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Toxicological Review of Benzo[a]pyrene

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

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ABBREVIATIONS

1-OH-Py	1-hydroxypyrene
AchE	acetylcholine esterase
ADAF	age-dependent adjustment factor
Ah	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
AhR	aryl hydrocarbon receptor
AIC	Akaike's Information Criterion
AKR	aldo-keto reductase
AMI	acute myocardial infarction
ANOVA	analysis of variance
ARNT	Ah receptor nuclear translocator
AST	aspartate transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose, 95% lower bound
BMDS	Benchmark Dose Software
BMR	benchmark response
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide
BPQ	benzo[a]pyrene semiquinone
BrdU	bromodeoxyuridine
BSM	benzene-soluble matter
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAL/EPA	California Environmental Protection Agency
CASRN	Chemical Abstracts Service Registry Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHO	Chinese hamster ovary
CI	confidence interval
CYP	cytochrome
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
dbcAMP	dibutyl cyclic adenosine monophosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC	European Commission
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EROD	7-ethoxyresorufin-O-deethylase
ETS	environmental tobacco smoke
EU	European Union
Fe_2O_3	ferrous oxide
FSH	follicle stimulating hormone
GABA	gamma-aminobutyric acid
GD	gestational day
GI	gastrointestinal
GJIC	gap junctional intercellular communication
GSH	reduced glutathione
GST	glutathione-S-transferase
GSTM1	glutathione-S-transferase M1
hCG	human chorionic gonadotropin
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HFC	high-frequency cell
HPLC	high-performance liquid chromatography
hprt	hypoxanthine guanine phosphoribosyl transferase
HR	hazard ratio
Hsp90	heat shock protein 90
i.p.	intraperitoneal
i.v.	intravenous
Ig	immunoglobulin
IHD	ischemic heart disease
IRIS	Integrated Risk Information System
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MAP	mitogen-activated protein
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MIAME	Minimum Information About a Microarray Experiment
MLE	maximum likelihood estimate
MMAD	mass median aerodynamic diameter
MN	micronucleus
MPPD	Multi-Path Particle Deposition
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NCE	normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NIOSH	National Institute for Occupational Safety and Health
NK	natural-killer

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

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PREFACE

This Toxicological Review, prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) program, critically reviews the publicly available studies on benzo[a]pyrene in order to identify potential adverse health effects and to characterize exposure-response relationships. Benzo[a]pyrene is found in the environment and in food. Benzo[a]pyrene occurs in conjunction with other structurally related chemical compounds known as polycyclic aromatic hydrocarbons (PAHs).[1] Benzo[a]pyrene is universally present in these mixtures and is routinely analyzed and detected in environmental media contaminated with PAH mixtures: thus it is often used as an indicator chemical to measure exposure to PAH mixtures (Boström et al., 2002), and as an index chemical for deriving potency factors for PAH mixtures.

Benzo[a]pyrene is listed as a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), is found at 524 hazardous waste sites on the National Priorities List (NPL) and is ranked number 8 out of 275 chemicals on the Priority List of Hazardous Substances for CERCLA (ATSDR, 2011). This ranking is based on a combination of factors that include the frequency of occurrence at NPL sites, the potential for human exposure, and the potential health hazard. Benzo[a]pyrene is also listed as a drinking water contaminant under the Safe Drinking Water Act and a Maximum Contaminant Level Goal (MCLG) and enforceable Maximum Contaminant Level (MCL) have been established[2]. It is also one of the chemicals included in EPA's Persistent Bioaccumulative and Toxic Chemical Program (http://www.epa.gov/pbt/pubs/benzo.htm). In air, benzo[a]pyrene is regulated as a component in a class of chemicals referred to as Polycyclic Organic Matter, defined as a Hazardous Air Pollutant by the 1990 amendments to the Clean Air Act.

This assessment updates IRIS assessment of benzo[a]pyrene that was developed in 1987. The previous assessment included a cancer descriptor and oral slope factor. New information has become available, and this assessment reviews information on all health effects by all exposure routes. Organ/system-specific reference values are calculated based on developmental, reproductive, and immune system toxicity data. These reference values may be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system. In addition, in consideration of the Agency's need to estimate the potential

[1]PAHs are a large class of chemical compounds formed during the incomplete combustion of organic matter. They consist of only carbon and hydrogen arranged in two or more fused rings.
[2]MCLG = 0, MCL = 0.0002 mg/L.

for skin cancer from dermal exposure (U.S. EPA, 2004), especially in children exposed to contaminated soil, this assessment includes the IRIS Program's first dermal slope factor.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to Toxicological Reviews. Appendices for chemical and physical properties, toxicokinetic information, and summaries of toxicity studies are provided as *Supplemental Information* to this assessment.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

Chemical Properties

Benzo[a]pyrene is a five-ring PAH. It is a pale yellow crystalline solid with a faint aromatic odor. It is relatively insoluble in water and has low volatility. Benzo[a]pyrene is released to the air from both natural and anthropogenic sources and removed from the atmosphere by photochemical oxidation; reaction with nitrogen oxides, hydroxy and hydroperoxy radicals, ozone, sulfur oxides, and peroxyacetyl nitrate; and wet and dry deposition to land or water. In air, benzo[a]pyrene is predominantly adsorbed to particulates but may also exist as a vapor at high temperatures (ATSDR, 1995).

Uses and Pathways of Exposure

There is no known commercial use for benzo[a]pyrene; it is only produced as a research chemical. Benzo[a]pyrene is ubiquitous in the environment primarily as a result of incomplete combustion emissions. It is found in fossil fuels, crude oils, shale oils, and coal tars (HSDB, 2012). 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. Several studies have reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for a large proportion of PAHs in many watersheds (Rowe and O'Connor, 2011; Van Metre and Mahler, 2010; Mahler et al., 2005). Benzo[a]pyrene exposure can also occur to workers involved in the production of aluminum, coke, graphite, and silicon carbide, and in coal tar distillation. The major sources of non-occupational exposure are tobacco products, inhalation of polluted air, ingestion of contaminated food and water, and through cooking processes that involve smoke (HSDB, 2012). Dermal exposure can occur through contact with materials containing soot, tar, or crude petroleum, including pharmaceutical products containing coal tar, such as coal tar-based shampoos and treatments for eczema and psoriasis (Cal/EPA, 2010; IARC, 2010).

It persists for a long period of time in the atmosphere in the particulate phase and is thus efficiently transported over long distances. It is lipophilic with low water solubility; therefore, once deposited in water or sediments, it adsorbs strongly to sediments and particulate matter and

degrades slowly over several years (Cal/EPA, 2010; GLC, 2007). Because of its presence in high concentrations in the waters and sediments of the Great Lakes and St. Lawrence river ecosystem, it is 1 of the 12 level I substances identified and targeted for reduction in the Great Lakes Region (GLC, 2007).

Most aquatic organisms metabolize benzo[a]pyrene, eliminating it in days, and thus, it is not expected to bioconcentrate in these organisms; however, several aquatic organisms such as plankton, oysters, and some fish cannot metabolize benzo[a]pyrene (U.S. EPA, 2010a). Thus, the data on benzo[a]pyrene bioconcentration in aquatic organisms varies from low to very high (HSDB, 2012). Biomagnification of benzo[a]pyrene in the food chain has not been reported (ATSDR, 1995). Additional information on benzo[a]pyrene exposure and chemical properties can be found in Appendix A.

Implementation of the 2011 National Research Council Recommendations

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

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

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

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

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more

tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS benzo[a]pyrene assessment is in Phase 2 and represents a significant advancement in implementing the NRC recommendations shown in Table F-1 in Appendix F. The Program is implementing all of these recommendations, but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined in Table F-2 in Appendix F, including the development of a standardized approach to describe the strength of evidence for noncancer effects. In May 2014, the NRC released their report reviewing the IRIS assessment development process. As part of this review, the NRC reviewed current methods for evidence-based reviews and made several recommendations with respect to integrating scientific evidence for chemical hazard and dose-response assessments. In their report, the NRC states that EPA should continue to improve its evidence-integration process incrementally and enhance the transparency of its process. The committee did not offer a preference but suggests that EPA consider which approach best fits its plans for the IRIS process. The NRC recommendations will inform the IRIS Program's efforts in this area going forward. This effort is included in Phase 3 of EPA's implementation plan.

Assessments by Other National and International Health Agencies

Toxicity information on benzo[a]pyrene has been evaluated by the World Health Organization, Health Canada, the International Agency for Research on Cancer, and the European Union. The results of these assessments are presented in Appendix B. It is important to recognize that these assessments were prepared at different times, for different purposes, using different guidelines and methods, and that newer studies have been included in the IRIS assessment.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after the EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that the EPA provide "an ample margin of safety to protect public health;" the Safe Drinking Water Act, that "no adverse effects on the health of persons may reasonably be anticipated to occur, allowing an adequate margin of safety." Accordingly, the EPA uses information on the adverse effects of chemicals and on exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects from exposure to chemicals and to characterize exposure-response relationships. In terms set forth by the National Research Council (NRC, 1983), IRIS assessments cover the hazard identification and dose-response assessment steps of risk assessment, not the exposure assessment or risk characterization steps that are conducted by the EPA's program and regional offices and by other federal, state, and local health agencies that evaluate risk in specific populations and exposure scenarios. IRIS assessments are distinct from and do not address political, economic, and technical considerations that influence the design and selection of risk management alternatives.

An IRIS assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. These agents may be found in air, water, soil, or sediment. Exceptions are chemicals currently used exclusively as pesticides, ionizing and non-ionizing radiation, and criteria air pollutants listed under Section 108 of the Clean Air Act (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state health agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. Agents may be considered for reassessment as significant new studies are published. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. Other agents may also be assessed in response to an urgent public health need.

2. Process for developing and peer reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009 and enhanced in July 2013) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. The EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2014).

Before beginning an assessment, the IRIS Program discusses the scope with other EPA programs and regions to ensure that the assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the studies and analytical approaches that might contribute to their resolution.

Step 1. Development of a draft Toxicological Review. The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate study quality, identify health effects, identify

mechanistic events and pathways, integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

Step 2. Internal review by scientists in EPA programs and regions. The draft assessment is revised to address the comments from within the EPA.

Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President. The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and the EPA's response to major comments become part of the public record.

Step 4. Public review and comment, followed by external peer review. The EPA releases the draft assessment for public review and comment. A public meeting provides an opportunity to discuss the assessment prior to peer review. Then the EPA releases a draft for external peer review. The peer reviewers also receive written and oral public comments and the peer review meeting is open to the public. The peer reviewers assess whether the evidence has been assembled and evaluated according to guidelines and whether the conclusions are justified by the evidence. The peer review draft, written public comments, and peer review report become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary. The draft assessment is revised to reflect the peer review comments, public comments, and newly published studies that are critical to the conclusions of the assessment. The disposition of peer review comments and public comments becomes part of the public record.

Step 6. Final EPA review and interagency science discussion with other federal agencies and the Executive Offices of the President. The draft assessment and summary are revised to address the EPA and interagency comments. The science discussion draft, written interagency comments, and the EPA's response to major comments become part of the public record.

Step 7. Completion and posting. The Toxicological Review and IRIS summary are posted on the IRIS website (http://www.epa.gov/iris/).

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (U.S. EPA, 2005a, b, 2000b, 1998, 1996, 1991c, 1986a, b) and other methods (U.S. EPA, 2012a, c, 2011, 2006a, b, 2002, 1994). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

3. Identifying and selecting pertinent studies

3.1. Identifying studies

Before beginning an assessment, the EPA conducts a comprehensive search of the primary scientific literature. The literature search follows standard practices and includes the PubMed and ToxNet databases of the National Library of Medicine, Web of Science, and other databases listed in the EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Searches for information on

mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.

Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. The EPA posts the results of the literature search on the IRIS website and requests information from the public on additional studies and ongoing research.

The EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act. Material submitted as Confidential Business Information is considered only if it includes health and safety data that can be publicly released. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, the EPA will have it peer-reviewed.

The EPA also examines the toxicokinetics of the agent to identify other chemicals (for example, major metabolites of the agent) to include in the assessment if adequate information is available, in order to more fully explain the toxicity of the agent and to suggest dose metrics for subsequent modeling.

In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components. The literature search seeks, in decreasing order of preference (U.S. EPA, 2000b, §2.2; 1986c, §2.1):

- Studies of the mixture being assessed.
- Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.
- Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

3.2. Selecting pertinent epidemiologic studies

Study design is the key consideration for selecting pertinent epidemiologic studies from the results of the literature search.

- Cohort studies, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological studies (geographic correlation studies) relate exposures and effects by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.
- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare effect or about the relevance of analogous results in animals.

The assessment briefly reviews ecological studies and case reports but reports details only if they suggest effects not identified by other studies.

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental animal studies or human clinical studies.

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered most pertinent to human environmental exposure.
- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may

be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

Exposure duration is also a key design consideration for selecting pertinent experimental animal studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from less-than-chronic exposure are pertinent but less preferred for identifying effects from lifetime human exposure. Such studies may be indicative of effects from less-than-lifetime human exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information.

For developmental toxicity and reproductive toxicity, irreversible effects may result from a brief exposure during a critical period of development. Accordingly, specialized study designs are used for these effects (U.S. EPA, 2006b, 1998, 1996, 1991c).

4. Evaluating the quality of individual studies

After the subsets of pertinent epidemiologic and experimental studies have been selected from the literature searches, the assessment evaluates the quality of each individual study. This evaluation considers the design, methods, conduct, and documentation of each study, but not whether the results are positive, negative, or null. The objective is to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

4.1. Evaluating the quality of epidemiologic studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each epidemiologic study in the overall evaluation (U.S. EPA, 2005a, 1998, 1996, 1994, 1991c):

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error (can lead to misclassification of exposure, health outcomes, and other factors) and other types of information bias.
- Potential confounding and other sources of bias addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is related to both exposure and outcome and is sufficiently prevalent to result in bias.

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating epidemiologic studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991c).

4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each

experimental animal study, in vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991c). Research involving human subjects is considered only if conducted according to ethical principles.

- Documentation of study design, animals or study population, methods, basic data, and results.
- Nature of the assay and validity for its intended purpose.
- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.
- Sample sizes and statistical power to detect dose-related differences or trends.
- Ascertainment of survival, vital signs, disease or effects, and cause of death.
- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (U.S. EPA, 2005a, §2.2.2.1.3).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991c). In multigeneration studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (U.S. EPA, 1998, §3.1.2.4.5.4; 1991c, §3.1.1.4).

4.3. Reporting study results

The assessment uses evidence tables to present the design and key results of pertinent studies. There may be separate tables for each site of toxicity or type of study.

If a large number of studies observe the same effect, the assessment considers the study quality characteristics in this section to identify the strongest studies or types of study. The tables present details from these studies and the assessment explains the reasons for not reporting details of other studies or groups of studies that do not add new information. Supplemental information provides references to all studies considered, including those not summarized in the tables.

The assessment discusses strengths and limitations that affect the interpretation of each study. If the interpretation of a study in the assessment differs from that of the study authors, the assessment discusses the basis for the difference.

As a check on the selection and evaluation of pertinent studies, the EPA asks peer reviewers to identify studies that were not adequately considered.

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the

agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

Causal inference involves scientific judgment, and the considerations are nuanced and complex. Several health agencies have developed frameworks for causal inference, among them the U.S. Surgeon General (CDC, 2004; HEW, 1964), the International Agency for Research on Cancer (IARC, 2006) , the Institute of Medicine (IOM, 2008), and the U.S. EPA. (2010b, §1.6; 2005a, §2.5). Although developed for different purposes, the frameworks are similar in nature and provide an established structure and language for causal inference. Each considers aspects of an association that suggest causation, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998), and U.S. EPA (2005a, §2.2.1.7; 1994, §2.2.1.7).

Strength of association: The finding of a large relative risk with narrow confidence intervals strongly suggests that an association is not due to chance, bias, or other factors. Modest relative risks, however, may reflect a small range of exposures, an agent of low potency, an increase in an effect that is common, exposure misclassification, or other sources of bias.

Consistency of association: An inference of causation is strengthened if elevated risks are observed in independent studies of different populations and exposure scenarios. Reproducibility of findings constitutes one of the strongest arguments for causation. Discordant results sometimes reflect differences in study design, exposure, or confounding factors.

Specificity of association: As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less informative aspect of causation, unless the effect is rare or unlikely to have multiple causes.

Temporal relationship: A causal interpretation requires that exposure precede development of the effect.

Biologic gradient (exposure-response relationship): Exposure-response relationships strongly suggest causation. A monotonic increase is not the only pattern consistent with causation. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causation is strengthened by data demonstrating plausible biologic mechanisms, if available. Plausibility may reflect subjective prior beliefs if there is insufficient understanding of the biologic process involved.

Coherence: An inference of causation is strengthened by supportive results from animal experiments, toxicokinetic studies, and short-term tests. Coherence may also be found in other lines of evidence, such as changing disease patterns in the population.

"Natural experiments": A change in exposure that brings about a change in disease frequency provides strong evidence, as it tests the hypothesis of causation. An example would be an intervention to reduce exposure in the workplace or environment that is followed by a reduction of an adverse effect.

Analogy: Information on structural analogues or on chemicals that induce similar mechanistic events can provide insight into causation.

These considerations are consistent with guidelines for systematic reviews that evaluate the quality and weight of evidence. Confidence is increased if the magnitude of effect is large, if there is evidence of an exposure-response relationship, or if an association was observed and the plausible biases would tend to decrease the magnitude of the reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al., 2008b; Guyatt et al., 2008a).

5.2. Evaluating evidence in humans

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole. The objective is to determine whether a credible association has been observed and, if so, whether that association is consistent with causation. In doing this, the assessment explores alternative explanations (such as chance, bias, and confounding) and draws a conclusion about whether these alternatives can satisfactorily explain any observed association.

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may select a standard descriptor to characterize the epidemiologic evidence of association between exposure to the agent and occurrence of a health effect.

Sufficient epidemiologic evidence of an association consistent with causation: The evidence establishes a causal association for which alternative explanations such as chance, bias, and confounding can be ruled out with reasonable confidence.

Suggestive epidemiologic evidence of an association consistent with causation: The evidence suggests a causal association but chance, bias, or confounding cannot be ruled out as explaining the association.

Inadequate epidemiologic evidence to infer a causal association: The available studies do not permit a conclusion regarding the presence or absence of an association.

Epidemiologic evidence consistent with no causal association: Several adequate studies covering the full range of human exposures and considering susceptible populations, and for which alternative explanations such as bias and confounding can be ruled out, are mutually consistent in not finding an association.

5.3. Evaluating evidence in animals

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Several concepts discussed by Hill (1965) are pertinent to the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 2005a, §2.2.1.7; 1994, Appendix C)}.

In weighing evidence from multiple experiments, U.S. EPA (2005a), §2.5 distinguishes:

Conflicting evidence (that is, mixed positive and negative results in the same sex and strain using a similar study protocol) from

Differing results (that is, positive results and negative results are in different sexes or strains or use different study protocols).

Negative or null results do not invalidate positive results in a different experimental system. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological differences (for example, relative sensitivity of the tests, differences in

dose levels, insufficient sample size, or timing of dosing or data collection).

It is well established that there are critical periods for some developmental and reproductive effects (U.S. EPA, 2006b, 2005a, b, 1998, 1996, 1991c). Accordingly, the assessment determines whether critical periods have been adequately investigated. Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

In evaluating evidence of genetic toxicity:

- Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (in vivo) provides the strongest evidence.
- This is followed by positive results in lower organisms or in cultured cells (in vitro) or for other genetic events.
- Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC, 2006).

For germ-cell mutagenicity, the EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (U.S. EPA, 1986a, §2.3).

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

- The biologic plausibility of a causal interpretation of human studies.
- The generalizability of animal studies to humans.
- The susceptibility of particular populations or lifestages.

The focus of the analysis is to describe, if possible, mechanistic pathways that lead to a health effect. These pathways encompass:

- *Toxicokinetic processes* of absorption, distribution, metabolism, and elimination that lead to the formation of an active agent and its presence at the site of initial biologic interaction.
- *Toxicodynamic processes* that lead to a health effect at this or another site (also known as a *mode of action*).

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. Information on mode of action is not required for a conclusion that the agent is causally related to an effect (U.S. EPA, 2005a, §2.5).

The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a, §2.4.3.4).

1) **Is the hypothesized mode of action sufficiently supported in test animals?** Strong support for a key event being necessary to a mode of action can come from experimental challenge to the hypothesized mode of action, in which studies that suppress a key event observe suppression of the effect. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, much more so than by replicate experiments in the same model. The assessment may consider various aspects of causation in addressing this question.

2) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold

for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

3) **Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?** The assessment reviews the key events to identify populations and lifestages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or lifestages.

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (U.S. EPA, 2005a, §2.4.3.3). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008a).

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity relationships to mutagenic carcinogens (U.S. EPA, 2005a ,§2.3.5).

5.5. Characterizing the overall weight of the evidence

After evaluating the human, animal, and mechanistic evidence pertinent to an effect, the assessment answers the question: Does the agent cause the adverse effect (NRC, 2009, 1983)? In doing this, the assessment develops a narrative that integrates the evidence pertinent to causation. To provide clarity and consistency, the narrative includes a standard hazard descriptor. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a, §2.5).

Carcinogenic to humans: There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive animal evidence, identification of key precursor events in animals, and strong evidence that they are anticipated to occur in humans.

Likely to be carcinogenic to humans: The evidence demonstrates a potential hazard to humans but does not meet the criteria for *carcinogenic.* There may be a plausible association in humans, multiple positive results in animals, or a combination of human, animal, or other experimental evidence.

Suggestive evidence of carcinogenic potential: The evidence raises concern for effects in humans but is not sufficient for a stronger conclusion. This descriptor covers a range of evidence, from a positive result in the only available study to a single positive result in an extensive database that includes negative results in other species.

Inadequate information to assess carcinogenic potential: No other descriptors apply. *Conflicting evidence* can be classified as *inadequate information* if all positive results are opposed by negative studies of equal quality in the same sex and strain. *Differing results*, however, can be classified as *suggestive evidence* or as *likely to be carcinogenic*.

Not likely to be carcinogenic to humans: There is robust evidence for concluding that there is no basis for concern. There may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

Multiple descriptors may be used if there is evidence that carcinogenic effects differ by dose range or exposure route (U.S. EPA, 2005a, §2.5).

Another example of standard descriptors comes from EPA's Integrated Science Assessments, which evaluate causation for the effects of the criteria pollutants in ambient air (U.S. EPA, 2010b, §1.6).

Causal relationship: Sufficient evidence to conclude that there is a causal relationship. Observational studies cannot be explained by plausible alternatives, or they are supported by other lines of evidence, for example, animal studies or mechanistic information.

Likely to be a causal relationship: Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but coexposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

Suggestive of a causal relationship: At least one high-quality epidemiologic study shows an association but other studies are inconsistent.

Inadequate to infer a causal relationship: The studies do not permit a conclusion regarding the presence or absence of an association.

Not likely to be a causal relationship: Several adequate studies, covering the full range of human exposure and considering susceptible populations, are mutually consistent in not showing an effect at any level of exposure.

