrikova, A; Aleksandrov, VA. (1974) Comparative penetration of polycyclic hydrocarbons through the rat placenta into the fetus. Bull Exp Biol Med 77:169–171.

Sherson, D; Sabro, P; Sigsgaard, T; et al. (1990) Biological monitoring of foundry workers exposed to polycyclic aromatic hydrocarbons. Br J Ind Med 47(7):448–453.

Shimizu, Y; Nakatsuru, Y; Ichinose, M; et al. (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci USA 97(2):779–782.

Shukla, Y, Yadav, A, Arora, A. (2002). Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin. Cancer Letters 182:33–41.

Shultz, MA; Morin, D; Chang, AM; et al. (2001) Metabolic capabilities of CYP2F2 with various pulmonary toxicants and its relative abundance in mouse lung subcompartments. J Pharmacol Exp Ther 296(2):510–519.

Shvedova, A; Kisin, ER; Murray, AR; et al. (2002). Antioxidant Balance and Free Radical Generation in Vitamin E . Chem. Res. Toxicol., 2002, 15 (11), pp 1451–1459.

Silverstone, AE; Cantor, H; Goldstein, G; et al. (1976) Terminal deoxynucleotidyl transferase is found in prothymocytes. J Exp Med 144(2):543-548.

Singh, VK; Singh, J; Anand, M; et al. (2008) Comparison of polycyclic aromatic hydrocarbon levels in placental tissues of Indian women with full- and preterm deliveries. Int J Hyg Environ Health 211:639-647.

Sinha, R; Kulldorff, M; Gunter, MJ; et al. (2005) Dietary benzo[a]pyrene intake and risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 14(8):2030–2034.

Sivak A, Niemeier R, Lynch D, et al., (1997). Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. Cancer Lett. 117(1):113-23.

Slaga, TJ; Bracken, WM; Viaje, A; et al. (1977) Comparison of the tumor-initiating activities of benzo(a)pyrene arene oxides and diol-epoxides. Cancer Res 37(11):4130–4133.

Slaga, TJ; Huberman, E; Selkirk, JK; et al. (1978) Carcinogenicity and mutagenicity of benz(a)anthracene diols and diol-epoxides. Cancer Res 38(6):1699–1704.

Slaga, TJ; Iyer, RP; Lyga, W; et al. (1980) Comparison of the skin tumor-initiating activities of dihydrodiols, diol-epoxides, and methylated derivatives of various polycyclic aromatic hydrocarbons. In: Bjorseth, A; Dennis, AJ; eds. Polynuclear aromatic hydrocarbons: chemistry and biological effects. Columbus, OH: Battelle Press; pp. 753–769.

Soares, SR; Melo, MA. 2008. Cigarette smoking and reproductive function. Curr Opin Obstet Gynecol. 20:281-91.

Soyka, LF. (1980) Hepatic drug metabolizing enzyme activity and tumorigenesis in mice following perinatal exposure to benzo(a)pyrene. Pediatr Pharmacol 1(1):85–96.

Spinelli, JJ; Band, PR; Svirchev, LM; et al. (1991) Mortality and cancer incidence in aluminum reduction plant workers. J Occup Med 33(11):1150–1155.

Spinelli, JJ; Demers, PA; Le, ND; et al. (2006) Cancer risk in aluminum reduction plant workers (Canada). Cancer Causes Control 17(7):939–948.

Stansbury, KH; Noll, DM; Groopman, JD; et al. (2000) Enzyme-mediated dialdehyde formation: an alternative pathway for benzo[a]pyrene 7,8-dihydrodiol bioactivation. Chem Res Toxicol 13(11):1174–1180.

Stavric, B; Klassen, R. (1994) Dietary effects on the uptake of benzo[a]pyrene. Food Chem Toxicol 32(8):727–734.

Steiner, PE. (1955) Carcinogenicity of multiple chemicals simultaneously administered. Cancer Res 15(9):632–635.

Straif, K; Baan, R; Grosse, Y; et al. (2005) Carcinogenicity of polycyclic aromatic hydrocarbons. Lancet Oncology 6:931–932.

Sugiyama, T. (1973) Chromosomal aberrations and carcinogenesis by various benz(a)anthracene derivatives. Gann 64(6):637–639.

Sun, JD; Wolff, RK; Kanapilly, GM. (1982) Deposition, retention, and biological fate of inhaled benzo(a)pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol Appl Pharmacol 65(2):231–244.

Surh, YJ; Tannenbaum, SR. (1995) Sulfotransferase-mediated activation of 7,8,9,10-tetrahydro-7-ol, 7,8-dihydrodiol, and 7,8,9,10-tetraol derivatives of benzo[a]pyrene. Chem Res Toxicol 8(5):693–698.

Swartz, WJ; Mattison, DR.(1985) Benzo(a)pyrene inhibits ovulation in C57BL/6N mice. Anat Rec 212:268-276.

Taioli, E; Sram, RJ; Binkova, B; et al. (2007) Biomarkers of exposure to carcinogenic PAHs and their relationship with environmental factors. Mutat Res 620(1–2):16–21.

Takizawa, K; Yagi, H; Jerina, DM; Mattison, DR. Murine strain differences in ovotoxicity following intraovarian injection with benzo(a)pyrene, (+)-(7R,8S),-oxide, (–)-(7R,*R)-dihydrodiol, or (+)-(7R,8S)-diol-(9S,10R)-epoxide-2. Cancer Res 44:2571-2576.

Talaska, G; Jaeger, M; Reilman, R; et al. (1996) Chronic, topical exposure to benzo[a]pyrene induces relatively high steady-state levels of DNA adducts in target tissues and alters kinetics of adduct loss. Proc Natl Acad Sci USA 93(15):7789–7793.

Talaska, G; Ginsburg, D; LaDow, K; et al. (2006) Impact of Cyp1a2 or Ahr gene knockout in mice: implications for biomonitoring studies. Toxicol Lett 162(2-3):246–249.

Tamaki, A; Hayashi, H; Nakajima, H; et al. (2004) Polycyclic aromatic hydrocarbon increases mRNA level for interleukin 1 beta in human fibroblast-like synoviocyte line via aryl hydrocarbon receptor. Biol Pharm Bull 27(3):407–410.

Tang, D; Li, TY; Liu, JJ; et al. (2006) PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. Environ Health Perspect 114(8):1297–1300.

Tang, D; Li, TY; Liu, JJ, et al. (2008) Effects of prenatal exposure to coal-burning pollutants on children's development in China. Environ Health Perspect 116:674–679.

Tarantini, A; Maitre, A; Lefebvre, E; et al. (2009) Relative contribution of DNA strand breaks and DNA adducts to the genotoxicity of benzo[a]pyrene as a pure compound and in complex mixtures. Mutat Res 671:67-75.

Terracini, B; Shubik, P; Dellaporta, A. (1960). A Study of Skin Carcinogenesis in the Mouse with Single Applications of 9,10-Dimethyl-l,2-benzanthracene at Different Dosages. Cancer Research Vol. 20:1538-1542.

Theriault, G; Tremblay, C; Cordier, S; et al. (1984) Bladder cancer in the aluminium industry. Lancet 1(8383):947–950.

Thirman, MJ; Albrecht, JH; Krueger, MA; et al. (1994) Induction of cytochrome CYPIA1 and formation of toxic metabolites of benzo[a]pyrene by rat aorta: a possible role in atherogenesis. Proc Natl Acad Sci U S A 91(12):5397–5401.

Thyssen, J; Althoff, J; Kimmerle, G; et al. (1981) Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J Natl Cancer Inst 66(3):575–577.

Topping, DC; Martin, DH; Nettesheim, P. (1981) Determination of cocarcinogenic activity of benzo[e]pyrene for respiratory tract mucosa. Cancer Lett 11(4):315–321.

Tremblay, C; Armstrong, B; Theriault, G; et al. (1995) Estimation of risk of developing bladder cancer among workers exposed to coal tar pitch volatiles in the primary aluminum industry. Am J Ind Med 27(3):335–348.

Temple, L; Kawabata, TT; Munson, AE; et al. (1993) Comparison of ELISA and plaque-forming cell assays for measuring the humoral immune response to SRBC in rats and mice treated with benzo[a]pyrene or cyclophosphamide. Fundam Appl Toxicol 21(4):412-419.

Triolo, AJ; Aponte, GE; Herr, DL. (1977) Induction of aryl hydrocarbon hydroxylase and forestomach tumors by benzo(a)pyrene. Cancer Res 37:3018–3021.

Tsuruda, L; Hou, Y; Penning, TM. (2001) Stable expression of rat dihydrodiol dehydrogenase (AKR1C9) in human breast MCF-7 cells results in the formation of PAH-o-quinones and enzyme mediated cell death. Chem Res Toxicol 14(7):856–862.

Tuntawiroon, J; Mahidol, C; Navasumrit, P; et al. (2007) Increased health risk in Bangkok children exposed to polycyclic aromatic hydrocarbons from traffic-related sources. Carcinogenesis 28(4):816–822.

Turusov, VS; Nikonova, TV; Parfenov, Y. (1990) Increased multiplicity of lung adenomas in five generations of mice treated with benz(a)pyrene when pregnant. Cancer Lett 55(3):227–231.

Tuttle, AM; Stämpfli, M; Foster, WG. (2009) Cigarette smoke causes follicle loss i nmice ovaries at concentrations representative of human exposure. Human Repro 24:1452-1459.

Uno, S; Dalton, TP; Shertzer, HG; et al. (2001) Benzo[a]pyrene-induced toxicity: paradoxical protection in Cyp1a1(-/-) knockout mice having increased hepatic benzo[a]pyrene-DNA adduct levels. Biochem Biophys Res Commun 289:1049-1056.

Uno, S; Dalton, TP; Dragin, N; et al. (2006) Oral benzo[a]pyrene in Cyp1 knockout mouse lines: CYP1A1 important in detoxication, CYP1B1 metabolism required for immune damage independent of total-body burden and clearance rate. Mol Pharmacol 69(4):1103–1114.

Urso, P; Gengozian, N. (1980) Depressed humoral immunity and increased tumor incidence in mice following in utero exposure to benzo[alpha]pyrene. J Toxicol Environ Health 6(3):569–576.

Urso, P; Gengozian, N. (1982) Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[a]pyrene and x-rays before or after birth. J Toxicol Environ Health 10(4-5):817-835.

Urso, P; Gengozian, N. (1984) Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after in utero exposure to benzo[a]pyrene. J Toxicol Environ Health 14(4):569–584.

Urso, P; Johnson, RA. (1987) Early changes in t lymphocytes and subsets of mouse progeny defective as adults in controlling growth of a syngeneic tumor after in utero insult with benzo(a)pyrene. Immunopharmacology 14(1):1-10.

Urso, P; Johnson, RA. (1988) Quantitative and functional change in T cells of primiparous mice following injection of benzo(a)pyrene at the second trimester of pregnancy. Immunopharmacol Immunotoxicol 10(2):195–217.

Urso, P; Ryan, MC; Bennett, JS. (1988) Changes in peripheral blood cells in mice after injection with benzo(a)pyrene during pregnancy. Immunopharmacol Immunotoxicol 10(2):179–193.

Urso, P; Zhang, W; Cobb, JR. (1992) Immunological consequences from exposure to benzo(a)pyrene during pregnancy. Scand J Immunol Suppl 11:203-206.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6–87/008. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1991a) Dose-response analysis of ingested benzo(a)pyrene (CAS no 50-32-8). Cincinnati, OH: U.S. Environmental Protection Agency. EPA600R92045.

U.S. EPA (Environmental Protection Agency). (1991b) Drinking water criteria document for polycyclic aromatic hydrocarbons (PAHs). Cincinnati, OH: Agency, Environmental Protection Agency; PB92173459.

U.S. EPA (Environmental Protection Agency). (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8–90/066F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R–94/007. Available online at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000a) Science policy council handbook: risk characterization. Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-00-002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000b) Benchmark dose technical guidance document. External review draft. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000c) Supplementary guidance for conducting for health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001B. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2006a) Science policy council handbook: peer review. Third edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2006b) A Framework for Assessing Health Risk of Environmental Exposures to Children. National Center for Environmental Assessment, Washington, DC, EPA/600/R-05/093F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2007) Framework for determining a mutagenic mode of action for carcinogenicity: Using EPA's 2005 cancer guidelines and supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. External Review Draft. EPA 120/R-07/002-A. Available online at http://www.epa.gov/osa/mmoaframework/pdfs/MMOA-ERD-FINAL-83007.pdf (accessed April 21, 2009).

Vainio, H; Uotila, P; Hartiala, J; et al. (1976) The fate of intratracheally installed benzo(a)pyrene in the isolated perfused rat lung of both control and 20-methylcholanthrene pretreated rats. Res Commun Chem Pathol Pharmacol 13(2):259–271.

Van Agen, B; Maas, LM; Zwingmann, IH; et al. (1997) B[a]P-DNA adduct formation and induction of human epithelial lung cell transformation. Environ Mol Mutagen 30(3):287–292.

van Delft, JH; Steenwinkel, MS; van Asten, JG; et al. (2001) Biological monitoring the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for GSTM1 and GSTT1. Ann Occup Hyg 45(5):395–408.

van den Berg, FA; Baken, KA; Vermeulen, JP; et al. (2005) Use of the local lymph node assay in assessment of immune function. Toxicology 211(1-2):107–114.

van der Bijl, P; van Eyk, AD. (1999) Penetration of benzo[a]pyrene through human buccal and vaginal mucosa. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 87(4):452–455.

Vandebriel, RJ; Spiekstra, SW; Hudspith, BN; et al. (1999) In vitro exposure effects of cyclosporin a and bis(tri-n-butyltin)oxide on lymphocyte proliferation, cytokine (receptor) mRNA expression, and cell surface marker expression in rat thymocytes and splenocytes. Toxicology 135(1):49-66.

Van Lipzig, MMH; Vermeulen, NPE; Gusinu, R; et al. (2005) Formation of estrogenic metabolites of benzo[a]pyrene and chrysene by cytochrome P450 activity and their combined and supra-maximal estrogenic activity. Environ Toxicol Pharmacol 19(1):41–55.

van Rooij, JG; Bodelier-Bade, MM; Jongeneelen, FJ. (1993) Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. Br J Ind Med 50(7):623–632.

Van Schooten, FJ; Hillebrand, MJ; Scherer, E; et al. (1991) Immunocytochemical visualization of DNA adducts in mouse tissues and human white blood cells following treatment with benzo[a]pyrene or its diol epoxide. A quantitative approach. Carcinogenesis 12(3):427–433.

Verschueren, K. (2001) Handbook of environmental data on organic chemicals. New York, NY: John Wiley & Sons, Inc; pp. 295-305.

Vesselinovitch, SD; Kyriazis, AP; Mihailovich, N. (1975) Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. Cancer Res 35:2948–2953.

Vesselinovitch, SD, Rao, KVN; Mihailovich, N. (1979) Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. Natl Cancer Inst Monogr 51: 239–250.

Vijayalakshmi, KP; Suresh, CH. (2008) Theoretical studies on the carcinogenicity of polycyclic aromatic hydrocarbons. J Comput Chem 29:1108–1117.

Vineis, P; Perera, F. (2007) Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. Cancer Epidemiol Biomarkers Prev 16(10):1954–1965.

Vineis, P; Anttila, S; Benhamou, S; et al. (2007a) Evidence of gene gene interactions in lung carcinogenesis in a large pooled analysis. Carcinogenesis 28(9):1902–1905.

Vineis, P; Veglia, F; Garte, S; et al. (2007b) Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol 18(7):1230–1242.

Vinggaard, AM; Hnida, C; Larsen, JC. (2000) Environmental polycyclic aromatic hydrocarbons affect androgen receptor activation in vitro. Toxicology 145(2–3):173–183.

Vogel, EW; Zijlstra, JA; Blijleven, WG. (1983) Mutagenic activity of selected aromatic amines and polycyclic hydrocarbons in Drosophila melanogaster. Mutat Res 107(1):53–77.

Vondracek, J; Kozubik, A; Machala, M. (2002) Modulation of estrogen receptor-dependent reporter construct activation and G0/G1-S-phase transition by polycyclic aromatic hydrocarbons in human breast carcinoma MCF-7 cells. Toxicol Sci 70(2):193–201.

Wachsmuth, E. (1983) Evaluating immunopathological effects of new drugs. Immunotoxicology 237-250.

Waidyanatha, S; Zheng, Y; Rappaport, SM. (2003) Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography-mass spectrometry. Chem Biol Interact 145(2):165–174.

Wang, JJ; Frazer, DG; Law, B; et al. (2003) Identification and quantification of urinary benzo[a]pyrene and its metabolites from asphalt fume exposed mice by microflow LC coupled to hybrid quadrupole time-of-flight mass spectrometry. Analyst 128(7):864–870.

Warshawsky, D; Barkley, W. (1987) Comparative carcinogenic potencies of 7H-dibenzo[c,g]carbazole, dibenz[a,j]acridine and benzo[a]pyrene in mouse skin. Cancer Lett 37(3):337–344.

Watanabe, S; Kamiguchi, Y. (2001) Chromosome analysis of human spermatozoa following in vitro exposure to cyclophosphamide, benzo(a)pyrene and N-nitrosodimethylamine in the presence of rat liver S9. Mutat Res 491(1–2):57–63.

Waylen, AL; Metwally, M; Jones, GL; Wilkinson, AJ; Ledger. WL. (2009) Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis. Hum Reprod Update. 15:31-44.

Wei, H; Tye, L; Bresnick, E; et al. (1990). Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer Research 50:499-502.

Wei, Q; Cheng, L; Amos, CI; et al. (2000) Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. J Natl Cancer Inst 92(21):1764–1772.

Wei, C; Cacavale, RJ; Kehoe, JJ; et al. (2001) CYP1A2 is expressed along with CYP1A1 in the human lung. Cancer Lett 164(1):25–32.

Wei, C; Caccavale, RJ; Weyand, EH; et al. (2002) Induction of CYP1A1 and CYP1A2 expressions by prototypic and atypical inducers in the human lung. Cancer Lett 178(1):25–36.

Wester, RC; Maibach, HI; Bucks, DA; et al. (1990) Percutaneous absorption of [14C]DDT and [14C]benzo[a]pyrene from soil. Fundam Appl Toxicol 15(3):510–516.

Weyand, EH; Bevan, DR. (1986) Benzo(a)pyrene disposition and metabolism in rats following intratracheal instillation. Cancer Res 46(11):5655–5661.

Weyand, EH; Bevan, DR. (1987) Species differences in disposition of benzo[a]pyrene. Drug Metab Dispos 15(4):442–448.

Weyand, EH; LaVoie, EJ. (1988) Comparison of PAH DNA adduct formation and tumor initiating activity in newborn mice. Proc Am Assoc Cancer Res 29(10):98.

Weyand, EH; He, ZM; Ghodrati, F; et al. (1992) Effect of fluorine substitution on benzo[j]fluoranthene genotoxicity. Chem Biol Interact 84(1):37–53.

Weyand, EH; Wu, Y; Patel, S; et al. (1994) Biochemical effects of manufactured gas plant residue following ingestion by B6C3F1 mice. J Toxicol Environ Health 42(1):89–107.

Weyand, EH; Chen, YC; Wu, Y; et al. (1995) Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[a]pyrene vs manufactured gas plant residue. Chem Res Toxicol 8(7):949–954.

Whitlock, JP, Jr. (1999) Induction of cytochrome P4501A1. Annu Rev Pharmacol Toxicol 39:103–125.

Whitwell, J; Fowler, P; Allars, S; et al. (2010) 5-Fluorouracil, colchicine, benzo[a]pyrene and cytosine arabinoside tested in the in vitro mammalian cell micronucleus test (MNvit) in Chinese hamster V79 cells at Covance Laboratories, Harrogate, UK in support of OECD draft Test Guideline 487. Mutat Res.

WHO (World Health Organizatino). (1998) Environmental health criteria. Vol. 202. Selected non-heterocyclic polycyclic aromatic hydrocarbons. International Programme on Chemical Safety, Geneva, Switzerland.

Wiersma, DA; Roth, RA. (1983a) Total body clearance of circulating benzo(a)pyrene in conscious rats: effect of pretreatment with 3-methylcholanthrene and the role of liver and lung. J Pharmacol Exp Ther 226(3):661–667.

Wiersma, DA; Roth, RA. (1983b) The prediction of benzo[a]pyrene clearance by rat liver and lung from enzyme kinetic data. Mol Pharmacol 24(2):300–308.

Wijnhoven, SW; Kool, HJ; van Oostrom, CT; et al. (2000) The relationship between benzo[a]pyrene-induced mutagenesis and carcinogenesis in repair-deficient Cockayne syndrome group B mice. Cancer Res 60(20):5681–5687.

Williams, JA; Martin, FL; Muir, GH; et al. (2000) Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. Carcinogenesis 21(9):1683–1689.

Wilson, JS; Holland, LM. (1988) Periodic response difference in mouse epidermis chronically exposed to crude-oils or benzo[a]pyrene: males vs. females. Toxicology 50(1):83–94.

Wislocki, PG; Bagan, ES; Lu, AY; et al. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. Carcinogenesis 7(8):1317–1322.

Withey, JR; Shedden, J; Law, FC; et al. (1993) Distribution of benzo[a]pyrene in pregnant rats following inhalation exposure and a comparison with similar data obtained with pyrene. J Appl Toxicol 13(3):193–202.

Wolff, RK; Griffith, WC; Henderson, RF; et al. (1989) Effects of repeated inhalation exposures to 1-nitropyrene, benzo[a]pyrene, Ga2O3 particles, and SO2 alone and in combinations on particle clearance, bronchoalveolar lavage fluid composition, and histopathology. J Toxicol Environ Health 27(1):123–138.

Wood, AW; Levin, W; Chang, RL; et al. (1980) Mutagenicity and tumor-initiating activity of cyclopenta(c,d)pyrene and structurally related compounds. Cancer Res 40(3):642–649.

Wormhoudt, LW; Commandeur, JN; Vermeulen, NP. (1999) Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit Rev Toxicol 29(1):59–124.

Wormley, DD; Chirwa, S; Nayyar, T; et al. (2004) Inhaled benzo(a)pyrene impairs long-term potentiation in the F1 generation rat dentate gyrus. Cell Mol Biol 50(6):715–721.

Wu, J; Hou, H; Ritz, B; Chen, Y. (2010) Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population. Sci Total Environ 408:2312-2318.

Wu, Y; Kim, SJ; Weyand, EH. (1994) Bioavailability of manufactured gas plant residue (MPG) components in mice following ingestion: comparison of adulterated powder and gel diets. Polycyclic Aromatic Hydrocarbons 7:175–182.

Wu, MT; Simpson, CD; Christiani, DC; et al. (2002) Relationship of exposure to coke-oven emissions and urinary metabolites of benzo(a)pyrene and pyrene in coke-oven workers. Cancer Epidemiol Biomarkers Prev 11(3):311–314.

Wu, J; Ramesh, A; Nayyar, T; et al. (2003) Assessment of metabolites and AhR and CYP1A1 mRNA expression subsequent to prenatal exposure to inhaled benzo(a)pyrene. Int J Dev Neurosci 21(6):333–346.

Wu, X; Roth, JA; Zhao, H; et al. (2005) Cell cycle checkpoints, DNA damage/repair, and lung cancer risk. Cancer Res 65(1):349–357.

Wu, X; Gu, J; Spitz, MR. (2007) Mutagen sensitivity: a genetic predisposition factor for cancer. Cancer Res 67(8):3493–3495.

Wu, Q; Suzuki, JS; Zaha, H; et al. (2008) Differences in gene expression and benzo[a]pyrene-induced DNA adduct formation in the liver of three strains of female mice with identical AhRb2 genotype treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin and/or benzo[a]pyrene. J Appl Toxicol 28:724-733.

Wynder, EL; Hoffmann, D. (1959) A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. Cancer 12:1079–1086.

Wynder, EL; Fritz, L; Furth, N. (1957) Effect of concentration of benzopyrene in skin carcinogenesis. J Natl Cancer Inst 19(3):361–370.

Xu, Z; Brown, LM; Pan, GW; et al. (1996) Cancer risks among iron and steel workers in Anshan, China, Part II: Case-control studies of lung and stomach cancer. Am J Ind Med 30(1):7–15.

Xu, D; Penning, TM; Blair, IA; et al. (2009) Synthesis of phenol and quinone metabolites of benzo[a]pyrene, a carcinogenic component of tobacco smoke implicated in lung cancer. J Org Chem 74(2):597–604.

Xue, W; Warshawsky, D. (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 206(1):73–93.

Yamasaki, H. (1990) Gap junction intercellular communication and carcinogenesis. Carcinogenesis 11(7):1051–1058.

Yang, SK; Roller, PP; Fu, PP; et al. (1977) Evidence for a 2,3-epoxide as an intermediate in the microsomal metabolism of benzo[a]pyrene to 3-hydroxybenzo[a]pyrene. Biochem Biophys Res Commun 77(4):1176–1182.

Yang, JJ; Roy, TA; Krueger, AJ; et al. (1989) In vitro and in vivo percutaneous absorption of benzo[a]pyrene from petroleum crude-fortified soil in the rat. Bull Environ Contam Toxicol 43(2):207–214.

Yang, H; Mazur-Melnyk, M; de Boer, JG; et al. (1999) A comparison of mutational specificity of mutations induced by S9-activated B[a]P and benzo[a]pyrene-7,8-diol-9,10-epoxide at the endogenous aprt gene in CHO cells. Mutat Res 423:23-32.

Yu, D; Berlin, JA; Penning, TM; et al. (2002) Reactive oxygen species generated by PAH o-quinones cause change-in-function mutations in p53. Chem Res Toxicol 15(6):832–842.

Zhao, J; Lahiri-Chatterjee, M; Y Sharma, Y. et al; (2000). Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. Carcinogenesis 21(4): 811-816.

Zeilmaker, MJ; van Eijkeren, JCH. (1997) Modeling Ah-receptor dependent P450 induction I. Cellular model definition and its corporation in a PBPK model of 2,3,7,8-TCDD. RIVM Report 604138 001. National Institute of Public Health and the Environment, B, The Netherlands.

Zeilmaker, MJ; van Eijkeren, JCH; Kroese, ED. (1999a) PBPK simulated DNA adduct formation: relevance for the risk assessment of benzo(a)pyrene. Bilthoven, The Netherlands.

Zeilmaker, MJ; van Eijkeren, JCH; Olling, M. (1999b) A PBPK-model for B(a)P in the rat relating dose and liver DNA-adduct level. Bilthoven, The Netherlands.

Zenzes, MT; Bielecki, R; Reed, T.E. (1999a) Detection of benzo[a]pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. adducts in ovarian cells of women exposed to cigarette smoke. Fertil Steril 1999;72:330-335.

Zenzes, MT; Puy, LA; Bielecki, R; et al. (1999b) Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol Hum Reprod 5(2):125–131.

Zenzes, MT; Puy, LA; Bielecki, R; et al. (1999b) Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol Hum Reprod 5(2):125–131.

Zhang, Y; Ramos, KS. (1997) The induction of proliferative vascular smooth muscle cell phenotypes by benzo[a]pyrene does not involve mutational activation of ras genes. Mutat Res 373(2):285–292.

Zhang, YJ; Hsu, TM; Santella, RM. (1995) Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in oral mucosa cells of smokers and nonsmokers. Cancer Epidemiol Biomarkers Prev 4:133–138.

Zhang, YJ; Weksler, BB; Wang, L; et al. (1998) Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis 140:325–331.

Zheng, Z; Fang, JL; Lazarus, P. (2002) Glucuronidation: an important mechanism for detoxification of benzo[a]pyrene metabolites in aerodigestive tract tissues. Drug Metab Dispos 30(4):397–403.

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

[THIS PAGE INTENTIONALLY LEFT BLANK]

APPENDIX B. BENCHMARK DOSE MODELING RESULTS FOR NONCANCER

Increased liver weight (Kroese et al, 2001) male

Table B-1. Liver weight (±SD)[a] in male F344 rats administered benzo[a]pyrene by gavage for 90 days

Organ	Dose (mg/kg-d)			
	0	**3**	**10**	**30**
Liver weight (g) Males	7.49 ± 0.97	8.00 ± 0.85	8.62 ± 1.30[b]	9.67 ± 1.17[b]

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

Table B-2. BMD modeling results for increased liver weight in male rats, with BMR=10%

Study	Endpoint	Model	AIC	Goodness-of-fit *p* value	BMD	BMDL
Kroese et al., 2001	Liver weight	**Linear (1° polynomial), Power**	**49.51**	**0.58**	**8.11**	**5.8**
		Polynomial (2°)[a]	50.53	0.74	4.53	2.29
		Hill	50.48	0.82	4.1	1.24

[a] In order to consider apparent curvature in the dose-response data, the polynomial coefficients were allowed to be negative; a satisfactory fit was achieved, with monotonically increasing predictions within the observed data range. Since the AIC was higher than for the linear model, the 2-degree polynomial was not selected as the best fit.

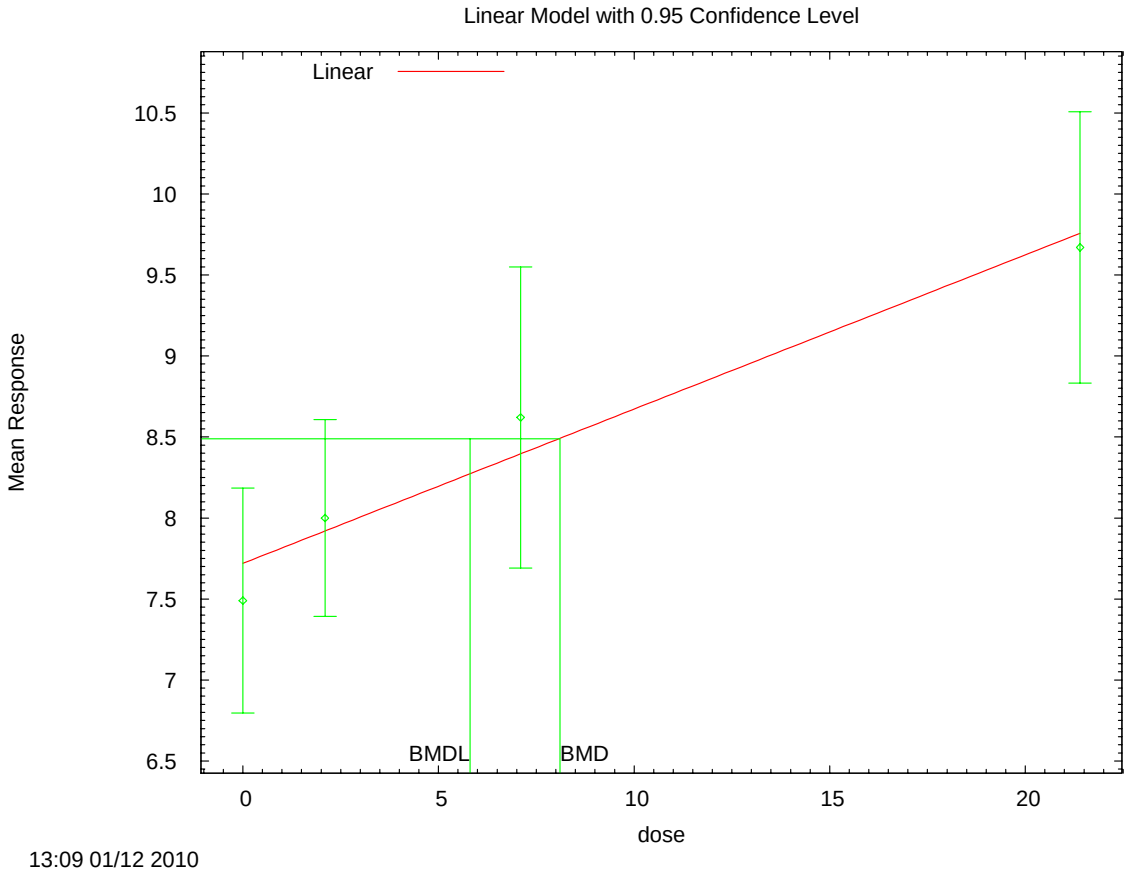


Figure B-1. Fit of polynomial model to data on increased liver weight in male Wistar rats—90 days.

Model output:

```
 ================================================================
         Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
         Input Data File: C:\USEPA\BMDS21\Data\linLiverwtKroeseLinearDefault.(d)
         Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\linLiverwtKroeseLinearDefault.plt
                                                 Tue Jan 12 13:09:58 2010
 ================================================================

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

   The form of the response function is:

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


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



                  Default Initial Parameter Values
                          alpha =      1.18058
```

```
                            rho =            0   Specified
                         beta_0 =      7.71695
                         beta_1 =    0.0951703


           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    -3.6e-011       1e-010

      beta_0    -3.6e-011            1        -0.68

      beta_1       1e-010        -0.68            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
           alpha          1.09179         0.244132            0.613302             1.57028
         beta_0           7.71695         0.224102             7.27772             8.15618
         beta_1         0.0951703        0.0197929           0.0563769            0.133964


      Table of Data and Estimated Values of Interest

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

    0    10       7.49         7.72         0.97         1.04         -0.687
  2.1    10          8         7.92         0.85         1.04          0.252
  7.1    10       8.62         8.39          1.3         1.04          0.688
 21.4    10       9.67         9.75         1.17         1.04         -0.253


 Model Descriptions for likelihoods calculated


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

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

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

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


                         Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -21.212822            5      52.425644
              A2          -20.156688            8      56.313377
              A3          -21.212822            5      52.425644
          fitted          -21.756413            3      49.512826
               R          -30.879511            2      65.759022


                    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              21.4456          6        0.001525
   Test 2              2.11227          3          0.5494
   Test 3              2.11227          3          0.5494
   Test 4              1.08718          2          0.5807

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 =           0.1

Risk Type        =     Relative risk

Confidence level =          0.95

              BMD =        8.10857


             BMDL =        5.80436
```

Decreased thymus weight (males) Kroese et al., 2001

Table B-3. Means ± SD[a] for thymus weight in male Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 90 days

Organ	Dose (mg/kg-d)			
	0	**3**	**10**	**30**
Thymus weight (mg) Males	380 ± 60	380 ± 110	330 ± 60	270 ± 40[b]

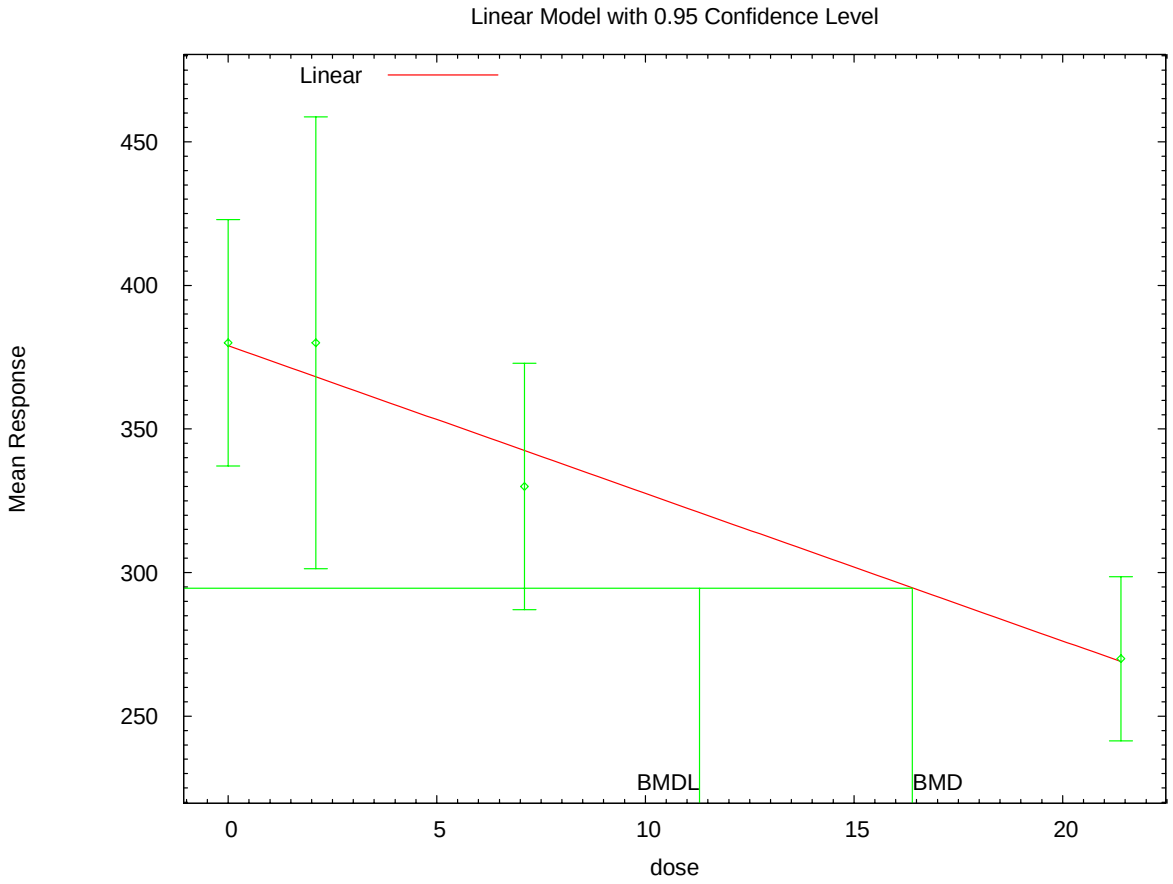
[a] Reported as SE, but judged to be SD (and confirmed by study authors).
[b]Significantly ($p < 0.05$) different from control mean; student t-test (unpaired, two-tailed); n = 10/sex/group.

Source: Kroese et al. (2001).

Table B-4. Model predictions for decreased thymus weight in male Wistar rats—90 days

Model	Variance *p*-value[a]	Goodness-of-fit *p*-value	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Constant variance					
Linear	0.01	0.74	384.84	12.97	8.97
Nonconstant variance					
Hill[c]	NA				
Linear, Polynomial (2-degree), Power[c]	**0.30**	**0.23**	**380.71**	**16.40**	**11.30**

NA = not applicable, model failed;



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

Source: Kroese et al. (2001).

Figure B-2. Fit of linear model (nonconstant variance) to data on decreased thymus weight in male Wistar rats—90 days.

Linear (nonconstant variance)

```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2
Linkrolin.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\90day\thymusweight\male\durationadjusted\2
Linkrolin.plt
                                          Thu Oct 15 15:33:37 2009
 ================================================================

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

   The form of the response function is:

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

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

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



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


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho       beta_0       beta_1

    lalpha            1           -1        0.048       -0.061

       rho           -1            1       -0.048        0.061

    beta_0        0.048       -0.048            1        -0.84

    beta_1       -0.061        0.061        -0.84            1


                                Parameter Estimates

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


     Table of Data and Estimated Values of Interest

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

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


 Model Descriptions for likelihoods calculated
```

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

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

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

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


                      Likelihoods of Interest

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


                   Explanation of Tests

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

                     Tests of Interest

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

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

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

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

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

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


              Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =         0.95
```

```
1
2                BMD =        16.4008
3
4
5               BMDL =        11.2965
6
7
8
9
10
11
12
```

Decreased thymus weight females (Kroese et al., 2001)

Table B-5. Means ± SD[a] for thymus weight in female Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 90 days

Organ	Dose (mg/kg-d)			
	0	3	10	30
Thymus weight (mg) Females	320 ± 60	310 ± 50	300 ± 40	230 ± 30[b]

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

Source: Kroese et al. (2001).

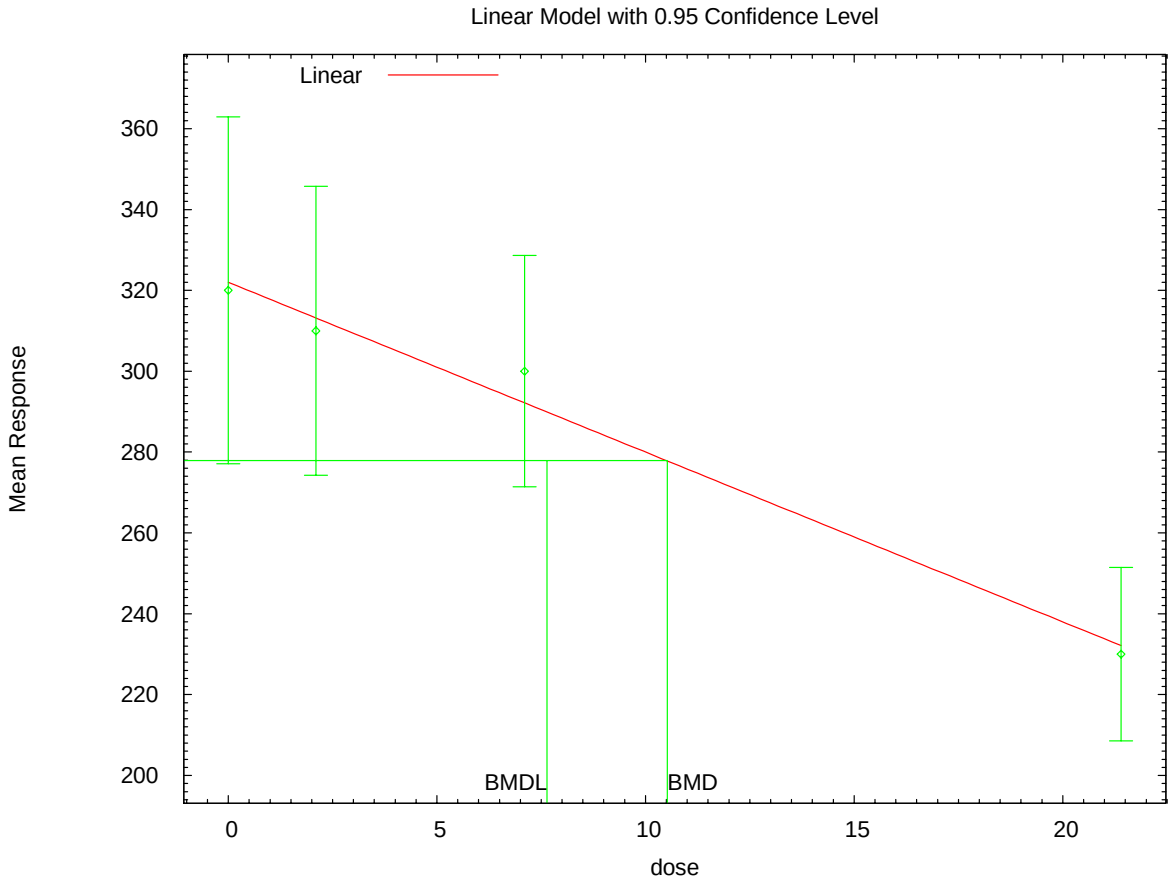
Table B-6. Model predictions for decreased thymus weight in female Wistar rats—90 days

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

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

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

Source: Kroese et al. (2001).



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

Source: Kroese et al. (2001).

Figure B-3. Fit of linear model (constant variance) to data on decreased thymus weight in female Wistar rats—90 days.

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

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

   The form of the response function is:

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


   Dependent variable = mean
```

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

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



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


           Asymptotic Correlation Matrix of Parameter Estimates

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

                   alpha       beta_0       beta_1

     alpha             1     2.4e-008    -2.3e-008

    beta_0     2.4e-008            1        -0.68

    beta_1    -2.3e-008        -0.68            1



                                Parameter Estimates

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


     Table of Data and Estimated Values of Interest

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

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


 Model Descriptions for likelihoods calculated

```

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

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

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

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


                        Likelihoods of Interest

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


                    Explanation of Tests

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

                      Tests of Interest

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

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

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

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


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

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


              Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =         0.95
```

```
1
2                BMD =        10.5228
3
4
5               BMDL =        7.64037
6
7
8
```

Decreased thymus weight males (DeJong et al., 1999)

Table B-7. Means ± SD for thymus weight in male Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 35 days

Organ	Dose (mg/kg-d)				
	0	**3**	**10**	**30**	**90**
Thymus weight (mg) (means; n = 7–8)	517 ± 47	472 ± 90	438 ± 64[a]	388 ± 71[a]	198 ± 65[a]

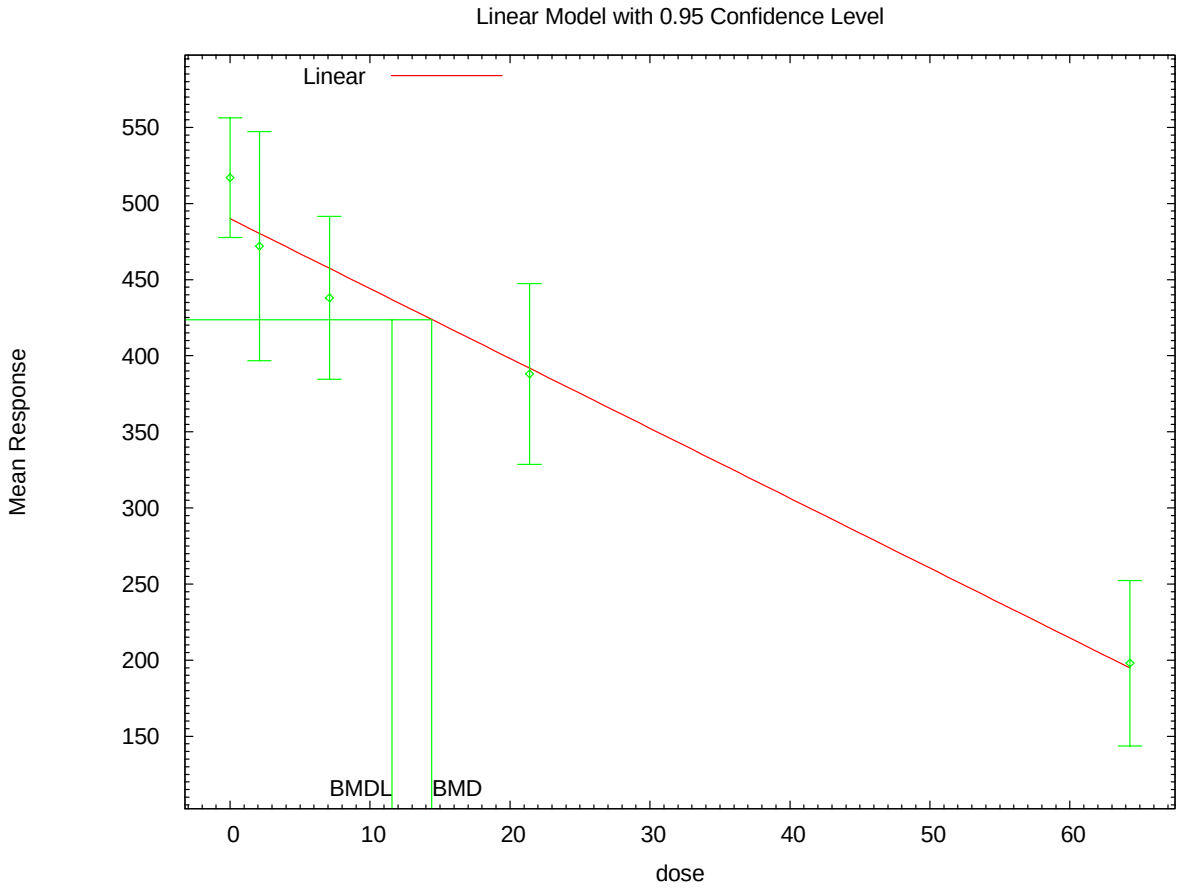
Table B-8. Model predictions for decreased thymus weight in male Wistar rats—35 days

Model (constant variance)	Variance *p*-value[b]	Means *p*-value[b]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Hill[c]	0.50	0.42	382.91	11.15	6.19
Linear[d], Polynomial (2-degree)[d,e]	**0.50**	**0.52**	**381.41**	**14.41**	**11.58**
Power[d]	NA				

[a]Number of animals was reported to be 7–8 per dose group, and was not specified for each group; forBMD modeling purposes, n = 8 was used.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Coefficients restricted to be negative.
[e]Lowest degree polynomial with an adequate fit is reported.

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

Source: De Jong et al. (1999).



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

Source: De Jong et al. (1999).

Figure B-4. Fit of linear model (constant variance) to data on decreased thymus weight in male Wistar rats—35 days.

Model Output:

```
================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\dejong1999\35day\thymusweightmale\durationadjusted\2L
indejlin.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\dejong1999\35day\thymusweightmale\durationadjusted\2L
indejlin.plt
                                          Mon Oct 19 04:31:24 2009
 ================================================================

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

   The form of the response function is:

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

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

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =            1
                            rho =            0   Specified
                         beta_0 =      489.769
                         beta_1 =      -4.5927


           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    -3.1e-009    -3.2e-009

    beta_0    -3.1e-009            1        -0.62

    beta_1    -3.2e-009        -0.62            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha           4382.3          979.911             2461.71
6302.89
         beta_0          489.769          13.3751             463.555
515.984
         beta_1          -4.5927         0.438716            -5.45257            -
3.73283


     Table of Data and Estimated Values of Interest

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

    0     8        517          490           47         66.2           1.16
  2.1     8        472          480           90         66.2         -0.347
  7.1     8        438          457           64         66.2         -0.819
 21.4     8        388          391           71         66.2         -0.149
 64.3     8        198          194           65         66.2          0.151
```

```
 Model Descriptions for likelihoods calculated


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

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

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

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


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1         -186.580733            6     385.161466
              A2         -184.896632           10     389.793264
              A3         -186.580733            6     385.161466
          fitted         -187.706565            3     381.413130
               R         -214.086904            2     432.173809


                    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              58.3805          8          <.0001
   Test 2               3.3682          4          0.4982
   Test 3               3.3682          4          0.4982
   Test 4              2.25166          3          0.5218

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 =        14.4139


             BMDL =         11.577

```

Decreased Splenic B cells

Table B-9. Exposure-related effects in male Wistar rats exposed to benzo[a]pyrene by gavage 5 days/week for 5 weeks

Effect	Dose (mg/kg-d)				
	0	**3**	**10**	**30**	**90**
Spleen cell distribution (%)					
B cells	39± 4	36 ± 2	34 ± 3[a]	32 ± 4[a]	23 ± 4[a]
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

[a]Significantly ($p < 0.05$) different from control mean.

Source: De Jong et al. (1999).

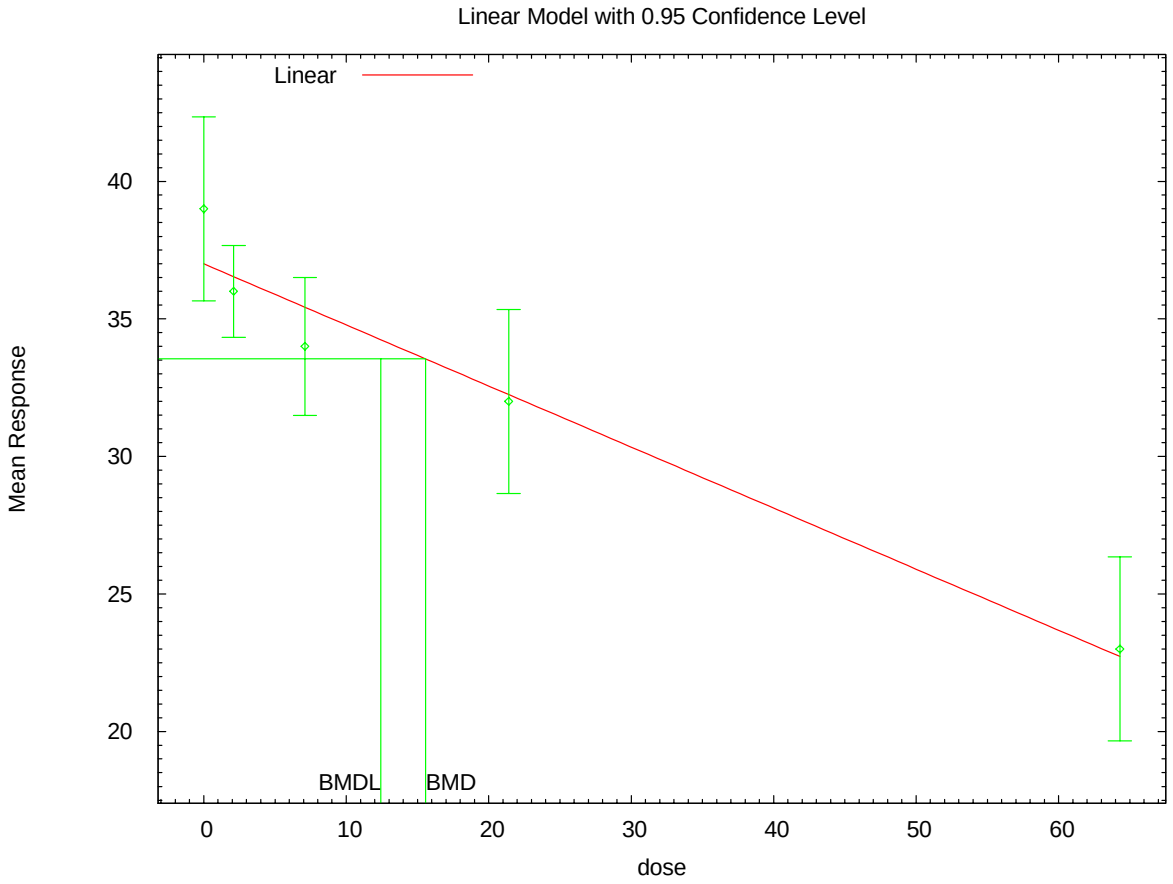
Table B-10. Model predictions for decreased spleen B-cells in male Wistar rats—35 days

Model	Variance *p*-value[a]	Means *p*-value[a]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Constant variance					
Hill[b]	0.30	0.18	146.18	10.24	5.31
Linear[c], Polynomial (2-degree)[c,d], Power[b]	**0.30**	**0.21**	**145.28**	**15.58**	**12.43**

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

BMD/BMC = maximum likelihood estimate of the dose/concentration associated with the selected BMR; NA = not applicable; model failed to generate these values

Source: De Jong et al. (1999).



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

Source: De Jong et al. (1999).

Figure B-5. Fit of linear model to data on decreased spleen B-cells in male Wistar rats—35 days.