The EPA is investigating and may on a trial basis use these or other standard descriptors to characterize the overall weight of the evidence for effects other than cancer.

6. Selecting studies for derivation of toxicity values

For each effect where there is credible evidence of an association with the agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The decision to derive toxicity values may be linked to the hazard descriptor.

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal:

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.
- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model can be used to extrapolate across exposure routes.
- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.

- Studies with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with nonmonotonic exposure-response relationships are not necessarily excluded from the analysis. A diminished effect at higher exposure levels may be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, exposure misclassification, or selection bias.

If a large number of studies are suitable for dose-response analysis, the assessment considers the study characteristics in this section to focus on the most informative data. The assessment explains the reasons for not analyzing other groups of studies. As a check on the selection of studies for dose-response analysis, the EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

The EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a, §3).

Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis. The modeling yields a *point of departure* (an exposure level near the lower end of the observed range, without significant extrapolation to lower doses; see Sections 7.2 and 7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (see Sections 7.4 and 7.5). If response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors to the point of departure in order to account for sources of uncertainty and variability (see Section 7.6).

For a group of agents that induce an effect through a common mode of action, the dose-response analysis may derive a *relative potency factor* for each agent. A full dose-response analysis is conducted for one well-studied *index chemical* in the group, then the potencies of other members are expressed in relative terms based on relative toxic effects, relative absorption or metabolic rates, quantitative structure-activity relationships, or receptor binding characteristics (U.S. EPA, 2005a, §3.2.6; 2000b, §4.4).

Increasingly, EPA is basing toxicity values on combined analyses of multiple data sets or multiple responses. The EPA also considers multiple dose-response approaches if they can be supported by robust data.

7.2. Modeling dose to sites of biologic effects

The preferred approach for analysis of dose is toxicokinetic modeling because of its ability to incorporate a wide range of data. The preferred dose metric would refer to the active agent at the site of its biologic effect or to a close, reliable surrogate measure. The active agent may be the administered chemical or a metabolite. Confidence in the use of a toxicokinetic model depends on the robustness of its validation process and on the results of sensitivity analyses (U.S. EPA, 2006a; 2005a, §3.1; 1994, §4.3).

Because toxicokinetic modeling can require many parameters and more data than are typically available, the EPA has developed standard approaches that can be applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a

longer duration (U.S. EPA, 2005a, §3.1.1; 1991c, §3.2).

- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.
  - Oral doses are scaled allometrically using mg/kg$^{3/4}$-d as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011; 2005a, §3.1.3).
  - Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 2012a; 1994, §3).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a, §3.1.4).

In the absence of study-specific data on, for example, intake rates or body weight, the EPA has developed recommended values for use in dose-response analysis (U.S. EPA, 1988).

7.3. Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to an effect. Such models require sufficient data to ascertain a mode of action and to quantitatively support model parameters associated with its key events. Because different models may provide equivalent fits to the observed data but diverge substantially at lower doses, critical biologic parameters should be measured from laboratory studies, not by model fitting. Confidence in the use of a toxicodynamic model depends on the robustness of its validation process and on the results of sensitivity analyses. Peer review of the scientific basis and performance of a model is essential (U.S. EPA, 2005a, §3.2.2).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, the EPA has developed a standard set of empirical ("curve-fitting") models (http://www.epa.gov/ncea/bmds/) that can be applied to typical data sets, including those that are nonlinear. The EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2012c). Additional judgment or alternative analyses are used if the procedure fails to yield reliable results, for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses (U.S. EPA, 2005a, §3.2.3).

Modeling is used to derive a point of departure (U.S. EPA, 2012c; 2005a, §3.2.4). (See Section 7.6 for alternatives if a point of departure cannot be derived by modeling.)

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).
- For nonlinear approaches, both statistical and biologic considerations are taken into account.
  - For dichotomous data, a response level of 10% extra risk is generally used for minimally adverse effects, 5% or lower for more severe effects.
  - For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for

minimally adverse effects, one-half standard deviation for more severe effects.

The point of departure is the 95% lower bound on the dose associated with the selected response level.

7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data, considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1 and §3.3.2).

1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model below the observed range if significant model uncertainty can be ruled out with reasonable confidence.

2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:

   - Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.
   - Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

   Linear extrapolation is also used when data are insufficient to establish mode of action and when scientifically plausible.

   The result of linear extrapolation is described by an oral slope factor or an inhalation unit risk, which is the slope of the dose-response curve at lower doses or concentrations, respectively.

3) Nonlinear models are used for extrapolation if there are sufficient data to ascertain the mode of action and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. Nonlinear approaches generally should not be used in cases where mode of action has not been ascertained. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

4) Both linear and nonlinear approaches may be used if there are multiple modes of action. For example, modeling to a low response level can be useful for estimating the response at doses where a high-dose mode of action would be less important.

If linear extrapolation is used, the assessment develops a candidate slope factor or unit risk for each suitable data set. These results are arrayed, using common dose metrics, to show the distribution of relative potency across various effects and experimental systems. The assessment then derives or selects an overall slope factor and an overall unit risk for the agent, considering the various dose-response analyses, the study preferences discussed in Section 6, and the possibility of basing a more robust result on multiple data sets.

7.5. Considering susceptible populations and lifestages

The assessment analyzes the available information on populations and lifestages that may be particularly susceptible to each effect. A tiered approach is used (U.S. EPA, 2005a, §3.5).

5) If an epidemiologic or experimental study reports quantitative results for a susceptible population or lifestage, these data are analyzed to derive separate toxicity values for susceptible individuals.

6) If data on risk-related parameters allow comparison of the general population and susceptible individuals, these data are used to adjust the general-population toxicity values for application to susceptible individuals.

7) In the absence of chemical-specific data, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential carcinogens that have a mutagenic mode of action. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, the EPA recommends (U.S. EPA, 2005b, §5):

   - 10-fold adjustment for exposures before age 2 years.
   - 3-fold adjustment for exposures between ages 2 and 16 years.

7.6. Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002, §4.2). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a necessary key event does not occur below a specific dose. Reference values provide no information about risks at higher exposure levels.

The assessment characterizes effects that form the basis for reference values as adverse, considered to be adverse, or a precursor to an adverse effect. For developmental toxicity, reproductive toxicity, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1998, 1996, 1991c).

To account for uncertainty and variability in the derivation of a lifetime human exposure where adverse effects are not anticipated to occur, reference values are calculated by applying a series of *uncertainty factors* to the point of departure. If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level or a lowest-observed-adverse-effect level is used instead. The assessment discusses scientific considerations involving several areas of variability or uncertainty.

Human variation: The assessment accounts for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. A factor of 10 is generally used to account for this variation. This factor is reduced only if the point of departure is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991c, §3.4).

Animal-to-human extrapolation: If animal results are used to make inferences about humans, the assessment adjusts for cross-species differences. These may arise from differences in toxicokinetics or toxicodynamics. Accordingly, if the point of departure is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. In most other cases, a factor of 10 is applied (U.S. EPA, 2011; 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991c, §3.4).

Adverse-effect level to no-observed-adverse-effect level: If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making

this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991c, §3.4).

Subchronic-to-chronic exposure: If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure. This factor may also be applied for developmental or reproductive effects if exposure covered less than the full critical period. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1994, §4.3.9.1).

Incomplete database: If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991c, §3.4). The size of the factor depends on the nature of the database deficiency. For example, the EPA typically follows the suggestion that a factor of 10 be applied if both a prenatal toxicity study and a two-generation reproduction study are missing and a factor of $10^{1/2}$ if either is missing (U.S. EPA, 2002, §4.4.5).

In this way, the assessment derives candidate values for each suitable data set and effect that is credibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (U.S. EPA, 1994, §4.3.9).

The assessment derives or selects an *organ- or system-specific reference value* for each organ or system affected by the agent. The assessment explains the rationale for each organ/system-specific reference value (based on, for example, the highest quality studies, the most sensitive outcome, or a clustering of values). By providing these organ/system-specific reference values, IRIS assessments facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (NRC, 2009).

The assessment then selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur. This is generally the most sensitive organ/system-specific reference value, though consideration of study quality and confidence in each value may lead to a different selection.

7.7. Confidence and uncertainty in the reference values

The assessment selects a standard descriptor to characterize the level of confidence in each reference value, based on the likelihood that the value would change with further testing. Confidence in reference values is based on quality of the studies used and completeness of the database, with more weight given to the latter. The level of confidence is increased for reference values based on human data supported by animal data (U.S. EPA, 1994, §4.3.9.2).

High confidence: The reference value is not likely to change with further testing, except for mechanistic studies that might affect the interpretation of prior test results.

Medium confidence: This is a matter of judgment, between high and low confidence.

Low confidence: The reference value is especially vulnerable to change with further testing.

These criteria are consistent with guidelines for systematic reviews that evaluate the quality of evidence. These also focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al., 2008b).

All assessments discuss the significant uncertainties encountered in the analysis. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

References

CDC (Centers for Disease Control and Prevention). (2004). The health consequences of smoking: A report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services. http://www.surgeongeneral.gov/library/smokingconsequences/

Guyatt, GH; Oxman, AD; Kunz, R; Vist, GE; Falck-Ytter, Y; Schünemann, HJ. (2008a). GRADE: What is "quality of evidence" and why is it important to clinicians? [Review]. BMJ 336: 995-998. http://dx.doi.org/10.1136/bmj.39490.551019.BE

Guyatt, GH; Oxman, AD; Vist, GE; Kunz, R; Falck-Ytter, Y; Alonso-Coello, P; Schünemann, HJ. (2008b). GRADE: An emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336: 924-926. http://dx.doi.org/10.1136/bmj.39489.470347.AD

HEW (U.S. Department of Health, Education and Welfare). (1964). Smoking and health: Report of the advisory committee to the surgeon general of the public health service. Washington, DC: U.S. Department of Health, Education, and Welfare. http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/NNBBMQ

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300.

IARC (International Agency for Research on Cancer). (2006). Preamble to the IARC monographs. Lyon, France. http://monographs.iarc.fr/ENG/Preamble/

IOM (Institute of Medicine). (2008). Improving the presumptive disability decision-making process for veterans. In JM Samet; CC Bodurow (Eds.). Washington, DC: National Academies Press. http://www.nap.edu/openbook.php?record_id=11908

NRC (National Research Council). (1983). Risk assessment in the federal government: Managing the process. Washington, DC: National Academies Press. http://www.nap.edu/openbook.php?record_id=366&page=R1

NRC (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: National Academies Press. http://www.nap.edu/catalog/12209.html

Rothman, KJ; Greenland, S. (1998). Modern epidemiology (2nd ed.). Philadelphia, PA: Lippincott, Williams, & Wilkins.

U.S. EPA (U.S. Environmental Protection Agency). (1986a). Guidelines for mutagenicity risk assessment. (EPA/630/R-98/003). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/iris/backgrd.html

U.S. EPA (U.S. Environmental Protection Agency). (1986b). Guidelines for the health risk assessment of chemical mixtures. (EPA/630/R-98/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=22567

U.S. EPA (U.S. Environmental Protection Agency). (1986c). Guidelines for the health risk assessment of chemical mixtures. Fed Reg 51: 34014-34025.

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

U.S. EPA (U.S. Environmental Protection Agency). (1991c). Guidelines for developmental toxicity risk assessment. (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/guidelines-dev-toxicity-risk-assessment.htm

U.S. EPA (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993

U.S. EPA (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment. (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/REPRO51.PDF

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment. (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF

U.S. EPA (U.S. Environmental Protection Agency). (2000b). Supplementary guidance for conducting health risk assessment of chemical mixtures. (EPA/630/R-00/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=51717

U.S. EPA (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment. (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/cancerguidelines/

U.S. EPA (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. In US Environmental Protection Agency, Risk Assessment Forum (pp. 1125-1133). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/cancerguidelines/guidelines-carcinogen-supplement.htm

U.S. EPA (U.S. Environmental Protection Agency). (2006a). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (Final Report) [EPA Report]. (EPA/600/R-05/043F). Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668

U.S. EPA (U.S. Environmental Protection Agency). (2006b). A framework for assessing health risk of environmental exposures to children. (EPA/600/R-05/093F). Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363

U.S. EPA (U.S. Environmental Protection Agency). (2010b). Integrated science assessment for carbon monoxide [EPA Report]. (EPA/600/R-09/019F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=218686

U.S. EPA (U.S. Environmental Protection Agency). (2011). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/interspecies-extrapolation.htm

U.S. EPA (U.S. Environmental Protection Agency). (2012a). Advances in inhalation gas dosimetry for derivation of a reference concentration (RFC) and use in risk assessment. (EPA/600/R-12/044). Washington, DC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=244650

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2014). EPAs Integrated Risk Information System: Assessment development process [EPA Report]. Washington, DC. http://epa.gov/iris/process.htm

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EXECUTIVE SUMMARY

Occurrence and Health Effects

Benzo[a]pyrene is a five-ring polycyclic aromatic hydrocarbon (PAH). Benzo[a]pyrene (along with other PAHs) is released into the atmosphere as a component of smoke from forest fires, industrial processes, vehicle exhaust, cigarettes, and through the burning of fuel (such as wood, coal, and petroleum products). Oral exposure to benzo[a]pyrene can occur by eating certain food products, such as charred meats, where benzo[a]pyrene is formed during the cooking process or by eating foods grown in areas contaminated with benzo[a]pyrene (from the air and soil). Dermal exposure may occur from contact with soils or materials that contain soot, tar, or crude petroleum products or by using certain pharmaceutical products containing coal tars, such as those used to treat the skin conditions, eczema and psoriasis. The magnitude of human exposure to benzo[a]pyrene and other PAHs depends on factors such as lifestyle (e.g., diet, tobacco smoking), occupation, and living conditions (e.g., urban versus rural setting, domestic heating, and cooking methods).

Animal studies demonstrate that exposure to benzo[a]pyrene may be associated with developmental, reproductive, and immunological effects. In addition, epidemiology studies involving exposure to PAH mixtures have reported associations between internal biomarkers of exposure to benzo[a]pyrene (benzo[a]pyrene diol epoxide-DNA adducts) and adverse birth outcomes (including reduced birth weight, postnatal body weight, and head circumference) and decreased fertility.

Studies in multiple animal species demonstrate that benzo[a]pyrene is carcinogenic at multiple tumor sites (alimentary tract, liver, kidney, respiratory tract, pharynx, and skin) by all routes of exposure. In addition, there is strong 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. An increasing number of occupational studies demonstrate a positive exposure-response relationship with cumulative benzo[a]pyrene exposure and lung cancer.

Effects Other Than Cancer Observed Following Oral Exposure

In animals, oral exposure to benzo[a]pyrene has been shown to result in developmental toxicity, reproductive toxicity, and immunotoxicity. Developmental effects in rats and mice include neurobehavioral changes and cardiovascular effects following gestational exposures. Reproductive and immune effects include decreased sperm counts, ovary weight, and follicle numbers, and decreased immunoglobulin and B-cell numbers and thymus weight following oral exposures in adult animals. In humans, benzo[a]pyrene exposure occurs in conjunction with other PAHs and, as

such, attributing the observed effects to benzo[a]pyrene is complicated. However, human studies report associations between particular health endpoints and internal measures of exposure, such as benzo[a]pyrene-deoxyribonucleic acid (DNA) adducts, or external measures of benzo[a]pyrene exposure. Overall, the human studies report developmental and reproductive effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for hazards associated with benzo[a]pyrene exposure.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ- or system-specific RfDs were derived for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by neurobehavioral changes following neonatal exposure, was chosen as the basis for the proposed overall oral RfD as the available data indicate that neurobehavioral changes represent the most sensitive hazard of benzo[a]pyrene exposure. The neurodevelopmental study by Chen et al. (2012) was used to derive the RfD. The endpoint of altered anxiety-like behavior, as measured in the elevated plus maze, was selected as the critical effect due to the sensitivity of this endpoint and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling was utilized to derive the $BMDL_{1SD}$ of 0.09 mg/kg-day that was used as the point of departure (POD) for RfD derivation.

The proposed overall RfD was calculated by dividing the POD for altered anxiety-like behavior as measured in the elevated plus maze by a composite uncertainty factor (UF) of 300 to account for the extrapolation from animals to humans (10), for interindividual differences in human susceptibility (10), and for deficiencies in the toxicity database (3).

Table ES-1. Organ/system-specific RfDs and proposed overall RfD for benzo[a]pyrene

Effect	Basis	RfD (mg/kg-d)	Confidence
Developmental	Neurobehavioral changes Gavage neurodevelopmental study in rats (postnatal days [PNDs] 5–11) Chen et al. (2012)	3×10^{-4}	Medium
Reproductive	Decreased ovary weight Gavage subchronic (60 d) reproductive toxicity study in rats Xu et al. (2010)	4×10^{-4}	Medium
Immunological	Decreased thymus weight and serum IgM Gavage subchronic (35 d) study in rats De Jong et al. (1999)	2×10^{-3}	Low
Proposed Overall RfD	**Developmental toxicity**	3×10^{-4}	**Medium**

Confidence in the Overall Oral RfD

The overall confidence in the RfD is medium. Confidence in the principal study (Chen et al., 2012) is medium-to-high. The design, conduct, and reporting of this neurodevelopmental study was good and a wide variety of neurotoxicity endpoints were measured. Some informative experimental details were, however, omitted including the sensitivity of some assays at the indicated developmental ages and lack of reporting gender-specific data for all outcomes. Several subchronic and developmental studies covering a wide variety of endpoints are also available; however, the lack of a multigeneration toxicity study with exposure throughout development is not available. Therefore, confidence in the database is medium.

Effects Other Than Cancer Observed Following Inhalation Exposure

In animals, inhalation exposure to benzo[a]pyrene has been shown to result in developmental and reproductive toxicity. Studies in rats following inhalation exposure show decreased fetal survival and brain effects in offspring, and decreased testes weight and sperm counts in adult animals. Overall, the available human PAH mixtures studies report developmental and reproductive effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for the hazards associated with benzo[a]pyrene exposure.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

An attempt was made to derive organ- or system-specific RfCs for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-2). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by decreased fetal survival, was chosen as the basis for the proposed inhalation RfC as the available data indicate that developmental effects represent a sensitive hazard of benzo[a]pyrene exposure. The developmental inhalation study in rats by Archibong et al. (2002) and the observed decreased fetal survival following exposure to benzo[a]pyrene on gestation days (GDs) 11–20 were used to derive the overall RfC. The lowest-observed-adverse-effect level (LOAEL) of 25 μg/m^3 based on decreased fetal survival was selected as the POD. The LOAEL was adjusted to account for the discontinuous daily exposure to derive the POD_{ADJ} and the human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by 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, 1994). These adjustments resulted in a POD_{HEC} of 4.6 μg/m^3, which was used as the POD for RfC derivation.

The RfC was calculated by dividing the POD by a composite UF of 3,000 to account for toxicodynamic differences between animals and humans (3), interindividual differences in human susceptibility (10), LOAEL-to-no-observed-adverse-effect level (NOAEL) extrapolation (10), and deficiencies in the toxicity database (10).

Table ES-2. Organ/system-specific RfCs and proposed overall RfC for benzo[a]pyrene

Effect	Basis	RfC (mg/m^3)	Confidence
Developmental	Decreased fetal survival Developmental toxicity study in rats (GDs 11–20) Archibong et al. (2002)	2×10^{-6}	Low-medium
Reproductive	Reductions in testes weight and sperm parameters Subchronic (60 d) reproductive toxicity study in rats (Archibong et al. (2008); Ramesh et al. (2008))	Not calculated[a]	NA
Proposed Overall RfC	**Developmental toxicity**	2×10^{-6}	Low-medium

[a]Not calculated due to UF >3,000.

Confidence in the Overall Inhalation RfC

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 inhalation study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study and the lack of information on varied toxicity endpoints following subchronic and chronic inhalation exposure. However, confidence in the RfC is bolstered by consistent systemic effects observed by the oral route (including reproductive and developmental effects) and similar effects observed in human populations exposed to PAH mixtures.

Evidence for Human Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans" based on strong and consistent evidence in animals and humans. The evidence includes an extensive number of studies demonstrating carcinogenicity in multiple animal species exposed via all routes of administration and increased cancer risks, particularly in the lung and skin, in humans exposed to different PAH mixtures containing benzo[a]pyrene. Mechanistic studies provide strong supporting evidence that links the metabolism of benzo[a]pyrene to DNA-reactive agents with key mutational events in genes that can lead to tumor development. These events include formation of specific DNA adducts and characteristic mutations in oncogenes and tumor suppressor genes that have been observed in humans exposed to PAH mixtures. This combination of human, animal, and mechanistic evidence provides the basis for characterizing benzo[a]pyrene as "carcinogenic to humans."

Quantitative Estimate of Carcinogenic Risk From Oral Exposure

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,

forestomach tumors in male and female Sprague-Dawley rats, and forestomach, esophagus, tongue, and larynx tumors in female B6C3F$_1$ mice (male mice were not tested). Less-than-lifetime oral exposure to benzo[a]pyrene has also been associated with forestomach tumors in more than 10 additional bioassays with several strains of mice. The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. 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 up to 2 years, and included detailed reporting methods and results (including individual animal data).

Time-weighted, average daily doses were converted to human equivalent doses (HEDs) on the basis of (body weight)$^{3/4}$ scaling (U.S. EPA, 1992). EPA then used the multistage-Weibull model for the derivation of the oral slope factor. This model was used because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups. 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$_{10}$) reported by Kroese et al. (2001) and Beland and Culp (1998). The oral slope factor of **1 per mg/kg-day** based on the tumor response in the alimentary tract (forestomach, esophagus, tongue, and larynx) of female B6C3F$_1$ mice (Beland and Culp, 1998) was selected as the factor with the highest value (most sensitive) among a range of slope factors derived.

Quantitative Estimate of Carcinogenic Risk From Inhalation Exposure

Inhalation exposure to benzo[a]pyrene has been associated with squamous cell neoplasia in the larynx, pharynx, trachea, nasal cavity, esophagus, and forestomach of male Syrian golden hamsters exposed for up to 130 weeks to benzo[a]pyrene condensed onto NaCl particles (Thyssen et al., 1981). Supportive evidence for the carcinogenicity of inhaled benzo[a]pyrene comes from additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation. The Thyssen et al. (1981) bioassay represents the only study of lifetime exposure to inhaled benzo[a]pyrene.

A time-to-tumor dose-response model was fit to the time-weighted average (TWA) continuous exposure concentrations and the individual animal incidence data for the overall incidence of tumors in the upper respiratory tract or pharynx. The inhalation unit risk of **6×10^{-4} per µg/m^3** was calculated by linear extrapolation (slope factor = 0.1/BMCL$_{10}$) from a BMCL$_{10}$ of 0.16 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).

Quantitative Estimate of Carcinogenic Risk From 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, unrefined

mineral oils, shale oils, and soot. In animal models, numerous dermal bioassays have demonstrated an increased incidence of skin tumors with increasing dermal exposure of benzo[a]pyrene in all species tested (mice, rabbits, rats, and guinea pigs), although most benzo[a]pyrene bioassays have been conducted in mice. Due to the evidence supporting a hazard from exposure to benzo[a]pyrene by the dermal route (see Section 1.1.5) and the availability of quantitative information, a cancer slope factor for the dermal route was developed. The analysis in this assessment focuses on lifetime carcinogenicity bioassays in several strains of mice demonstrating increasing incidence of benign and malignant skin tumors following repeated dermal exposure to benzo[a]pyrene.