Model Output:

```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\dejong1999\35day\spleenBcell\durationadjusted\2Lindej
lin.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\dejong1999\35day\spleenBcell\durationadjusted\2Lindej
lin.plt
                                                Mon Oct 19 05:06:33 2009
 ================================================================

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

   The form of the response function is:

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

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

   Total number of dose groups = 5
   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 =         12.2
                            rho =            0   Specified
                         beta_0 =      37.0148
                         beta_1 =    -0.222068


           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-009     3.8e-009

    beta_0    -2.4e-009            1        -0.62

    beta_1     3.8e-009        -0.62            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          11.9647          2.6754             6.72106
17.2084
         beta_0          37.0148         0.698873             35.6451
38.3846
         beta_1        -0.222068        0.0229237           -0.266997           -
0.177138


     Table of Data and Estimated Values of Interest

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

    0     8         39           37            4         3.46           1.62
  2.1     8         36         36.5            2         3.46         -0.449
  7.1     8         34         35.4            3         3.46          -1.18
 21.4     8         32         32.3            4         3.46         -0.215
 64.3     8         23         22.7            4         3.46          0.216


```

```
 Model Descriptions for likelihoods calculated


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

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

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

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


                       Likelihoods of Interest

             Model      Log(likelihood)   # Param's      AIC
              A1          -67.358091            6     146.716182
              A2          -64.934513           10     149.869025
              A3          -67.358091            6     146.716182
          fitted          -69.639287            3     145.278575
               R          -93.795081            2     191.590163


                    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              57.7211          8          <.0001
   Test 2              4.84716          4          0.3033
   Test 3              4.84716          4          0.3033
   Test 4              4.56239          3          0.2068

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 =        15.5764


             BMDL =        12.4286
```

Forestomach hyperplasia

All available dichotomous models in the EPA BMDS (version 2.1) were fit to the incidence data shown in Table B-9 for forestomach hyperplasia in rats and mice orally exposed to benzo[a]pyrene for 2 years (Kroese et al., 2001; Beland and Culp, 1998). In accordance with U.S. EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated for all models.

Adequate model fit was judged by three criteria: goodness-of-fit *p*-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all of the models providing adequate fit to the data, the lowest BMDL is selected as the POD when the difference between the BMDLs estimated from these models are more than threefold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) is chosen. If an adequate fit to the data was not achieved using the protocol above for the full dataset, doses were dropped (starting with the highest dose) until an adequate fit was achieved.

Table B-11. Dose-response data for forestomach hyperplasia in Wistar rats and B6C3F$_1$ rats orally exposed to benzo[a]pyrene for 2 years

	Administered dose (mg/kg-day)			
	0	**3**	**10**	**30**
	Duration-adjusted dose (× 5/7)			
Species, sex (reference)	**0**	**2.1**	**7.14**	**21.4**
Wistar rat, female (Kroese et al., 2001)	1/52	8/51	13/51	2/52
Wistar rat, male (Kroese et al., 2001)	2/50	8/52	8/52	0/52
	Administered dose (mg/kg-day)			
	0	**0.7**	**3.3**	**16.5**
B6C3F$_1$ mice, female (Beland and Culp, 1998)	13/48	23/47	33/46	46/47

All data sets provided adequate descriptions of the dose-response relationship for forestomach hyperplasia from chronic oral exposure to benzo[a]pyrene, but at the highest dose level in rats, the incidence of hyperplasia was not increased. It is possible that the forestomach hyperplasia observed following benzo[a]pyrene exposure may be a precursor to the development of forestomach tumors, but specific data supporting this conclusion are unavailable. Regardless, the male and female data sets in rats (Kroese et al., 2001) were modeled without the data from the highest dose group due to the nonmontonic increase in response to increasing dose (Kroese et al., 2001).

Table B-12. Summary of BMDs and BMDLs from the best fitting model forestomach hyperplasia—oral exposure

Endpoint/data	Strain/species	Exposure duration	Best fitting model	BMD (mg/kg-d)	BMDL (mg/kg-d)	Reference
Forestomach hyperplasia (highest dose excluded)[a]	Wistar rat (male)	2 yrs	Log-logistic	5.31	2.39	Kroese et al., 2001
Forestomach hyperplasia (highest dose excluded)[a]	Wistar rat (female)	2 yrs	Log-logistic	2.15	1.35	Kroese et al., 2001
Forestomach hyperplasia	B6C3F$_1$ mouse (female)	2 yrs	Log-logistic	0.33	0.12	Beland and Culp, 1998

[a]Data for the high-dose group were excluded in the modeled datset due to the absence of an increase in incidence at the high dose.

Forestomach hyperplasia- Male Wistar Rats, 2 yrs (Kroese et al., 2001)

Table B-13. Model predictions for forestomach hyperplasia in male Wistar rats in a 2-year study

Model	Degrees of freedom	χ^2	χ^2 Goodness-of-fit *p*-value[a]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Highest dose excluded[b]						
Gamma[c], Multistage[d], Weibull[c]	1	2.39	0.12	112.37	5.63	2.67
Logistic	1	2.83	0.09	112.93	7.25	4.35
LogLogistic	**1**	**2.28**	**0.13**	**112.27**	**5.31**	**2.39**
LogProbit	1	3.64	0.06	113.88	8.36	4.52
Probit	1	2.78	0.10	112.87	7.09	4.13

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b] Data for the high-dose group were excluded in the modeled datset due to the absence of an increase in incidence at the high dose; likely related to the statistically significantly increased incidence of forestomach tumors in these animals.
[c]Power restricted to ≥1.
[d]Betas restricted to ≥0; lowest degree polynomial with an adequate fit is reported (1-degree polynomial).

Source: Kroese et al. (2001).

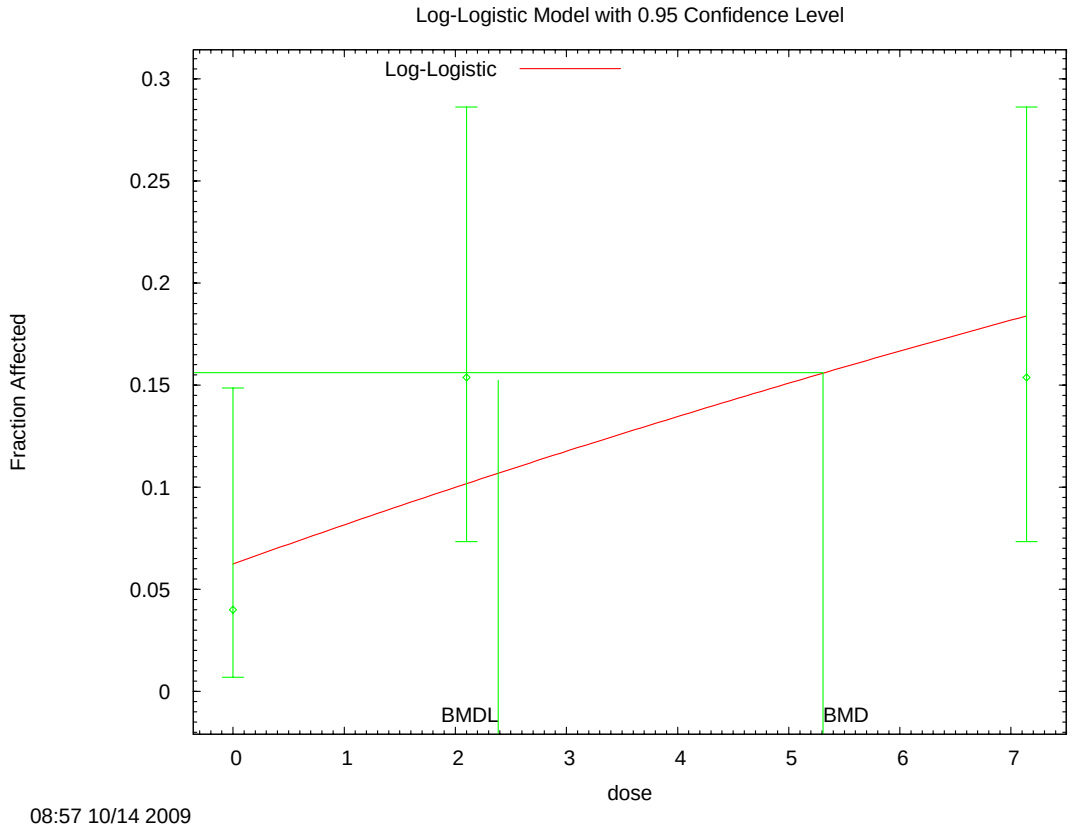


Figure B-6. Fit of log logistic model to data on forestomach hyperplasia in male Wistar rats in a 2-year study.

Model output:

```
 ===============================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\chronic\forestomachhyperplasia\male\durati
onadjusted\3Logkrolog.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\chronic\forestomachhyperplasia\male\durati
onadjusted\3Logkrolog.plt
                                          Tue Oct 13 21:42:15 2009
 ===============================================================

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

   The form of the probability function is:

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


   Dependent variable = incidence
   Independent variable = dose
   Slope parameter is restricted as slope >= 1
```

```
   Total number of observations = 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



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =         0.04
                     intercept =     -3.27842
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

             background    intercept

background            1         -0.7

 intercept         -0.7            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background        0.0623861            *                *                 *
      intercept         -3.86644            *                *                 *
          slope                1            *                *                 *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -53.0468         3
   Fitted model        -54.1335         2       2.17337      1          0.1404
  Reduced model        -55.5429         1       4.99229      2          0.0824

           AIC:         112.267


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
    0.0000     0.0624         3.119     2.000          50       -0.654
    2.1000     0.1019         5.297     8.000          52        1.239
    7.1400     0.1843         9.584     8.000          52       -0.566

 Chi^2 = 2.28      d.f. = 1        P-value = 0.1306
```

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =          5.308

             BMDL =        2.38692
```

Forestomach hyperplasia- Female Wistar Rats, 2 yrs (Kroese et al., 2001)

Table B-14. Model predictions for forestomach hyperplasia in female Wistar rats in a 2-year study

Model	Degrees of freedom	χ^2	χ^2 Goodness-of-fit *p*-value[a]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Highest dose excluded[b]						
Logistic	1	3.68	0.06	120.02	4.23	3.28
LogLogistic	**1**	**0.98**	**0.32**	**117.04**	**2.15**	**1.35**
LogProbit	1	5.09	0.02	121.13	3.91	2.57
Gamma[c], Multistage[d], Weibull[c]	1	1.40	0.24	117.42	2.40	1.59
Probit	1	3.47	0.06	119.74	3.99	3.06

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Data for the high-dose group were excluded in the modeled datset due to the absence of an increase in incidence at the high dose; likely related to the statistically significantly increased incidence of forestomach tumors in these animals.
[c]Power restricted to ≥1.
[d]Betas restricted to ≥0; lowest degree polynomial with an adequate fit is reported (1-degree polynomial).

BMD = maximum likelihood estimate of the dose/concentration associated with the selected BMR

Source: Kroese et al. (2001).

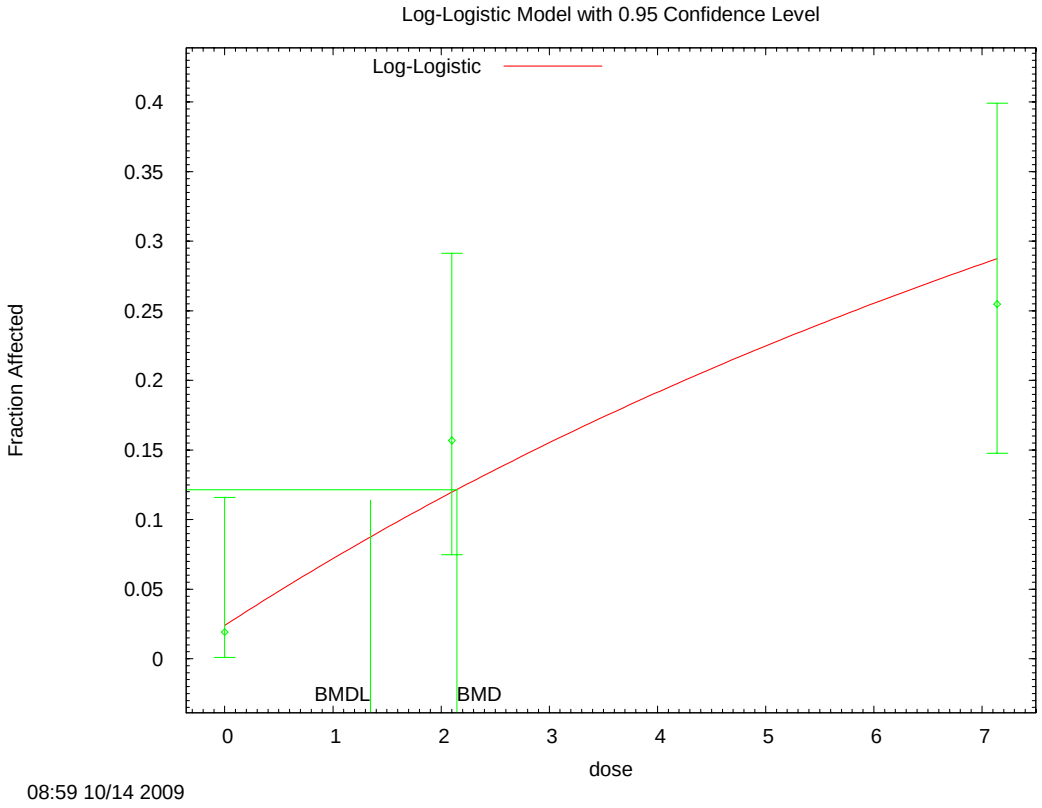


Figure B-7. Fit of log logistic model to data on forestomach hyperplasia in female Wistar rats in a 2-year study.

Model output:

```
================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\chronic\forestomachhyperplasia\female\dura
tionadjusted\3Logkrolog.(d)
         Gnuplot Plotting File:
C:\USEPA\IRIS\benzo[a]pyrene\RfD\Kroese2001\chronic\forestomachhyperplasia\female\dura
tionadjusted\3Logkrolog.plt
                                          Wed Oct 14 08:59:23 2009
 ================================================================

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

   The form of the probability function is:

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


   Dependent variable = incidence
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 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
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.0192308
intercept = -2.7983
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.46
intercept	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0238694	*	*	*
intercept	-2.96044	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-56.048	3			
Fitted model	-56.5181	2	0.940072	1	0.3323
Reduced model	-63.1579	1	14.2198	2	0.000817

AIC: 117.036

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0239	1.241	1.000	52	-0.219
2.1000	0.1196	6.101	8.000	51	0.819
7.1400	0.2874	14.658	13.000	51	-0.513

Chi^2 = 0.98 d.f. = 1 P-value = 0.3216

Benchmark Dose Computation

Specified effect = 0.1

```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.14515

            BMDL =        1.34776
```

Forestomach hyperplasia- female mice, 2 yrs (Beland and Culp, 1998)

Table B-15. Model predictions for forestomach hyperplasia in female B6C3F1 mice in a 2-year study

Model	Degrees of freedom	χ^2	χ^2 Goodness-of-fit *p*-value[a]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Logistic	2	5.71	0.06	194.7	0.757	0.545
LogLogistic	**2**	**1.55**	**0.21**	**193.3**	**0.329**	**0.115**
LogProbit	2	2.49	0.29	192.1	0.670	0.448
Multistage[c], Weibull[b], Gamma[b]	2	1.74	0.42	191.3	0.421	0.295
Probit	2	7.04	0.03	196.6	0.946	0.711

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0; lowest degree polynomial with an adequate fit is reported (1-degree polynomial).

BMD = maximum likelihood estimate of the dose/concentration associated with the selected BMR
Source: Beland and Culp (1998).

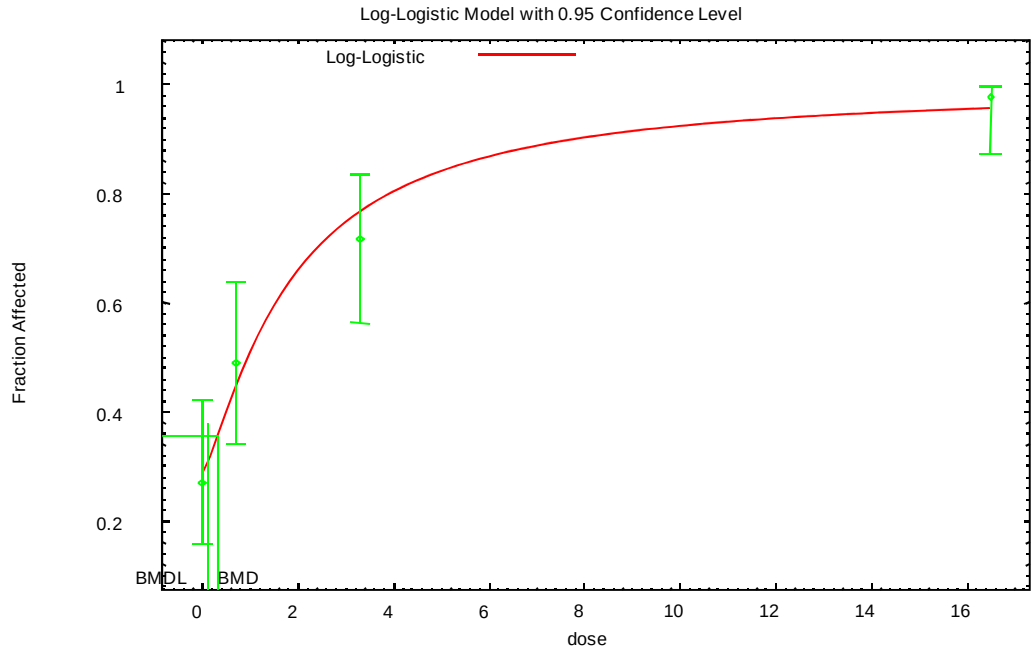


Figure B-8. Fit of log logistic model to data on forestomach hyperplasia in female B6C3F$_1$ mice in a 2-year study.

```
Model output:

 ================================================================
         Logistic Model. (Version: 2.12; Date: 05/16/2008)
         Input Data File:
C:\Usepa\BMDS21\Data\lnl_benzo[a]pyrene_BelandCulp_mice_4s_hyperplasia_generic_dich_10
.(d)
         Gnuplot Plotting File:
C:\Usepa\BMDS21\Data\lnl_benzo[a]pyrene_BelandCulp_mice_4s_hyperplasia_generic_dich_10
.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 = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 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 = 0.270833
intercept = -0.637972
slope = 1.38091

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.66	0.46
intercept	-0.66	1	-0.8
slope	0.46	-0.8	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.286381	*	*	*
intercept	-0.789676	*	*	*
slope	1.26641	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.8312	4			
Fitted model	-93.6497	3	1.63686	1	0.2008
Reduced model	-125.58	1	65.4982	3	<.0001

AIC: 193.299

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2864	13.746	13.000	48	-0.238
0.7000	0.4464	20.979	23.000	47	0.593
3.3000	0.7667	35.270	33.000	46	-0.791
16.5000	0.9575	45.005	46.000	47	0.720

Chi^2 = 1.55 d.f. = 1 P-value = 0.2127

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.329083

```
1                BMDL =       0.115446
```

Decreased ovary weight- female rats, 60 days (Xu et al., 2010)

Table B-16. Means ± SDa for ovary weight in female SD-rats

Organ	Dose (mg/kg-d)[a]		
	0	**2.5**	**5**
Ovary weight (mg)	0.160 ± 0.0146	0.143 ± 0.0098[b]	0.136 ± 0.0098[b]

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

Table B-17. Model predictions for decreased ovary weight in female SD-rats—60 days

Model	Goodness-of-fit *p*-value	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Power	N/A			
Linear, Polynomial (1°)	**0.39**	**-138.67**	**2.27**	**1.49**

NA = not applicable, model failed;

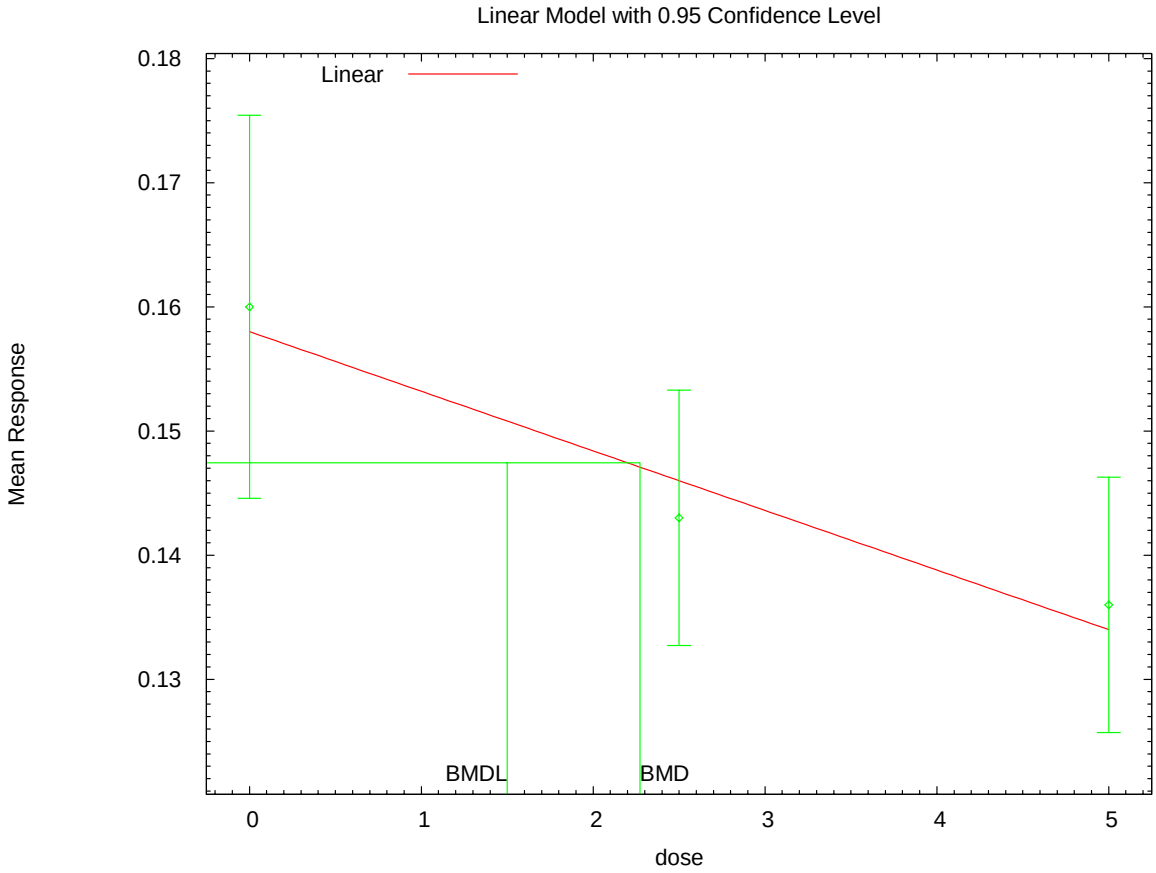


Figure B-9. Fit of linear/polynomial (1°) model to data on decreased ovary weight

```
Model Output:

========================================================================
         Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
         Input Data File:
C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1
SD.(d)
         Gnuplot Plotting File:
C:/USEPA/BMDS212/Data/benzo[a]pyrene/Bap_AbsOvaryWeight/Xu2010_AbsOvaryWeight_Linear_1
SD.plt
                                                Tue Dec 14 13:51:32 2010
 ========================================================================
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

    The form of the response function is:

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


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

    Total number of dose groups = 3
```

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



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


            Asymptotic Correlation Matrix of Parameter Estimates

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

                 alpha       beta_0       beta_1

     alpha           1       4e-010    -4.5e-010

    beta_0       4e-010            1        -0.77

    beta_1    -4.5e-010        -0.77            1


                               Parameter Estimates

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


      Table of Data and Estimated Values of Interest

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

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


 Model Descriptions for likelihoods calculated


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

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

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

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

Likelihoods of Interest

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

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	12.9201	4	0.01167
Test 2	1.40394	2	0.4956
Test 3	1.40394	2	0.4956
Test 4	0.861408	1	0.3533

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

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

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

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2.27159

BMDL = 1.49968

APPENDIX C. ADDITIONAL CALCULATIONS FOR THE RfC

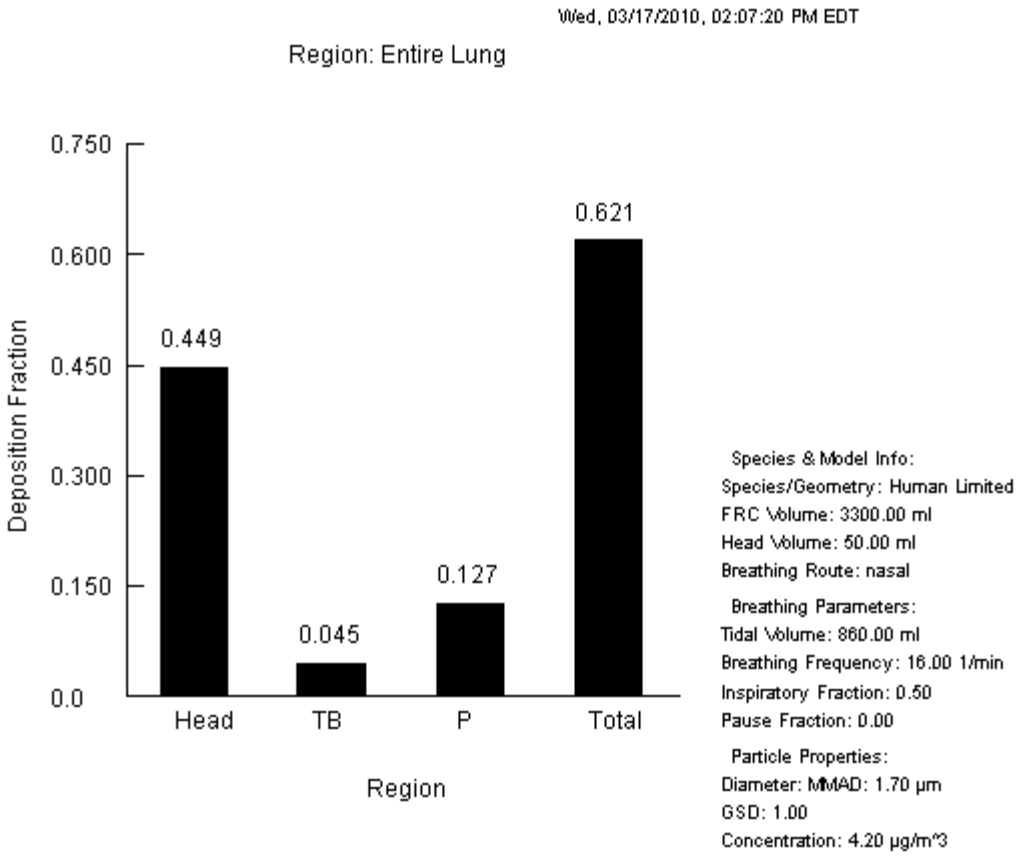


Figure C-1. Human Fractional Deposition

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

Region: Entire Lung
Region: Entire Lung
Region   Deposition Fraction
```

```
--    --
Head  0.449
TB    0.045
 P    0.127
Total 0.621
```

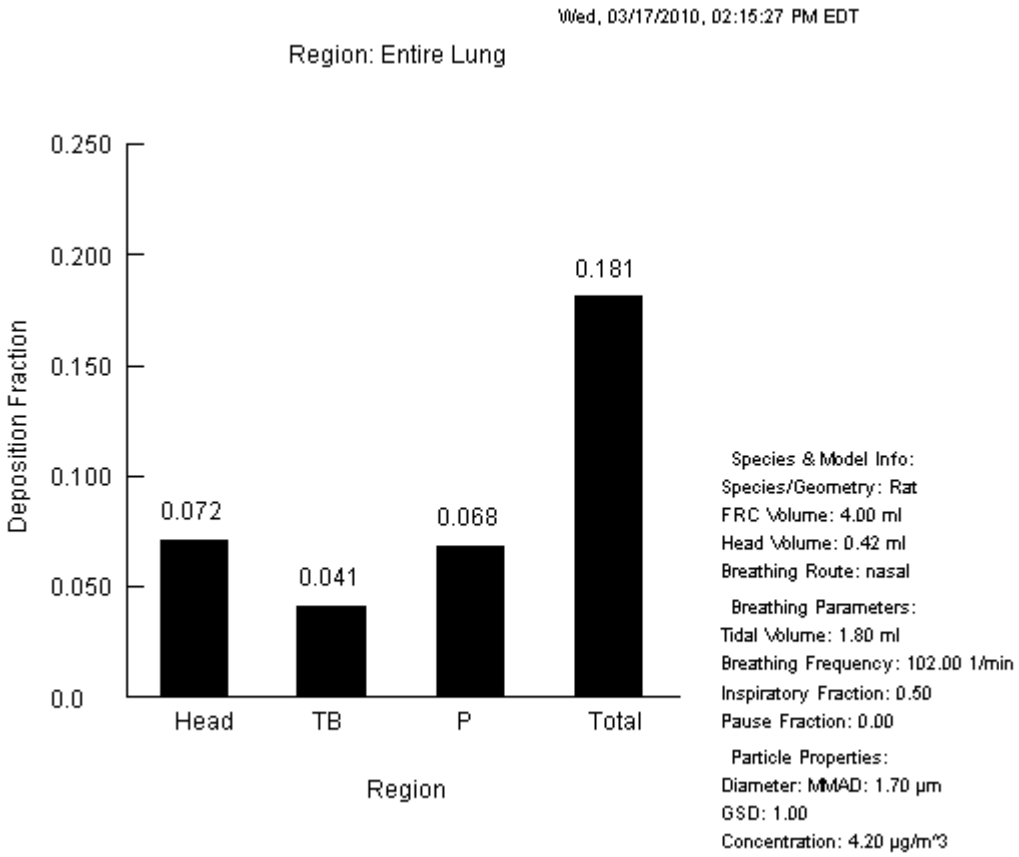


Figure C-2. Rat Fractional Deposition

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

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

APPENDIX D. TIME-TO-TUMOR MODELING FOR THE ORAL SLOPE FACTOR

Table D-1. Tumor incidence data, with time to death with tumor; male rats exposed by gavage to Benzo[a]pyrene (Kroese et al., 2001)

Dose Group (mg/kg-day)	Week of Death	Total Examined	Numbers of Animals with							
			Oral Cavity or Forestomach Tumors		Liver Tumors		Duodenum or Jejunum Tumors	Skin or Mammary Gland		Kidney Urothelial Carcinoma
								Basal Cell Tumors	Squamous Cell Tumors	
			Incid.[a]	Fatal[a]	Incid.	Fatal	Incid.	Incid.	Incid.	Incid.
0	44	1	0	0	0	0	0	1	0	0
	80	1	0	0	0	0	0	0	0	0
	82	1	0	0	0	0	0	0	0	0
	84	1	0	0	0	0	0	0	0	0
	89	1	0	0	0	0	0	0	0	0
	90	3	0	0	0	0	0	0	0	0
	91	1	0	0	0	0	0	0	0	0
	92	1	0	0	0	0	0	0	0	0
	93	1	0	0	0	0	0	0	0	0
	94	1	0	0	0	0	0	0	0	0
	95	2	0	0	0	0	0	0	0	0
	96	2	0	0	0	0	0	0	0	0
	97	1	0	0	0	0	0	0	0	0
	98	1	0	0	0	0	0	0	0	0
	100	3	0	0	0	0	0	1	0	0
	104	1	0	0	0	0	0	0	0	0
	105	1	0	0	0	0	0	0	0	0
	108	7	0	0	0	0	0	0	0	0
	109	22	0	0	0	0	0	0	0	0
3	29	1	0	0	0	0	0	0	0	0
	40	1	1	0	0	0	0	0	0	0
	74	1	0	0	0	0	0	0	0	0
	76	1	0	0	0	0	0	0	0	0
	79	1	0	0	0	0	0	0	0	0
	82	1	0	0	0	0	0	0	0	0
	92	2	0	0	0	0	0	0	0	0
	93	1	0	0	0	0	0	0	0	0
	94	1	0	0	0	0	0	0	0	0
	95	2	0	0	0	0	0	0	0	0
	98	1	0	0	0	0	0	0	0	0
	107	10	4	0	1	0	0	0	0	0
	108	15	2	0	3	0	0	1	1	0
	109	14	1	0	0	0	0	0	0	0
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

Table D-1. Tumor incidence data, with time to death with tumor; male rats exposed by gavage to Benzo[a]pyrene (Kroese et al., 2001)

Dose Group (mg/kg-day)	Week of Death	Total Examined	Numbers of Animals with							
			Oral Cavity or Forestomach Tumors		Liver Tumors		Duodenum or Jejunum Tumors	Skin or Mammary Gland		Kidney Urothelial Carcinoma
								Basal Cell Tumors	Squamous Cell Tumors	
			Incid.[a]	Fatal[a]	Incid.	Fatal	Incid.	Incid.	Incid.	Incid.
30	32	1	1	0	0	0	0	0	0	0
	35	1	1	0	1	0	0	0	0	0
	37	1	1	0	0	0	0	0	0	0
	44	1	0	1	1	0	0	0	0	0
	45	2	2	0	2	0	0	0	0	0
	47	1	1	0	1	0	0	0	0	0
	48	1	1	0	1	0	0	0	0	0
	49	1	1	0	1	0	0	0	0	0
	50	1	1	0	1	0	0	0	0	0
	51	1	1	0	1	0	1	0	0	0
	52	4	3	1	3	1	0	1	1	0
	53	1	1	0	1	0	0	1	0	0
	56	2	1	1	1	1	0	0	0	0
	58	2	2	0	2	0	0	1	0	0
	59	2	2	0	2	0	0	0	0	0
	60	2	1	1	1	1	1	0	0	0
	61	3	2	1	1	2	1	0	0	0
	62	5	5	0	0	4	3	0	0	0
	63	5	5	0	4	1	1	2	1	2
	64	2	2	0	1	1	0	0	0	1
	65	3	2	1	1	2	0	3	2	0
	66	1	1	0	0	1	0	0	0	0
	67	3	1	2	2	1	1	1	1	0
	68	1	1	0	1	0	0	0	0	0
	70	2	2	0	1	1	1	1	0	0
	71	1	1	0	1	0	0	1	1	0
	73	1	0	1	1	0	0	1	0	0
	76	1	1	0	0	1	0	1	0	0

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

Table D-2. Tumor incidence data, with time to death with tumor; female rats exposed by gavage to Benzo[a]pyrene (Kroese et al., 2001)

Dose Group (mg/kg-day)	Week of Death	Total Examined	Numbers of Animals with				
			Oral Cavity or Forestomach Tumors		Liver Tumors		Duodenum or Jejunum Tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental
0	64	1	0	0	0	0	0
	69	1	0	0	0	0	0
	75	1	0	0	0	0	0
	104	1	0	0	0	0	0
	106	4	0	0	0	0	0
	107	7	0	0	0	0	0
	108	7	0	0	0	0	0
	109	30	1	0	0	0	0
3	8	1	0	0	0	0	0
	47	1	0	0	0	0	0
	52	1	0	0	0	0	0
	60	1	0	0	0	0	0
	65	1	0	0	0	0	0
	76	1	0	0	0	0	0
	77	1	0	0	0	0	0
	83	2	0	0	0	0	0
	85	1	0	0	0	0	0
	86	1	0	0	0	0	0
	88	1	0	0	0	0	0
	93	2	0	0	0	0	0
	94	1	0	0	0	0	0
	97	1	1	0	0	0	0
	107	6	2	0	1	0	0
	108	9	2	0	0	0	0
	109	21	1	0	0	0	0
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

Table D-2. Tumor incidence data, with time to death with tumor; female rats exposed by gavage to Benzo[a]pyrene (Kroese et al., 2001)

Dose Group (mg/kg-day)	Week of Death	Total Examined	Numbers of Animals with				
			Oral Cavity or Forestomach Tumors		Liver Tumors		Duodenum or Jejunum Tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal	Incidental
30	26	1	0	0	0	0	0
	44	4	4	0	3	1	0
	47	3	3	0	2	1	0
	48	1	1	0	0	1	0
	54	1	0	0	1	0	0
	55	3	3	0	1	2	0
	56	2	2	0	0	2	0
	57	2	2	0	2	0	0
	58	4	3	1	0	4	0
	59	2	1	1	0	2	0
	60	1	0	1	1	0	0
	61	2	2	0	0	2	0
	62	2	2	0	1	1	0
	63	3	3	0	0	3	0
	64	5	5	0	0	5	3
	66	3	3	0	0	3	0
	67	2	1	1	0	2	0
	68	1	1	0	0	1	0
	69	4	3	1	1	3	1
	71	4	3	1	1	3	0
	72	2	1	1	0	2	0

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

Table D-3. Tumor incidence data, with time to death with tumor; female mice exposed to Benzo[a]pyrene via diet (Beland and Culp, 1998)

Dose Group (ppm in diet)	Week of Death	Total Examined	Number of Animals With Alimentary Tract squamous cell tumors	
			Fatal[a]	Incidental
0	31	1	0	0
	74	1	0	0
	89	2	0	0
	91	1	0	0
	93	2	0	0
	94	2	0	0
	97	2	0	0
	98	2	0	0
	99	1	0	0
	100	2	0	0
	101	2	0	0
	104	1	0	0
	105	29	0	1
5	25	1	0	0
	55	1	0	0
	83	1	0	0
	86	1	0	0
	87	2	0	0
	88	2	0	0
	90	1	0	0
	94	1	0	0
	95	2	0	0
	96	1	0	0
	97	2	0	0
	98	2	0	0
	101	2	0	0
	102	2	0	0
	105	27	0	3
25	44	1	1	0
	47	1	0	0
	64	1	0	0
	70	1	1	0
	77	1	1	0
	80	1	0	0
	81	1	1	0
	84	2	1	1
	85	1	1	0
	86	1	1	0
	88	1	1	0
	89	1	0	0
	90	4	4	0
	93	3	2	1
	94	2	2	0
	96	3	0	2
	97	1	1	0
	98	1	1	0
	99	2	1	1
	100	1	1	0
	101	1	0	0
	102	2	2	0
	104	1	1	0
	105	13	0	10

Table D-3. Tumor incidence data, with time to death with tumor; female mice exposed to Benzo[a]pyrene via diet (Beland and Culp, 1998)

Dose Group (ppm in diet)	Week of Death	Total Examined	Number of Animals With Alimentary Tract squamous cell tumors	
			Fatal[a]	Incidental
100	39	1	1	0
	40	1	1	0
	42	1	1	0
	47	2	2	0
	49	1	0	0
	50	1	1	0
	53	1	0	0
	55	3	3	0
	56	1	1	0
	57	1	1	0
	58	1	1	0
	59	3	3	0
	60	1	1	0
	61	3	3	0
	62	5	5	0
	63	4	4	0
	64	3	3	0
	65	2	2	0
	66	3	3	0
	68	1	1	0
	69	2	2	0
	70	2	2	0
	71	1	1	0
	72	1	1	0
	73	1	1	0
	74	1	1	0
	79	1	1	0

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

Table D-4. Derivation of HEDs to use for BMD modeling of Wistar rat tumor incidence data from Kroese et al. (2001)

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

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

Table D-5. Derivation of HEDs for BMD modeling of B6C3F1 female mouse tumor incidence data from Beland and Culp (1998)

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

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

Table D-6. Summary of Model Selection and Modeling Results for best-fitting multistage-Weibull models, using time-to-tumor data for rats (Kroese et al., 2001)

Sex	EndPoints	Model stages	LL[a]	χ^{2} [b]	Number of param.	AIC	BMD_{10}	Responses at mg/kg-d levels[c] 0	0.54	1.8	5.2	Selected model parameter estimates c	t0	Model Selection Rationale
Males	Oral Cavity and							*0*	*8*	*45*	*52*			
	Forestomach:	1	-284.891	NR	4	577.8	0.104	0.0	21.1	39.8	38.7	2.2	44	
	Squam. Cell	2	-198.795	172.2	5	407.6	0.678	0.0	3.3	25.4	48.0	1.3	44	
	Tumors	**3**	**-108.512**	**180.6**	**6**	**229.0**	**0.453**	**0.0**	**6.8**	**41.7**	**50.0**	**3.7**	**41**	**Lowest AIC, best fit to low dose data**
	Hepatocellular							*0*	*4*	*38*	*49*			
	Tumors	1	-179.664	NR	4	367.3	0.181	0	13.6	32.1	42	1	52	
		2	-145.749	67.8	5	301.5	0.472	0.0	6.5	37.2	49	2.3	48.4	
		3	**-138.544**	**14.4**	**6**	**289.1**	**0.651**	**0.0**	**3.4**	**36.8**	**49.6**	**3.5**	**40.2**	**Lowest AIC, best fit to low dose data**
	Duodenum and							*0*	*0*	*1*	*9*			
	Jejunum	1	-31.781	NR	3	69.6	2.64	0.0	1.1	3.4	5.7	1	NR	
	Tumors	2	-28.941	5.7	4	65.9	3.04	0.0	0.2	1.8	8.2	1	NR	
		3	**-28.439**	**1.0**	**5**	**66.9**	**3.03**	**0.0**	**0.0**	**1.0**	**9.0**	**1.8**	**NR**	**Best fit to data**
	Kidney:							*0*	*0*	*0*	*3*			
	Uroethelial	1	-12.956	NR	3	31.9	9.16	0.0	0.3	1	1.7	1	NR	
	Carcinoma	2	-11.837	2.2	4	31.7	5.71	0.0	0.1	0.5	2.5	1	NR	
		3	**-11.398**	**0.9**	**5**	**32.8**	**4.65**	**0.0**	**0.0**	**0.3**	**2.7**	**1.7**	**NR**	**Best fit to data**
	Skin and							*2*	*1*	*1*	*13*			
	Mammary	1	-52.314	NR	3	110.6	1.88	1.0	2.5	5.5	8.3	1	NR	
	Gland: Basal	2	-48.570	7.5	4	105.1	2.58	1.1	1.3	3.4	11.4	1	NR	
	Cell Tumors	**3**	**-47.362**	**2.4**	**5**	**104.7**	**2.86**	**1.2**	**1.2**	**2.3**	**12.5**	**1.4**	**NR**	**Lowest AIC, best fit to low dose data**
	Skin and							*0*	*1*	*1*	*6*			
	Mammary	**1**	**-28.745**	**NR**	**3**	**63.5**	**3.36**	**0.0**	**0.9**	**2.7**	**4.5**	**1**	**NR**	**Lowest AIC, best fit to low dose data**
	Gland: Squam.	2	-28.145	1.2	4	64.3	2.75	0.0	0.4	2.4	5.2	1.9	NR	
	Cell Tumors	3	-27.652	1.0	5	65.3	2.64	0.0	0.4	2.1	5.5	3.0	NR	
Females	Oral Cavity and							*1*	*6*	*30*	*50*			
	Forestomach:	1	-134.532	NR	4	277.1	0.245	1.0	10.1	23.7	35.1	1	58	
	Squam. Cell	2	-100.809	67.4	5	211.6	0.428	0.8	6.8	33.2	47.9	2.5	52	
	Tumors	3	**-94.512**	**12.6**	**6**	**201.0**	**0.539**	**1.1**	**4.9**	**31.8**	**49.4**	**3.5**	**47**	**Lowest AIC, best fit to low dose data**
	Hepatocellular							*0*	*1*	*39*	*51*			
	Tumors	1	-293.771	NR	4	595.5	0.146	0.0	14.6	32.6	43.8	1	44	
		2	-382.470	177.4	5	774.9	0.370	0.0	8.1	38.5	50.1	2.2	44	
		3	**-228.170**	**308.6**	**6**	**468.3**	**0.575**	**0.0**	**3.0**	**38.4**	**51.4**	**3.1**	**39**	**Lowest AIC, best fit to low dose data**
	Duodenum and							*0*	*0*	*0*	*4*			
	Jejunum	1	-15.948	NR	3	37.9	6.00	0.0	0.4	1.3	2.4	1	NR	
	Tumors	2	-14.518	2.9	4	37.0	4.33	0.0	0.0	0.7	3.3	1.1	NR	
		3	**-13.878**	**1.3**	**5**	**37.8**	**3.43**	**0.0**	**0.0**	**0.4**	**3.6**	**2.3**	**NR**	**Best fit to low dose data**

[a] LL=log-likelihood.

[b] χ^{2} = chi-squared statistic for testing the difference between 2 model fits, from $2 \times |(LL_i - LL_j)|$ evaluated for i-j degrees of freedom. In all cases the difference was evaluated for consecutive numbers of stages; i-j = 1, and χ^{2} at $\alpha = 0.05$ is 3.84.

[c] **"**Responses" describes the number of animals with each tumor type; observed responses are in italics, and expected responses (predicted by each model fit) are given to one decimal place for comparison with the observed data.

Table D-6. Summary of Model Selection and Modeling Results for best-fitting multistage-Weibull models, using time-to-tumor data for rats (Kroese et al., 2001)

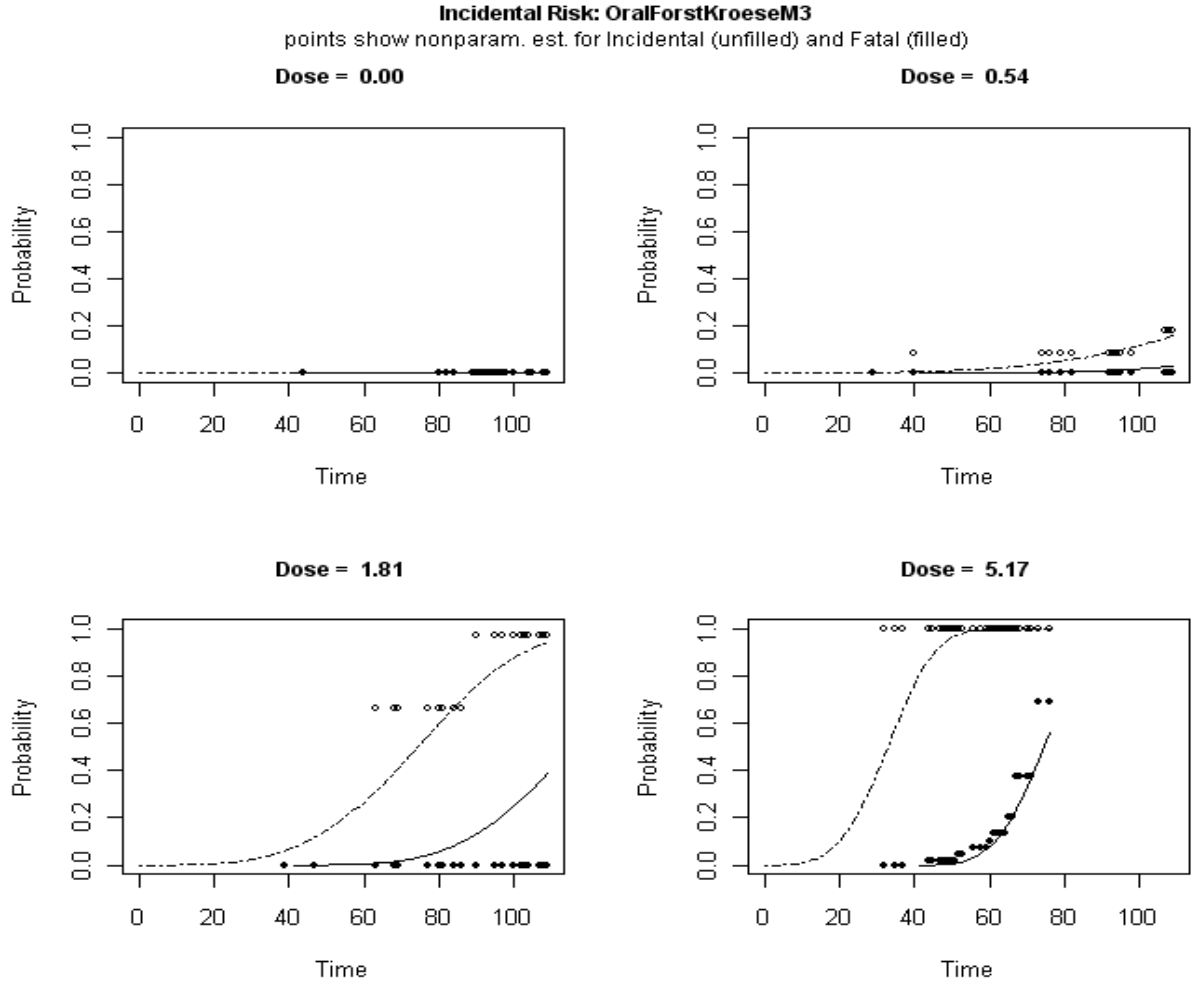
Sex	EndPoints	Model stages	LL[a]	χ^2 [b]	Number of param.	AIC	BMD_{10}	Responses at mg/kg-d levels[c] 0	0.54	1.8	5.2	Selected model parameter estimates c	t0	Model Selection Rationale
Males	Oral Cavity and							*0*	*8*	*45*	*52*			
	Forestomach:	1	-284.891	NR	4	577.8	0.104	0.0	21.1	39.8	38.7	2.2	44	
	Squam. Cell	2	-198.795	172.2	5	407.6	0.678	0.0	3.3	25.4	48.0	1.3	44	
	Tumors	**3**	**-108.512**	**180.6**	**6**	**229.0**	**0.453**	**0.0**	**6.8**	**41.7**	**50.0**	**3.7**	**41**	**Lowest AIC, best fit to low dose data**
	Hepatocellular							*0*	*4*	*38*	*49*			
	Tumors	1	-179.664	NR	4	367.3	0.181	0	13.6	32.1	42	1	52	
		2	-145.749	67.8	5	301.5	0.472	0.0	6.5	37.2	49	2.3	48.4	
		3	**-138.544**	**14.4**	**6**	**289.1**	**0.651**	**0.0**	**3.4**	**36.8**	**49.6**	**3.5**	**40.2**	**Lowest AIC, best fit to low dose data**
	Duodenum and							*0*	*0*	*1*	*9*			
	Jejunum	1	-31.781	NR	3	69.6	2.64	0.0	1.1	3.4	5.7	1	NR	
	Tumors	2	-28.941	5.7	4	65.9	3.04	0.0	0.2	1.8	8.2	1	NR	
		3	**-28.439**	**1.0**	**5**	**66.9**	**3.03**	**0.0**	**0.0**	**1.0**	**9.0**	**1.8**	**NR**	**Best fit to data**
	Kidney:							*0*	*0*	*0*	*3*			
	Uroethelial	1	-12.956	NR	3	31.9	9.16	0.0	0.3	1	1.7	1	NR	
	Carcinoma	2	-11.837	2.2	4	31.7	5.71	0.0	0.1	0.5	2.5	1	NR	
		3	**-11.398**	**0.9**	**5**	**32.8**	**4.65**	**0.0**	**0.0**	**0.3**	**2.7**	**1.7**	**NR**	**Best fit to data**
	Skin and							*2*	*1*	*1*	*13*			
	Mammary	1	-52.314	NR	3	110.6	1.88	1.0	2.5	5.5	8.3	1	NR	
	Gland: Basal	2	-48.570	7.5	4	105.1	2.58	1.1	1.3	3.4	11.4	1	NR	
	Cell Tumors	**3**	**-47.362**	**2.4**	**5**	**104.7**	**2.86**	**1.2**	**1.2**	**2.3**	**12.5**	**1.4**	**NR**	**Lowest AIC, best fit to low dose data**
	Skin and							*0*	*1*	*1*	*6*			
	Mammary	**1**	**-28.745**	**NR**	**3**	**63.5**	**3.36**	**0.0**	**0.9**	**2.7**	**4.5**	**1**	**NR**	**Lowest AIC, best fit to low dose data**
	Gland: Squam.	2	-28.145	1.2	4	64.3	2.75	0.0	0.4	2.4	5.2	1.9	NR	
	Cell Tumors	3	-27.652	1.0	5	65.3	2.64	0.0	0.4	2.1	5.5	3.0	NR	
Females	Oral Cavity and							*1*	*6*	*30*	*50*			
	Forestomach:	1	-134.532	NR	4	277.1	0.245	1.0	10.1	23.7	35.1	1	58	
	Squam. Cell	2	-100.809	67.4	5	211.6	0.428	0.8	6.8	33.2	47.9	2.5	52	
	Tumors	3	**-94.512**	**12.6**	**6**	**201.0**	**0.539**	**1.1**	**4.9**	**31.8**	**49.4**	**3.5**	**47**	**Lowest AIC, best fit to low dose data**
	Hepatocellular							*0*	*1*	*39*	*51*			
	Tumors	1	-293.771	NR	4	595.5	0.146	0.0	14.6	32.6	43.8	1	44	
		2	-382.470	177.4	5	774.9	0.370	0.0	8.1	38.5	50.1	2.2	44	
		3	**-228.170**	**308.6**	**6**	**468.3**	**0.575**	**0.0**	**3.0**	**38.4**	**51.4**	**3.1**	**39**	**Lowest AIC, best fit to low dose data**
	Duodenum and							*0*	*0*	*0*	*4*			
	Jejunum	1	-15.948	NR	3	37.9	6.00	0.0	0.4	1.3	2.4	1	NR	
	Tumors	2	-14.518	2.9	4	37.0	4.33	0.0	0.0	0.7	3.3	1.1	NR	
		3	**-13.878**	**1.3**	**5**	**37.8**	**3.43**	**0.0**	**0.0**	**0.4**	**3.6**	**2.3**	**NR**	**Best fit to low dose data**

NR = not relevant.