The National Institute for Occupational Safety and Health (NIOSH) study (Sivak et al., 1997; NIOSH, 1989) was selected as the best available study for dose-response analysis and extrapolation to lifetime cancer risk following dermal exposure to benzo[a]pyrene. This study used three exposure levels that highlighted the low-dose region and reported a number of attributes not available for the older studies, including single housing of mice, blinded assessment of tumor status, and time of appearance of tumors for each animal.

This mouse skin tumor incidence data was modeled using the multistage-Weibull model. The resulting $BMDL_{10}$ was adjusted for interspecies differences by allometric scaling. The dermal slope factor of **0.006 per µg/day** was calculated by linear extrapolation (slope factor = $0.1/BMDL_{10\text{-HED}}$) from the human equivalent POD for the occurrence of skin tumors in male mice exposed dermally to benzo[a]pyrene for 104 weeks. 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.

Susceptible Populations and Lifestages

Benzo[a]pyrene has been determined to be carcinogenic by a mutagenic mode of action in this assessment. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to have an increased risk for cancer. The oral slope factor of 1 per mg/kg-day, inhalation unit risk of 0.0006 per µg/m^3, and dermal slope factor of 0.006 per µg/day for benzo[a]pyrene, calculated from data applicable to adult exposures, do not reflect presumed early life susceptibility to this chemical. Although some chemical-specific data exist for benzo[a]pyrene that demonstrate increased early life susceptibility to cancer, these data were not considered sufficient to develop separate risk estimates for childhood exposure. 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 ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to benzo[a]pyrene.

Regarding effects other than cancer, there are epidemiological studies that report associations between developmental effects (decreased postnatal growth, decreased head

circumference, and neurodevelopmental delays), reproductive effects and internal biomarkers of exposure to benzo[a]pyrene. Studies in animals also indicate alterations in neurological development and heightened susceptibility to reproductive effects following gestational or early postnatal exposure to benzo[a]pyrene.

Key Issues Addressed in Assessment

The overall RfD and RfC were developed based on effects observed following exposure to benzo[a]pyrene during a critical window of development. The derivation of a general population toxicity value based on exposure during development has implications regarding the evaluation of populations exposed outside of the developmental period and the averaging of exposure to durations outside of the critical window of susceptibility. Discussion of these considerations is provided in Sections 2.1.5 and 2.2.5.

The dermal slope factor was developed based on data in animals. Because there is no established methodology for extrapolating dermal toxicity from animals to humans, several alternative approaches were evaluated (see Appendix D in Supplemental Information). Allometric scaling using body weight to the ¾ power was selected based on known species differences in dermal metabolism and penetration of benzo[a]pyrene.

LITERATURE SEARCH STRATEGY|STUDY SELECTION

The literature search strategy used to identify primary, peer-reviewed literature pertaining to benzo[a]pyrene was conducted using the databases listed in Table LS-1 (see Appendix C for the complete list of keywords used). References from previous assessments by the U.S. Environmental Protection Agency (EPA) and other national and international health organizations were also examined. EPA conducted a comprehensive, systematic literature search for benzo[a]pyrene through February, 2012. In addition, a search of the online database PubMed was conducted for the timeframe January 2012 through August 2014, to ensure inclusion of critical studies published since the initial literature search.

Table LS-1. Summary of the search strategy employed for benzo[a]pyrene

Database	Keywords
Pubmed Toxcenter Toxline	Chemical name (CASRN): benzo[a]pyrene (50-32-8) Synonyms: benzo[d,e,f]chrysene, benzo[def]chrysene, 3,4-benzopyrene, 1,2-benzpyrene, 3,4-bp, benz(a)pyrene, 3,4-benzpyren, 3,4-benzpyrene, 4,5-benzpyrene, 6,7-benzopyrene, benzopirene, benzo(alpha)pyrene Standard toxicology search keywords Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors
TSCATS ChemID Chemfinder CCRIS HSDB GENETOX RTECS	Searched by CASRNs and chemical names (including synonyms)

[a]Primary and secondary keywords used for the Pubmed, Toxcenter, and Toxline databases can be found in the Supplemental Information.

Figure LS-1 depicts the literature search, study selection strategy, and number of references obtained at each stage of literature screening. Approximately 20,700 references were identified with the initial keyword search. Based on a secondary keyword search followed by a preliminary manual screen of titles or abstracts by a toxicologist, approximately 1,190 references were identified that provided information potentially relevant to characterizing the health effects or physical and chemical properties of benzo[a]pyrene. A more detailed manual review of titles, abstracts, and/or papers was then conducted. Notable exclusions from the Toxicological Review

are large numbers of animal in vivo or in vitro studies designed to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of benzo[a]pyrene and toxicity studies of benzo[a]pyrene in nonmammalian species (e.g., aquatic species, plants).

For the updated literature search conducted for the timeframe January 2012 through August 2014, the search terms included benzo(a)pyrene AND (rat OR mouse OR mice) and results were screened manually by title, abstract, and/or full text using the exclusion criteria outlined in Figure LS-1. Relevant studies that could potentially impact the hazard characterization and dose-response assessment were identified and considered. No studies were identified that would impact the assessment's major conclusions. Several pertinent studies, published since the last comprehensive literature search, were identified and incorporated into the text where relevant.

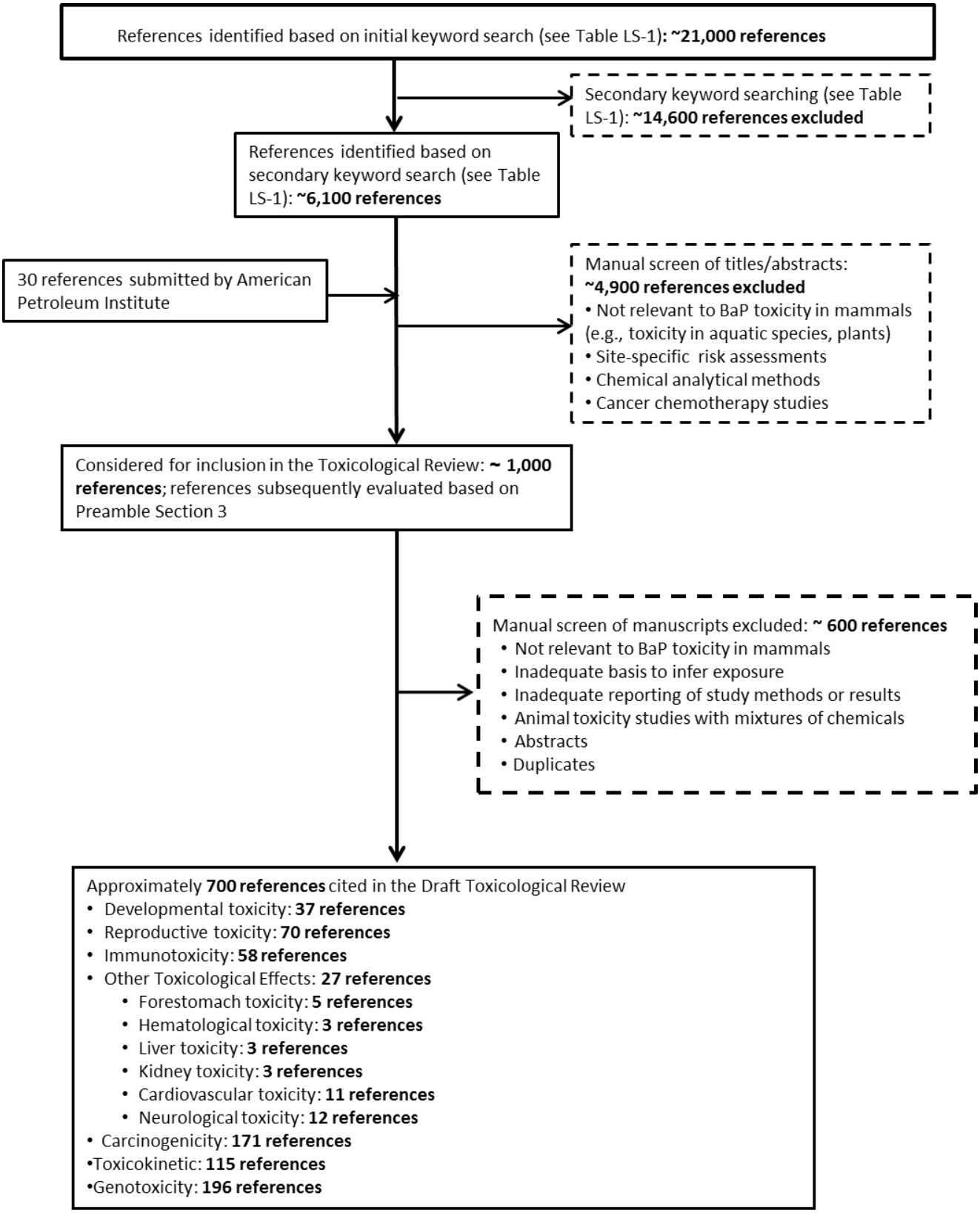

Figure LS-1. Study selection strategy.

Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment and general study quality considerations. In general, the relevance of health effect studies was evaluated as outlined in the Preamble and EPA guidance (*A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* (U.S. EPA, 1994)). The reasons for excluding epidemiological and animal studies from the references identified by the keyword search are provided in Figure LS-1.

The available studies examining the health effects of benzo[a]pyrene exposure in humans are discussed and evaluated in the hazard identification sections of the assessment (Section 1), with specific limitations of individual studies and of the collection of studies noted. The common major limitation of the human epidemiological studies (with respect to identifying potential adverse health outcomes specifically from benzo[a]pyrene) is that they all involve exposures to complex mixtures containing other PAHs and other compounds. The evaluation of the epidemiological literature focuses on studies in which possible associations between external measures of exposure to benzo[a]pyrene or biomarkers of exposure to benzo[a]pyrene (e.g., benzo[a]pyrene-DNA adducts or urinary biomarkers) and potential adverse health outcomes were evaluated. Pertinent mechanistic studies in humans (e.g., identification of benzo[a]pyrene-DNA adducts and characteristics of mutations in human tumors) were also considered in assessing the weight of evidence for the carcinogenicity of benzo[a]pyrene.

The health effects literature for benzo[a]pyrene is extensive. All animal studies of benzo[a]pyrene involving repeated oral, inhalation, or dermal exposure that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to benzo[a]pyrene. These studies were evaluated for aspects of design, conduct, or reporting that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of hazard potential using the study quality considerations outlined in the Preamble. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant.

Animal toxicity studies involving short-term duration and other routes of exposure were also evaluated to inform conclusions about health hazards, especially regarding mode of action. The references considered and cited in this document, including bibliographic information and abstracts, can be found on the Health and Environmental Research Online (HERO) website[2] (http://hero.epa.gov/benzoapyrene).

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[2]HERO (Health and Environmental Research On-line) is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

1. HAZARD IDENTIFICATION

1.1. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

NOTE: In the environment, benzo[a]pyrene occurs in conjunction with other structurally related chemical compounds known as polycyclic aromatic hydrocarbons (PAHs).[3] Accordingly, there are few epidemiologic studies designed to solely investigate the effects of benzo[a]pyrene. There are, however, many epidemiologic studies that have investigated the effects of exposure to PAH mixtures. Benzo[a]pyrene is universally present in these mixtures and is routinely analyzed and detected in environmental media contaminated with PAH mixtures, thus, it is often used an an indicator chemical to measure exposure to PAH mixtures (Boström et al., 2002).

1.1.1. Developmental Toxicity

Human and animal studies provide evidence for PAH- and benzo[a]pyrene-induced developmental effects. Effects on fetal survival, postnatal growth, and development have been demonstrated in human populations exposed to PAH mixtures during gestation. Animal studies demonstrate various effects including changes in fetal survival, pup weight, blood pressure, fertility, reproductive organ weight and histology, and neurological function in gestationally and/or early postnatally treated animals.

Altered Birth Outcomes

Human and animal studies provide evidence that benzo[a]pyrene exposure may lead to altered outcomes reflecting growth and development in utero or in early childhood. Two cohort studies in pregnant women in China and the United States examined cord blood levels of benzo[a]pyrene-7,8-diol-9,10 epoxide (BPDE)-deoxyribonucleic acid (DNA) adducts in relation to measures of child growth following exposure to PAH mixtures (Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004) (Table 1-1). In the Chinese cohort, high benzo[a]pyrene-adduct levels were associated with reduced weight at 18, 24, and 30 months of age, but not at birth (Tang et al., 2006). In the U.S. cohort, an independent effect on birth weight was not observed with either benzo[a]pyrene-adducts or environmental tobacco smoke (ETS) exposure; however, a doubling of cord blood adducts in combination with ETS exposure in utero was seen, corresponding to an 8% reduction in birth weight (Perera et al., 2005b). ETS, also called secondhand smoke, is the smoke given off by a burning tobacco product and the smoke exhaled by a smoker that contains over

[3]PAHs are a large class of chemical compounds formed during the incomplete combustion of organic matter.

7,000 chemicals including benzo[a]pyrene. No associations were seen with birth length (or height at later ages) in either of these cohort studies.

A Chinese case-control study indicated that PAH exposure may be associated with increased risk of fetal death (Wu et al., 2010). A strong association was seen between maternal blood benzo[a]pyrene-DNA adduct levels and risk of delayed miscarriage (fetal death before 14 weeks of gestation), with a fourfold increased risk for levels above compared with below the median. However, no significant difference in adduct levels was detected between fetal tissue from cases compared to controls.

Decreased fetal survival has also been noted in gestationally treated animals at relatively high doses by the oral and inhalation routes. An approximate 40% decrease in fetal survival was noted in mouse dams treated by gavage on GDs 7–16 at doses of 160 mg/kg-day, but no decreases were observed at 10 or 40 mg/kg-day (Mackenzie and Angevine, 1981). Several lower dose studies of rats treated on GDs 14–17 with doses of up to 1.2 mg/kg-day benzo[a]pyrene did not observe any difference in fetal survival (Jules et al., 2012; McCallister et al., 2008; Brown et al., 2007). By the inhalation route, fetal survival was decreased by 19% following exposure to 25 μg/m^3 benzo[a]pyrene on GDs 11–20 in F344 rats (Archibong et al., 2002). Another publication from the same group of collaborators Wu et al. (2003a) graphically reported fetal survival as part of a study analyzing metabolites of benzo[a]pyrene and activation of the aryl hydrocarbon receptor (AhR) and cytochrome P450 (CYP450) 1A1 (Wu et al., 2003a). This study did not report the number of dams or litters, and no numerical data were reported. The study authors reported statistically significant decreases in fetal survival at 75 and 100 μg/m^3 benzo[a]pyrene on GDs 11–20 compared to the 25 μg/m^3 group. An apparent decrease in fetal survival was also seen at 25 μg/m^3, but it was unclear whether or not this change was statistically significant compared to the vehicle control.

In animals (Table 1-2 and Figure 1-1), reduced body weight in offspring has also been noted in some developmental studies. Decreases in body weight (up to 13%) were observed in mice following prenatal gavage exposure (gestation days [GDs] 7–16), and as time from exposure increased (postnatal days [PNDs] 20–42), the dose at which effects were observed decreased (from 40 to 10 mg/kg-day, respectively) (Mackenzie and Angevine, 1981). In addition, decreases in body weight (approximately 10–15%) were observed in rats on PNDs 36 and 71 following gavage exposure at only 2 mg/kg-day on PNDs 5–11 (Chen et al., 2012). At doses up to 1.2 mg/kg-day and follow-up to PND 30, two developmental studies in rats did not observe decrements in pup body weight following treatment from GD 14 to 17 (Jules et al., 2012; McCallister et al., 2008). Maternal toxicity was not observed in mouse or rat dams exposed to up to 160 mg/kg-day benzo[a]pyrene (Jules et al., 2012; McCallister et al., 2008; Brown et al., 2007; Kristensen et al., 1995; Mackenzie and Angevine, 1981).

Fertility in Offspring

Several studies suggest that gestational exposure to maternal tobacco smoke decreases the future fertility of female offspring (Ye et al., 2010; Jensen et al., 1998; Weinberg et al., 1989)

(Table 1-1). In animal models, marked effects on the development of male and female reproductive organs and the fertility of animals exposed gestationally has also been demonstrated (Kristensen et al., 1995; Mackenzie and Angevine, 1981) (Table 1-2 and Figure 1-1). In two studies examining reproductive effects in mice, decreased fertility and fecundity in F1 animals was observed following exposure to doses ≥10 mg/kg-day during gestation (Kristensen et al., 1995; Mackenzie and Angevine, 1981). When F1 females were mated with untreated males, a dose-related decrease in fertility of >30% was observed, in addition to a 20% decrease in litter size starting at the lowest dose tested of 10 mg/kg-day (Mackenzie and Angevine, 1981). A dose-related decrease in fertility was also observed in male mice treated gestationally with benzo[a]pyrene. At the lowest dose tested (10 mg/kg-day), a 35% decrease in fertility was observed when gestationally exposed animals were mated with untreated females (Mackenzie and Angevine, 1981). Similar effects on fertility were observed in another developmental study in mice (Kristensen et al., 1995). F1 females (bred continuously for 6 months) in this study had 63% fewer litters, and litters were 30% smaller as compared to control animals. The fertility of male offspring was not assessed in this study.

Reproductive Organ Effects in Offspring

The above-mentioned studies also demonstrated dose-related effects on male and female reproductive organs in animals exposed gestationally to benzo[a]pyrene (Table 1-2 and Figure 1-1). Testicular weight was decreased and atrophic seminiferous tubules and vacuolization were increased at ≥10 mg/kg-day in male mice exposed to benzo[a]pyrene gestationally from GD 7 to 16; severe atrophic seminiferous tubules were observed at 40 mg/kg-day (Mackenzie and Angevine, 1981). Testicular weight was also statistically significantly decreased in Sprague-Dawley rats treated on PNDs 1-7 at doses ≥10 mg/kg-day, when examined at PND 8 (Liang et al., 2012).

In female mice treated with doses ≥10 mg/kg-day during gestation, ovarian effects were observed including decreases in ovary weight, numbers of follicles, and corpora lutea (Kristensen et al., 1995; Mackenzie and Angevine, 1981). Specifically, ovary weight in F1 offspring was reduced 31% following exposure to 10 mg/kg-day benzo[a]pyrene (Kristensen et al., 1995), while in another gestational study at the same dose level, ovaries were so drastically reduced in size (or absent) that they were not weighed (Mackenzie and Angevine, 1981). Hypoplastic ovaries with few or no follicles and corpora lutea (numerical data not reported), and ovaries with few or no small, medium, or large follicles and corpora lutea (numerical data not reported) have also been observed in mouse offspring exposed gestationally to benzo[a]pyrene (Kristensen et al., 1995; Mackenzie and Angevine, 1981).

Cardiovascular Effects in Offspring

Increased systolic and diastolic blood pressure was observed in adult animals following gestational treatment with benzo[a]pyrene (Jules et al., 2012) (Table 1-2 and Figure 1-1). Approximate elevations in systolic and diastolic blood pressure of 20–30 and 50–80% were noted

in the 0.6 and 1.2 mg/kg-day dose groups, respectively. Heart rate was decreased at 0.6 mg/kg-day, but was increased at 1.2 mg/kg-day.

Immune Effects in Offspring

Several injection studies in laboratory animals suggest that immune effects may occur following gestational or early postnatal exposure to benzo[a]pyrene. These studies are discussed in Section 1.1.3.

Table 1-1. Evidence pertaining to developmental effects of benzo[a]pyrene in humans

Study design and reference	Results
Tang et al. (2006) (Tongliang, China) Birth cohort 150 nonsmoking women who delivered babies between March 2002 and June 2002 Exposure: Mean hours per day exposed to ETS 0.42 (SD 1.19); lived within 2.5 km of power plant that operated from December 2001 to May 2002; benzo[a]pyrene-DNA adducts from maternal and cord blood samples; cord blood mean 0.33 (SD 0.14) (median 0.36) adducts/10^{-8} nucleotides; maternal blood mean 0.29 (SD 0.13) adducts/10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed weight and height Weight Beta (*p*-value) / Length (height) Beta (*p*-value) Birth: −0.007 (0.73) / −0.001 (0.89) 18 mo: −0.048 (0.03) / −0.005 (0.48) 24 mo: −0.041 (0.027) / −0.007 (0.28) 30 mo: −0.040 (0.049) / −0.006 (0.44) Adjusted for ETS, sex of child, maternal height, maternal weight, and gestational age (for measures at birth)
(Perera et al. (2005b); Perera et al. (2004)) (New York, United States) Birth cohort 265 pregnant African-American and Dominican nonsmoking women who delivered babies between April 1998 and October 2002 (214 and 208 for weight and length analysis, respectively); approximately 40% with a smoker in the home Exposure: Benzo[a]pyrene-DNA adducts in cord blood samples; mean 0.22 (SD 0.14) adducts/10^{-8} nucleotides; median of detectable values 0.36 adducts/ 10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed weight and length Weight Beta (*p*-value) / Length Beta (*p*-value) Interaction term: −0.088 (0.05) / −0.014 (0.39) Benzo[a]-pyrene-DNA adducts: −0.020 (0.49) / −0.005 (0.64) ETS in home: −0.003 (0.90) / −0.007 (0.32) Adjusted for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age

Study design and reference	Results
Wu et al. (2010) (Tianjin, China) Case control study: 81 cases (96% participation rate)—fetal death confirmed by ultrasound before 14 wks of gestation; 81 controls (91% participation rate)—elective abortions; matched by age, gestational age, and gravidity; excluded smokers and occupational PAH exposure Exposure: Benzo[a]pyrene in aborted tissue and maternal blood samples (51 cases and controls; 2 of 4 hospitals)	*Benzo[a]pyrene adduct levels (/10^8 nucleotides), mean (± SD)* Cases: Maternal blood 6.0 (± 4.7); Aborted tissue 4.8 (± 6.0) Controls: Maternal blood 2.7 (± 2.2); Aborted tissue 6.0 (± 7.4) (*p*-value): Maternal blood (<0.001); Aborted tissue (0.29) Low correlation between blood and tissue levels (r = −0.02 in cases, r = −0.21 in controls) Association between benzo[a]pyrene adducts and miscarriage[a] Per unit increase in adducts: OR 1.37 (95% CI) (1.12, 1.67) Dichotomized at median: OR 4.56 (95% CI) (1.46, 14.3) [a]Conditional logistic regression, adjusted for maternal education, household income, and gestational age; age also considered as potential confounder

CI = confidence interval; OR = odds ratio; SD = standard deviation.

Table 1-2. Evidence pertaining to developmental effects of benzo[a]pyrene in animals

Study design and reference	Results
Birth outcomes and postnatal growth	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/ dose 0, 10, 40, or 160 mg/kg-d by gavage GDs 7−16	↓ number of F0 females with viable litters: 46/60, 21/30, 44/60, and 13/30* ↓ F1 body weight at PND 20 % change from control: 0, 4, −7*, and −13* ↓ F1 body weight at PND 42 % change from control: 0, −6*, −6*, and −10* (no difference in pup weight at PND 4)
Kristensen et al. (1995) NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GDs 7−16	Exposed F0 females showed no gross signs of toxicity and no effects on fertility (data not reported)
Jules et al. (2012) Long-Evans rats, 6−17 F0 females/dose 0, 0.15, 0.3, 0.6, or 1.2 mg/kg-d by gavage GDs 14−17	No overt signs of toxicity in dams or offspring, differences in pup body weight, or number of pups/litter

Study design and reference	Results
McCallister et al. (2008) Long-Evans Hooded rats, 5–6/group 0 or 0.3 mg/kg-d by gavage GDs 14–17	No difference in number of pups/litter No overt maternal or pup toxicity No difference in liver:body weight Increased brain:body weight ratio at PNDs 15 and 30 (data not shown)
Brown et al. (2007) Long-Evans Hooded rats, 6/group 0, 0.025, or 0.15 mg/kg-d by gavage GDs 14–17	No difference in number of pups/litter or overt maternal or pup toxicity
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Statistically significant decrease in pup body weight (approximate 10–15% decrease) at 2 mg/kg-d measured on PNDs 36 and 71 (no significant alteration of pup weight during treatment period) No differences among treatment groups in developmental milestones: incisor eruption, eye opening, development of fur, testis decent, or vaginal opening
Archibong et al. (2002) F344 rats, 10 females/group 0, 25, 75, or 100 µg/m^3 nose-only inhalation for 4 hrs/d GDs 11–20	↓ fetal survival ([pups/litter]/[implantation sites/litter] × 100) % fetal survival: 97, 78*, 38*, and 34*%
Reproductive effects in offspring	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/dose 0, 10, 40, or 160 mg/kg-d by gavage GDs 7–16	↓ number of F1 females with viable litters: 35/35, 23/35*, 0/55*, and 0/20* ↓F1 female fertility index (females pregnant/females exposed to males × 100): 100, 66*, 0*, and 0* ↓ F1 male fertility index (females pregnant/females exposed to males × 100): 80, 52*, 5*, and 0* ↓ F2 litter size from F1 dams (20%) at 10 mg/kg-d (no litters were produced at high doses) ↓ size or absence of F1 ovaries (weights not collected) hypoplastic ovaries with few or no follicles and corpora lutea (numerical data not reported) ↓ testicular weight in F1 offspring % change from control: 0, −42, −82, and ND (statistical significance not reported) ↑ atrophic seminiferous tubules and vacuolization at ≥10 mg/kg-d; severe atrophic seminiferous tubules at 40 mg/kg-d (numerical data not reported)

Study design and reference	Results
Kristensen et al. (1995) NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GDs 7–16	↓ number of F2 litters (−63%) ↓ F2 litter size (−30%) ↓ ovary weight (−31%) in F1 females Few or no small, medium, or large follicles and corpora lutea
Cardiovascular effects in offspring	
Jules et al. (2012) Long-Evans rats, 6–17 F0 females/dose 0, 0.15, 0.3, 0.6, or 1.2 mg/kg-d by gavage GDs 14–17	↑ systolic blood pressure (measured at PND 53) 15%* increase at 0.6 mg/kg-d 52%* increase at 1.2 mg/kg-d (other dose groups not reported) ↑ diastolic blood pressure (measured at PND 53) 33%* increase at 0.6 mg/kg-d 83% *increase at 1.2 mg/kg-d (other dose groups not reported) Altered heart rate 10%* increase at 0.6 mg/kg-d 8%* decrease at 1.2 mg/kg-d

*Statistically significantly different from the control ($p < 0.05$).
[a]% change from control calculated as: (treated value − control value)/control value × 100.

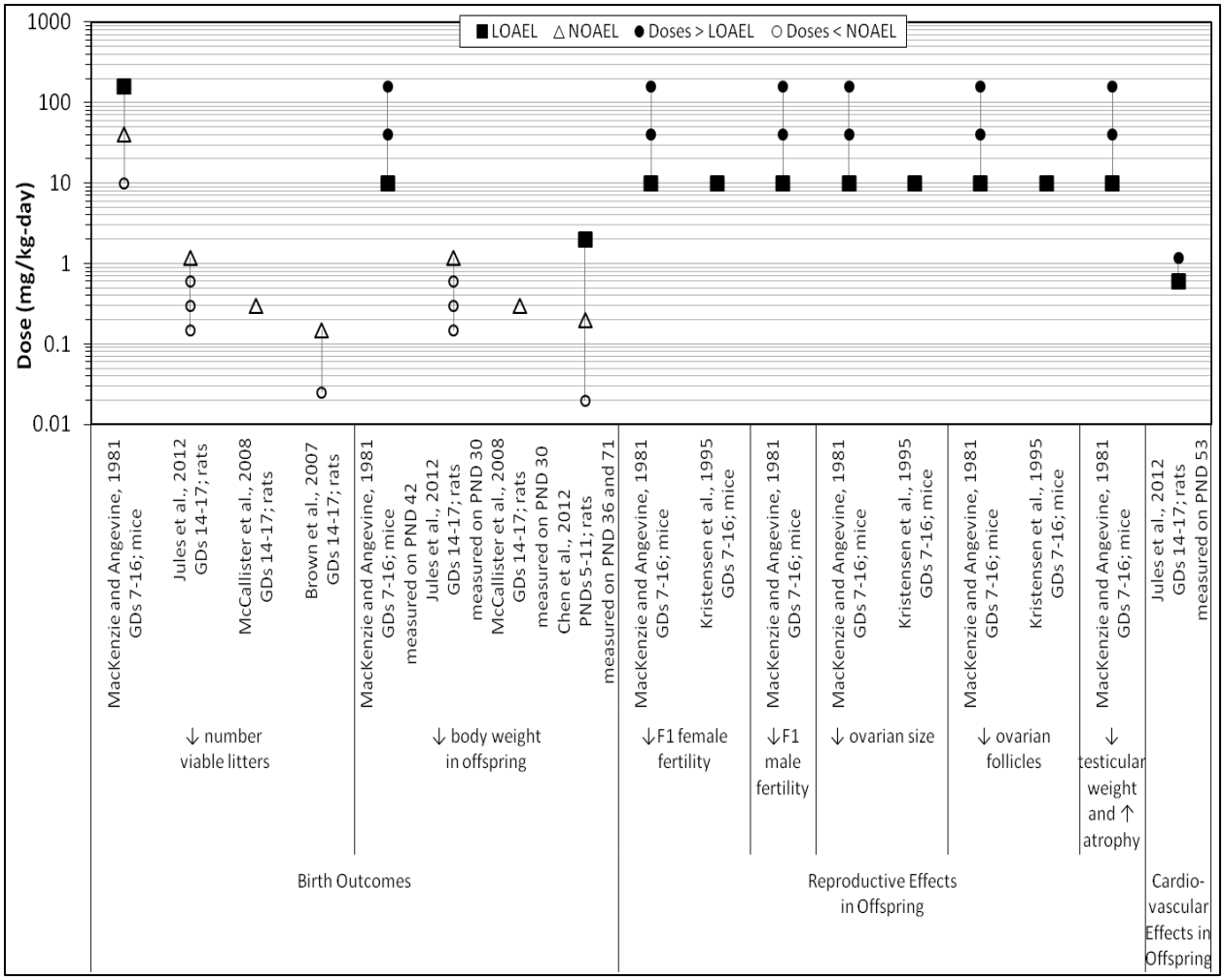

Figure 1-1. Exposure-response array for developmental effects following oral exposure to benzo[a]pyrene.

Neurodevelopmental Effects

There is evidence in humans and animals that benzo[a]pyrene induces developmental neurotoxicity. In addition to the persistent reductions in cognitive ability observed in epidemiology studies of prenatal PAH exposure, the two epidemiology studies that examined benzo[a]pyrene-specific measures observed effects on neurodevelopment and behavior in young children. Altered learning and memory, motor activity, anxiety-like behavior, and electrophysiological changes have also been observed in animals following oral and inhalation exposure to benzo[a]pyrene.

The mammalian brain undergoes periods of rapid brain growth, particularly during the last 3 months of pregnancy in humans, which has been compared to the first 1–2 weeks of life in the rat and mouse neonate (Dobbing and Sands, 1979, 1973). This period is characterized by axonal and dendritic outgrowth and the establishment of mature neuronal connections. Also during this critical period, animals acquire many new motor and sensory abilities (Kolb and Whishaw, 1989). There is a growing literature of animal studies that shows subtle changes in motor and cognitive

function following acute or repeated perinatal or lactational exposure to benzo[a]pyrene (Bouayed et al., 2009a; McCallister et al., 2008; Wormley et al., 2004). These effects are described below.

Cognitive function

Head circumference at birth is associated with measures of intelligence in children, even among term infants (Broekman et al., 2009; Gale et al., 2006). The two birth cohort studies that examined maternal or cord blood levels of benzo[a]pyrene-DNA adducts in relation to head circumference provide some evidence of an association, most strongly within the context of an interaction with ETS (Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004) (Table 1-3). The cohort in Tongliang, China also examined intelligence quotient scores at age 5 years (Perera et al., 2012a). An interaction with ETS was seen in this analysis, with larger decrements seen on the full scale and verbal scales with increased benzo[a]pyrene-DNA adduct levels in the presence of prenatal exposure to ETS compared to the effects seen in the absence of prenatal exposure.

Animal studies have also provided evidence of altered learning and memory behaviors following lactational or direct postnatal exposure to benzo[a]pyrene (Chen et al., 2012; Bouayed et al., 2009a) (Table 1-4). In mice, spatial working memory was measured using the Y-maze spontaneous alternation test (Bouayed et al., 2009a). This test records alternations between arm entries in a Y-shaped maze as a measure of memory, as rodents typically prefer to investigate a new arm of the maze. To a lesser extent, this test can also reflect changes in sensory processing, novelty preference, and anxiety-related responses in rodents. An improvement in working memory was evident in mice, as exhibited by significant increases in spontaneous alternations in the Y-maze test in mice on PND 40 following lactational exposure to 2 mg/kg-day benzo[a]pyrene (but not 20 mg/kg-day) from PND 0 to 14 (Bouayed et al., 2009a). The total number of arm entries in the Y-maze was unaffected by lactational exposure, suggesting that changes in motor function were not driving this response. Similarly, $CPR^{lox/lox}$ transgenic mice (a mouse model with low brain expression of CYP450 reductase) exhibit deficiencies in novel object recognition tests following in utero exposure to benzo[a]pyrene during late gestation (Li et al., 2012; Sheng et al., 2010). This finding suggests impairment in short-term memory, although these tests also reflect locomotor exploratory behavior, response to novelty, and attention. In rats, spatial learning and memory was measured using the Morris water maze, which measures the ability of a rat to navigate to a target platform using external spatial cues. Increased escape latency (time to find the hidden platform), as well as decreased time in the target quadrant and decreased number of platform crossings during a probe trial with the platform removed were observed in PND 39–40 rats following postnatal exposure to 2 mg/kg-day benzo[a]pyrene (Chen et al., 2012). These effects were more pronounced in animals tested at PNDs 74–75, with effects observable at ≥0.2 mg/kg-day. No difference in swim speed was observed during the probe trial tests (swim speed did not appear to be analyzed during the hidden platform trials) between treatment groups, suggesting that the observed changes are not attributable to general motor impairment. These observations may indicate primary effects of benzo[a]pyrene on learning and/or memory processes; however, the presented data were

insufficient to attribute these findings to learning and memory processes alone. Specifically, visual examinations of the improvements in escape latency (slopes) over the four learning trial days were not noticeably affected by treatment dose, suggesting that all groups learned at a similar rate. As four trials/day were averaged for each animal at each trial day, it is unclear whether the dose-related increases in escape latency already observable at trial day 1 reflect effects on learning across those first four trials or other effects (e.g., altered anxiety or vision responses). As it is not clear that the groups learned to a comparable extent in hidden platform tests, the results of the probe trial cannot be conclusively attributed to memory retention; however, the decreased performance of the benzo[a]pyrene-exposed rats in the Morris water maze still represents a persistent neurobehavioral change.

Neuromuscular function, coordination, and sensorimotor development

Motor behavior and coordination, assessed by locomotion, reaching, balance, comprehension, drawing, and hand control was one of the specific domains assessed in the Chinese birth cohort evaluated by Tang et al. (2008). In children aged 2 years, decreased scores were seen in relation to increasing benzo[a]pyrene-DNA adducts measured in cord blood, with a Beta per unit increase in adducts of −16 (p = 0.04), and an approximate twofold increased risk of development delay per unit increase in adducts (Table 1-3).

In laboratory animals (Table 1-4 and Figure 1-2), impaired performance in neuromuscular and sensorimotor tests have been consistently observed in mice lactationally exposed to ≥2 mg/kg-day benzo[a]pyrene from PND 0 to 14 (Bouayed et al., 2009a) and in rat pups postnatally exposed to ≥0.02 mg/kg-day benzo[a]pyrene from PND 5 to 11 (Chen et al., 2012). In the righting reflex test, significant increases in righting time were observed in PNDs 3−5 mice and in PNDs 12−16 rats. These decrements did not show a monotonic dose response. In another test of sensorimotor function and coordination, dose-dependent increases in latency in the negative geotaxis test were observed in PND 5−9 mice and in PND 12−14 rats. The forelimb grip strength test of neuromuscular strength was also evaluated in both mice and rats, but alterations were only observed in mice. In mice, a dose-dependent increase in duration of forelimb grip was observed on PNDs 9 and 11 during lactational exposure to benzo[a]pyrene. The Water Escape Pole Climbing test was also used to evaluate neuromuscular function and coordination in mice (Bouayed et al., 2009a). No effect on climbing time was observed, suggesting no change in muscle strength. However, increased latency in pole grasping and pole escape in PND 20 male pups was observed, highlighting potential decrements in visuomotor integration and/or coordination, although anxiety or fear-related responses cannot be ruled out. Treatment-dependent increases in pup body weight around the testing period complicate the interpretation of these results.

Chen et al. (2012) observed statistically significant delays on the order of ~0.2−0.3 seconds in the surface righting test and ~3−4 seconds in the negative geotaxis test. Differences due to exposure were notable between PND 12 and 16, with equal performance in righting observed by PND 18 and in geotaxis by PND 16. The authors found no effect on gender, therefore the data for

male and female rats was pooled for these measures. However, it should be noted that differences in the maturation of these developmental landmarks following challenge have been shown to exist between males and females. Negative geotaxis and surface righting are discrete endpoints routinely used as part of a neurobehavioral test battery to assess acquisition of behavioral reflexes. Chen et al. (2012) used the surface righting and negative geotaxis tests as quantitative measures of sensorimotor function at PND 12 and beyond. Typically in these tests, animals are observed on consecutive days (e.g., PNDs 3–12) and time to acquisition of these phenotypes is measured. Chen et al. (2012) did not measure performance over consecutive days of development or measure baseline acquisition of these behaviors. Although these tests as conducted by Chen et al. (2012) cannot discern a developmental delay, the data support a transient impairment of sensorimotor function in animals that have already developed this reflex (e.g., able to orient 180 degrees and able to right within 2 seconds). Thus, these data indicate that benzo[a]pyrene may affect sensorimotor function in developing animals.

Anxiety and activity

Anxiety/depression and attention/hyperactivity symptoms in children ages 6–7 years were examined via questionnaire in relation to prenatal air monitoring of benzo[a]pyrene and other PAHs, and in relation to benzo[a]pyrene-specific DNA adducts measured at birth in a follow-up of a birth cohort study conducted in New York City (Perera et al., 2012b). PAH exposure levels (based on personal air monitoring, n = 253) and benzo[a]pyrene-specific DNA adducts measured in cord blood samples (n = 138) were both positively associated with symptoms of anxiety/depression and attention problems (see Table 1-3). Given the limited sample size, however, the cord blood results are based on relatively sparse data (<5 in the borderline or clinical range in the low exposure referent group). Associations with maternal blood adducts were similar to or slightly smaller than those seen with cord blood adducts. Exposure was treated as a dichotomy (i.e., for adducts, detectable compared with non-detectable levels) in these analyses.

Decreased anxiety-like behavior was reported in both rats and mice weeks to months following postnatal oral exposure to benzo[a]pyrene (Chen et al., 2012; Bouayed et al., 2009a) (Table 1-4). Anxiety-like behaviors were measured in both species using an elevated plus maze, where an increase in the time spent in the closed arms of the maze is considered evidence of anxious behavior. In mice, significant increases in the percent open arm entries and percent time spent in open arms of the maze, as well as significantly decreased entries into closed arms of the maze (in the 2 mg/kg-day group), were observed on PND 32 following lactational exposure to ≥2 mg/kg-day benzo[a]pyrene (Bouayed et al., 2009a). To rule out potential differences in total activity or general motivation and exploration, the authors expressed the open arm data as percentages, and they also demonstrated that there were no exposure-related effects on the total number of arm entries. The mice also exhibited decreased latency of the first entry into an open arm following lactational exposure to 20 mg/kg-day benzo[a]pyrene. Similar results were reported for rats, with decreased anxiety-like behavior following oral benzo[a]pyrene exposure from PND 5

to 11, although sex-specific differences were observed (Chen et al., 2012). In females, postnatal exposure to ≥0.2 mg/kg-day benzo[a]pyrene was associated with a significant increase in the number of open arm entries and a significant decrease in the number of closed arm entries on PND 70. Significantly increased time in open arms of the maze was reported in PND 70 female rats following postnatal exposure to ≥0.02 mg/kg-day. Male rats also showed decreased anxiety-like behavior on PND 70, although the doses needed to detect these responses were higher than females (i.e., increases at ≥2 mg/kg-day for open arm entries and ≥0.2 mg/kg-day for time spent in open arms). A significant decrease in latency to enter an open arm of the maze was observed in both male and female rat pups exposed to 2 mg/kg-day benzo[a]pyrene. Similar to the observations in mice, exposure did not appear to have an effect on total activity or general motivation of the rats, as total arm entries were unchanged by treatment.

Increased spontaneous locomotor activity in the open field on PNDs 34 and 69 has been reported in rats postnatally exposed to 2 and ≥0.2 mg/kg-day, respectively (Chen et al., 2012), but not in mice exposed lactationally to doses up to 20 mg/kg-day and tested on PND 15 (Bouayed et al., 2009a). Interestingly, no differences in the open field test were observed in rats that were postnatally exposed and tested on PNDs 18 and 20, suggesting either that longer latencies between exposure and testing may be required, or that these developmental effects may only manifest in more mature rats (Chen et al., 2012). An apparent increased sensitivity of older animals was also present in the elevated plus maze and Morris water maze tests performed by Chen et al. (2012). Elevated activity in an open field is attributable primarily to either increased motor activity or decreased anxiety-like behavior. However, the relative contributions of these two components could not be separated in either of these studies, as the authors did not evaluate activity in central versus peripheral regions of the field (i.e., anxious rodents will spend less time in the center of the field).

Electrophysiological changes

Electrophysiological effects of gestational exposure to benzo[a]pyrene have been examined in two studies (by the same research group) through implanted electrodes in the rat cortex and hippocampus (Table 1-4). Maternal inhalation exposure to 0.1 mg/m^3 resulted in reduced long-term potentiation in the dentate gyrus of male offspring between PND 60 and 70 (Wormley et al., 2004); however, significant fetal toxicity at this exposure level complicates interpretation of these results. Oral exposure of dams to 0.3 mg/kg-day for 4 days during late gestation resulted in decreased evoked neuronal activity in male offspring following mechanical whisker stimulation between PND 90 and 120 (McCallister et al., 2008). Specifically, the authors noted reduced spike numbers in both short and long latency responses following whisker stimulation. These effects were observed several months post-exposure, suggesting that gestational benzo[a]pyrene exposure may have long-lasting functional effects on neuronal activity elicited by sensory stimuli.