Male Rat (Kroese et al., 2001): Squamous Cell Papilloma or Carcinoma in Oral Cavity or Forestomach

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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

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


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


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

                 c            t_0          beta_1       beta_3

    c                 1        -0.53        -0.93        -0.99

    t_0            -0.53            1         0.47         0.57

    beta_1         -0.93         0.47            1          0.9

    beta_3         -0.99         0.57          0.9            1


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

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


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


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

    Minimum observation time for F tumor context =         44
```

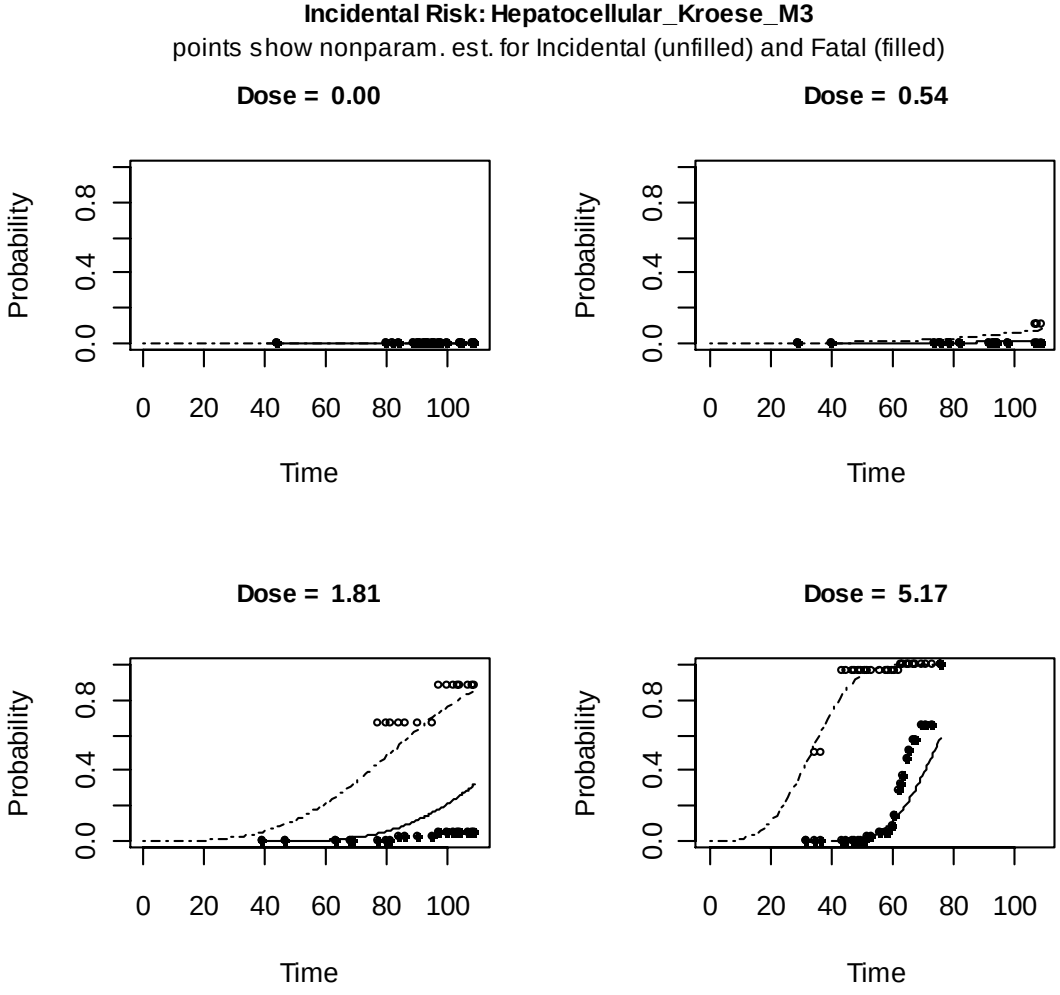
```
   Benchmark Dose Computation
Risk Response     =     Incidental
Risk Type         =          Extra
Confidence level =            0.9
Time              =            104


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



Male Rat (Kroese et al., 2001): Hepatocellular Adenoma or Carcinoma

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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


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


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


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

                 c            t_0          beta_1       beta_3

    c                 1        -0.84        -0.88           -1

    t_0           -0.84            1         0.71         0.86

    beta_1        -0.88         0.71            1         0.86

    beta_3           -1         0.86         0.86            1


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

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


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


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

    Minimum observation time for F tumor context =         52
```

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


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

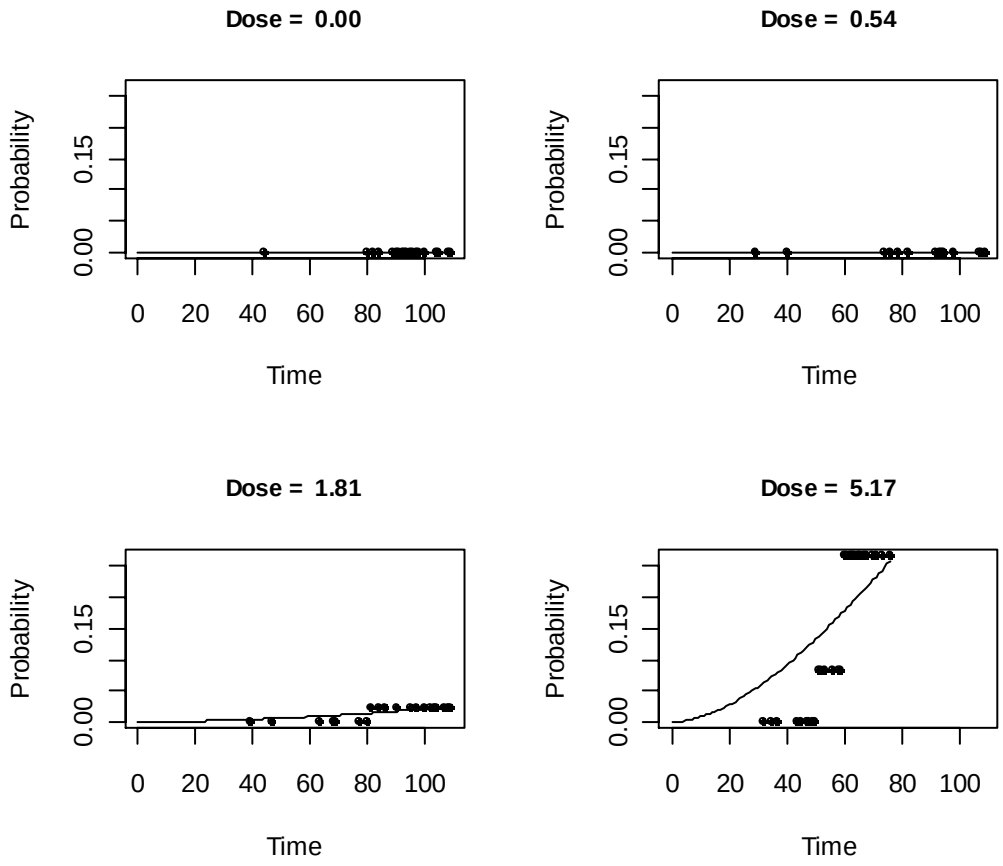


Male Rat (Kroese et al., 2001): Duodenum or Jejunum Adenocarcinoma

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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



   User specifies the following parameters:
          t_0    =          0

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


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


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

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


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

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


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


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


   Benchmark Dose Computation
Risk Response    =     Incidental
```

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


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

Incidental Risk: DuoJej_Kroese_M3



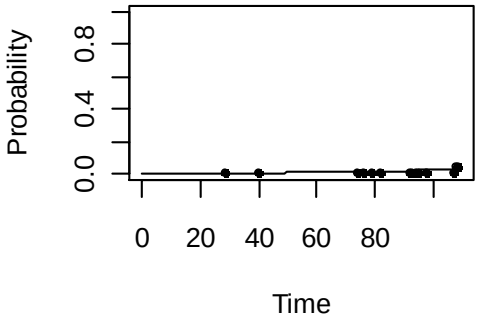
Male Rat (Kroese et al., 2001): Skin or Mammary Gland Basal Cell Tumors

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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



   User specifies the following parameters:
          t_0    =          0

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


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


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

                 c            beta_0       beta_3

    c                1           -1           -1

    beta_0          -1            1         0.99

    beta_3          -1         0.99            1


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

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


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


                    Data Summary
                       CONTEXT
               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
```

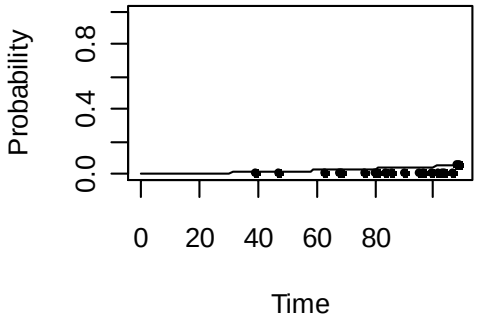
```
      Benchmark Dose Computation
  Risk Response    =     Incidental
  Risk Type        =          Extra
  Confidence level =            0.9
  Time             =            104


Specified effect =   0.1           0.01          0.001
             BMD =   2.86276       1.30804       0.606222
            BMDL =   2.35118       0.415897      0.0424277
            BMDU =   3.62258       1.69571       0.761447
```

Incidental Risk: Skin_Mam_Basal_Kroese_M3

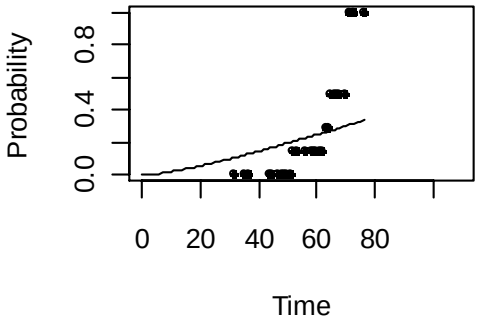
Dose = 0.54



Dose = 1.81



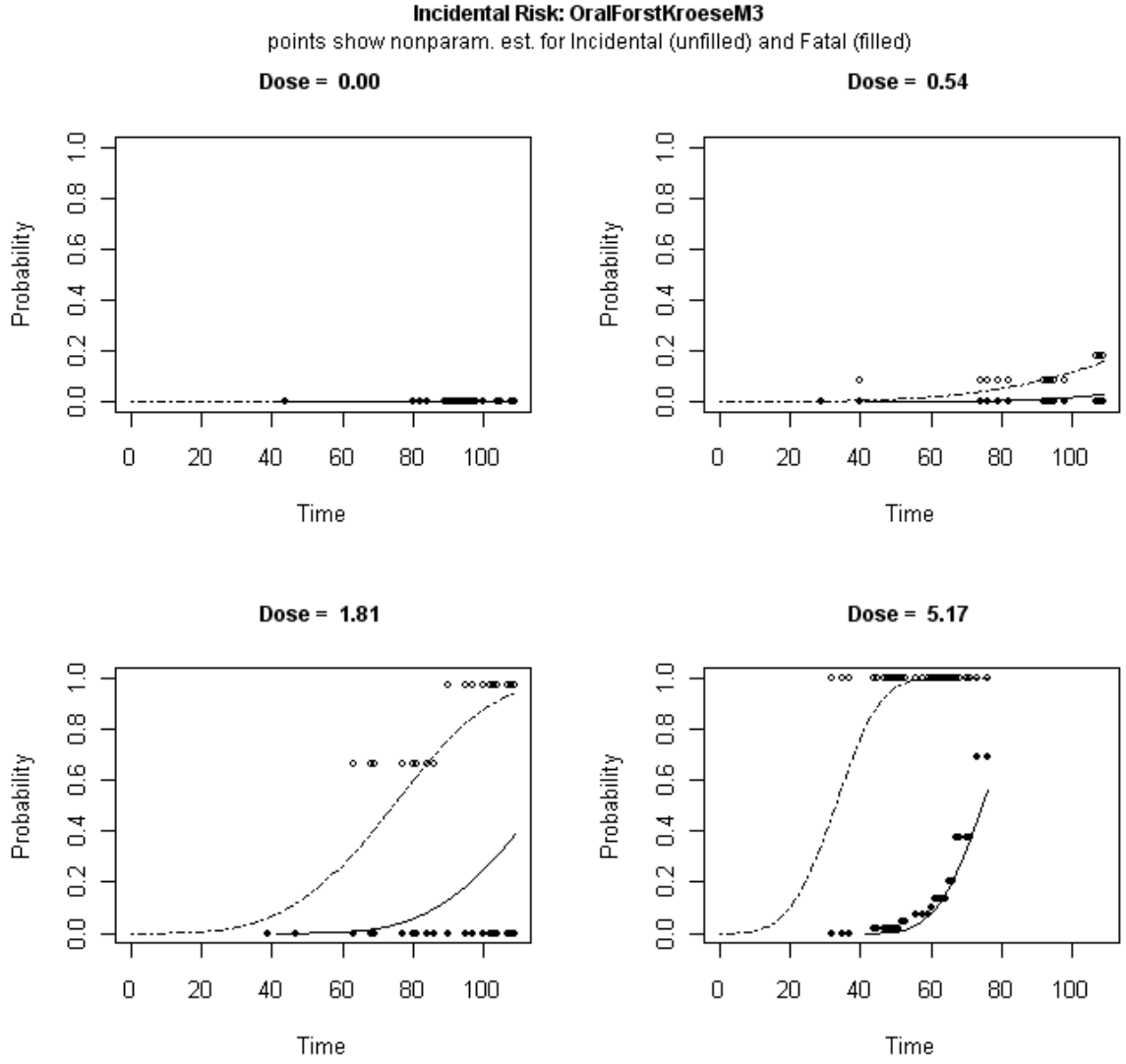
Dose = 5.17



Male Rat (Kroese et al., 2001): Skin or Mammary Gland Squamous Cell Tumors

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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



   User specifies the following parameters:
          t_0    =          0

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


                  Default Initial Parameter Values
                        c      =            3
                        t_0    =            0   Specified
                        beta_0 =            0
                        beta_1 = 1.25256e-008
                        beta_2 = 1.25627e-030
                        beta_3 = 3.34696e-009


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

                 c            beta_1       beta_3

    c                 1        -0.99           -1

    beta_1        -0.99            1         0.99
    beta_3           -1         0.99            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               2.96213            2.591           -2.11613             8.04039
         beta_0                0               NA
         beta_1     1.50104e-008     1.86972e-007       -3.51447e-007        3.81468e-007
         beta_2                0               NA
         beta_3      3.9084e-009     4.15374e-008       -7.75033e-008        8.53201e-008

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


                Log(likelihood)   # Param            AIC
   Fitted Model         -27.652         5          65.304


                    Data Summary
                        CONTEXT
               C      F      I      U  Total  Expected Response
    DOSE
       0      52      0      0      0     52     0.00
     0.54     51      0      1      0     52     0.42
      1.8     51      0      1      0     52     2.12
      5.2     46      0      6      0     52     5.51


   Benchmark Dose Computation
 Risk Response    =     Incidental
```

```
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


 Specified effect =   0.1           0.01          0.001
              BMD =   2.6414        0.64109       0.070558
            BMDL =   1.76931       0.211043      0.0210552
            BMDU =   4.42145       2.03605       > 0.564463
```

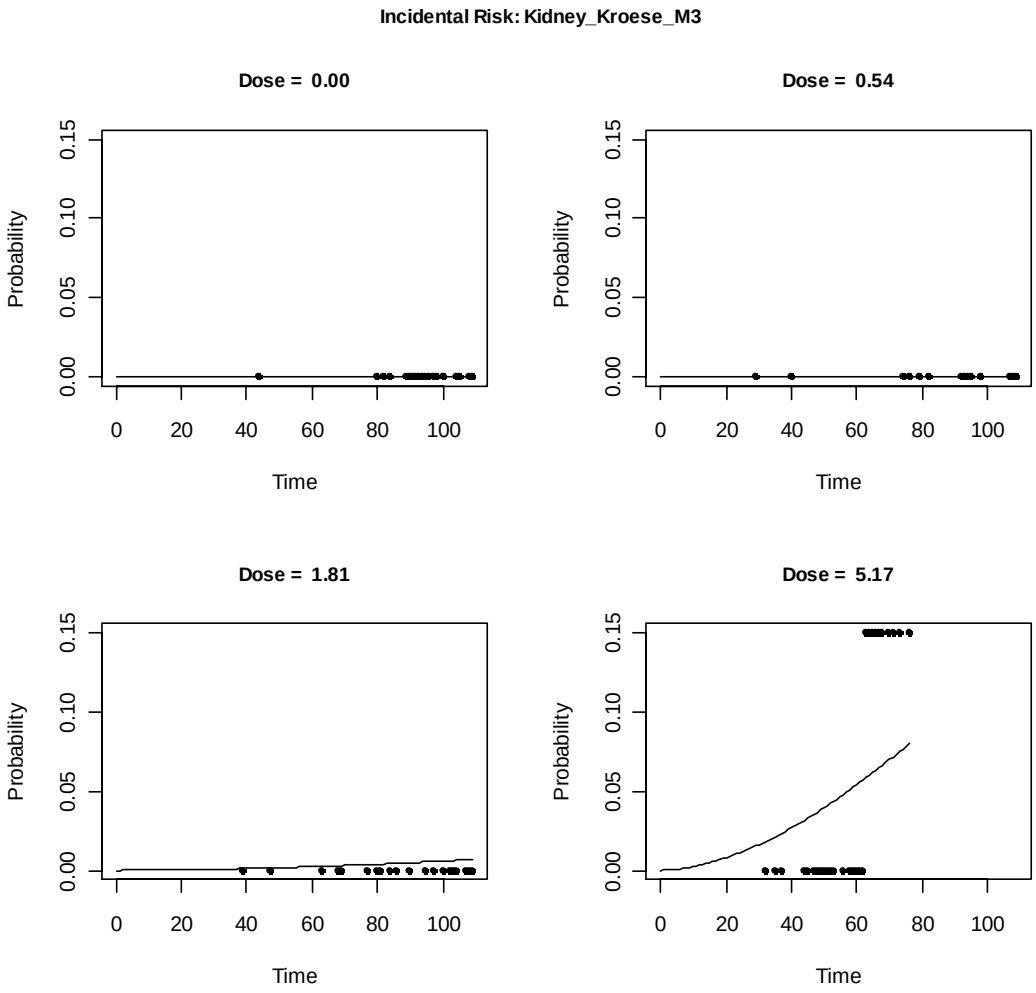


Dose Response plot

Male Rat (Kroese et al., 2001): Kidney Urothelial Carcinomas

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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



   User specifies the following parameters:
          t_0    =          0

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


                  Default Initial Parameter Values
                        c      =      1.63636
                        t_0    =            0   Specified
                        beta_0 = 3.78734e-027
                        beta_1 = 1.59278e-027
                        beta_2 =   2.718e-024
                        beta_3 = 4.96063e-007


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

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               1.74897          3.79403           -5.68719            9.18512
         beta_0                0               NA
         beta_1                0               NA
         beta_2                0               NA
         beta_3     3.11107e-007     4.90313e-006       -9.29885e-006        9.92107e-006

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


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


                   Data Summary
                       CONTEXT
              C      F      I      U  Total  Expected Response
    DOSE
        0     52      0      0      0     52     0.00
      0.54    52      0      0      0     52     0.01
       1.8    52      0      0      0     52     0.29
       5.2    49      0      3      0     52     2.71


   Benchmark Dose Computation
```

```
Risk Response    =     Incidental
Risk Type        =          Extra
Confidence level =            0.9
Time             =            104


 Specified effect =   0.1           0.01          0.001
              BMD =   4.64886       2.12413       0.984449
             BMDL =   2.49972       0.734665      0.0748097
             BMDU =   9.01023       3.49311       1.61892
```



Female Rat (Kroese et al., 2001): Oral Cavity or Forestomach, Squamous Cell Papilloma or Carcinoma

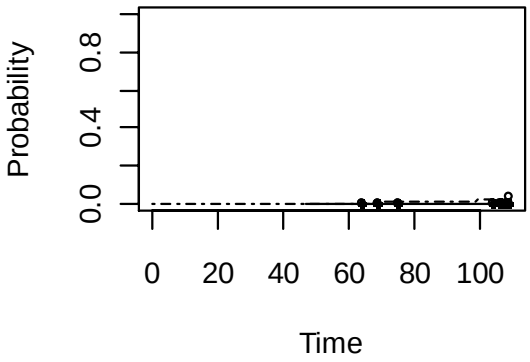
```
=====================================================================
     Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
     Solutions are obtained using donlp2-intv, (c) by P. Spellucci
     Input Data File: OralForstKroeseF3.(d)
=====================================================================

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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


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


                  Default Initial Parameter Values
                        c      =          3.6
                        t_0    =      45.1111
                        beta_0 = 1.11645e-009
                        beta_1 = 4.85388e-009
                        beta_2 =            0
                        beta_3 = 1.95655e-008

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

                 c            t_0          beta_0       beta_1       beta_3

    c                 1        -0.79        -0.92        -0.93           -1

    t_0           -0.79            1         0.73         0.72          0.8

    beta_0        -0.92         0.73            1         0.79         0.92

    beta_1        -0.93         0.72         0.79            1         0.91

    beta_3           -1          0.8         0.92         0.91            1


                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.52871         0.701117            2.15454             4.90287
         t_0             46.553          5.93306            34.9244             58.1816
         beta_0     1.53589e-009     5.40523e-009       -9.05817e-009        1.21299e-008
         beta_1     7.57004e-009      2.9647e-008       -5.05369e-008         6.5677e-008
         beta_2                0               NA
         beta_3     2.53126e-008     7.66404e-008         -1.249e-007        1.75525e-007

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


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


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

```
      Minimum observation time for F tumor context =         58

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

 Specified effect =   0.1            0.01           0.001
              BMD =   0.538801       0.0981283      0.0100797
             BMDL =   0.328135       0.0345104      0.00344714
             BMDU =   0.717127       0.325909       > 0.0806373
```

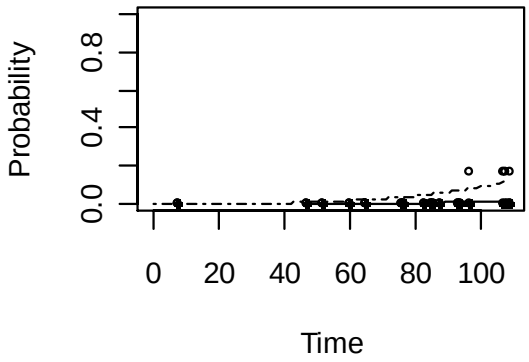
Incidental Risk: OralForstKroeseF3

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

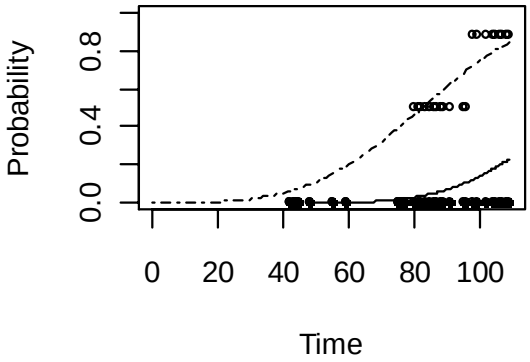
Dose = 0.00



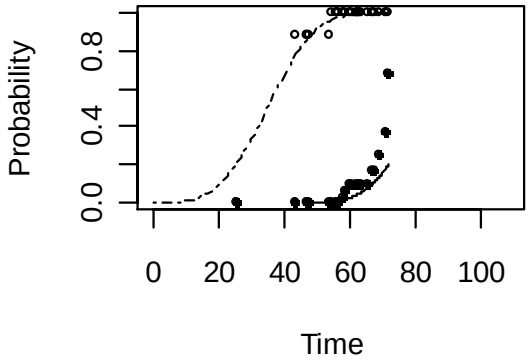
Dose = 0.49



Dose = 1.62



Dose = 4.58



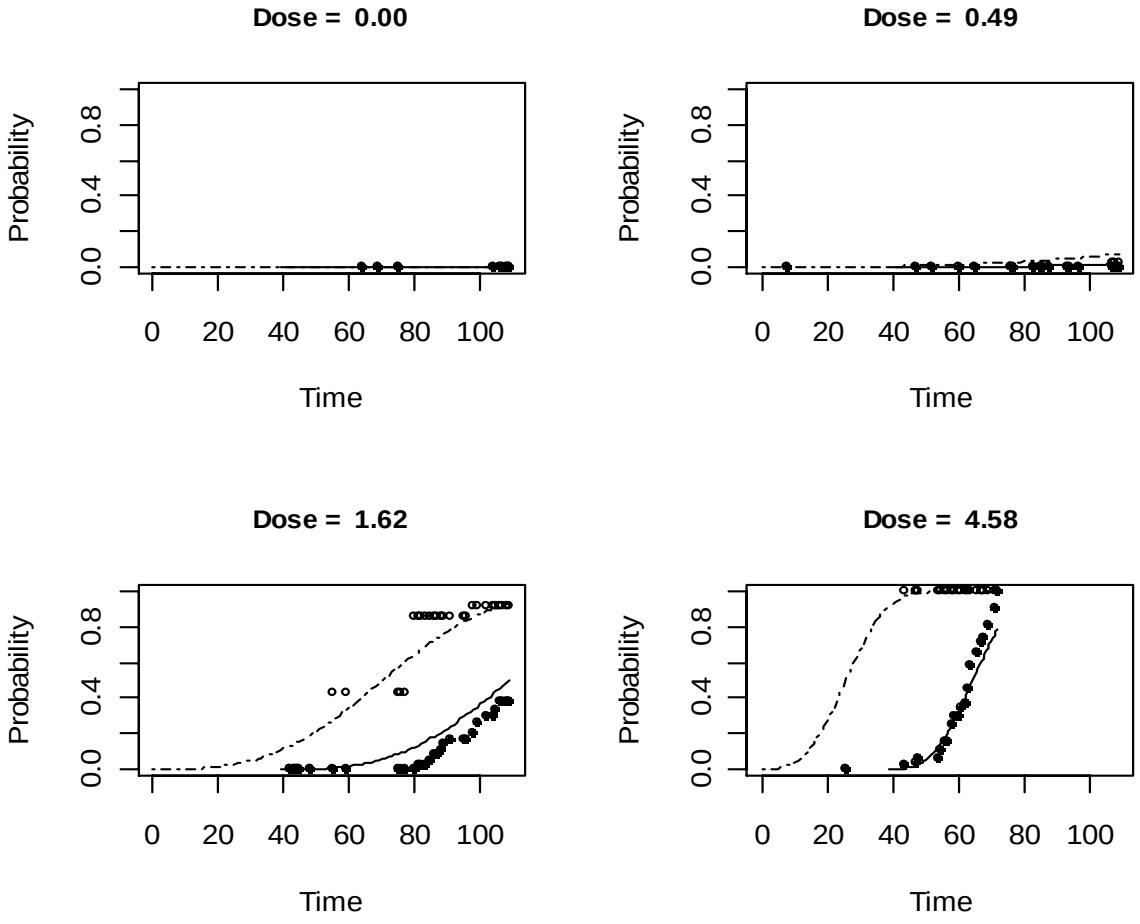
Female Rat (Kroese et al., 2001): Hepatocellular Adenoma or Carcinoma

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

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

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

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

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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


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


                  Default Initial Parameter Values
                         c      =          3.6
                         t_0    =      31.7778
                         beta_0 =            0
                         beta_1 =  4.9104e-031
                         beta_2 = 5.45766e-030
                         beta_3 = 3.44704e-008


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

                 c            t_0          beta_3

    c                 1         -0.9           -1

    t_0            -0.9            1         0.92

    beta_3           -1         0.92            1


                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              3.11076         0.549208            2.03434             4.18719
         t_0            38.6965          5.21028            28.4846             48.9085
         beta_0               0               NA
         beta_1               0               NA
         beta_2               0               NA
         beta_3     2.94354e-007    7.19418e-007       -1.11568e-006        1.70439e-006