Table 1-3. Evidence pertaining to the neurodevelopmental effects of benzo[a]pyrene from PAH mixtures

Reference and study design	Results
(Tang et al. (2008); Tang et al. (2006)) (Tongliang, China) Birth cohort 150 nonsmoking women, delivered March 2002–June 2002; lived within 2.5 km of power plant that operated from December 2001 to May 2002 Outcomes: Head circumference at birth; Gesell Developmental Schedule, administered by physicians at 2 yrs of age (four domains: motor, adaptive, language, and social); standardized mean score = 100 ± SD 15 (score <85 = developmental delay) Exposure: Mean hrs/d exposed to ETS 0.42 (SD 1.19); lived within 2.5 km of power plant that operated from December 2001 to May 2002; benzo[a]pyrene-DNA adducts from maternal and cord blood samples; cord blood mean 0.33 (SD 0.14) (median 0.36) adducts/10^{-8} nucleotides; maternal blood mean 0.29 (SD 0.13) adducts/10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed head circumference Beta (*p*-value) Birth: −0.011 (0.057) 18 mo: −0.012 (0.085) 24 mo: −0.006 (0.19) 30 mo: −0.005 (0.31) High versus low, dichotomized at median, adjusted for ETS, sex of child, maternal height, maternal weight, Cesarean section delivery, maternal head circumference, and gestational age (for measures at birth)
(Tang et al. (2008); Tang et al. (2006)) (see above for population and exposure details) n = 110 for Developmental Quotient analysis; no differences between the 110 participants in this analysis and the nonparticipants with respect to maternal age, gestational age, birth weight, birth length, or birth head circumference; higher maternal education (direction not reported, *p* = 0.056) Outcomes: Gesell Developmental Schedule, administered by physicians at 2 yrs of age (four domains: motor, adaptive, language, and social); standardized mean score = 100 ± SD 15 (score <85 = developmental delay)	Association between benzo[a]pyrene adducts and development Motor: Beta (95% CI)[a] −16.0 (−31.3, −0.72)*; OR (95% CI)[b] 1.91 (1.22, 2.97)* Adaptive: −15.5 (−35.6, 4.61); 1.16 (0.76, 1.76) Language: −16.6 (−33.7, 0.46); 1.31 (0.84, 2.05) Social: −9.29 (−25.3, 6.70); 1.52 (0.93, 2.50) Average: −14.6 (−28.8, −0.37)*; 1.67 (0.93, 3.00) [a]Linear regression of change in Developmental Quotient per unit increase in benzo[a]pyrene adducts [b]Logistic regression of risk of developmental delay (defined as normalized score <85) per 1 unit (0.1 adducts/10^{-8} nucleotides) increase in adducts Both analyses adjusted for sex, gestational age, maternal education, ETS, and cord lead levels

Reference and study design	Results
Perera et al. (2012a); (Tang et al. (2008); Tang et al. (2006)) (see above for population and exposure details) 132 (83%) followed through age 5; 100 of these had complete data for analysis; no differences between the 100 participants in this analysis and the nonparticipants with respect to adduct levels, ETS exposure, IQ measures, maternal age, gestational age, or infant gender; higher maternal education (60 and 35% with ≥ high school, respectively, in participants and nonparticipants, $p < 0.05$) Outcomes: Wechsler Preschool and Primary Intelligence Quotient scale (Shanghai version)	ETS measure not correlated with benzo[a]pyrene adduct measures (i.e., absolute value of Spearman r < 0.10) Relation between cord blood benzo[a]pyrene-DNA adducts, ETS exposure, and IQ measures Beta (95% CI) Main effect / With ETS interaction term Full scale: −2.42 (−7.96, 3.13) / −10.10 (−18.90, −1.29) Verbal: −1.79 (−7.61, 4.03) / −10.35 (−19.61, −1.10) Performance: −2.57 (−8.92, 3.79) / −7.78 (−18.03,2.48) Beta per 1 unit increase in log-transformed cord adducts, adjusted for ETS exposure, gestational age, maternal education, cord lead, maternal age, and gender
(Perera et al. (2012b); Perera et al. (2005b); Perera et al. (2004)) (United States, New York) Birth cohort 265 pregnant women: African-American and Dominican nonsmoking women who delivered babies between April 1998 and October 2002 (253 and 207 for behavior and head circumference analysis, respectively); approximately 40% with a smoker in the home Outcomes: Head circumference at birth Exposure: Benzo[a]pyrene-DNA adducts from maternal and cord blood samples; mean 0.22 (SD 0.14) adducts/10^{-8} nucleotides; median of detectable values 0.36 adducts/10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts, environmental tobacco smoke exposure (ETS), and log-transformed head circumference Beta (*p*-value) Interaction term: −0.032 (0.01) benzo[a]pyrene-DNA adducts: −0.007 (0.39) ETS in home: −0.005 (0.43) High versus low, dichotomized at 0.36 adducts/10^{-8} nucleotides, adjusted for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age

Reference and study design	Results
Perera et al. (2012b) n = 215 with outcome data and no missing covariate data); no differences between the participants in this analysis and the nonparticipants with respect to adduct levels, ETS exposure, maternal age, gestational age, and socioeconomic variables; participants more likely to be female and African-American Outcomes: Child Behavior Checklist (118 items), completed by mothers for children ages 6–7 yrs. Two domains: anxious/depression, attention problems (normalized T-score ≤65 = borderline or clinical syndrome); also used for scales of anxiety problems and attention deficit hyperactivity problems based on DSM classification	Logistic regression of risk of borderline or clinical status in relation PAH levels and to detectable levels of benzo[a]pyrene adducts PAH: Anxious/depressed — Prevalence 6.3 %; PAH OR (95% CI) 8.9 (1.7, 46.5); Cord blood OR (95% CI) 2.6 (0.69, 9.4) Attention problems — Prevalence 6.7%; PAH OR (95% CI) 3.8 (1.1, 12.7); Cord blood OR (95% CI) 4.1 (0.99, 16.6) Anxiety (DSM) — Prevalence 9.5%; PAH OR (95% CI) 4.6 (1.5, 14.3); Cord blood OR (95% CI) 2.5 (0.84, 7.7) Attention deficit – hyperactivity (DSM) — Prevalence 7.9%; PAH OR (95% CI) 2.3 (0.79, 6.7); Cord blood OR (95% CI) 2.6 (0.68, 10.3) Exposure dichotomized for PAH as above and below median (2.273 ng/m^3) for parent population and for cord blood benzo[a]pyrene adducts as detectable (n = 56 cord blood samples) versus non-detectable (n = 92); adjusted for sex, gestational age, maternal education, maternal IQ, prenatal ETS, ethnicity, age, heating season, prenatal demoralization, and HOME inventory

*Statistically significantly different from the control ($p < 0.05$).

DSM = Diagnostic and Statistical Manual of Mental Disorders; HOME = Home Observation for Measurement of the Environment; IQ = intelligence quotient.

Table 1-4. Evidence pertaining to the neurodevelopmental effects of benzo[a]pyrene in animals

Reference and study design	Results[a]
Cognitive function	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Hidden Platform test in Morris water maze: Adolescent test period (PNDs 36–39): significant increase in escape latency at 2 mg/kg-d only Adult test period (PNDs 71–74): significant increase in escape latency at ≥0.2 mg/kg-d Increases in latency were already ~30% greater than controls at 2 mg/kg-d on the first trial day (i.e., on PND 36 or 71) All experimental groups exhibited similar improvements in escape latency, as slopes were visually equivalent across the 4 trial days Probe test in the Morris water maze (d 5): Time spent in the target quadrant: PND 40: significant decrease at 2 mg/kg-d only PND 75: significant decrease at ≥0.2 mg/kg-d Number of platform crossings: PND 40: significant decrease at 2 mg/kg-d only PND 75: significant decrease at ≥0.2 mg/kg-d (in females) and

Reference and study design	Results[a]
	2 mg/kg-d (in males)
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d maternal gavage PNDs 0–14 (lactational exposure)	Significant increase in the percent of spontaneous alternations in the Y-maze alternation test at 2 mg/kg-d but not at 20 mg/kg-d No effect on the total number of arm entries in the Y-maze alternation test
Neuromuscular function, coordination, and sensorimotor development	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Latency in the surface righting reflex test PND 12: significant increase at 0.2 mg/kg-d only PND 14: significant increase at 0.02 and 2 mg/kg-d only PND 16: significant difference at 2 mg/kg-d only PND 18: no significant difference Latency in the negative geotaxis test PND 12: significant increase at all doses PND 14: significant increase at 2 mg/kg-d only PNDs 16 and 18: no significant difference No effect on duration of forelimb grip in forelimb grip strength test No effect on the latency to retract from the edge in cliff aversion test Note: Males and females were pooled for all analyses
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d by maternal gavage PNDs 0–14 (lactational exposure)	Significant increase in righting time in the surface righting reflex test at both doses on PNDs 3 and 5 (but not PNDs 7 and 9) Significant increase in latency in the negative geotaxis time for 20 mg/kg-d dose group at PNDs 5, 7, and 9 (no significant difference at PND 11) Significant increase in duration of forelimb grip in forelimb grip strength test at both dose groups on PND 9 (statistically significant at PND 11 only at high dose) Significant increase in pole grasping latency in male pups in the water escape pole climbing test at 20 mg/kg-d No effect on climbing time in the water escape pole climbing test Significant increase in pole escape latency in the water escape pole climbing test in male rats at 20 mg/kg-d
Anxiety and/or motor activity	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Elevated plus maze: Significant increase in the number of entries into open arms at PND 70 at ≥0.2 mg/kg-d (in females) and 2 mg/kg-d (in males) (no difference at PND 35) Significant decrease in the number of entries into closed arms at PND 70 at ≥0.2 mg/kg-d (in females) and 2 mg/kg-d (in males) (no

Reference and study design	Results[a]
	difference at PND 35) Significant increase in the time spent in open arms at PND 35 at 2 mg/kg-d in females and at PND 70 at doses ≥0.02 mg/kg-d in females and ≥0.2 mg/kg-d in males Significant decrease in latency time to first enter an open arm on PND 70 at ≥0.2 mg/kg-d (no difference at PND 35) No effect on the total number of arm entries between treatment groups (calculated by EPA from graphically reported open and closed arm entries) Open field test: Significant increase in the number of squares: PND 34, significant increase at 2 mg/kg-d; PND 69, significant increase at ≥0.02 mg/kg-d (no difference at PNDs 18 and 20) Significant increase in rearing activity at 0.2 mg/kg-d on PND 69 (no difference at PNDs 18, 20, and 34)
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d by maternal gavage PNDs 0–14 (lactational exposure)	Elevated plus maze: Significantly increased time in open arms at ≥2 mg/kg-d Significantly increased percentage of entries into open arms at ≥2 mg/kg-d Significantly decreased entries into closed at 2 mg/kg-d, but not at 20 mg/kg-d Significantly decreased latency time to enter an open arm at 20 mg/kg-d No effect on the total number of arm entries No significant effect of gender on performance was detected, so males and females were pooled for analyses Open field test: No significant change in activity on PND 15, but data not provided
Electrophysiological changes	
McCallister et al. (2008) Long-Evans Hooded rats, 5–6/group 0 or 0.3 mg/kg-d by gavage GDs 14–17	Statistically significant decreases in stimulus-evoked cortical neuronal activity on PNDs 90–120 Reduction in the number of spikes in both the short and long latency periods on PNDs 90–120 (numerical data not presented)

Reference and study design	Results[a]
Wormley et al. (2004) F344 rats, 10 females/group 0 or 100 µg/m^3 by nose-only inhalation for 4 hrs/d GDs 11–21	Electrophysiological changes in the hippocampus: Consistently lower long term potentiation following gestational exposure (statistical analysis not reported) % change relative to control: -26% Note: significant fetal toxicity observed (99 versus 34% birth index)

[a]% change from control calculated as: (treated value − control value)/control value × 100.

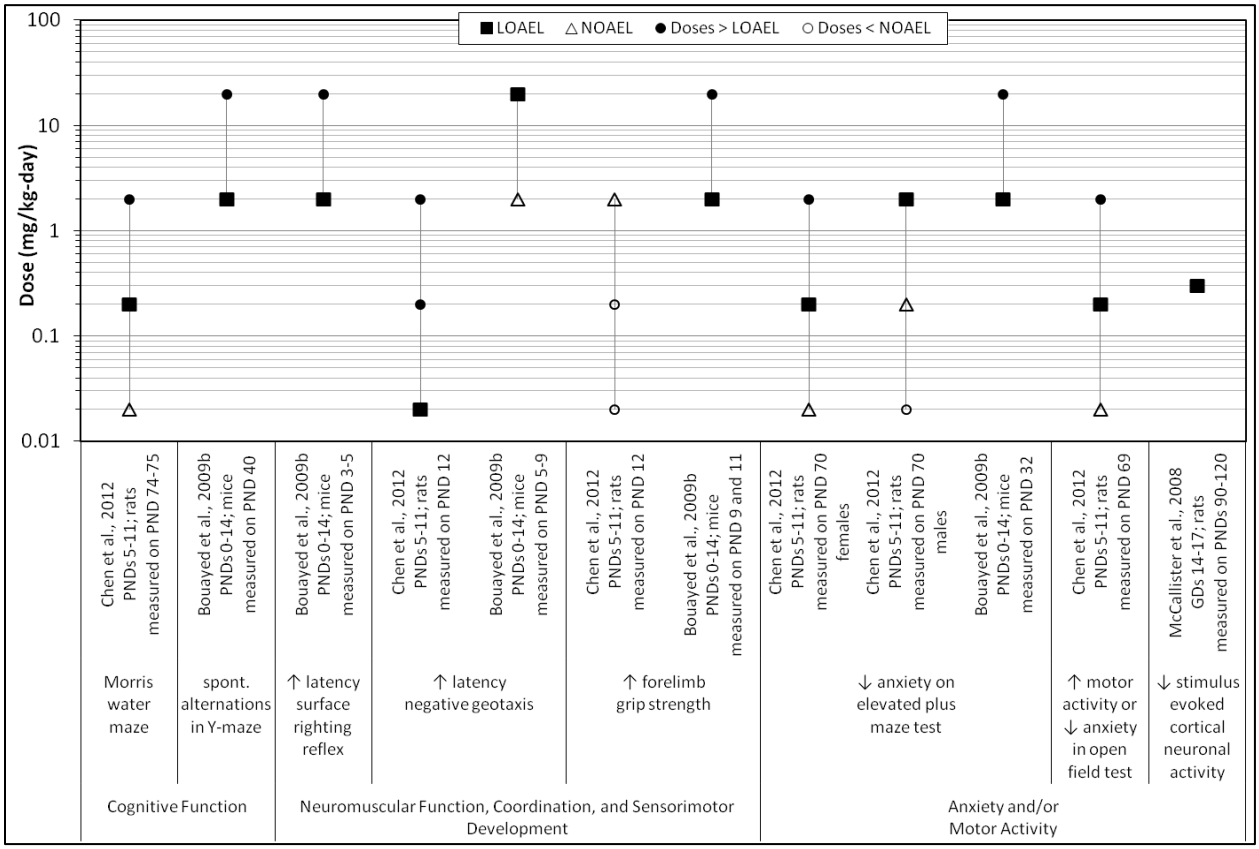

Figure 1-2. Exposure-response array for neurodevelopmental effects following oral exposure.

Mode of Action Analysis—Developmental Toxicity and Neurodevelopmental Toxicity

Data regarding the potential mode of action for the various manifestations of developmental toxicity associated with benzo[a]pyrene exposure are limited, and the mode of action for developmental toxicity is not known. General hypothesized modes of action for the various observed developmental effects include, but are not limited to, altered cell signaling, cytotoxicity, and oxidative stress.

It is plausible that developmental effects of benzo[a]pyrene may be mediated by altered cell signaling through the AhR. Benzo[a]pyrene is a ligand for the AhR, and activation of this receptor

regulates downstream gene expression including the induction of CYP enzymes important in the conversion of benzo[a]pyrene into reactive metabolites. Studies in AhR knock-out mice indicate that AhR signaling during embryogenesis is essential for normal liver, kidney, vascular, hematopoietic, and immune development (Schmidt et al., 1996; Fernandez-Salguero et al., 1995). In experiments in AhR responsive and less-responsive mice, the mice with the less-responsive AhR were protected from renal injury as adults following gavage treatment with 0.1 or 0.5 mg/kg-day benzo[a]pyrene from GD 10 to 13. Renal injury was indicated by an increase in urinary albumin and a decrease in glomerular number (Nanez et al., 2011).

Low birth weight has been associated with prenatal exposure to PAHs in human populations (Perera et al., 2005b). Several epidemiology studies have revealed an inverse association between low birth weight and increased blood pressure, hypertension, and measures of decreased renal function as adults (Zandi-Nejad et al., 2006). It has been hypothesized that this may be attributable to a congenital nephron deficit associated with intrauterine growth restriction (Zandi-Nejad et al., 2006).

No clear mode(s) of action for the observed neurodevelopmental changes following benzo[a]pyrene exposure have been demonstrated. General hypothesized mechanisms with limited support are related to altered central nervous system neurotransmission. These mechanisms involve altered neurotransmitter gene expression, neurotransmitter levels, and neurotransmitter receptor signaling in regions associated with spatial learning, anxiety, and aggression, such as the hippocampus, striatum, amygdala, and hypothalamus (Li et al., 2012; Qiu et al., 2011; Tang et al., 2011; Xia et al., 2011; Bouayed et al., 2009a; Grova et al., 2008; Brown et al., 2007; Grova et al., 2007; Stephanou et al., 1998).

Mechanistic studies in rodents exposed as adults, which exhibit some of the same behavioral changes as animals exposed during development, may also inform potential mode(s) of action for the observed neurodevelopmental changes. Specifically regarding potential changes in spatial learning and memory processes, multiple studies in developing (Li et al., 2012; McCallister et al., 2008; Wormley et al., 2004) and adult (Maciel et al., 2014; Qiu et al., 2013; Tang et al., 2011; Grova et al., 2008; Grova et al., 2007) rodents suggest that changes in N-methyl-D-aspartate (NMDA) receptor signaling seen with benzo[a]pyrene exposure (e.g., changes in expression patterns of NR2A and NR2B subunits) may be responsible for apparent effects on learning and memory.

In relation to potential changes in anxiety-like behaviors (and also relevant to effects on learning and memory processes), many commonly used anti-anxiety medications work by increasing brain serotonin levels (e.g., selective serotonin reuptake inhibitors), increasing brain dopamine levels (e.g., dopamine reuptake inhibitors), or by targeting gamma-aminobutyric acid (GABA) receptors (e.g., benzodiazepines). Although $GABA_A$ receptor messenger ribonucleic acid (mRNA) in whole-brain homogenates was unchanged following lactational benzo[a]pyrene exposure, exposure at ≥2 mg/kg-day from PND 1 to 14 caused dose-dependent decreases in

serotonin receptor ($5HT_{1A}$) expression (Bouayed et al., 2009a; Stephanou et al., 1998). Additional support for identifying changes in monoamine neurotransmitter signaling (serotonin and dopamine signaling, in particular) as a potential mechanism(s) in the altered anxiety-like behaviors observed following benzo[a]pyrene exposure is provided in multiple studies of rodents exposed as adults (Bouayed et al., 2012; Qiu et al., 2011; Xia et al., 2011; Stephanou et al., 1998; Jayasekara et al., 1992) and in a single study of blood neurotransmitter levels in occupationally-exposed men (Niu et al., 2010). Overall, these data suggest possible effects of benzo[a]pyrene exposure on NMDA receptor expression and regulation of monoamine neurotransmitters including serotonin and dopamine, but these findings require additional studies to clarify and extend understanding of these events.

Summary of Developmental Effects

Developmental effects following in utero exposure to PAH mixtures or benzo[a]pyrene alone have been reported in humans and in animal models. In human populations, decreased head circumference, decreased birth weight, and decreased postnatal weight have been reported. Analogous effects in laboratory animals, including decreased pup weight and decreased fetal survival, have been noted following gestational or early postnatal exposure to benzo[a]pyrene by the oral or inhalation route (Chen et al., 2012; Archibong et al., 2002; Mackenzie and Angevine, 1981). Reproductive function is also altered in mice treated gestationally with benzo[a]pyrene (Kristensen et al., 1995; Mackenzie and Angevine, 1981). These effects include impaired reproductive performance in F1 offspring (male and female) and alterations of the weight and histology of reproductive organs (ovaries and testes).

The available human and animal data also support the conclusion that benzo[a]pyrene is a developmental neurotoxicant. Human studies of environmental PAH exposure in two cohorts have observed neurotoxic effects, including suggestions of reduced head circumference (Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004), impaired cognitive ability (Perera et al., 2009; Tang et al., 2008), impaired neuromuscular function (Tang et al., 2008), and increased attention problems and anxious/depressed behavior following prenatal exposure (Perera et al., 2012b). These effects were seen in birth cohort studies in different populations (New York City and China), in studies using specific benzo[a]pyrene measures (i.e., adduct levels measured in cord blood samples) (Perera et al., 2012b; Tang et al., 2008; Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004). The available evidence from mice and rats also demonstrates significant and persistent developmental impairments following exposure to benzo[a]pyrene. Impaired learning and memory behaviors, decreased anxiety-like behaviors, and impaired neuromuscular function were consistently observed in multiple neurobehavioral tests in two separate species at comparable oral doses, and in the absence of maternal or neonatal toxicity (Chen et al., 2012; Bouayed et al., 2009a).

A decrease in anxiety, indicative of a change in nervous system function, can impair an organism's ability to react to a potentially harmful situation. This decreased ability of an organism to adapt to the environment is considered to be an adverse effect according to EPA's Neurotoxicity

guidelines (U.S. EPA, 1998). EPA's Developmental Toxicity guidelines state that an alteration in a functional outcome following developmental exposure indicates the potential for altered development in humans, although the types of developmental effects in animals will not necessarily be the same as those produced in humans (U.S. EPA, 1991c).

Studies in humans also suggest that behavior is adversely affected by benzo[a]pyrene exposure. In studies where symptoms were self-reported, exposure to benzo[a]pyrene was positively associated with increased symptoms of anxiety/depression and/or attention problems in prenatally-exposed children (Perera et al., 2012b). In addition, one study of an occupationally-exposed cohort of coke oven workers also reported increased symptoms of anxiety/depression and/or attention problems (Qiu et al., 2013); however, these results were inconsistent with a second cohort of occupationally exposed male workers (Niu et al., 2010). While these results could suggest that benzo[a]pyrene exposure may adversely affect anxiety-related processes in a different manner across species (i.e., increasing anxiety in humans, but decreasing anxiety in rodents), additional studies in humans are necessary to draw such a conclusion. Importantly, however, it is recognized that animals and humans may not necessarily experience the same effects or functional changes, and that limitations in the information available across species can sometimes prevent endpoint-specific comparisons (Francis et al., 1990).

In conclusion, EPA identified developmental toxicity and developmental neurotoxicity as human hazards of benzo[a]pyrene exposure.

Susceptible Populations and Lifestages

Childhood susceptibility to benzo[a]pyrene toxicity is indicated by 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 (Perera et al., 2012b; Perera et al., 2009; Tang et al., 2008; Tang et al., 2006; Perera et al., 2005b; Perera et al., 2005a; Perera et al., 2004). The occurrence of benzo[a]pyrene-specific DNA adducts in maternal and umbilical cord blood in conjunction with exposure to ETS was associated with reduced birth weight and head circumference in offspring of pregnant women living in New York City (Perera et al., 2005b). In other studies, elevated levels of BPDE-DNA adducts in umbilical cord blood were associated with: (1) reduced birth weights or reduced head circumference (Perera et al., 2005a; Perera et al., 2004); and (2) decreased body weight at 18, 24, and 30 months (Tang et al., 2008; Tang et al., 2006).

Studies in humans and experimental animals indicate that exposure to PAHs in general, and benzo[a]pyrene in particular, may impact neurological development. 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). In the Perera et al. (2009) study, the exposure measures are based on a composite of eight PAHs measured in air. In Tang et al., (2008), increased levels of benzo[a]pyrene-DNA adducts in cord blood were associated with decreased developmental quotients in offspring (Tang et al., 2008).

Evidence in animals of the effects of benzo[a]pyrene on neurological development includes: (1) decrements in reflex-related behaviors associated with neuromuscular coordination and sensorimotor function (Chen et al., 2012; Bouayed et al., 2009a); (2) disrupted learning and/or short-term memory processes (Chen et al., 2012; Li et al., 2012; Sheng et al., 2010; Bouayed et al., 2009a); and (3) decreased anxiety-related responses (Chen et al., 2012; Bouayed et al., 2009a). Mechanistic studies also support findings of benzo[a]pyrene induced alterations in electrophysiological response to stimulation of the dentate gyrus of the hippocampus (Wormley et al., 2004; Wu et al., 2003a) and decreased evoked response in the field cortex (McCallister et al., 2008).

1.1.2. Reproductive Toxicity

Human and animal studies provide evidence for benzo[a]pyrene-induced male and female reproductive toxicity. Effects on sperm quality and male fertility have been demonstrated in human populations highly exposed to PAH mixtures (Soares and Melo, 2008; Hsu et al., 2006). The use of internal biomarkers of exposure in humans (e.g., BPDE-DNA adducts) support associations between benzo[a]pyrene exposure and these effects. In females, numerous epidemiological studies indicate that cigarette smoking reduces fertility; however, few studies have specifically examined levels of benzo[a]pyrene exposure and female reproductive outcomes. Animal studies demonstrate decrements in sperm quality, changes in testicular histology, and hormone alterations following benzo[a]pyrene exposure in adult male animals, and decreased fertility and ovotoxic effects in adult females following exposure to benzo[a]pyrene.

Male Reproductive Effects

Fertility

Effects on male fertility have been demonstrated in populations exposed to mixtures of PAHs. Spermatozoa from smokers have reduced fertilizing capacity, and embryos display lower implantation rates (Soares and Melo, 2008). Occupational PAH exposure has been associated with higher levels of PAH-DNA adducts in sperm and male infertility (Gaspari et al., 2003). In addition, men with higher urinary levels of PAH metabolites have been shown to be more likely to be infertile (Xia et al., 2009). Studies were not identified that directly examined the reproductive capacity of adult animals following benzo[a]pyrene exposure. However, a dose-related decrease in fertility was observed in male mice treated in utero with benzo[a]pyrene, as discussed in Section 1.1.1.