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


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


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

```
      Minimum observation time for F tumor context =         44


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

 Specified effect =   0.1           0.01          0.001
              BMD =   0.575127      0.262783      0.12179
            BMDL =   0.506633      0.134213      0.0152934
            BMDU =   0.629806      0.287232      0.133064
```

Incidental Risk: Hepatocellular_Kroese_F3

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



Female Rat (Kroese et al., 2001): Duodenum or Jejunum Adenocarcinoma

```
=====================================================================
      Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
      Solutions are obtained using donlp2-intv, (c) by P. Spellucci
      Input Data File: DuoJejKroeseF3.(d)
 =====================================================================
   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

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


   User specifies the following parameters:
          t_0    =          0

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


                  Default Initial Parameter Values
                        c       =         2.25
                        t_0     =            0   Specified
                        beta_0  =            0
                        beta_1  =            0
                        beta_2  =            0
                        beta_3  =   7.289e-008


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

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                  Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               2.32531          3.58729            -4.70565             9.35626
         beta_0                0               NA
         beta_1                0               NA
         beta_2                0               NA
         beta_3     5.32209e-008     7.98487e-007       -1.51178e-006        1.61823e-006

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


               Log(likelihood)   # Param            AIC
   Fitted Model        -13.8784         5         37.7569


                    Data Summary
                        CONTEXT
               C      F      I      U  Total  Expected Response
    DOSE
        0     52      0      0      0     52     0.00
     0.49     52      0      0      0     52     0.01
      1.6     52      0      0      0     52     0.44
      4.6     48      0      4      0     52     3.57


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

```
Time             =           104

 Specified effect =   0.1           0.01          0.001
              BMD =   3.43129       1.56781       0.726615
             BMDL =   1.94745       0.560867      0.0584891
             BMDU =   5.70108       2.61447       1.21046
```

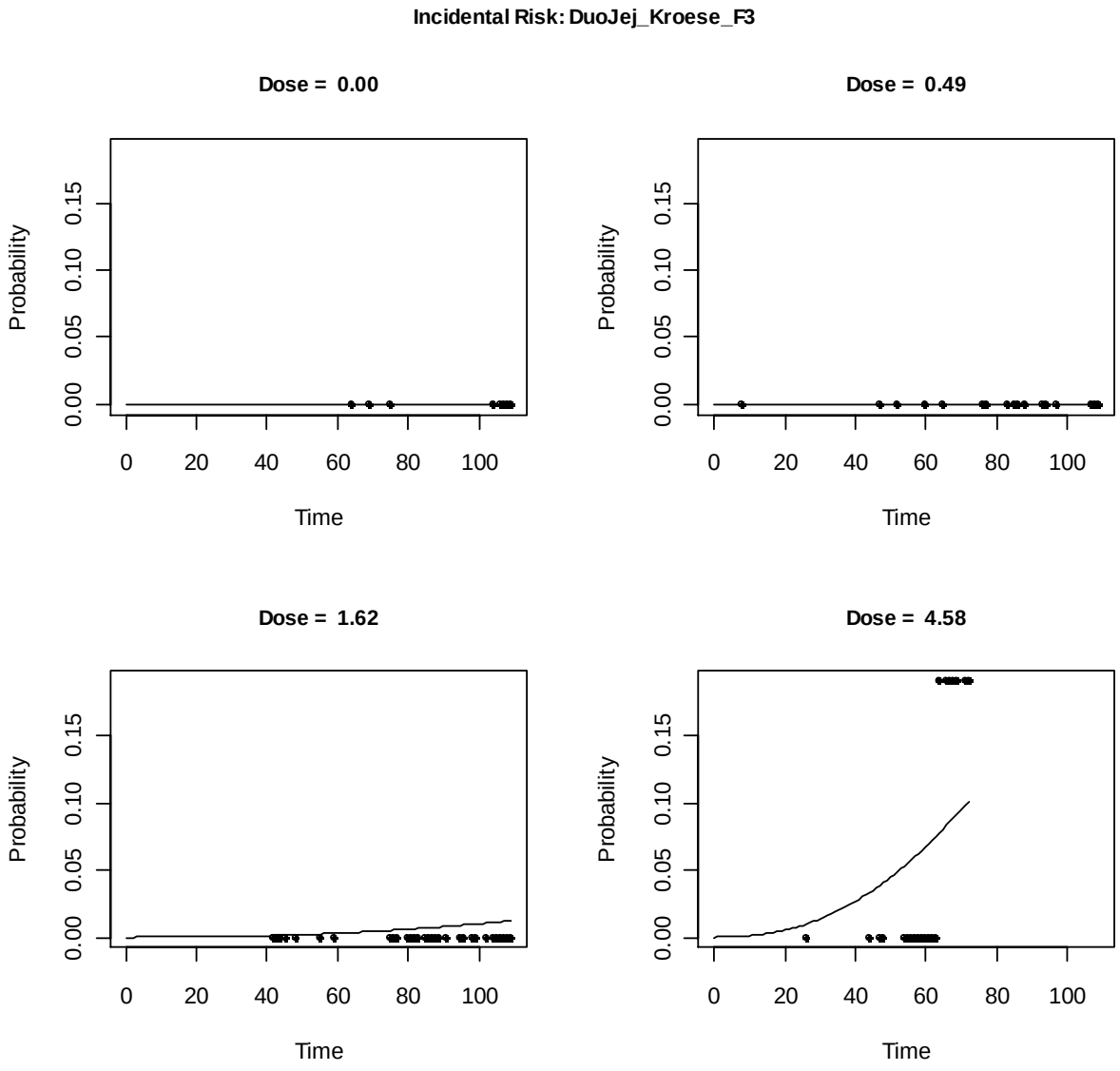


Table D-7. Summary of human equivalent overall cancer risk values, based on male and female rat tumor incidence (Kroese et al., 2001)

Data set	Tumor Site	BMD_{001}	$BMDL_{001}$	Risk value[a] at BMD_{001}	Risk value[a] at $BMDL_{001}$	SD	SD^2	Prop. of total variance
Males	Oral cavity/forestomach	6.37E-03	2.86E-03	1.57E-01	3.50E-01	1.17E-01	1.38E-02	0.64
	Liver	2.00E-02	5.30E-03	5.00E-02	1.89E-01	8.42E-02	7.09E-03	0.33
	Duodenum/ jejunum	6.42E-01	4.21E-02	1.56E-03	2.38E-02	1.35E-02	1.82E-04	0.01
	Skin/mammary gland: basal cell	6.06E-01	4.24E-02	1.65E-03	2.36E-02	1.33E-02	1.78E-04	0.01
	Skin/mammary gland: squam. cell	7.06E-02	2.11E-02	1.42E-02	4.75E-02	2.03E-02	4.10E-04	0.02
	Kidney	9.84E-01	7.48E-02	1.02E-03	1.34E-02	7.51E-03	5.64E-05	0.00
			Sum, risk values at BMD_{001}:	2.25E-01		Sum, SD^2:	2.17E-02	
						Overall SD[b]:	1.47E-01	
			Upper bound on sum of risk estimates[c]:		4.68E-01			
Females	Oral cavity/forestomach	3.45E-03	1.01E-02	2.90E-01	9.92E-02	1.16E-01	1.35E-02	0.91
	Liver	1.53E-02	1.22E-01	6.54E-02	8.21E-03	3.48E-02	1.21E-03	0.08
	Duodenum/ jejunum	5.85E-02	7.27E-01	1.71E-02	1.38E-03	9.56E-03	9.13E-05	0.01
			Sum, risk values at BMD_{001}:	1.09E-01		Sum, SD^2:	1.48E-02	
						Overall SD:	1.22E-01	
			Upper bound on sum of risk estimates[c]:		3.09E-01			

[a] Risk value=$0.001/BMDL_{001}$
[b] Overall SD = $(\text{Sum, } SD^2)^{0.5}$
[c] Upper bound on the overall risk estimate = Sum of BMD_{001} risk values + 1.645 × Overall SD.

Table D-8. Summary of model selection among multistage-Weibull models fit to alimentary tract tumor data for female mice (Beland and Culp, 1998)

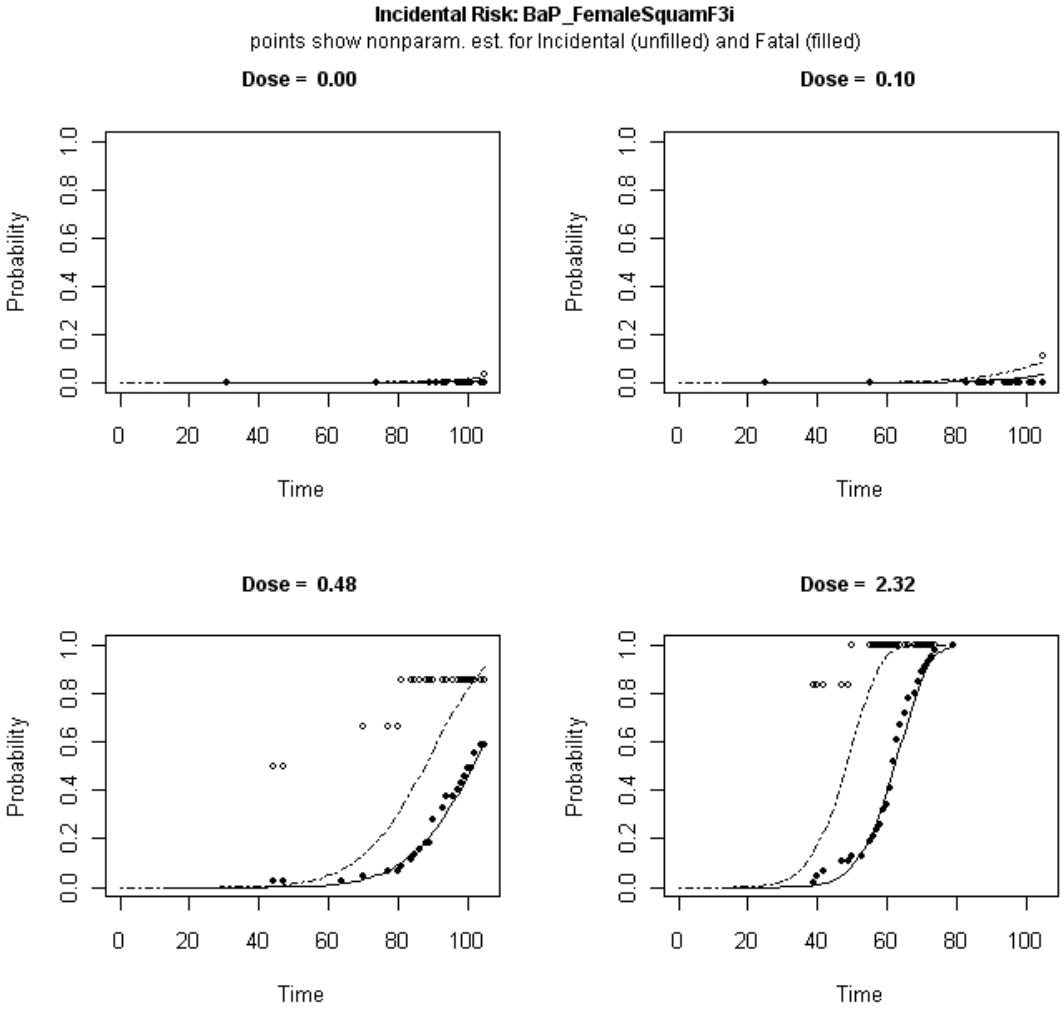
Model stages	LL[a]	χ^2 [b]	Number of param.	AIC	BMD_{10}	Responses @ mg/kg-d levels[c] 0	0.1	0.48	2.3	Selected model parameter estimates c	t0	Model Selection Rationale
						1	*3*	*38*	*46*			
1	-340.271	NR	4	688.5	0.104	0.6	14.6	34.1	36.3	3.4	18	
2	-309.620	61.3	5	629.2	0.102	0.7	4.5	33.2	41.8	5.5	16	
3	-306.265	**6.7**	**6**	624.5	**0.127**	**0.9**	**3.2**	**30.8**	**41.9**	**6.9**	**14**	**Lowest AIC, best fit to low dose data**

[a] LL=log-likelihood.
[b] χ^2 = chi-squared statistic for testing the difference between 2 model fits, from $2 \times |(LL_i - LL_j)|$ evaluated for i-j degrees of freedom. In all cases the difference was evaluated for consecutive numbers of stages; i-j = 1, and χ^2 at $\alpha = 0.05$ is 3.84.
[c] "Responses" describes the number of animals with each tumor type; observed responses are in italics, and expected responses (predicted by each model fit) are given to one decimal place for comparison with the observed data.
NR = not relevant.

Female Mice (Beland and Culp, 1998): Alimentary Tract Squamous Cell Tumors

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

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

   The parameter betas are restricted to be positive

   Dependent variable = Class
   Independent variables = Dose, time

 Total number of observations = 191
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3


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



                 User Inputs Initial Parameter Values
                        c      =            2
                        t_0    =           15
                        beta_0 =     1.6e-014
                        beta_1 =            0
                        beta_2 =     5.5e-012
                        beta_3 =     4.4e-012


           Asymptotic Correlation Matrix of Parameter Estimates
                 c            t_0          beta_0       beta_1       beta_2       beta_3

    c               1        -0.78        -0.97        -0.42        -0.99        -0.99

    t_0          -0.78            1         0.76         0.39         0.74         0.84

    beta_0       -0.97         0.76            1         0.33         0.97         0.96

    beta_1       -0.42         0.39         0.33            1         0.31         0.46

    beta_2       -0.99         0.74         0.97         0.31            1         0.97

    beta_3       -0.99         0.84         0.96         0.46         0.97            1


                                 Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              6.92317          1.33874            4.29929             9.54705
         t_0            13.9429          4.96646            4.20881              23.677
         beta_0     2.46916e-016     1.47619e-015       -2.64636e-015       3.14019e-015
         beta_1                0     1.30525e-014       -2.55825e-014       2.55825e-014
         beta_2     5.85452e-014     3.75144e-013       -6.76723e-013       7.93813e-013
         beta_3     9.76542e-014     5.62017e-013       -1.00388e-012       1.19919e-012


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


                  Data Summary
                      Class
             C      F      I      U  Total  Expected Response
    Dose
        0     47      0      1      0     48     0.93
      0.1     45      0      3      0     48     3.21
     0.48      8     23     15      1     47    30.82
      2.3      1     46      0      1     48    41.91

    Minimum observation time for F tumor context =         39


   Benchmark Dose Computation
Risk Response    =     Incidental
```

```
Risk Type        =          Extra
Specified effect =            0.1
Confidence level =            0.9

Time             =            104

             BMD =       0.126983
            BMDL =      0.0706103
            BMDU =       0.179419
```



APPENDIX E. TIME-TO-TUMOR MODELING FOR THE INHALATION UNIT RISK

Table E-1. Individual pathology and tumor occurrence data for male Syrian hamsters exposed to benzo[a]pyrene via inhalation for lifetime (Thyssen et al., 1981)

Admin. Exposure Conc. (mg/m^3)	Time on Study	Number Examined	Larynx	Pharynx	Trachea	Esophagus	Forestomach	Nasal Cavity
0	17	1	0	0[a]	0	0	0	0
	39	1	0	0	0	0	0	0
	45	1	0	0	0	0	0	0
	79	1	0	0	0	0	0	0
	83	1	0	0	0	0	0	0
	85	1	0	0[a]	0	0	0	0
	86	1	0	0	0	0	0	0
	88	2	0	0	0	0	0	0
	89	2	0	0	0	0	0	0
	90	1	0	0	0	0	0	0
	101	1	0	0	0	0	0	0
	102	1	0	0	0	0	0	0
	103	1	0	0	0	0	0	0
	106	1	0	0	0	0	0	0
	108	1	0	0	0	0	0	0
	109	1	0	0	0	0	0	0
	112	1	0	0	0	0	0	0
	115	1	0	0	0	0	0	0
	116	1	0	0[a]	0	0	0	0
	122	1	0	0	0	0	0	0
	123	1	0	0	0	0	0	0
	124	1	0[a]	0	0	0	0	0
	125	1	0	0	0	0	0	0
	127	1	0	0[a]	0	0	0	0
	132	1	0	0	0	0	0	0
2	14	1	0[a]	0[a]	0	0	0	0
	35	1	0	0	0	0	0	0
	53	1	0	0	0	0	0	0
	59	1	0	0	0	0	0	0
	71	1	0	0	0	0	0	0
	78	1	0	0	0	0	0	0
	80	1	0	0	0	0	0	0
	85	1	0	0	0	0	0	0
	87	1	0	0	0	0	0	0
	88	1	0	0	0	0	0	0
	93	1	0	0	0	0	0	0
	98	1	0	0[a]	0	0	0	0
	99	1	0	0	0	0	0	0
	102	1	0	0	0	0	0	0
	103	1	0	0	0	0	0	0
	108	1	0	0	0	0	0	0
	111	1	0	0	0	0	0	0
	113	1	0	0	0	0	0	0
	114	1	0	0	0	0	0	0
	115	1	0	0	0	0	0	0
	116	1	0	0	0	0	0	0
	117	1	0	0	0	0	0	0
	120	1	0	0	0	0	0	0
	122	2	0[a]	0[a]	0	0	0	0
	133	2	0	0	0	0	0	0

Table E-1. Individual pathology and tumor occurrence data for male Syrian hamsters exposed to benzo[a]pyrene via inhalation for lifetime (Thyssen et al., 1981)

Admin. Exposure Conc. (mg/m^3)	Time on Study	Number Examined	Larynx	Pharynx	Trachea	Esophagus	Forestomach	Nasal Cavity
10	31	1	0	0	0	0	0	0
	32	1	0	0	0	0	0	0
	52	1	0	0	0	0	0	0
	67	1	0	0	0	0	0	0
	73	1	0	0	0	0	0	0
	76	2	0	2	0	0	0	0
	80	1	1	0	0	0	0	0
	85	1	0	0	0	0	0	0
	94	1	1	0	0	0	0	0
	100	1	0	0	0	0	0	0
	102	1	0	1	0	0	0	0
	105	1	1	1	0	0	0	0
	111	1	0	1	0	0	0	0
	113	1	0	1	0	0	0	0
	114	1	1	1	0	0	0	0
	115	1	1	0[a]	1	0	0	1
	116	1	0	0	1	0	0	1
	117	1	1	0	0	0	0	0
	118	4	3	1[b]	0	0	1	1
	122	1	1	0	0	0	0	0
	124	1	1	1	0	0	0	0
	125	1	0	0	0	0	0	1
50	20	1	0[a]	0[a]	0[a]	0	0	0
	21	1	0[a]	0[a]	0[a]	0	0	0
	25	2	0[a]	0[a]	0[a]	0	0	0
	29	1	0[a]	0[a]	0[a]	0	0	0
	30	1	0[a]	0[a]	0[a]	0	0	0
	34	1	0[a]	0[a]	0[a]	0	0	0
	36	2	0[a]	0[a]	0[a]	0	0	0
	37	1	0[a]	0[a]	0[a]	0	0	0
	40	2	1[a]	1[a]	1[a]	0	0	0
	41	1	0	0	0	0	0	0
	43	1	0	0	0	0	0	0
	47	1	1	1	0	0	0	0
	48	1	0	1	0	0	0	0
	51	1	0	0[a]	0	0	0	0
	56	1	1	1	0	0	0	0
	57	1	0	1	0	0	0	0
	60	1	0	1	0	0	0	0
	63	1	0	0	0	0	0	0
	64	1	0	1	0	0	1	0
	66	1	1	1	0	0	0	0
	68	1	0	1	0	0	0	0
	70	1	1	1	0	1	0	0
	71	1	1	1	1	0	0	0
	72	1	1	1	0	0	0	0
	73	2	2	2	0	0	0	0
	79	4	3	4	1	1	0	1

[a] Tissue was not examined for one animal of total examined.
[b] Tissue was not examined for two animals of total examined.

Table E-2. Summary of model selection among multistage-Weibull models fit to tumor data for male hamsters[a] (Thyssen et al., 1981)

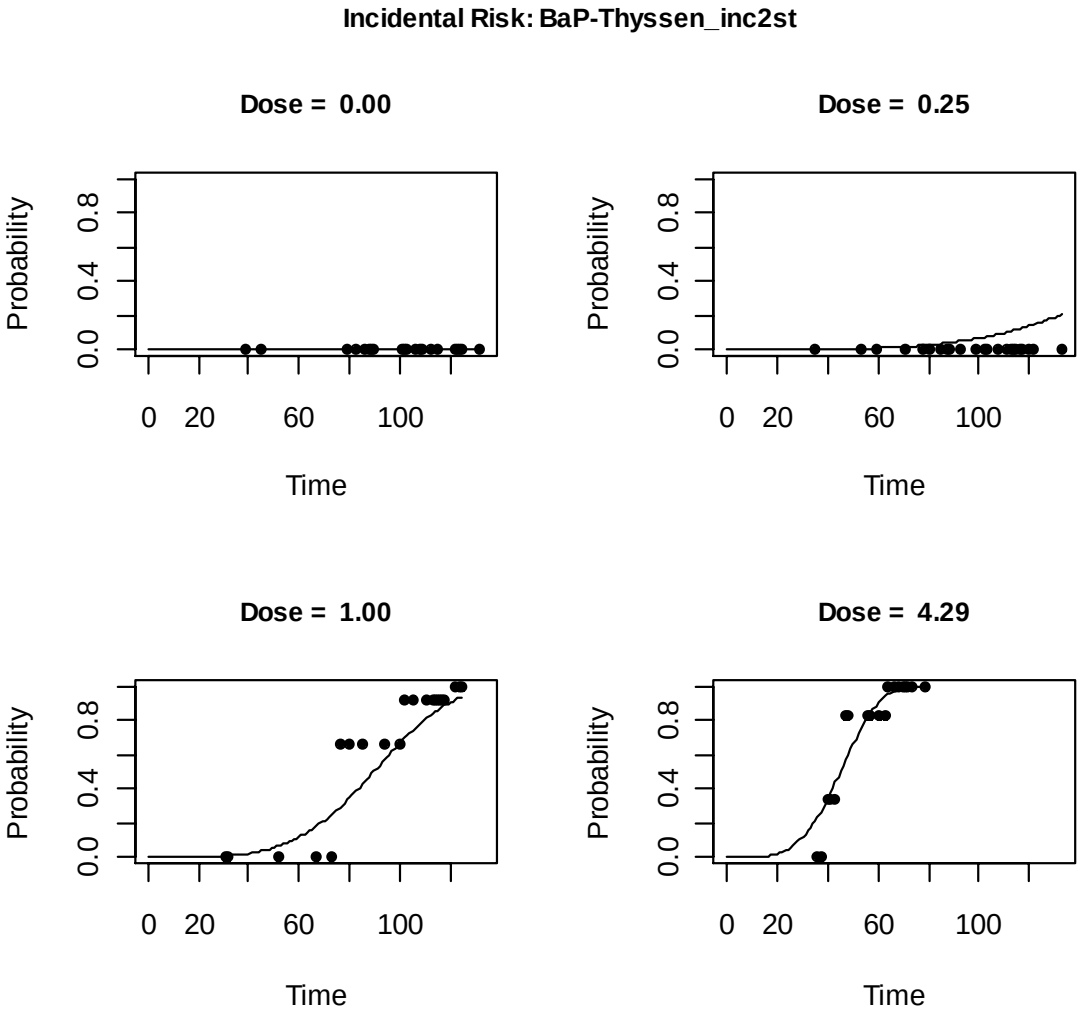
Model stages	LL[b]	χ^2 [c]	Number of param.	AIC	BMD_{10}	Responses @ mg/kg-d levels[d] 0	0.25	1.01	4.29	Selected model parameter estimates c	t_0	Model Selection Rationale
Oral tract tumors: all tumors considered incidental to cause of death						*0*	*0*	*18*	*18*			
1	-26	NR	3	58	0.090	0.0	5.6	15.9	17.2	2.2	NR	
2	**-19.967**	**12.1**	**4**	**47.9**	**0.285**	**0.0**	**1.9**	**16.0**	**18.2**	**4.2**	**NR**	**Lowest AIC, best fit to data; maximum number of stages that could be fit**
Oral tract tumors: all tumors considered to be cause of death						*0*	*0*	*18*	*18*			
1	-160.646	NR	3	327.292	0.136	Not available				4.9	NR	
2	-147.428	26.4	4	302.857	0.421	Not available				6.7	NR	
3	**-144.522**		**5**	**299.043**	**0.648**	Not available				**9.0**	**NR**	**Lowest AIC; best fit to data (see graphs)**

[a] All animals with missing tissues were omitted.
[b] LL=log-likelihood.
[c] χ^2 = chi-squared statistic for testing the difference between 2 model fits: $\chi^2 = 2 \times |(LL_i - LL_j)|$ evaluated for |i-j| degrees of freedom (df). In all cases the difference was evaluated for consecutive numbers of stages; i-j = 1, and χ^2 for 1 df at $\alpha = 0.05$ is 3.84.
[d] "Responses" describes the number of animals with each tumor type; observed responses are in italics, and expected responses (predicted by each model fit) are given to one decimal place for comparison with the observed data.
NR = not relevant.

Output for Oral tract tumors: all tumors considered incidental to cause of death

```
=====================================================================
      Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
      Solutions are obtained using donlp2-intv, (c) by P. Spellucci
      Input Data File: C:\msw\benzo[a]pyrene-Thyssen_inc2st.(d)
=====================================================================

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

   The parameter betas are restricted to be positive

   Dependent variable = Class
   Independent variables = Conc, Time

 Total number of observations = 96
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 32
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


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


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

                 c            beta_2

    c                 1           -1

    beta_2           -1            1


                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               4.21938         0.840997            2.57105              5.8677
         beta_0                0               NA
         beta_1                0               NA
         beta_2     4.00402e-009       1.495e-008       -2.52974e-008        3.33054e-008

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


               Log(likelihood)   # Param            AIC
   Fitted Model         -19.967         4         47.9339


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


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
```

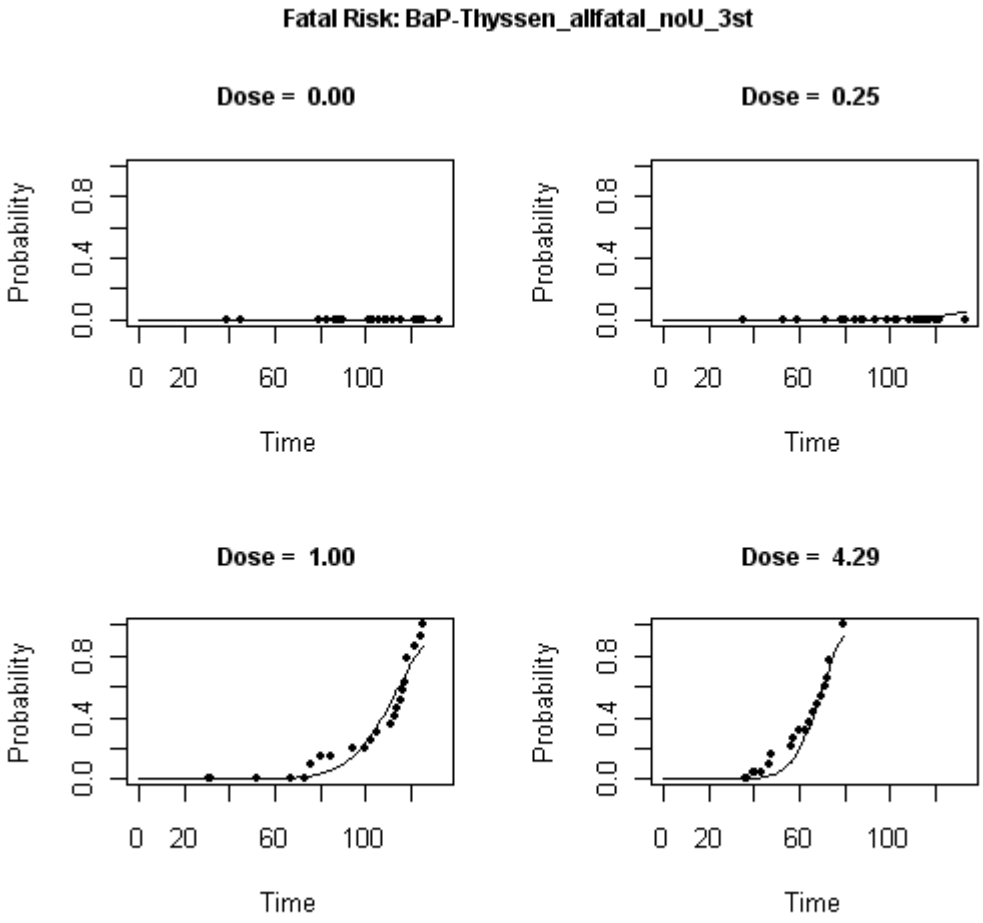
```
Specified effect =           0.1
Confidence level =           0.9

Time             =           104

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



Output for Oral tract tumors: all tumors considered to be cause of death

```
=====================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: C:\msw\benzo[a]pyrene-Thyssen_allfatal_noU_3st.(d)
=====================================================================

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

   The parameter betas are restricted to be positive

   Dependent variable = Class
   Independent variables = Conc, Time

 Total number of observations = 96
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 32
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


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


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

                 c            beta_3

    c                 1           -1

    beta_3           -1            1


                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              8.95016         0.896607            7.19284             10.7075
         beta_0               0               NA
         beta_1               0               NA
         beta_2               0               NA
         beta_3     3.43452e-019    1.39727e-018       -2.39515e-018        3.08205e-018

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


               Log(likelihood)   # Param            AIC
   Fitted Model        -144.522         5         299.043


                   Data Summary
                       Class
              C      F      I      U  Total
    Conc
        0     23      0      0      0     23
     0.25     24      0      0      0     24
        1      8     18      0      0     26
      4.3      5     18      0      0     23

    Minimum observation time for F tumor context =         40
```

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

Time             =            104

             BMD =       0.647659
            BMDL =       0.461415
            BMDU =       0.719325
```



APPENDIX F. BENCHMARK MODELING FOR THE DERMAL SLOPE FACTOR

Table F-1. Summary of model selection and modeling results for best-fitting multistage models, for multiple data sets of skin tumors in mice following dermal benzo[a]pyrene exposure

Data Set (See Section 5.4.3.2., Tables 5-7 to 5-10)		Degree of Model	df	Goodness-of-fit *p*-value	LL[b]	χ^{2} [c]	BMD_{10} (µg/d)	$BMDL_{10}$ (µg/d)	Model selection rationale, with best fitting model in boldface[a]	Figure number
Poel (1959)		2	2	0.027	-91.28	NR	NA	NA	(Inadequate fit for 1-2 stage models)	
male C57L		**3**	**2**	**0.053**	**-90.43**	1.7	**0.127**	**0.078**	**Most parsimonious fit**	F-1
		4	2	0.068	-90.12	0.58	0.122	0.077	Improvement in fit not statistically significant over 2-stage fit	
Roe et al. (1970)		1	5	0.110	-64.56	NR	0.299	0.233		
female Swiss		**2-5**	**3**	**0.463**	-58.81	11.5	**0.689**	**0.394**	**Most parsimonious fit**; significant improvement over 1-stage fit	F-2
Schmidt et al. (1973)		1	4	0.008	-80.34	NR	0.256	0.194		
female NMRI		**2**	**4**	**0.609**	**-72.68**	15.3	**0.329**	**0.287**	**Most parsimonious fit**	F-3
		3	4	0.999	-70.95	3.5	0.381	0.326	Improvement in fit not statistically significant over 2-stage fit	
Schmidt et al. (1973)		1	4	<0.01	-87.99	NR	0.116	0.093		
female Swiss		2	4	0.514	-75.66	24.7	0.216	0.192		
		3-4	**3**	**0.983**	**-73.66**	4.0	**0.282**	**0.223**	**Most parsimonious fit**; significant improvement over 2-stage fit	F-4
Schmähl et al. (1977)		1	2	0.136	-147.20	NR	0.140	0.117		
female NMRI		**2**	**1**	**0.939**	-145.13	4.14	**0.233**	**0.149**	**Most parsimonious fit**; significant improvement over 1-stage fit	F-5
Habs et al. (1980)		2	3	0.009	-41.18	NR	NA	NA	(Inadequate fit for 1-2 stage models)	
female NMRI		**3**	**3**	**0.207**	**-37.34**	7.7	**0.294**	**0.215**	**Most parsimonious fit**	F-6
Habs et al. (1984)		**1**	**2**	**0.577**	**-22.78**	NR	**0.078**	**0.056**	**Most parsimonious fit**	F-7
female NMRI		2	1	1.000	-22.22	1.1	0.171	0.060		
Grimmer et al. (1983)		**1**	**3**	**0.850**	**-108.94**	NR	**0.245**	**0.208**	**Most parsimonious fit**	F-8
female CFLP		2-3	2	0.972	-108.56	0.76	0.292	0.213	Improvement in fit not statistically significant over 1-stage fit	
Grimmer et al. (1984), female CFLP	Multistage	1-3	3	0.003	205.3[b]	NR	NA	NA	Inadequate fit	F-9
	Other: **LogLogistic**		**2**	**0.919**	**195.8**	**NR**	**1.07**	**0.479**	**Best fit**; slope parameter unrestricted	F-10
	Dich.-Hill		1	1.000	197.7	NR	0.902	0.533	Slope parameter unrestricted	
	LogProbit		3	0.047	200.2	NR	NA	NA	Inadequate fit	
	Gamma, Weibull		—	—	—	—	—	—	Same model as multistage (above)	
	Logistic		2	<0.01	250.5	NR	NA	NA	Inadequate fit	
	Probit		2	<0.01	255.4	NR	NA	NA	Inadequate fit	
	Multistage, high dose dropped[d]	**1**-2	**2**	**0.499**	NR	NR	**0.106**	**0.088**	**Best fit from multistage model**	F-11
Sivak et al. (1997)		1	3	0.059	-27.92		0.036	0.026		
male CeH/HeJ		**2**-3	**3**	**0.998**	**-23.30**	**9.2**	**0.109**	**0.058**	**Most parsimonious fit**	F-12

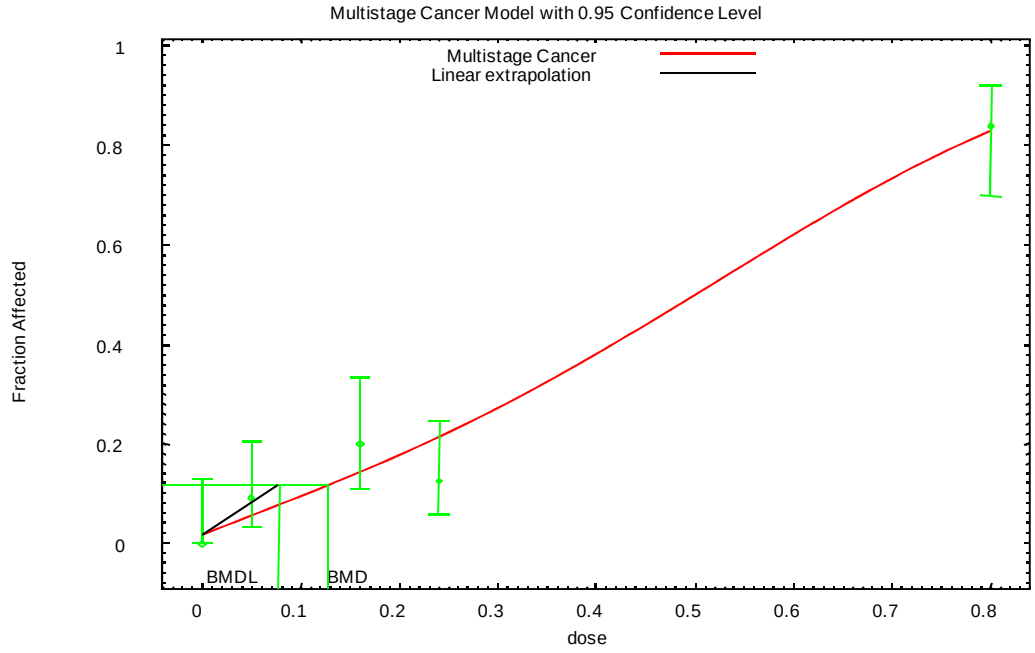
[a] Adequate fit: goodness-of-fit p>0.05, scaled residuals <2.0, good fit near BMR, lack of extreme curvature not reflected in the observed data.

[b] LL=Log-likelihood; values for Grimmer et al. (1984) are AICs, in order to compare across models.

[c] $\chi^{2} = 2 \times |(LL_{i} - LL_{j})|$, where i and j are consecutive numbers of stages. The test was evaluated for 1 degree of freedom (df). χ^{2} for 1 df at $\alpha = 0.05$ is 3.84.

[d] The preferred multistage model did not adequately fit the data for Grimmer et al. (1984), thus, the remaining suite of models were fit to the data. The POD for Grimmer et al. (1984) was based on the LogLogistic model. For comparison purposes, the multistage model was it fit to these data for Grimmer et al. (1984) with the highest dose dropped.

Figure F-1. Fit of multistage model to skin tumors in C57L mice exposed dermally to benzo[a]pyrene (Poel, 1959); graph and model output.




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

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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = NumAff
   Independent variable = LADD

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


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



                   Default Initial Parameter Values
                      Background =    0.0449589
                         Beta(1) =     0.490451
                         Beta(2) =            0
                         Beta(3) =      2.68146


            Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)	Beta(3)
Background	1	-0.87	0.74
Beta(1)	-0.87	1	-0.92
Beta(3)	0.74	-0.92	1

Parameter Estimates

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	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

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

AIC: 186.853

Goodness of Fit

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

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

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.126567

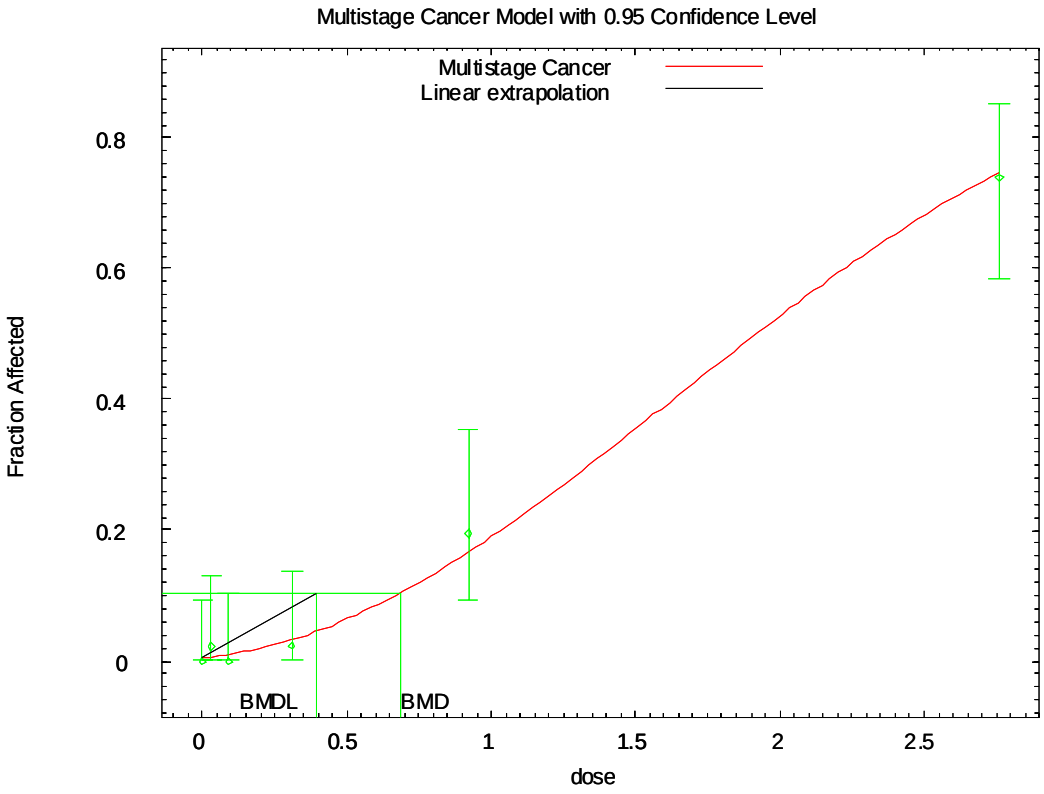
BMDL = 0.0777875

BMDU = 0.272961

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

Multistage Cancer Slope Factor = 1.28555

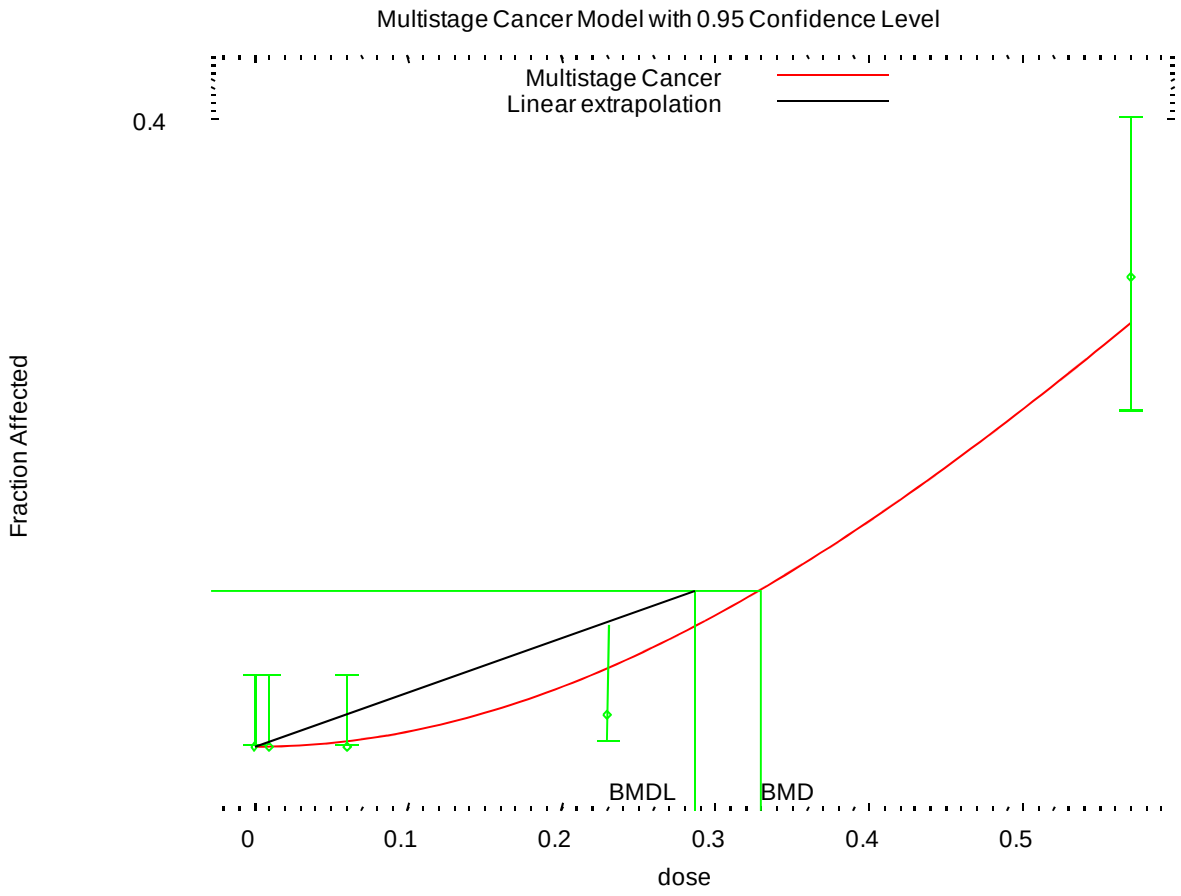
Figure F-2. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Roe et al., 1970); graph and model output.




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

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

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

    The form of the probability function is:

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

    The parameter betas are restricted to be positive


    Dependent variable = tumors
    Independent variable = LADD

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


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


                    Default Initial Parameter Values
                       Background =           0
```

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


            Asymptotic Correlation Matrix of Parameter Estimates

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

              Background      Beta(1)      Beta(2)

Background            1        -0.57         0.45

   Beta(1)        -0.57            1        -0.94

   Beta(2)         0.45        -0.94            1


                                 Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

            AIC:         121.139


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

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


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

              BMD =       0.689131

             BMDL =       0.393806

             BMDU =       0.952365

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

Multistage Cancer Slope Factor =      0.253932
```

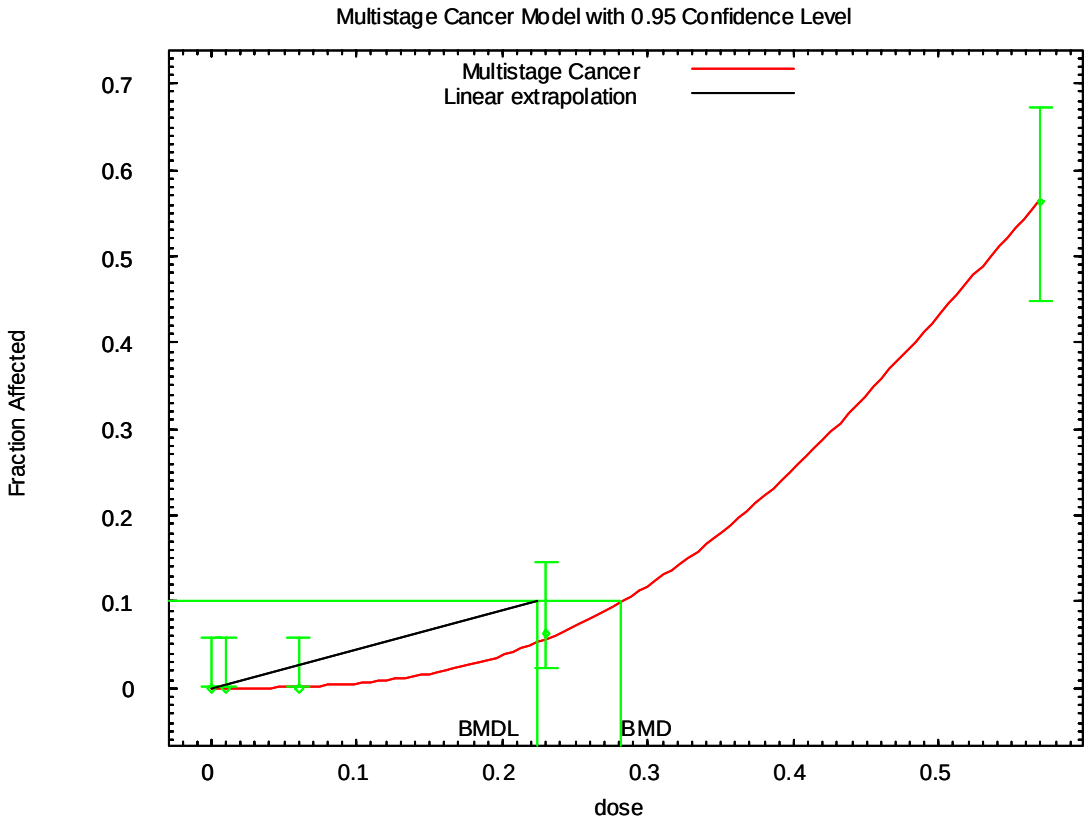
Figure F-3. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Schmidt et al., 1973); graph and model output.




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

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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

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


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

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


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


           Asymptotic Correlation Matrix of Parameter Estimates

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

               Beta(2)

   Beta(2)            1


                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

           AIC:         147.366


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

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


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.329464

            BMDL =       0.286624

            BMDU =       0.384046

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

Multistage Cancer Slope Factor =      0.348889
```

Figure F-4. Fit of multistage model to skin tumors in female Swiss mice exposed dermally to benzo[a]pyrene (Schmidt et al., 1973); graph and model output.




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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

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


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

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

**** incorporate these convergence criterion. Default values used. ****

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

Asymptotic Correlation Matrix of Parameter Estimates

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

	Beta(2)	Beta(3)
Beta(2)	1	-0.99
Beta(3)	-0.99	1

Parameter Estimates

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

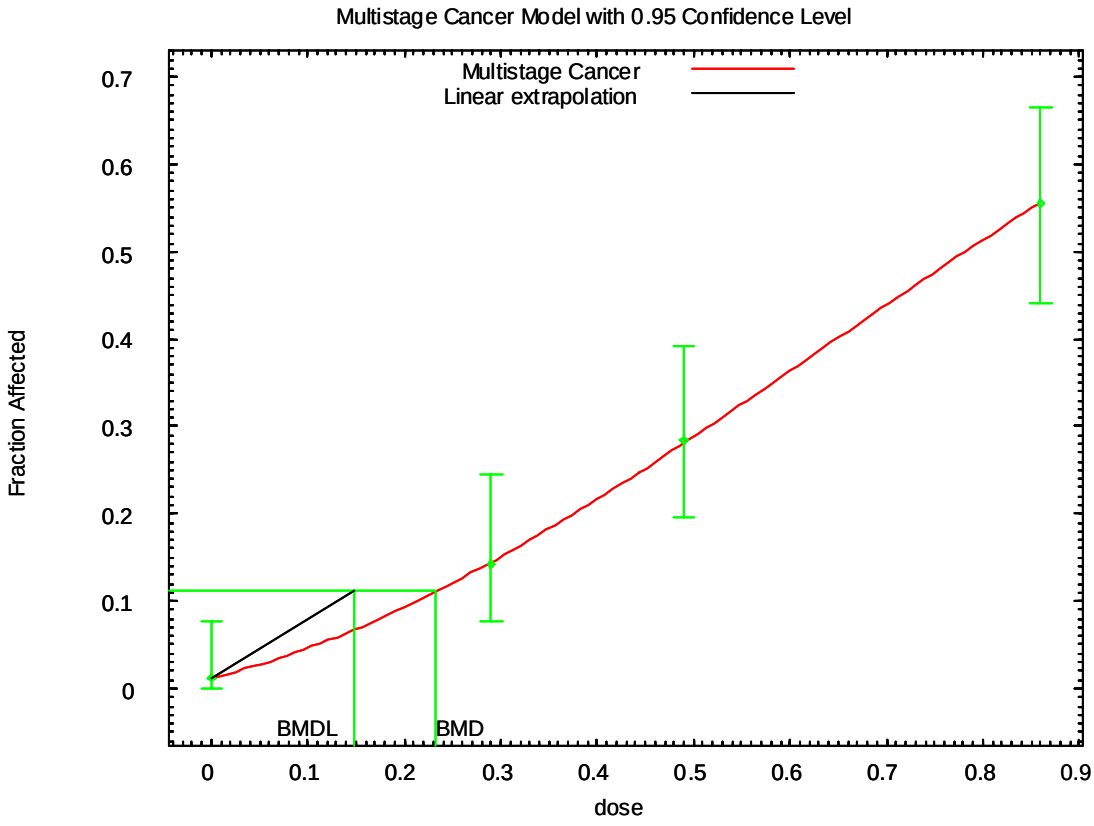
BMDL = 0.223401

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

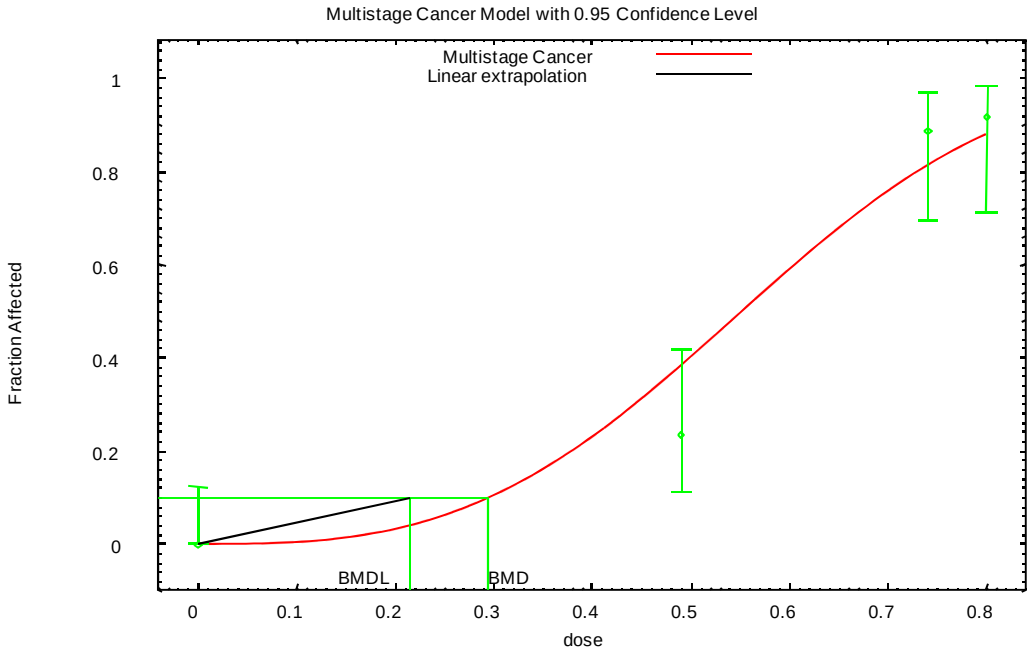
Figure F-5. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Schmähl et al., 1977); graph and model output.




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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

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


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

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

```
****  incorporate these convergence criterion.  Default values used.  ****


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


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)      Beta(2)

Background            1        -0.67         0.47

   Beta(1)        -0.67            1        -0.94

   Beta(2)         0.47        -0.94            1


                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

           AIC:         296.261


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

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


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.232893

            BMDL =       0.148895

            BMDU =       0.320396

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

Multistage Cancer Slope Factor =      0.671616
```

Figure F-6. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Habs et al., 1980); graph and model output.



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

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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = NumAff
   Independent variable = LADD

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


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


                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =      4.23649
                        Beta(3) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified
by the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1


                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

           AIC:         76.6745
```