Sperm parameters

Effects on semen quality have been demonstrated in populations exposed to mixtures of PAHs including coke oven workers and smokers (Soares and Melo, 2008; Hsu et al., 2006). Coke oven workers had higher frequency of oligospermia (19 versus 0% in controls) and twice the number of morphologically abnormal sperm (Hsu et al., 2006). Elevated levels of BPDE-DNA

adducts have been measured in the sperm of populations exposed to PAHs occupationally (Gaspari et al., 2003) and through cigarette smoke (Phillips, 2002; Zenzes et al., 1999). A higher concentration of BPDE-DNA adducts was observed in sperm not selected for intrauterine insemination or in vitro fertilization based on motility and morphology in patients of fertility clinics (Perrin et al., 2011b; Perrin et al., 2011a). An association between benzo[a]pyrene exposure levels and increased sperm DNA fragmentation using the sperm chromatin structure assay was observed by Rubes et al. (2010). However, it is currently unclear whether the sperm chromatin structure assay, which measures sperm fragmentation following denaturation, is predictive of fertility (Sakkas and Alvarez, 2010; ASRM, 2008).

In several studies in rats and mice, a decrease in sperm count, motility, and production and an increase in morphologically abnormal sperm have been reported (Table 1-5 and Figure 1-3). Alterations in these sperm parameters have been observed in different strains of rats and mice and across different study designs and routes of exposure.

Decreases in epididymal sperm counts (25–50% compared to controls) have been reported in Sprague-Dawley rats and C57BL6 mice treated with 1–5 mg/kg-day benzo[a]pyrene by oral exposure for 42 or 90 days (Chen et al., 2011; Mohamed et al., 2010). Another subchronic study noted a 44% decrease in epididymal sperm concentration at doses ≥50 mg/kg-day in Hsd:ICR (CD1) mice (Jeng et al., 2013). Additionally, a 15% decrease in epididymal sperm count was observed at a much lower dose in Sprague-Dawley rats exposed to benzo[a]pyrene for 90 days (Chung et al., 2011). However, confidence in this study is limited because the authors dosed the animals with 0.001, 0.01, and 0.1 mg/kg-day benzo[a]pyrene, but only reported on sperm parameters at the mid-dose, and no other available studies demonstrated findings in the range of the mid- and high-dose. In rats, an oral short-term study and a subchronic inhalation study lend support for the endpoint of decreased sperm count (Arafa et al., 2009; Archibong et al., 2008; Ramesh et al., 2008). Significantly decreased sperm count and daily sperm production (20–40% decrease from control in each parameter) were observed in rats following 10 days of gavage exposure to 50 mg/kg-day (Arafa et al., 2009) and following gavage dosing with 10 mg/kg-day on PNDs 1-7 (Liang et al., 2012). In addition, a 69% decrease from controls in sperm count was observed in rats following inhalation exposure to 75 μg/m^3 benzo[a]pyrene for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

Both oral and inhalation exposure of rodents to benzo[a]pyrene have been shown to lead to decreased epididymal sperm motility and altered morphology. Decreased motility of 20–30% compared to controls was observed in Hsd:ICR (CD1) mice (≥100 mg/kg-day), C57BL6 mice (≥1 mg/kg-day), and Sprague-Dawley rats (0.01 mg/kg-day) following subchronic oral exposure (Jeng et al., 2013; Chung et al., 2011; Mohamed et al., 2010). The effective doses spanned several orders of magnitude; however, as noted above, reporting is limited in the study that observed effects at 0.01 mg/kg-day benzo[a]pyrene (Chung et al., 2011). A short-term oral study in rats also reported a significantly decreased number of motile sperm (~40% decrease) following 10 days of gavage

exposure to 50 mg/kg-day benzo[a]pyrene (Arafa et al., 2009). In addition, decreased sperm motility was observed following inhalation exposure to 75 μg/m^3 benzo[a]pyrene in rats for 60 days (Archibong et al., 2008; Ramesh et al., 2008) and to ≥75 μg/m^3 for 10 days (Inyang et al., 2003). Abnormal sperm morphology was observed in Sprague-Dawley rats treated with 5 mg/kg-day benzo[a]pyrene by gavage for 84 days (Chen et al., 2011) and in rats exposed to 75 μg/m^3 benzo[a]pyrene by inhalation for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

Testicular changes

Several studies have demonstrated dose-related effects on male reproductive organs in adult animals exposed subchronically to benzo[a]pyrene (Table 1-5 and Figure 1-3). Decreases in testicular weight of approximately 35% have been observed in a 60-day gavage study in Hsd:ICR (CD1) mice at 100 mg/kg-day (Jeng et al., 2013), in a 10-day gavage study in adult Swiss albino rats at 50 mg/kg-day (Arafa et al., 2009), and following subchronic inhalational exposure of adult F344 rats to 75 μg/m^3 (Archibong et al., 2008; Ramesh et al., 2008). No effects on testes weight were observed in Wistar rats exposed for 35 days to gavage doses up to 50 mg/kg-day (Kroese et al., 2001), F344 rats exposed for 90 days to dietary doses up to 100 mg/kg-day (Knuckles et al., 2001), or Sprague-Dawley rats exposed for 90 days to gavage doses up to 0.1 mg/kg-day (Chung et al., 2011). Strain differences may have contributed to differences in response; however, F344 rats exposed to benzo[a]pyrene via inhalation showed effects on testicular weight (Archibong et al., 2008; Ramesh et al., 2008). In addition, decreased testicular weight has also been observed in offspring following in utero and early postnatal exposure to benzo[a]pyrene as discussed in Section 1.1.1.

Histological changes in the testis have often been reported to accompany decreases in testicular weight. Apoptosis, as evident by increases in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive germ cells and increases in caspase-3 staining, was evident in seminiferous tubules of Sprague-Dawley rats following 90 days of exposure to ≥0.001 and 0.01 mg/kg-day, respectively, benzo[a]pyrene by gavage (Chung et al., 2011). However, the study authors did not observe testicular atrophy or azospermia in any dose group. Seminiferous tubules were reported to look qualitatively similar between controls and animals exposed to benzo[a]pyrene by inhalation doses of 75 μg/m^3 for 60 days (Archibong et al., 2008; Ramesh et al., 2008). However, when histologically examined, statistically significantly reduced tubular lumen size and length were observed in treated animals. Seminiferous tubule diameters also appeared to be reduced in exposed animals, although this difference did not reach statistical significance (Archibong et al., 2008; Ramesh et al., 2008). In addition, histological changes in the seminiferous tubules have also been observed in offspring following in utero exposure to benzo[a]pyrene as discussed in Section 1.1.1.

Epididymal changes

In addition to testicular effects, histological effects in the epididymis have been observed following 90-day gavage exposure to benzo[a]pyrene (Chung et al., 2011) (Table 1-5 and Figure 1-3). Specifically, statistically significant decreased epididymal tubule diameter (for caput and cauda) was observed at doses ≥0.001 mg/kg-day. At the highest dose tested (0.1 mg/kg-day), diameters were reduced approximately 25%. A 60 day gavage study in Hsd:ICR(CD1) mice observed a 27% decrease in cauda epididymis weight at 100 mg/kg-day (Jeng et al., 2013); however, no change in epididymis weight was observed following an 84-day treatment in Sprague-Dawley rats of 5 mg/kg-day benzo[a]pyrene (Chen et al., 2011).

Hormone changes

Several animal models have reported decreases in testosterone following both oral and inhalation exposure to benzo[a]pyrene (Table 1-5 and Figure 1-3). In male Sprague-Dawley rats, decreases in testosterone have been observed following 90-day oral exposures (Chung et al., 2011; Zheng et al., 2010). Statistically significant decreases of 15% in intratesticular testosterone were observed at 5 mg/kg-day in one study (Zheng et al., 2010), while a second study in the same strain of rats reported statistically significant decreases of approximately 40% in intratesticular testosterone and 70% in serum testosterone at 0.1 mg/kg-day (Chung et al., 2011). In addition, Sprague-Dawley rats treated with 10 mg/kg-day by gavage on PNDs 1-7 exhibited statistically significantly decreased serum testosterone (≥40%) when examined at PND 8 and PND 35 (Liang et al., 2012). Statistically significant decreases in intratesticular testosterone (80%) and serum testosterone (60%) were also observed following inhalation exposure to 75 μg/m^3 benzo[a]pyrene in F344 rats for 60 days (Archibong et al., 2008; Ramesh et al., 2008). Statistically significant increases in serum luteinizing hormone (LH) have also been observed in Sprague-Dawley rats following gavage exposure to benzo[a]pyrene at doses of ≥0.01 mg/kg-day (Chung et al., 2011) and in F344 rats following inhalation exposure to 75 μg/m^3 benzo[a]pyrene for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

Table 1-5. Evidence pertaining to the male reproductive toxicity of benzo[a]pyrene in adult animals

Reference and study design	Results
Sperm quality	
Mohamed et al. (2010) C57BL/6 mice, 10 males/dose (treated before mating with unexposed females) 0, 1, or 10 mg/kg-d by gavage (F0 males only) 42 d	↓ epididymal sperm count in F0 mice Approximate % change from control (data reported graphically) : 0, −50*, and −70* ↓ epididymal sperm motility in F0 mice Approximate % change from control (data reported graphically): 0, −20*, and −50*

Reference and study design	Results
	↓ epididymal sperm count in untreated F1 and F2 generations (data reported graphically) No effects were observed in the F3 generation
Chen et al. (2011) Sprague-Dawley rats, 10 males/dose 0 or 5 mg/kg-d by gavage 84 d	↓ epididymal sperm count (% change from control) 0 and −29* ↑ % abnormal epididymal sperm 5 and 8*
Chung et al. (2011) Sprague-Dawley rats, 20−25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↓ epididymal sperm motility (% change relative to control; reported only for 0.01 mg/kg-d) 0 and −30* No statistically significant decrease in epididymal sperm count
(Archibong et al. (2008); Ramesh et al. (2008)) F344 rats, 10 males/group 0 or 75 µg/m^3, 4 hrs/d by inhalation 60 d	↓ epididymal sperm motility (% change from control) 0 and −73* ↓ epididymal sperm count (% change from control) 0 and −69* ↑ % abnormal epididymal sperm 33 and 87* ↓ spermatids/g testis (approximate % change from control; numerical data not reported) 0 and −45*
Testicular changes (weight, histology)	
Mohamed et al. (2010) C57BL/6 mice, 10 males/dose (treated before mating with unexposed females) 0, 1, or 10 mg/kg-d by gavage (F0 males only) 42 d	↓ seminiferous tubules with elongated spermatids (approximate % change from control; numerical data not reported) 0, −20*, and −35* No statistically significant change in area of seminiferous epithelium of testis (approximate % change from control; numerical data not reported) 0, 5, and 20
Chung et al. (2011) Sprague-Dawley rats, 20−25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↑ number of apoptotic germ cells per tubule (TUNEL or caspase 3 positive) No change in testis weight or histology
Chen et al. (2011) Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-d by gavage 84 d	↑ testicular lesions characterized as irregular arrangement of germ cells and absence of spermatocytes (numerical data not reported) No change in testis weight
(Archibong et al. (2008); Ramesh et al. (2008)) F344 rats, 10 adult males/group	↓ decreased testis weight (% change from control) 0 and 34*

Reference and study design	Results
0 or 75 µg/m^3, 4 hrs/d by inhalation 60 d	↓ size of seminiferous tubule lumens and reduced tubular length No change in % of tubules with elongated spermatids
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage, 5d/wk 35 d	No change in testis weight
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d in diet 90 d	No change in testis weight
Epididymal changes (weight, histology)	
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↓ diameter of caput epididymal tubule (n = 5; numerical data not reported) ↓ diameter of cauda epididymal tubule (n = 5; numerical data not reported)
Chen et al. (2011) Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-d by gavage 84 d	No change in epididymis weight
Hormone changes	
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, 0.1 mg/kg-d by gavage 90 d	↓ Intratesticular testosterone (approximate % change from control; data reported graphically) 0, −12, −25, and −40* ↓ Serum testosterone (approximate % change from control; numerical data not reported) 0, 0, −35, and −70* ↑ serum LH (approximate % change from control; numerical data not reported) 0, 33, 67*, and 87* ↓ Human chorionic gonadotropin (hCG) or dibutyl cyclic adenosine monophosphate (dbcAMP)-stimulated testosterone production in Leydig cells
Zheng et al. (2010) Sprague-Dawley rats, 8 males/dose 0, 1, or 5 mg/kg-d by gavage 90 d	↓ Intratesticular testosterone (approximate % change from control; numerical data not reported) 0, −15, and −15*
(Archibong et al. (2008); Ramesh et al. (2008)) F344 rats, 10 adult males/group 0 or 75 µg/m^3, 4 hrs/d by inhalation	↓ intratesticular testosterone (approximate % change from control; numerical data not reported) 0 and −80*

Reference and study design	Results
60 d	↓ serum testosterone (approximate % change from control) 0 and −60* ↑ serum LH (approximate % change from control) 0 and 50*

*Statistically significantly different from the control ($p < 0.05$).
[a]% change from control calculated as: (treated value − control value)/control value × 100.

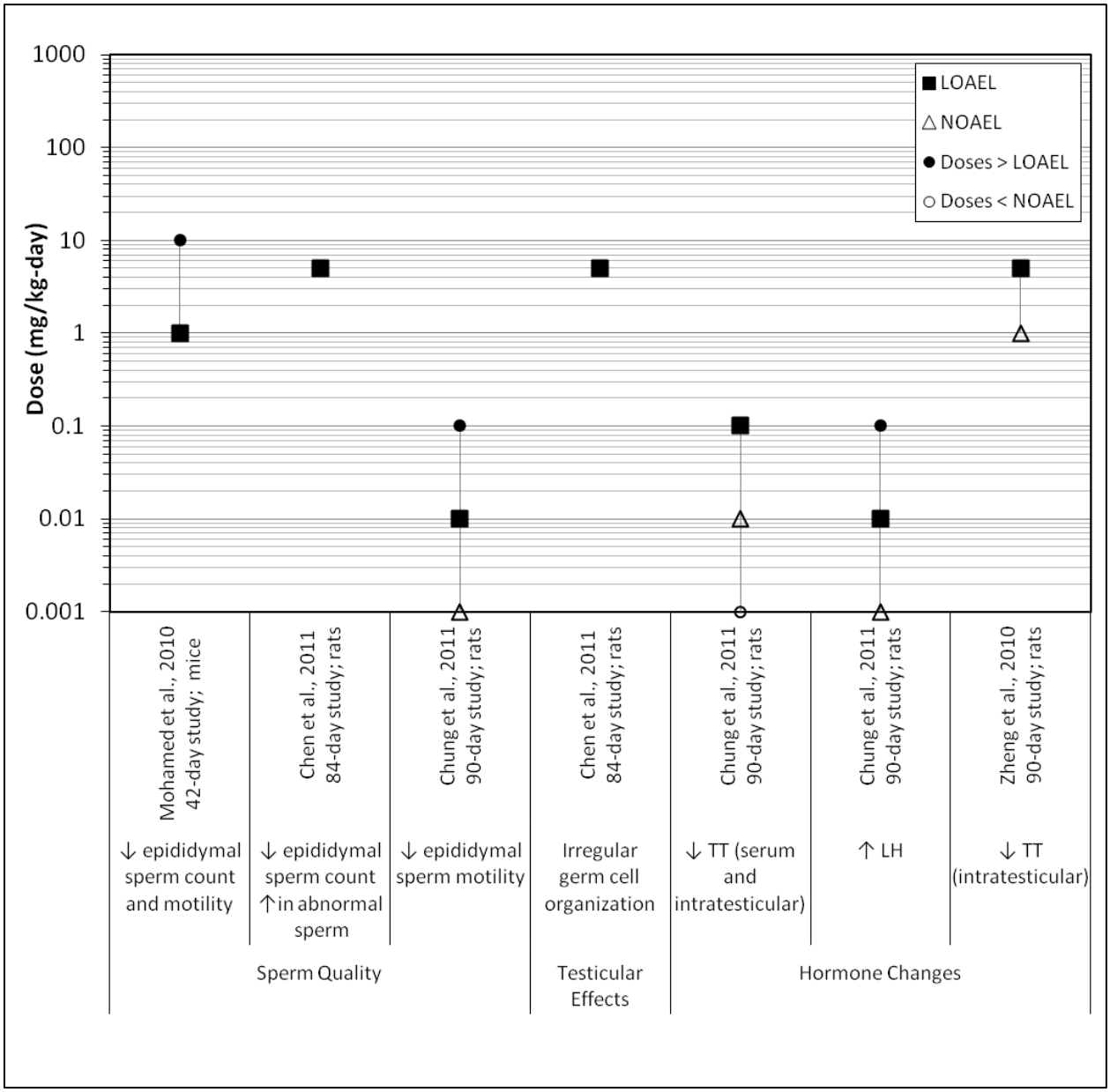

Figure 1-3. Exposure-response array for male reproductive effects following oral exposure in adult animals.

Mode-of-action analysis—male reproductive effects

Exposure to benzo[a]pyrene in laboratory animals induces male reproductive effects including decreased levels of testosterone and increased levels of LH, decreased sperm quality, and histological changes in the testis. Decrements in sperm quality and decreased fertility have also been demonstrated in populations highly exposed to PAH mixtures (Soares and Melo, 2008; Hsu et al., 2006).

Numerous studies have indicated that benzo[a]pyrene reduces testosterone levels following oral or inhalation exposure (Chung et al., 2011; Zheng et al., 2010; Archibong et al., 2008; Ramesh et al., 2008). It is plausible that the effects on sperm quality and histological changes of the reproductive organs are secondary to an insufficiency of testosterone (Inyang et al., 2003). One study has hypothesized that benzo[a]pyrene perturbs the production of testosterone by Leydig cells (Chung et al., 2011). This study found a statistically significant reduction in testicular testosterone in rats treated with 0.1 mg/kg-day benzo[a]pyrene for 90 days and found that testosterone production in isolated Leydig cells was also inhibited approximately 50%, even in cultures stimulated with human chorionic gonadotropin and dibutryl cyclic adenosine monophosphate.

Leydig cell function is thought to be regulated by testicular macrophages (Hales, 2002). When testicular macrophages are activated and produce inflammatory mediators, Leydig cell testosterone production is inhibited (Hales, 2002). Zheng et al. (2010) treated rats with 5 mg/kg-day benzo[a]pyrene for 90 days and reported a statistically significant increase in ED-1 type testicular macrophages and a statistically significant decrease in intratesticular testosterone.

Arafa et al. (2009) reported that male reproductive effects observed following benzo[a]pyrene exposure could be ameliorated by antioxidant pre-treatment. This study reported decreased sperm count, motility, and production, in addition to decreased testis weight following a 10 day oral administration in rats of 50 mg/kg-day benzo[a]pyrene. Pretreatment with the citris flavonoid hesperidin protected rats from all of these effects except the decrease in sperm motility.

A study in tobacco smokers suggests that direct DNA damage from the reactive metabolite BPDE may decrease sperm motility (Perrin et al., 2011a). In this study, motile sperm were separated from non-motile sperm using a "swim-up" self-migration technique. The investigators found that the motile sperm selected by this method had significantly fewer BPDE-adducts than non-selected sperm.

Other hypothesized modes of action of the observed male reproductive effects include benzo[a]pyrene-mediated DNA damage to male germ cells leading to genotoxicity, cytotoxicity, and apoptosis (Chung et al., 2011; Perrin et al., 2011b; Perrin et al., 2011a; Olsen et al., 2010; Revel et al., 2001), compromised function of Sertoli cells (Raychoudhury and Kubinski, 2003), and decreased embryo viability post-fertilization associated with sperm DNA damage (Borini et al., 2006; Seli et al., 2004).

Female Reproductive Effects

Fertility

In women, exposure to cigarette smoke has been shown to affect fertility, including effects related to pregnancy, ovulatory disorders, and spontaneous abortion (as reviewed in Waylen et al., 2009; Cooper and Moley, 2008; Soares and Melo, 2008). In addition, several studies suggest that in utero exposure to maternal tobacco smoke also decreases the future fertility of female offspring (Ye et al., 2010; Jensen et al., 1998; Weinberg et al., 1989). Benzo[a]pyrene levels in follicular fluid and benzo[a]pyrene-DNA adducts in granulosa-lutein cells and oocytes and in human cervical cells have been associated with smoking status and with amount smoked (Neal et al., 2008; Mancini et al., 1999; Melikian et al., 1999; Zenzes et al., 1998; Shamsuddin and Gan, 1988).

Few epidemiological studies have examined the specific influence of components of PAH mixtures on fertility or other reproductive outcomes; EPA identified only two studies with specific data on benzo[a]pyrene (Table 1-6). One of these studies addressed the probability of conception among women undergoing in vitro fertilization (Neal et al., 2008). Follicular fluid benzo[a]pyrene levels were significantly higher among the women who did not conceive compared with women who did get pregnant. No association was seen between conception and serum levels of benzo[a]pyrene. The other study examined risk of delayed miscarriage (fetal death before 14 weeks of gestation), using a case-control design with controls selected from women undergoing elective abortion (Wu et al., 2010). A strong association was seen between maternal blood benzo[a]pyrene-DNA adduct levels and risk of miscarriage, with a fourfold increased risk for levels above compared with below the median. Benzo[a]pyrene-DNA adduct levels were similar in the aborted tissue of cases compared with controls.

Experimental studies in mice also provide evidence that benzo[a]pyrene exposure affects fertility (Table 1-7 and Figure 1-4). Decreased fertility and fecundity (decreased number of F0 females producing viable litters at parturition) was statistically significantly reduced by about 35% in adult females exposed to 160 mg/kg-day of benzo[a]pyrene (Mackenzie and Angevine, 1981). In another study, F0 females showed no signs of general toxicity or effects on fertility following gavage exposure to 10 mg/kg-day on GDs 7–16 (Kristensen et al., 1995). Decrements in fertility were more striking in the offspring from these studies, as described in Section 1.1.1.

Ovarian effects

Human epidemiological studies that directly relate ovotoxicity and benzo[a]pyrene exposure are not available; however, smoking, especially during the time of the peri-menopausal transition, has been shown to accelerate ovarian senescence (Midgette and Baron, 1990). Benzo[a]pyrene-induced ovarian toxicity has been demonstrated in animal studies. In adult female rats treated by gavage, statistically significant, dose-related decreases in ovary weight have been observed in female rats treated for 60 days at doses ≥5 mg/kg (2.5 mg/kg-day adjusted) (Xu et al., 2010). At 10 mg/kg in adult rats (5 mg/kg-day adjusted), ovary weight was decreased 15% (Xu et

al., 2010). Changes in ovary weight were not observed in two subchronic studies in rats. Specifically, no changes in ovary weight were seen in Wistar rats exposed for 35 days to gavage doses up to 50 mg/kg-day (Kroese et al., 2001) or in F344 rats exposed for 90 days to dietary doses up to 100 mg/kg-day (Knuckles et al., 2001).