Goodness of Fit

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

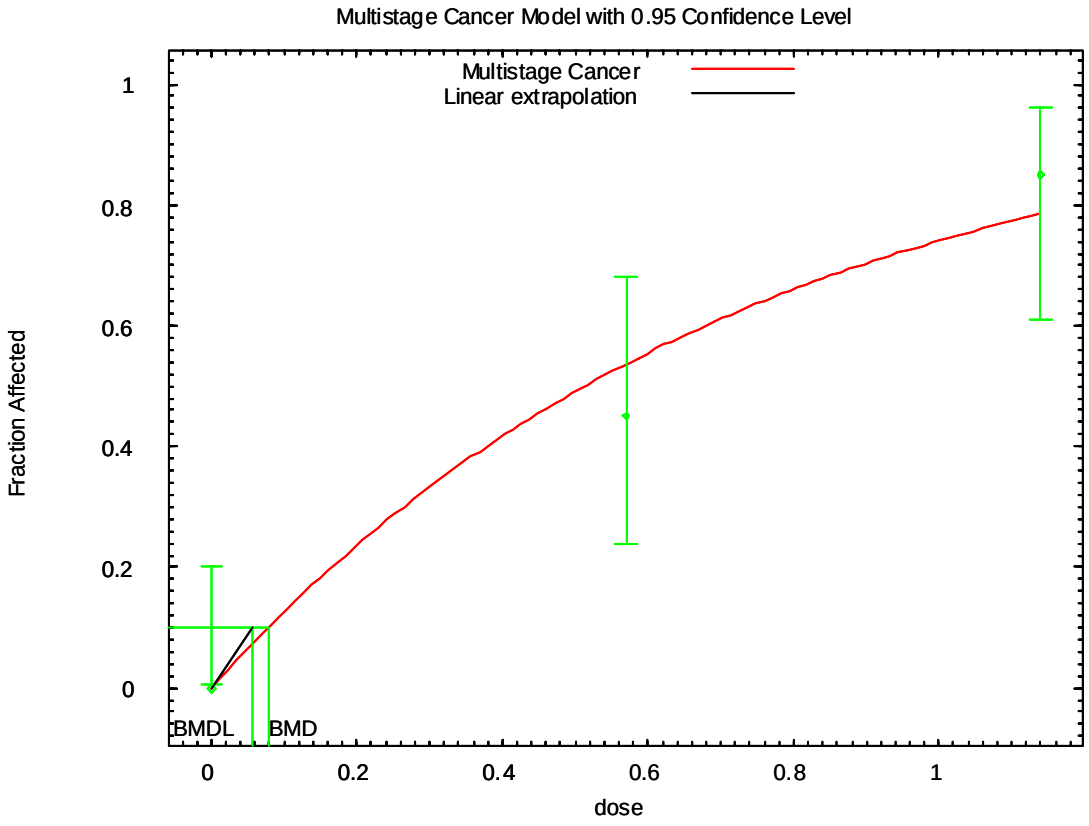
BMDL = 0.215151

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

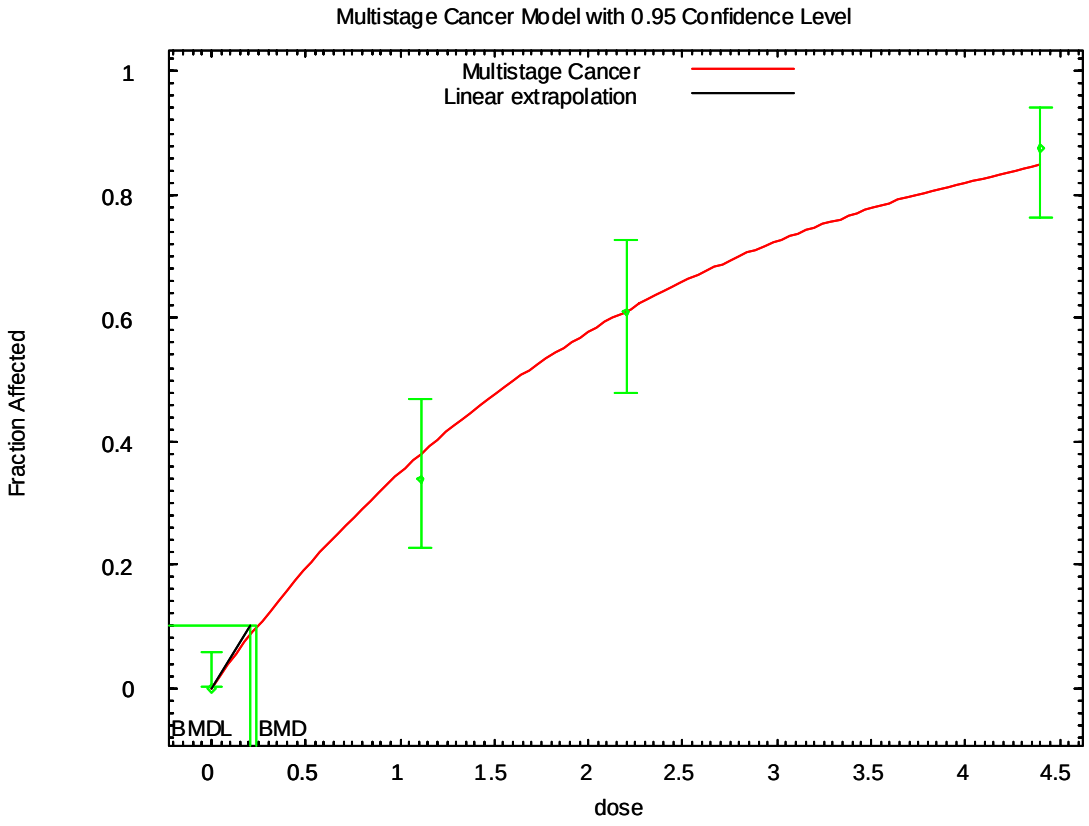
Figure F-7. Fit of multistage model to skin tumors in female NMRI mice exposed dermally to benzo[a]pyrene (Habs et al., 1984); graph and model output.




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

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

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

    The form of the probability function is:

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

    The parameter betas are restricted to be positive


    Dependent variable = tumors
    Independent variable = LADD

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


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

```
                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =      1.66414


            Asymptotic Correlation Matrix of Parameter Estimates

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

                 Beta(1)

    Beta(1)           1



                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

            AIC:         47.5757


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

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


    Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =      0.0778926

             BMDL =      0.0558466

             BMDU =       0.111853

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

Multistage Cancer Slope Factor =       1.79062
```

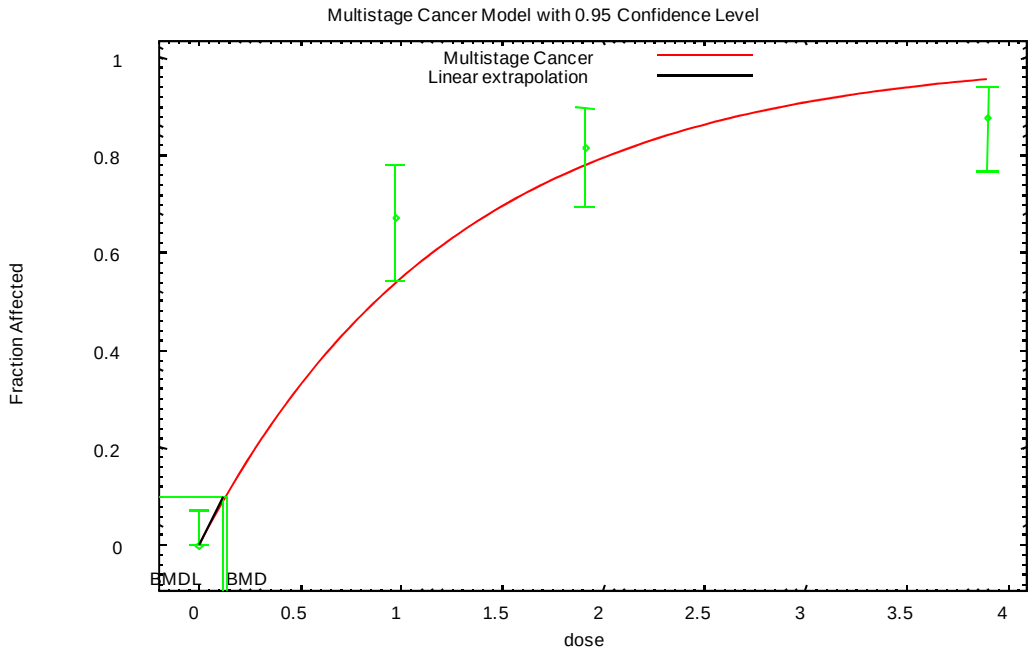
Figure F-8. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al, 1983); graph and model output.




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

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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

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


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

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



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


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

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

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

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

           AIC:         219.887


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

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


    Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =       0.244816

             BMDL =       0.208269

             BMDU =       0.289606

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

Multistage Cancer Slope Factor =      0.480148
```

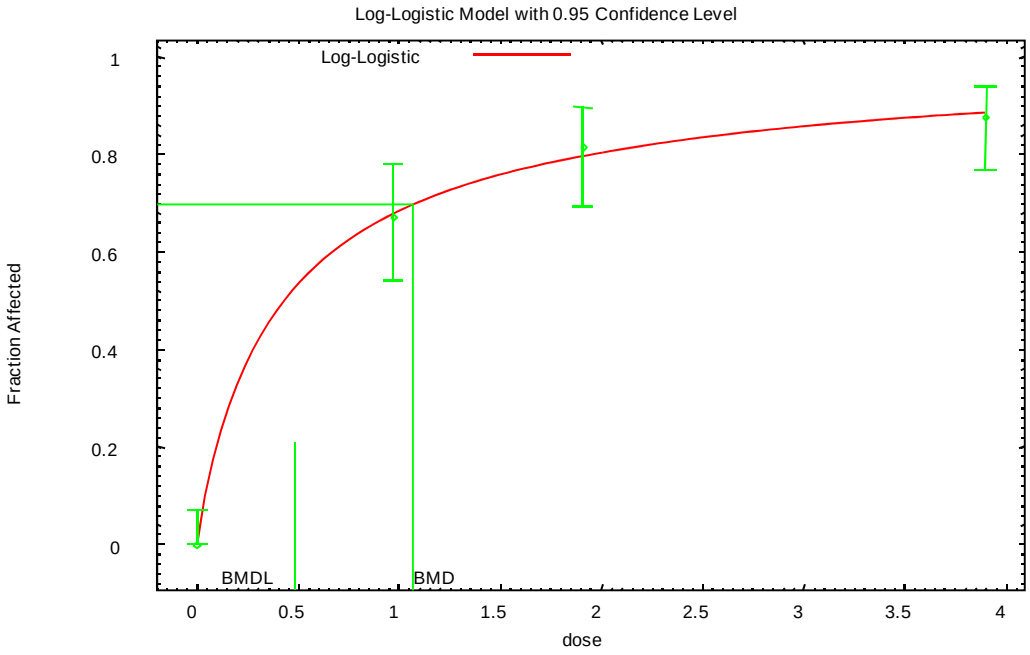
Figure F-9. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984); graph and model output.



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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = NumAff
   Independent variable = LADD

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


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



                  Default Initial Parameter Values
                     Background =     0.311241
                        Beta(1) =     0.502556


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

           AIC:         205.287


                                  Goodness  of  Fit
                                                              Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          65        0.000
```

```
    0.9700     0.5382        34.446    43.000          64        2.145
    1.9100     0.7816        50.804    53.000          65        0.659
    3.9000     0.9552        62.091    57.000          65       -3.054

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


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =       0.132272

             BMDL =       0.113427

             BMDU =       0.154848

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

Multistage Cancer Slope Factor =      0.881621
```

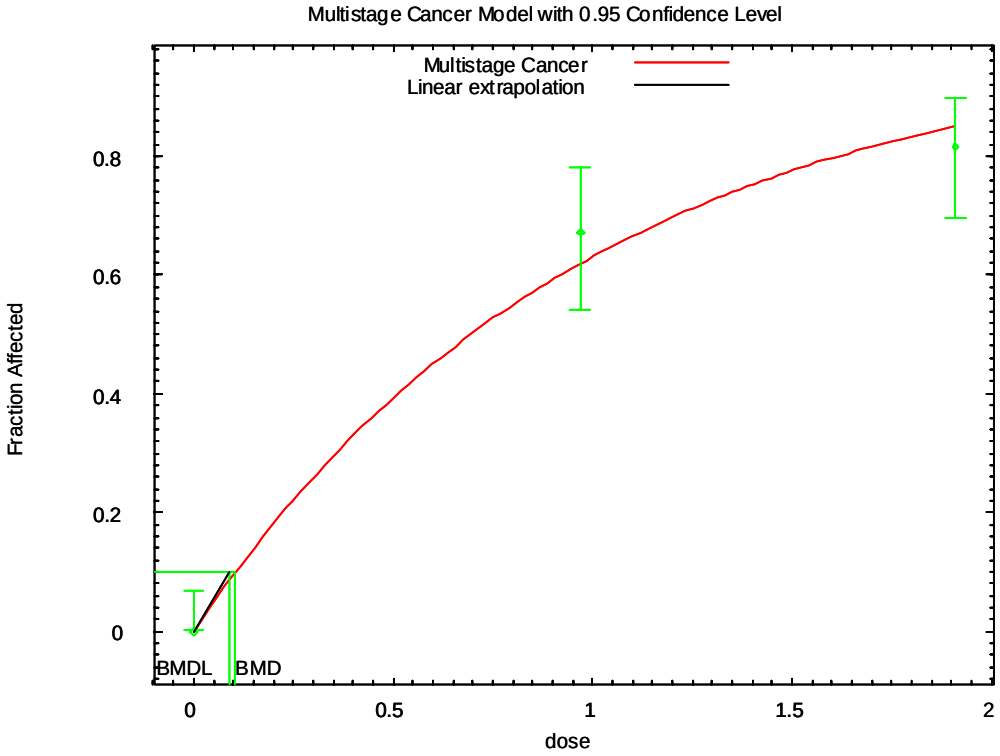
Figure F-9. Fit of log-logistic model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984); graph and model output.




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

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

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

   The form of the probability function is:

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


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

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



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =           0
                     intercept =    0.799142
                         slope =    0.894129


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified
by the user,
```

```
                 and do not appear in the correlation matrix )

              intercept        slope

 intercept            1        -0.68

     slope        -0.68            1


                                Parameter Estimates

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

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

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

           AIC:         195.847


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

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


   Benchmark Dose Computation

Specified effect =            0.7

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        1.07152

             BMDL =       0.478669
```

Figure F-11. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Grimmer et al., 1984), highest dose dropped; graph and model output.



```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File:
C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Grimmer1984_drophidose_Setting.(d)
         Gnuplot Plotting File:
C:\Usepa\BMDS21\Data\msc_benzo[a]pyrene_Grimmer1984_drophidose_Setting.plt

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

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

   The form of the probability function is:

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

   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 = 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 =    0.0806622
                        Beta(1) =      0.88595
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -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(1)

   Beta(1)           1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper
Conf. Limit
     Background                0            *                *                  *
        Beta(1)         0.997118            *                *                  *
        Beta(2)                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         -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
                                                        Scaled
```

Dose	Est._Prob.	Expected	Observed	Size	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 = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.105665

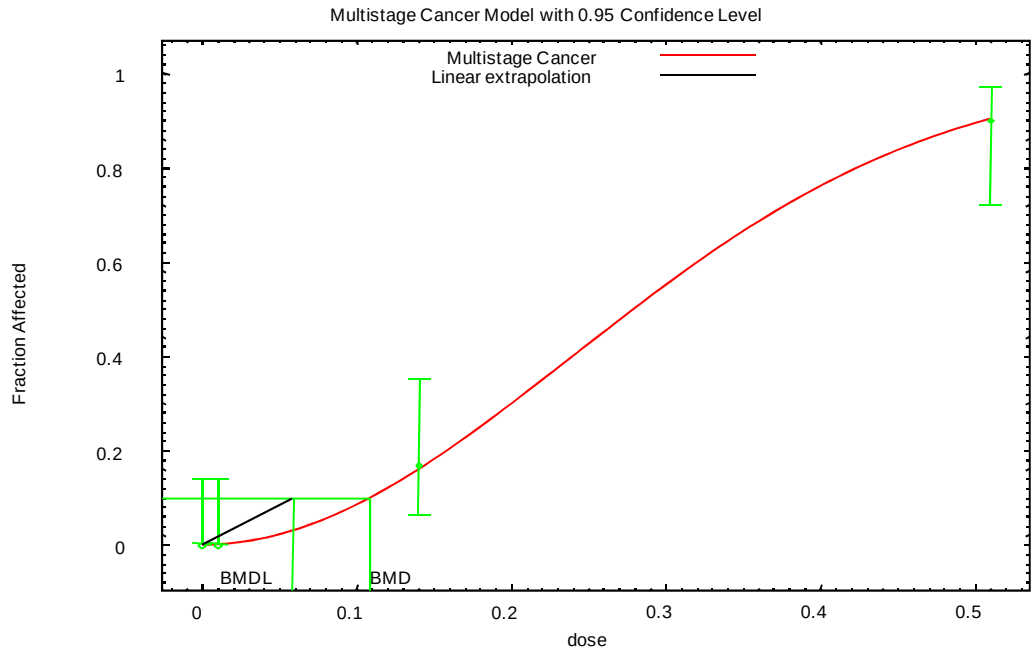
BMDL = 0.0881529

BMDU = 0.149328

Taken together, (0.0881529, 0.149328) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 1.13439

Figure F-12. Fit of multistage model to skin tumors in female CFLP mice exposed dermally to benzo[a]pyrene (Sivak et al., 1997); graph and model output.




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

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

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = NumAff
   Independent variable = LADD

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


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


                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =   0.0936505
                        Beta(2) =     8.67239
```

```
            Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(2)

   Beta(2)           1



                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     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
                                                                      Scaled
      Dose     Est._Prob.    Expected    Observed     Size       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
```

APPENDIX G. Additional Information in Support of the Dermal Slope factor

Mouse Dermal Carcinogenesis Exposure Methods

Studies which macroscopically examined systemic organs for tumors include Roe et al., 1970; Schmidt et al., 1973; Schmahl et al., 1977; Habs et al., 1980, 1984; Grimmer et al., 1983, 1984. The studies by Roe et al. 1970 and Habs et al. 1984 observed systemic tumors which the authors did not consider to be treatment related. The other studies which conducted post mortem macroscopic examinations of abnormal tissues, did not report any treatment related systemic effects.

Table G-1: Exposure methods for selected lifetime dermal exposure mouse cancer bioassays for benzo[a]pyrene-induced skin tumors

Mouse strain sex n	Applied dose of benzo[a]pyrene, times per week, vehicle	Application method, volume	Application region	Duration (weeks)	Comments	Reference
CeH/HeJ male 30	0, 0.05, 0.5, or 5 µg 2x/wk cyclohexane/acetone (1:1)	not specified 0.050 ml	shaved dorsal skin	104		Sivak et al., 1997
C57L male 13-55	0, 0.15, 0.38, 0.75, 3.8, 19, 94, 188, 376, or 752 µg 3x/wk toluene	calibrated needle dropper 0.0075 ml (solvent let dry between drops to limit spread)	shaved interscapular skin	103	mice were 18-20 weeks of age at the start of study "principal organs" examined	Poel, 1959
SWR male 14-25	0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 µg 3x/wk toluene	Calibrated needle pipette 0.0075 ml	Shaved interscapular skin	lifetime[1]	exposed until tumor development or death	Poel, 1960
C3HeB male 14-25	0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 µg benzo[a]pyrene 3x/wk toluene	Calibrated needle pipette 0.0075ml	Shaved interscapular skin	104 lifetime[1]	exposed until tumor development or death	Poel, 1960
A/He male 14-25	0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 µg benzo[a]pyrene 3x/wk toluene	Calibrated needle pipette 0.0075 ml	Shaved interscapular skin	104 lifetime[1]	exposed until tumor development or death	Poel, 1960
Swiss SPF female 50	0, 0.1, 0.3, 1, 3, or 9 µg 3x/wk acetone	Calibrated pipette 0.25 ml	Entire shaved dorsal area	93	systemic post-mortem exam	Roe et al., 1970

NMRI female 100	0, 0.05, 0.2, 0.8, or 2 µg 2x/wk acetone	Drop burette (dose delivered in single drop; volume not given)	Shaved back	104 lifetime[1]	macroscopic examination of internal organs	Schmidt et al., 1973
Swiss SPF female 80	0, 0.05, 0.2, 0.8, or 2 µg 2x/wk acetone	Drop burette (dose delivered in single drop; volume not given)	Shaved back	104 lifetime[1]	macroscopic examination of internal organs	Schmidt et al., 1973
NMRI female 100	0, 1, 1.7, or 3 µg 2x/wk acetone	Drop application with syringe 0.02 ml	Shaved back	104 lifetime[1]	macroscopic examination of internal organs	Schmahl et al., 1977
NMRI female 65	0, 1.7, 2.8, and 4.6 ug 2x/wk	Drop application by calibrated syringe, 0.02 ml	dorsal skin in interscapular area	104 lifetime[1]	macroscopic examination of internal organs	Habs et al., 1980
CFLP female 65	0, 3.9, 7.7 and 15.4 µg acetone/DMSO (1:3) 2x/wk	Drop 0.1 ml	Dorsal skin, interscapular area	104	macroscopic examination of internal organs	Grimmer et al., 1983
CFLP female 65	0, 3.4, 6.7, and 13.5 µg acetone:DMSO (1:3) 2x/wk	Drop 0.1 ml	Dorsal skin, interscapular area	104	macroscopic examination of internal organs	Grimmer et al., 1984
NMRI female 20	0, 2 or 4 µg acetone 2x/wk	drop application by calibrated syringe 0.01 ml	dorsal skin in interscapular area	lifetime[1]	macroscopic examination of internal organs	Habs et al., 1984

[1] Treated until natural death or sacrifice following tumor formation

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

Several publications which develop a dermal slope factor for benzo[a]pyrene are available in the peer reviewed literature (Knafla et al., 2010; 2006; Hussain et al., 1998; LaGoy and Quirk 1994; Sullivan et al., 1991). With the exception of the 2010 Knafla et al. publication, none of these approaches applied quantitative adjustments to account for interspecies differences, though the proposed slope factors were developed to account for human risk. Knafla et al. (2010) qualitatively discuss processes which could affect the extrapolation between mice and humans including skin metabolic activity adduct formation, stratum corneum thickness, epidermal thickness, etc. Ultimately, the authors apply an adjustment based on the increased epidermal thickness of human skin on the arms and hands compared to mouse interscapular epidermal thickness. They hypothesize that the carcinogenic potential of benzo[a]pyrene may be related to the thickness of the epidermal layer.

Because there is no established methodology for cross-species extrapolation of dermal toxicity, several alternative approaches were evaluated. Each approach begins with the POD of 0.066 µg/day that was based on a 10% extra risk for skin tumors in male mice (see Section 5.4.3). Based on the assumptions of each approach, a dermal slope factor for humans is calculated. The discussion of these approaches uses the following abbreviations:

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

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

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

$$DSF = 0.1/ POD_M$$

$$DSF= 0.1/0.066\ \mu g/day = \mathbf{2\ (\mu g/day)^{-1}}$$

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

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

Under this approach, animals and humans are assumed to have equal lifetime cancer risk with equal average whole body exposures in loading units (µg/cm^2-day). As long as doses are low enough that risk is proportional to the mass of applied compound, the daily dermal dose of benzo[a]pyrene can be normalized over the total surface area.

$$POD\ (\mu g/cm^2\text{-day}) = POD_{M/SA}\ (\mu g/cm^2\text{-day}) = POD_M\ (\mu g/day) / SA_M\ (cm^2)$$

$$POD = (0.066\ \mu g/day) / 100\ cm^2$$

= 0.00066 µg/cm^2-day

DSF = 0.1/(0.00066 µg/cm^2-day) ≈ **152 (µg/cm^2-day)$^{-1}$**

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

Approach 3. Cross-species adjustment based on body weight

Under this approach, a given mass of benzo[a]pyrene is normalized relative to the body weight of the animal or human. This approach has been used for oral doses for noncancer effects.

POD_M/ BW_M= 0.066 µg/0.035 kg-day = 1.9 µg/kg-day

DSF = 0.1/1.9 µg/kg-day = **0.05 (µg/kg-day)$^{-1}$**

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

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

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

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

$$POD\ (\mu g/day) = POD_M\ (\mu g/day) \times (BW_H / BW_M)^{3/4}$$

$$POD\ (\mu g/day) = 0.066\ \mu g/day \times (70\ kg / 0.035\ kg)^{3/4}$$
$$= 19.7\ \mu g/day$$

DSF = 0.1/(19.7 µg/day) ≈ **0.005 (µg/day)$^{-1}$**

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

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

Synthesis of the alternative approaches to cross-species scaling

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

Other potential interspecies adjustments

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

a. Quantitative information on interspecies differences in partitioning from exposure medium to the skin and absorption through the skin
b. Thickness of the stratum corneum between anatomical sites and between species
c. Thickness of epidermal layer
d. Skin permeability
e. Metabolic activity of skin
f. Formation of DNA adducts in skin

Table H-1. Alternative approaches to cross-species scaling

Approach	Assumptions	Dose metric	DSF	Risk at nominal exposure (0.0001 µg/day)*
1. Mass-per-day scaling	Equal mass per day (µg /d), if applied to equal areas of skin (cm^2), will affect similar numbers of cells across species. Cancer risk is proportional to the area (cm^2) exposed if the loading rate (µg /cm^2-d) is the same. This approach assumes that risk is proportional to dose expressed as mass per day. This approach implies that any combination of loading rate (µg /cm^2-day) and skin area exposed (cm^2) that have the same product when multiplied, will result in the same risk.	µg/day	1 per µg/day	2×10^{-4}
2. Surface-area scaling	Equal mass per day (µg /d), if applied to equal fractions of total skin surface (cm^2) will have similar cancer risks. That is, 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 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	152 per µg/cm^2-day	8×10^{-7}
3. Body-weight scaling	The skin is an organ with thickness and volume; benzo[a]pyrene is distributed within this volume of skin. Cancer risk is proportional to the concentration of benzo[a]pyrene in the exposed volume of skin. Equal mass per day (µg /d), if distributed within equal fractions of total body skin will have similar cancer risks. That is, whole-body lifetime exposure [e.g., 5%-of-the-body lifetime exposure] at the same loading rate (µg /cm^2-d) gives similar cancer risks across species. This approach assumes that risk is proportional to dose expressed as mass per kg body weight per day. This approach implies that any combination of dose (µg /day) and body weight (kg) that have the same result when divided, will result in the same risk.	µg/kg-day	0.05 per µg/kg-day	8×10^{-8}
4. Allometric scaling ($BW^{3/4}$)	Same as for body-weight scaling, except that benzo[a]pyrene distribution and metabolism takes place within this volume of skin. Allometric scaling is generally regarded as describing the relative rate of toxicokinetic processes across species. This approach also is used by EPA to scale oral exposures.	µg/day	0.005 per µg /day	5×10^{-7}

* Nominal exposure calculated as a geometric mean of average daily doses (µg/day) calculated from a range of benzo[a]pyrene soil concentrations (1- 1000 ppb) reported from non-contaminated rural/agricultural soils (ATSDR, 1995) and a range of standard exposure assumptions.