In adult female rats treated by gavage, dose-related decreases in the number of primordial follicles have been observed in female rats treated for 60 days at doses ≥2.5 mg/kg-day, with a statistically significant decrease of approximately 20% at the high dose (Xu et al., 2010) (Table 1-7 and Figure 1-4). No notable differences in other follicle populations and corpora lutea were observed. However, in utero studies exposing dams to the same doses produced offspring with few or no follicles or corpora lutea (Kristensen et al., 1995; Mackenzie and Angevine, 1981). Additional support for the alteration of female reproductive endpoints comes from intraperitoneal (i.p.) experiments in animals and in vitro experiments. Several studies have observed ovarian effects (decreased numbers of ovarian follicles and corpora lutea, absence of folliculogenesis, oocyte degeneration, and decreased fertility) in rats and mice exposed via i.p. injection (Borman et al., 2000; Miller et al., 1992; Swartz and Mattison, 1985; Mattison et al., 1980). Further evidence is available from in vitro studies showing inhibition of antral follicle development and survival, as well as decreased production of estradiol, in mouse ovarian follicles cultured with benzo[a]pyrene for 13 days (Sadeu and Foster, 2011). Likewise, follicle stimulating hormone (FSH)-stimulated growth of cultured rat ovarian follicles was inhibited by exposure to benzo[a]pyrene (Neal et al., 2007).

Hormone levels

Alteration of hormone levels has been observed in female rats following oral or inhalation exposure to benzo[a]pyrene (Table 1-7 and Figure 1-4). Inhalation exposure to benzo[a]pyrene:carbon black particles during gestation resulted in decreases in plasma progesterone, estradiol, and prolactin in pregnant rats (Archibong et al., 2002). In addition, statistically significant, dose-related decreases in estradiol along with altered estrus cyclicity was observed in female rats treated for 60 days at doses ≥2.5 mg/kg-day by gavage (Xu et al., 2010). Mechanistic experiments have also noted decreased estradiol output in murine ovarian follicles cultured with benzo[a]pyrene in vitro for 13 days, but did not find any decrease in progesterone (Sadeu and Foster, 2011).

Cervical effects

One subchronic animal study is available that investigated effects in the cervix following oral exposure to benzo[a]pyrene (Table 1-7 and Figure 1-4). Statistically-significant dose-related increases in the incidence of cervical inflammatory cells were observed in mice exposed twice a week for 98 days to benzo[a]pyrene via gavage at doses ≥2.5 mg/kg (Gao et al., 2011). Cervical effects of increasing severity, including epithelial hyperplasia, atypical hyperplasia, apoptosis, and necrosis, were observed at higher doses. There are no data on cervical effects in other species or in

other mouse strains. Gao et al. (2011) considered the hyperplasia responses to be preneoplastic lesions. Cervical neoplasia was not reported in the available chronic bioassays, but this tissue was not subjected to histopathology examination in either bioassay (Kroese et al., 2001; Beland and Culp, 1998). Thus, the relationship of the cervical lesions to potential development of neoplasia is uncertain.

Table 1-6. Evidence pertaining to the female reproductive effects of benzo[a]pyrene in humans

Reference and study design	Results
Probability of conception	
Neal et al. (2008) 36 women undergoing in vitro fertilization (19 smokers, 7 passive smokers, and 10 nonsmokers) Exposure: benzo[a]pyrene in serum and follicular fluid	Benzo[a]pyrene levels (ng/mL) Follicular fluid: Conceived 0.1; Did not conceive 1.7; *p*-value <0.001 Serum: Conceived 0.01; Did not conceive 0.05; *p*-value Not reported
Fetal death	
Wu et al. (2010) (Tianjin, China) Case control study: 81 cases (96% participation rate)—fetal death confirmed by ultrasound before 14 wks gestation; 81 controls (91% participation rate)—elective abortions; matched by age, gestational age, and gravidity; excluded smokers and occupational PAH exposure Exposure: benzo[a]pyrene in aborted tissue and maternal blood samples (51 cases and controls, 2 of 4 hospitals)	Benzo[a]pyrene adduct levels (/10^8 nucleotides), mean (±SD) Maternal blood: Cases 6.0 (± 4.7); Controls 2.7 (± 2.2); *p*-value <0.001 Aborted tissue: Cases 4.8 (± 6.0); Controls 6.0 (± 7.4); *p*-value 0.29 Low correlation between blood and tissue levels (r = −0.02 in cases, r = −0.21 in controls) Association between benzo[a]pyrene adducts and miscarriage[a] Per unit increase in adducts: OR 1.37; 95% CI 1.12, 1.67 Dichotomized at median: OR 4.56; 95% CI 1.46, 14.3 [a]Conditional logistic regression, adjusted for maternal education, household income, and gestational age; age also considered as potential confounder

Table 1-7. Evidence pertaining to the female reproductive effects of benzo[a]pyrene in adult animals

Reference and study design	Results[a]
Fertility	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/dose 0, 10, 40, or 160 mg/kg-d by gavage GDs 7–16	↓ number of F0 females with viable litters 46/60, 21/30, 44/60, and 13/30*
Kristensen et al. (1995) NMRI mice, 9 females/dose 0 or 10 mg/kg-d by gavage GDs 7–16	No changes in fertility of F0 females
Ovarian effects (weight, histology, follicle numbers)	
Xu et al. (2010) Sprague-Dawley rats, 6 females/dose 0, 5 or 10 mg/kg by gavage every other day (2.5 and 5 mg/kg-d, adjusted) 60 d	↓ ovary weight (% change from control) 0, −11*, and −15* ↓ number of primordial follicles (20%* decrease at high dose) ↑ increased apoptosis of ovarian granulosa cells (approximate % apoptosis) 2, 24*, and 14*
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d in diet 90 d	No changes in ovary weight
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk 35 d	No changes in ovary weight
Hormone levels	
Xu et al. (2010) Sprague-Dawley rats, 6 females/dose 0, 5, or 10 mg/kg by gavage every other day (2.5 and 5 mg/kg-d, adjusted) 60 d	↓ serum estradiol (approximate % change from control) 0, −16, and −25* Altered estrous cyclicity
Archibong et al. (2002) F344 rats, 10 females/group 0, 25, 75, or 100 µg/m^3 by inhalation 4 hrs/d GDs 11–20 (serum hormones tested at GD 15 and 17 in 0, 25, and 75 µg/m^3 dose groups)	↓ F0 estradiol, approximately 50% decrease at 75 µg/m^3 at GD 17 ↓ F0 prolactin, approximately 70% decrease at 75 µg/m^3 at GD 17 ↑ F0 plasma progesterone approximately 17% decrease at 75 µg/m^3 at GD 17

Reference and study design	Results[a]
Cervical effects	
Gao et al. (2011) ICR mice, 26 females/dose 0, 2.5, 5, or 10 mg/kg by gavage 2 d/wk 98 d	↑ cervical epithelial hyperplasia: 0/26, 4/26, 6/25*, and 7/24* ↑ cervical atypical hyperplasia: 0/26, 0/26, 2/25, and 4/24* ↑ inflammatory cells in cervical epithelium: 3/26, 10/26, 12/25*, and 18/24*

*Statistically significantly different from the control ($p < 0.05$).
[a]% change from control calculated as: (treated value − control value)/control value × 100.

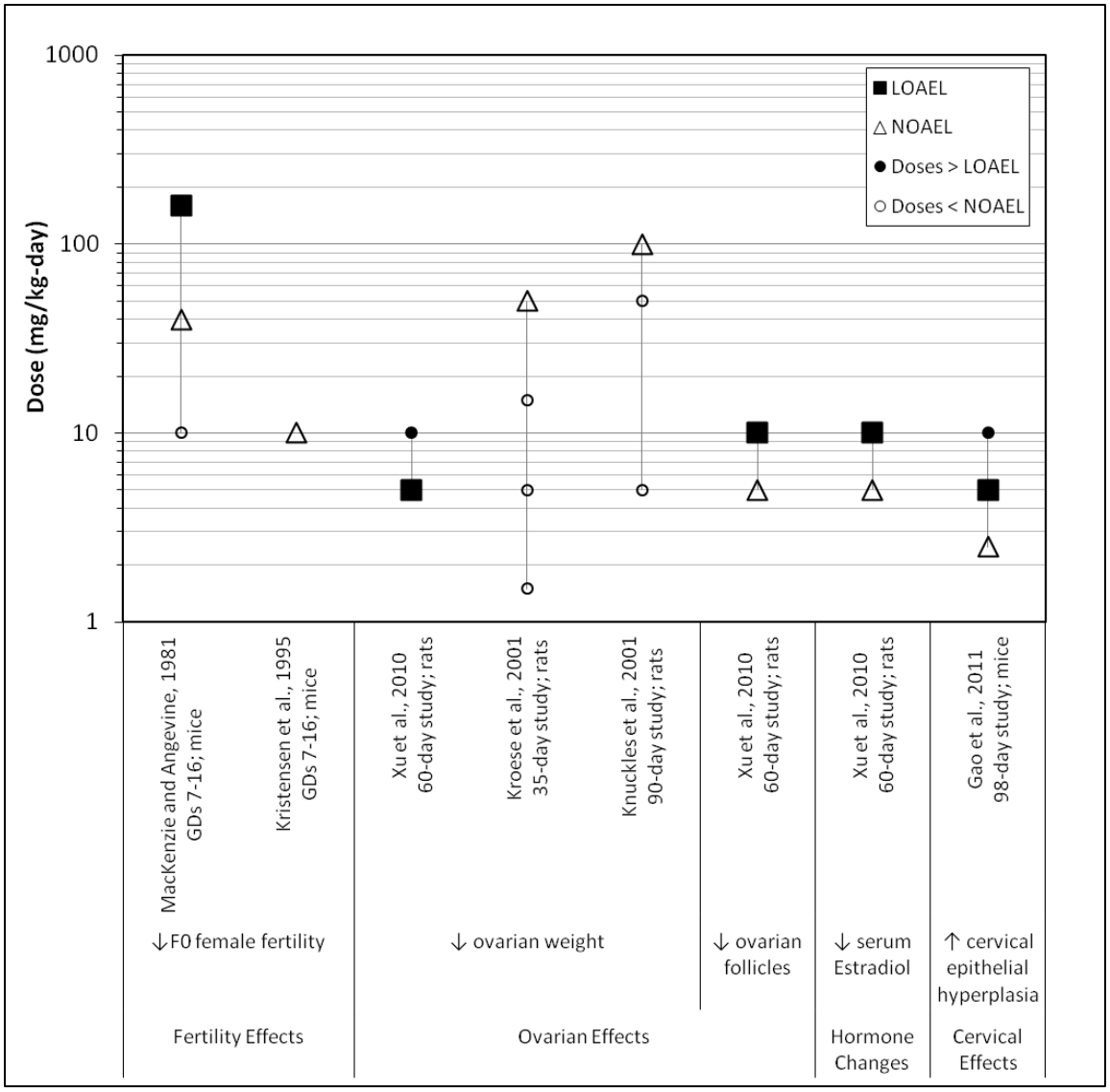

Figure 1-4. Exposure-response array for female reproductive effects following oral exposure in adult animals.

Mode-of-action analysis—female reproductive effects

Although the mechanisms underlying female reproductive effects following benzo[a]pyrene exposure are not fully established, associations with stimulation of apoptosis, impairment of steroidogenesis, and cytotoxicity have been made. Ovarian lesions in benzo[a]pyrene-exposed rats have been associated with increased apoptosis in ovarian granulosa cells and alteration in hormone-mediated regulation of folliculogenesis (Xu et al., 2010), and results from in vitro experiments provide support for an association between benzo[a]pyrene exposure and impaired folliculogenesis, steroidogenesis, and oocyte maturation (Sadeu and Foster, 2011; Neal et al., 2007). A growing body of research suggests that benzo[a]pyrene triggers the induction of apoptosis in oocytes through AhR-driven expression of pro-apoptotic genes, including Bax (Kee et al., 2010; Neal et al., 2010; Pru et al., 2009; Matikainen et al., 2002; Matikainen et al., 2001; Robles et al., 2000). Other proposed mechanisms include the impairment of folliculogenesis from reactive metabolites (Takizawa et al., 1984; Mattison and Thorgeirsson, 1979, 1977) or by a decreased sensitivity to FSH-stimulated follicle growth (Neal et al., 2007). Based on findings that an ERα antagonist counteracted effects of subcutaneously administered benzo[a]pyrene on uterine weight (decreased in neonatal rats and increased in immature rats), interactions with ERα have been proposed, possibly via occupation of ERα binding sites or via AhR-ER-crosstalk (Kummer et al., 2008; Kummer et al., 2007). However, 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; Vondrácek et al., 2002; Fertuck et al., 2001; Charles et al., 2000), so the role of the ER in benzo[a]pyrene-induced reproductive toxicity is unclear.

Summary of Reproductive Effects

Male reproductive effects

Exposure to benzo[a]pyrene in laboratory animals induces male reproductive effects including decreased levels of testosterone and increased levels of LH, decreased sperm count and motility, histological changes in the testis, and decreased reproductive success. These findings in animals are supported by decrements in sperm quality and decreased fertility in human populations exposed to PAH mixtures (Soares and Melo, 2008; Hsu et al., 2006). In laboratory animals, male reproductive toxicity has been observed after oral and inhalation exposure to rats or mice. Effects seen after oral exposures include impaired fertility, effects on sperm parameters, decreased reproductive organ weight, testicular lesions, and hormone alterations (Chen et al., 2011; Chung et al., 2011; Mohamed et al., 2010; Zheng et al., 2010; Mackenzie and Angevine, 1981). In addition to oral exposure, male reproductive effects of benzo[a]pyrene have also been observed following inhalation exposure in rats (Archibong et al., 2008; Ramesh et al., 2008; Inyang et al., 2003). The male reproductive effects associated with benzo[a]pyrene exposure are considered to be biologically plausible and adverse.

In conclusion, EPA identified male reproductive system effects as a human hazard of benzo[a]pyrene exposure.

Female reproductive effects

A large body of mechanistic data, both in vivo and in vitro, suggests that benzo[a]pyrene impacts fertility through the disruption of folliculogenensis. This finding is supported, albeit indirectly, by observations of premature ovarian senescence in women exposed to cigarette smoke (Midgette and Baron, 1990). Evidence for female reproductive toxicity of benzo[a]pyrene comes from studies of human populations exposed to PAH mixtures as well as laboratory animal and in vitro studies. In addition, two human studies observed associations specifically between benzo[a]pyrene measures and two fertility-related endpoints: decreased ability to conceive (Neal et al., 2008; Neal et al., 2007) and increased risk of early fetal death (i.e., before 14 weeks of gestation) (Wu et al., 2010). Studies in multiple strains of rats and mice indicate fertility-related effects including decreases in ovarian follicle populations and decreased fecundity. Decreased serum estradiol has also been noted in two different strains of rats exposed by oral or inhalation exposure. The reproductive effects associated with benzo[a]pyrene exposure are biologically supported and relevant to humans.

In conclusion, EPA identified female reproductive effects as a human hazard of benzo[a]pyrene exposure.

Susceptible Populations and Lifestages

Epidemiological studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (Neal et al., 2008; El-Nemr et al., 1998) 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 4 times more likely to have experienced a miscarriage (Wu et al., 2010).

Inhalation exposure of pregnant female rats to benzo[a]pyrene:carbon black aerosols on GDs 11–20 caused decreased fetal survival and number of pups per litter associated with decreased levels of plasma progesterone, estradiol, and prolactin (Archibong et al., 2002). Decreased numbers of live pups were also seen in pregnant mice following i.p. exposure to benzo[a]pyrene (Mattison et al., 1980). These results indicate that benzo[a]pyrene exposure can decrease the ability of females to maintain pregnancy.

Oral multigenerational studies of benzo[a]pyrene exposure in mice demonstrated effects on fertility and the development of reproductive organs (decreased ovary and testes weight) in both male and female offspring of pregnant mice exposed to 10–160 mg/kg-day on GDs 7–16 (Kristensen et al., 1995; Mackenzie and Angevine, 1981).

Reductions in female fertility associated with decreased ovary weight and follicle number following gestational exposure (as discussed in Section 1.1.1) are supported by observations of:

(1) destruction of primordial follicles (Borman et al., 2000; Mattison et al., 1980) and decreased corpora lutea (Miller et al., 1992; Swartz and Mattison, 1985) in adult female mice following i.p. exposure; (2) decreased ovary weight in adult female rats following oral exposure (Xu et al., 2010); and (3) stimulation of oocyte apoptosis (Matikainen et al., 2002; Matikainen et al., 2001) or by a decreased sensitivity to FSH-stimulated follicle growth (Neal et al., 2007).

Reductions in male fertility associated with decreased testes weight following gestational exposure (as discussed in Section 1.1.1) are supported by observations of: (1) decreased sperm count, altered serum testosterone levels, testicular lesions, and/or increased numbers of apoptotic germ cells in adult rats following repeated oral exposure to benzo[a]pyrene (Chung et al., 2011; Chen et al., 2010; Zheng et al., 2010; Arafa et al., 2009); (2) decreased epididymal sperm counts in adult F0 and F1 generations of male mice following 6 weeks of oral exposure of the F0 animals to benzo[a]pyrene (Mohamed et al., 2010); and (3) decreased testis weight, decreased testicular or plasma testosterone levels, and/or decreased sperm production, motility, and density in adult male rats following repeated inhalation exposure to aerosols of benzo[a]pyrene:carbon black (Archibong et al., 2008; Ramesh et al., 2008; Inyang et al., 2003).

1.1.3. Immunotoxicity

Human studies evaluating immune effects following exposure to benzo[a]pyrene alone are not available for any route of exposure. However, a limited number of occupational human studies, particularly in coke oven workers (Zhang et al., 2012; Wu et al., 2003b; Winker et al., 1997; Szczeklik et al., 1994), show effects on immune parameters associated with exposure to PAH mixtures. These studies are of limited utility because effects associated specifically with benzo[a]pyrene cannot be distinguished from other constituents of the PAH mixture. Subchronic and short-term animal studies have reported immunotoxic effects of benzo[a]pyrene by multiple routes of exposure (Table 1-8 and Figure 1-5). Effects include changes in thymus weight and histology, decreased B cell percentages and other alterations in the spleen, and immune suppression. Data obtained from subchronic oral gavage studies are supported by short-term, i.p., intratracheal, and subcutaneous (s.c.) studies. Additionally, there is evidence in animals for effects of benzo[a]pyrene on the developing immune system. No studies were located that examined immune system endpoints following inhalation exposure of animals to benzo[a]pyrene.

Thymus Effects

Decreased thymus weights (up to 62% compared to controls) were observed in male and female Wistar rats exposed by gavage to 10–90 mg/kg-day benzo[a]pyrene for 35 or 90 days (Kroese et al., 2001; De Jong et al., 1999). This effect may be due to thymic atrophy. The incidence of slight thymic atrophy was increased in males (6/10) and females (3/10) at a dose of 30 mg/kg-day in a 90-day study, although there was no evidence of atrophy at any lower dose (Kroese et al., 2001). Additionally, at the highest dose tested (90 mg/kg-day) in one of the 35-day studies, the relative cortex surface area of the thymus and thymic medullar weight were

significantly reduced (De Jong et al., 1999). Other histopathological changes in the thymus (increased incidence of brown pigmentation of red pulp; hemosiderin) were observed in Wistar rats of both sexes at 50 mg/kg-day in a 35-day study; however, this tissue was not examined in intermediate-dose groups (Kroese et al., 2001). Consistent with the effects observed in these studies, decreased thymus weights and reduced thymic cellularity were observed in i.p. injection studies that exposed mice to doses ranging from 50 to 150 mg/kg in utero (Holladay and Smith, 1995, 1994; Urso and Johnson, 1988).

Spleen Effects

Reduced splenic cellularity, indicated by decreased relative and absolute number of B cells in the spleen (decreased up to 41 and 61% compared to controls, respectively) and decreased absolute number of splenic cells (31% decrease at the highest dose), was observed in a subchronic study in male Wistar rats administered 3–90 mg/kg-day benzo[a]pyrene by gavage for 35 days (De Jong et al., 1999). While the effect on the relative number of B cells was dose-related, the lower doses did not affect the number of B cells or the absolute splenic cell number. The reduced splenic cell count at the highest dose was attributed by the study authors to the decreased B cells, and suggests a possible selective toxicity of benzo[a]pyrene to B cell precursors in the bone marrow. The spleen effects observed in De Jong et al. (1999) are supported by observations of reduced spleen cellularity and decreased spleen weights following i.p. injection or in utero benzo[a]pyrene exposure to doses ranging from 50 to 150 mg/kg (Holladay and Smith, 1995; Urso et al., 1988).

In addition to physical effects on the spleen, several studies have demonstrated functional suppression of the spleen following benzo[a]pyrene exposure. Dose-related decreases in sheep red blood cell (SRBC) specific serum IgM levels after SRBC challenge were reported in rats (10 or 40 mg/kg-day) and mice (5, 20,or 40 mg/kg-day) following s.c. injection of benzo[a]pyrene for 14 days (Temple et al., 1993). Similarly, reduced spleen cell responses, including decreased numbers of plaque forming cells and reduced splenic phagocytosis to SRBC and lipopolysaccharide challenge, 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).

Immunoglobulin Alterations

Alterations in immunoglobulin levels have been associated with exposure to PAH mixtures in a limited number of human studies. Some occupational studies have reported evidence of immunosuppression following PAH exposure. For example, reductions in serum IgM and/or IgA titers were reported in coke oven workers (Wu et al., 2003b; Szczeklik et al., 1994). Conversely, immunostimulation of immunoglobulin levels has also been observed in humans, specifically elevated IgG (Karakaya et al., 1999) and elevated IgE (Wu et al., 2003b) following occupational PAH exposure.

Decreases in serum IgM (13–33% compared to controls) and IgA levels (22–61% compared to controls) were observed in male Wistar rats exposed to 3–90 mg/kg-day benzo[a]pyrene by gavage for 35 days (De Jong et al., 1999); however, these reductions were not dose-dependent. Similarly, reductions in IgA (9–38% compared to controls) were also observed in male and female $B6C3F_1$ mice exposed to doses of 5–40 mg/kg benzo[a]pyrene by s.c. injection for 14 days (Munson and White, 1983). Reductions in serum IgG levels of 18–24%, although not statistically significant, were observed in female $B6C3F_1$ mice exposed to doses ≥50 mg/kg benzo[a]pyrene by i.p. injection for 14 days (Dean et al., 1983).

Immune Suppression and Sensitization

Some occupational studies of coke oven emissions have reported evidence of immunosuppression following PAH exposure. Reduced mitogenic responses in T cells (Winker et al., 1997) and reduced T-lymphocyte proliferative responses (Karakaya et al., 2004) have been observed following occupational exposure to PAH. Increased levels of apoptosis were observed in the peripheral blood mononuclear cells (a population of lymphocytes and monocytes) of occupationally exposed coke oven workers, which is a response that may contribute to immunodeficiency in this population (Zhang et al., 2012). However, a limitation of this study is that it does not attribute the proportion of apoptotic activity to a specific class of cells and does not include assessment of other potential markers of immunotoxicity in peripheral blood.

Results of functional immune assays in laboratory animals following short-term i.p. and s.c. exposures add to the evidence for benzo[a]pyrene immunotoxicity. Resistance to Streptococcus pneumonia or Herpes simplex type 2 was dose dependently reduced in $B6C3F_1$ mice following s.c. injection of ≥5 mg/kg-day benzo[a]pyrene for 14 days (Munson et al., 1985). Reduced cell proliferation, IFN-γ release, and IL-4 release were observed in male and female C56BL/6 mice following short-term exposure to a gavage dose of 13 mg/kg benzo[a]pyrene as measured in a modified local lymph node assay (van den Berg et al., 2005). A statistically significant decrease in natural killer cell activity was observed in male Wistar rats (Effector:Target cell ratio was 40.9 ± 28.4% that of controls) exposed to 90 mg/kg-day by gavage for 35 days (De Jong et al., 1999); however, splenic natural killer cell activity was not affected in $B6C3F_1$ mice after s.c. injection of 40mg/kg-day benzo[a]pyrene for 14 days (Munson et al., 1985). The magnitude of the dose and duration of the exposure may account for the discrepancy between these two studies. Single i.p. injections of 50 mg/kg benzo[a]pyrene decreased pro- and/or pre-B-lymphocytes and neutrophils in the bone marrow of C57BL/6J mice without affecting the numbers of immature and mature B-lymphocytes or GR-1+ myeloid cells (Galván et al., 2006).

In contrast to studies that have shown immunosuppression, benzo[a]pyrene may also induce sensitization responses. Epicutaneous abdominal application of 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).

Developmental Immunotoxicity

As noted above, several i.p. injection studies suggest that cell-mediated and humoral immunity may be altered by exposure to high doses of benzo[a]pyrene during gestation. Suppression of the mixed lymphocyte response, the graft-versus-host response, and suppression of the plaque-forming cell response to SRBCs was observed in mice exposed in utero to 150 mg/kg during mid (GDs 11–13), late (GDs 16–18), or both (GDs 11–17) stages of gestation; these effects persisted until 18 months of age (Urso and Gengozian, 1984, 1982, 1980). Fetal thymic atrophy, as assessed by reductions in cellularity (74–95%, compared to controls), was observed in mice exposed to 50–150 mg/kg benzo[a]pyrene from GD 13 to 17, when examined on GD 18 (Holladay and Smith, 1994). Analysis of cell surface markers (e.g., CD4, CD8) from the same study indicate that benzo[a]pyrene may inhibit and/or delay thymocyte maturation, possibly contributing to the observed thymic atrophy (Holladay and Smith, 1994). Consistent with these findings, several other studies have noted 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).

The fetal liver is the primary hematopoietic organ during gestation and a major source of thymocyte precursors beginning around GD 10 or 11 in mice (Landreth and Dodson, 2005; Penit and Vasseur, 1989). Statistically significant reductions in total cellularity in the fetal liver of 54 and 67% were reported in offspring after i.p. exposures of 50 or 100 mg/kg benzo[a]pyrene, respectively, to the dams on GDs 13–17 (Holladay and Smith, 1994). The decreased fetal liver cellularity was accompanied by decreased expression of terminal deoxynucleotidyl transferase and CD45R cellular markers, which are known to be present in cortical thymocyte progenitors in the fetal liver (Holladay and Smith, 1994; Fine et al., 1990; Silverstone et al., 1976). These data also suggest that benzo[a]pyrene disrupts liver hematopoiesis during gestation and may interfere with prolymphoid seeding of the thymus, possibly contributing to thymic atrophy and cell-mediated immunosuppression. Decreased numbers of $CD4^+$ T-cells have been reported in the spleen of 1-week-old mice following in utero benzo[a]pyrene exposure by i.p. injection to the dams, demonstrating the potential for downstream effects on T-cell development (Rodriguez et al., 1999). The decreased numbers of CD4+ T-cells correspond with observations of decreased proliferation in the presence of Concanavalin A and a weak response compared to controls in an allogeneic mixed lymphocyte reaction assay (Urso and Kramer, 2008).

Postnatal exposure to benzo[a]pyrene has also been suggested to cause immune effects. Dose-dependent decreases in erythrocytes (attributed to reduced bone marrow erythropoiesis), as well as reduced expression of IL-4 and IFN-γ were observed in the pups of Wistar rats exposed to 0.1–10 mg/kg-day benzo[a]pyrene by subcutaneous injection for 14 days (Matiasovic et al., 2008). This finding suggests that benzo[a]pyrene may alter the immune response to infection or vaccination in developing animals.

Table 1-8. Evidence pertaining to the immune effects of benzo[a]pyrene in animals

Reference and study design	Results[a]
Thymus effects	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d	↓ thymus weight Females (% change from control): 0, −3, −6, and −28* Males (% change from control): 0, 0, −13, and −29* ↑ slight thymic atrophy Females (incidence): 0/10, 0/10, 0/10, and 3/10 Males (incidence): 0/10, 2/10, 1/10, and 6/10*
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ thymus weight % change from control: 0, −9, −15*, −25*, and −62*
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk 35 d	↓ thymus weight Females (% change from control): 0, 13, 8, −3, and −17* Males (% change from control): 0, −8, −11, −27*, and −33*
Spleen effects	
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ relative number (%) of B cells in spleen % change from control: 0, −8, −13*, −18*, and −41* ↓ total number of B cells in spleen % change from control: 0, 13, −13, −13, and −61* Change in total cell number in the spleen % change from control: 0, 20, 0, +7, and −31*
Immunoglobulin alterations	
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ serum IgM % change from control: 0, −13, −14, −33*, and −19 ↓ serum IgA % change from control: 0, −27, −22, −28, and −61*

*Statistically significantly different from the control ($p < 0.05$).

[a]% change from control calculated as: (treated value − control value)/control value × 100.

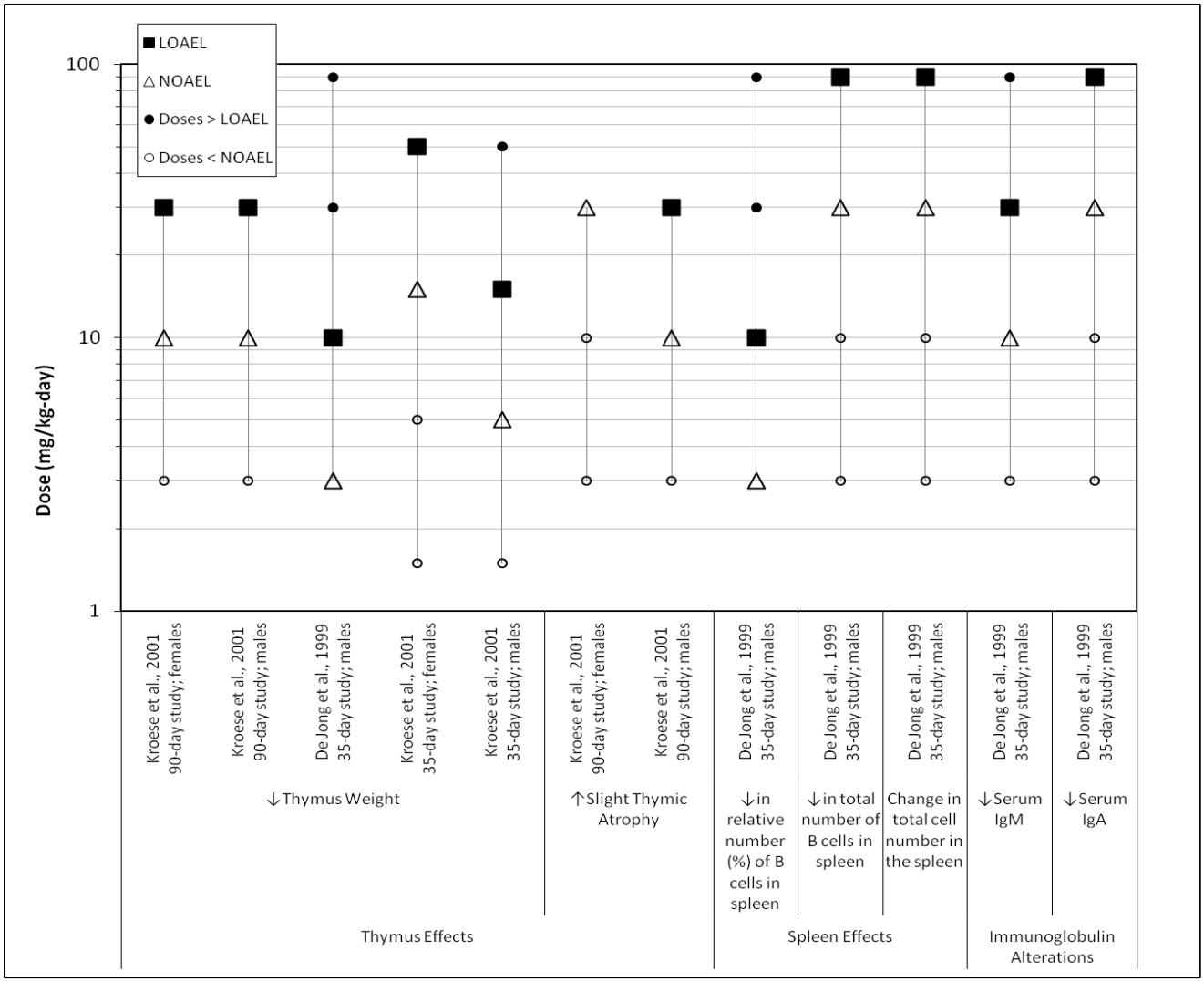

Figure 1-5. Exposure-response array for immune effects following oral exposure.

Mode-of-Action Analysis—Immune Effects

Exposure to benzo[a]pyrene induces immunosuppressive effects such as decreased numbers of B cells in the spleen and decreased thymus weight and cellularity following oral, i.p., s.c., or intratracheal exposure in experimental animals. However, the key events underlying benzo[a]pyrene immunotoxicity have not been identified.

Benzo[a]pyrene is a well-known ligand for the AhR (Okey et al., 1994; Nebert et al., 1993; Postlind et al., 1993). Ligands of the AhR have been shown to have a role in regulating hematopoietic stem cells in the bone marrow, a major site of B-cell proliferation and antibody production (Esser, 2009). Benzo[a]pyrene reduced B-cell lymphopoiesis at concentrations as low as 10^{-8}M (Hardin et al., 1992). Furthermore, Ah-responsive (C57BL/6) mice showed greater dose-dependent reductions in B-cell lymphopoiesis than those observed in Ah-nonresponsive (DBA/2)

mice (Hardin et al., 1992). Addition of the AhR antagonist and CYP450 inhibitor, α-naphthaflavone, inhibited the benzo[a]pyrene-induced suppression of B-cell lymphopoiesis in a concentration-dependent fashion. Similarly, the CYP1A1 inhibitor, 1-(1-propynyl) pyrene, blocked benzo[a]pyrene-induced B-cell growth inhibition but not growth inhibition caused by the benzo[a]pyrene metabolite, BPDE; these data suggest that a CYP1A1-dependent metabolite of benzo[a]pyrene is responsible for the B-cell growth suppressive effects observed after benzo[a]pyrene exposure (Allan et al., 2006). Altogether, these data suggest that benzo[a]pyrene may regulate B-cell proliferation and antibody production in the bone marrow via the AhR.

Summary of Immune Effects

Evidence for immunotoxic effects of benzo[a]pyrene exposure comes from animal studies that vary in route and duration of exposure. There are no human epidemiological studies that provide specific support for benzo[a]pyrene immunotoxicity; however, immunosuppression has been observed in studies following occupational exposure to PAH mixtures. However, these findings are limited by co-exposures to other constituents of PAH mixtures.

Effects such as altered thymus weight and histology, spleen effects, and altered immunoglobulin levels observed by the oral route reported in animal bioassays provide some evidence of immunotoxicity following benzo[a]pyrene exposure; however, in vivo functional assays provide stronger support for immunotoxicity (WHO, 2012). The immunological changes observed in the available subchronic gavage studies are supported by a larger database of in vivo studies of benzo[a]pyrene (by parenteral exposure) indicating functional immunosuppression such as decreased proliferative responses to antigens and decreased resistance to pathogens or tumor cells (Kong et al., 1994; Blanton et al., 1986; Munson et al., 1985; White et al., 1985; Dean et al., 1983; Munson and White, 1983). Although the key events underlying the mode of action of benzo[a]pyrene immunotoxicity are not firmly established, there is evidence of physical alterations to tissues/organs of the immune system, as well as decreases in immune function. Evidence of benzo[a]pyrene-associated immunotoxicity is supported by consistent thymic effects observed in two oral studies, as well as splenic effects, and varying immunosuppressive responses observed in short-term or in vitro tests.

EPA concluded there was suggestive evidence that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure.

Susceptible Populations and Lifestages

The severity and persistence of immune effects observed during in utero studies suggests that immunotoxicity may be greater during gestation than adulthood (Dietert and Piepenbrink, 2006; Holladay and Smialowicz, 2000; Urso and Gengozian, 1982). Urso and Gengozian (1982) provide experimental support demonstrating that 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 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, 2002; Dietert et al., 2000). In addition, chemical-specific studies show 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 et al., 2002; De Waal et al., 1997; Kuper et al., 1992). The thymus is believed to be critical for T lymphocyte production during early life and not in adulthood (Hakim et al., 2005; Schönland et al., 2003; Petrie, 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.

1.1.4. Other Toxicity

There is some evidence that benzo[a]pyrene can produce effects in the forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters. However, there is less evidence for these effects compared to organ systems described earlier in Sections 1.1.1–1.1.3. Overall, EPA concluded that the available evidence does not support these noncancer effects as potential human hazards.

Forestomach Toxicity

Lesions have been observed in the forestomach following subchronic and chronic oral exposure to benzo[a]pyrene (Table 1-9). Increases in the incidence of forestomach hyperplasia have been observed in Wistar rats following shorter-term, subchronic, and chronic gavage exposure (Kroese et al., 2001; De Jong et al., 1999) and in $B6C3F_1$ mice following chronic dietary exposure (Beland and Culp, 1998; Culp et al., 1998).

Following chronic gavage exposure, increased incidences of forestomach hyperplasia were observed in male and female rats at 3 and 10 mg/kg-day; at the highest dose, a lower incidence of hyperplasia was reported (Kroese et al., 2001). However, only the highest-level lesion (hyperplasia, papilloma, or carcinoma) observed in each organ was scored, such that hyperplasia observed in the forestomach, in which tumors were also observed, was not scored. The majority of animals in the high-dose group exhibited forestomach tumors; therefore, the hyperplasia was not scored and the incidence of forestomach hyperplasia in the study is more uncertain at the highest dose. Shorter-term studies (Kroese et al., 2001; De Jong et al., 1999) showed dose-related increases in forestomach hyperplasia at doses ≥10 mg/kg-day in Wistar rats. In addition, following chronic dietary exposure, a dose-dependent increase in the incidence of forestomach hyperplasia and hyperkeratosis was observed in female mice at ≥0.7 mg/kg-day (Beland and Culp, 1998; Culp et al.,

1998). Forestomach tumors were also observed at ≥0.7 mg/kg-day by Beland and Culp (1998) and Culp et al. (1998).

Although humans do not have a forestomach, forestomach effects observed in rodents are believed to be supportive of a human hazard, as humans have similar squamous epithelial tissue in their oral cavity (IARC, 2003; Wester and Kroes, 1988). Mechanistic investigations suggest that bioactivation of benzo[a]pyrene leads to reactive intermediates that can lead to mutagenic events, as well as to cytotoxic and apoptotic events. The available human, animal, and in vitro evidence best supports a mutagenic mode of action as the primary mode by which benzo[a]pyrene induces carcinogenesis. Available data indicate that forestomach hyperplasia may be a histological precursor to neoplasia observed at this site after chronic exposure to benzo[a]pyrene (Kroese et al., 2001; De Jong et al., 1999). Dose-response data show that forestomach hyperplasia occurs at shorter durations and at lower doses than tumors in rats and mice exposed to benzo[a]pyrene for up to 2 years (Kroese et al., 2001; Beland and Culp, 1998). Kroese et al. (2001) reported that the forestomach lesions demonstrated a progression over the course of intercurrent sacrifices; the authors described early lesions as focal or confluent basal hyperplasia, followed by more advanced hyperplasia with squamous cell papilloma, culminating in squamous cell carcinoma. The description of the progression of forestomach lesions provided by Kroese et al. (2001), coupled with the observation that hyperplasia occurs before tumors and at lower doses than tumors, suggests that forestomach hyperplasia induced by benzo[a]pyrene is likely a preneoplastic lesion.

Hematological Toxicity

Altered hematological parameters, including decreases in red blood cell (RBC) count, hemoglobin, and hematocrit have been observed in laboratory animals following benzo[a]pyrene exposure (Table 1-9). Statistically significant decreases in RBC count, hemoglobin, and hematocrit were observed in male Wistar rats at doses ≥10 mg/kg-day for 35 days (De Jong et al., 1999). A minimal, but statistically significant increase in mean cell volume and a decrease in mean cell hemoglobin were observed at the highest dose (90 mg/kg-day), which may indicate dose-related toxicity for the RBCs and/or RBC precursors in the bone marrow (De Jong et al., 1999). Similarly, male and female F344 rats also showed maximal decreases in RBC counts, hematocrit, and hemoglobin levels between 10 and 12% in a 90-day dietary study (Knuckles et al., 2001). Findings were significant for RBC counts and hematocrit in males at ≥50 mg/kg-day, while decreased RBC counts and hematocrit in females and hemoglobin levels in both sexes were only significant in the 100 mg/kg-day group (Knuckles et al., 2001). Small, but not statistically significant, decreases in RBC counts and hemoglobin were observed in both 35- and 90-day studies in Wistar rats (Kroese et al., 2001). It should be noted that when observed, the magnitudes of the decreases in RBCs, hemoglobin, and hematocrit were generally small; about 18% at 90 mg/kg-day and <10% at lower doses (De Jong et al., 1999) and about 10% in F344 rats (Knuckles et al., 2001). A decrease in white blood cells (WBCs), attributed to reduced numbers of lymphocytes and eosinophils, was also observed at 90 mg/kg-day following gavage exposure for 35 days (De Jong et al., 1999). The mode

of action by which benzo[a]pyrene exposure may lead to altered hematological parameters is undetermined.

Liver Toxicity

Liver effects other than cancer associated with benzo[a]pyrene exposure primarily include changes in liver weight and abnormal histopathology (Table 1-9). Increased liver weight was reported in a 90-day study in both male and female Wistar rats given benzo[a]pyrene by gavage (Kroese et al., 2001). Both females (17% increase) and males (29% increase) demonstrated statistically significant increased liver weights at the highest dose tested (30 mg/kg-day); a statistically significant increase (15%) was also reported in males at 10 mg/kg-day. Similar to the findings in the 90-day study by Kroese et al. (2001), increased liver:body weight ratios were observed at the highest dose in a 90-day dietary study in male F344 rats, although there was no change observed in female liver weights (Knuckles et al., 2001). Increased liver:body weight ratios were also observed in both sexes at high doses (600 and 1,000 mg/kg) in an accompanying acute study (Knuckles et al., 2001). A statistically significant increase in liver weight was also observed in male Wistar rats given 90 mg/kg-day benzo[a]pyrene by gavage for 35 days (De Jong et al., 1999). Consistent with the findings by De Jong et al. (1999), a statistically significant increased liver weight (about 18%) was also observed in both male and female Wistar rats at the highest dose (50 mg/kg-day) given by gavage in a 35-day study (Kroese et al., 2001).

Limited exposure-related differences in clinical chemistry parameters associated with liver toxicity were observed; no differences in alanine aminotransferase or serum aspartate transaminase levels were observed, and a small dose-related decrease in γ-glutamyl transferase was observed in males only exposed to benzo[a]pyrene for 90 days (Kroese et al., 2001).

Treatment-related lesions in the liver (oval cell hyperplasia) were identified as statistically significantly increased following exposure to 90 mg/kg-day benzo[a]pyrene for 35 days; however, incidence data were not reported (De Jong et al., 1999). A 2-year carcinogenicity study (Kroese et al., 2001) observed some histopathological changes in the liver; however, organs with tumors were not evaluated. Since many of the animals in the highest two doses developed liver tumors, the dose responsiveness of the histological changes is unclear.

A dose-dependent increase in liver microsomal ethoxyresorufin-o-deethylase (EROD) activity, indicative of CYP1A1 induction, was observed in both sexes at doses ≥1.5 mg/kg-day in a 35-day study (Kroese et al., 2001). However, at the highest dose tested, with the greatest fold induction in EROD activity, there was no evidence of associated adverse histopathologic findings. The finding of increased liver weight across multiple studies of varying exposure durations, as well as histopathological changes in the liver provide evidence of the liver as a target of benzo[a]pyrene-induced toxicity. The mode of action by which benzo[a]pyrene induces these effects is unknown.

Kidney Toxicity

There is minimal evidence of kidney toxicity following exposure to benzo[a]pyrene (Table 1-9). Statistically significant decreases in kidney weight were observed at doses of 3, 30, and 90 mg/kg-day, but not at 10 mg/kg-day, in a 35-day gavage study in male Wistar rats (De Jong et al., 1999). In a 35-day gavage study with a similar dose range in male and female Wistar rats, no statistically significant changes in kidney weights were observed at any dose (Kroese et al., 2001). Histopathological analysis of kidney lesions revealed an apparent dose-responsive increase in the incidence of abnormal tubular casts in the kidney in male F344 rats exposed by diet for 90 days (Knuckles et al., 2001). The casts were described as molds of distal nephrons lumen and were considered by the study authors to be indicative of renal dysfunction. However, the statistical significance of the kidney lesions is unclear. Several gaps and inconsistencies in the reporting make interpretation of the kidney effects difficult, including: (1) no reporting of numerical data; (2) no indication of statistical significance in the accompanying figure for kidney lesions; (3) discrepancies between the apparent incidences and sample sizes per dose group; and (4) uncertainty in how statistical analysis of histopathological data was applied. As such, the significance of the abnormal tubular casts is unclear.

Cardiovascular Toxicity

Atherosclerotic vascular disease and increased risk of cardiovascular mortality have been associated with cigarette smoking (Ramos and Moorthy, 2005; Miller and Ramos, 2001; Thirman et al., 1994) and, to a more limited degree, occupational exposure to PAH mixtures (Friesen et al., 2010; Friesen et al., 2009; Burstyn et al., 2005; Chau et al., 1993). Elevated mortality due to cardiovascular disease was observed in a PAH-exposed occupational population (coke oven plant workers), but elevated cardiovascular mortality was also observed in the non-exposed or slightly exposed populations (Chau et al., 1993). Elevated risks of ischemic heart disease (IHD) were associated with past cumulative benzo[a]pyrene exposure among aluminum smelter workers (with a 5-year lag), although the trend was not statistically significant; there was no observed association with more recent benzo[a]pyrene exposure (Friesen et al., 2010). Elevated risk of mortality from IHD was also associated with cumulative benzo[a]pyrene exposure in a cohort of male asphalt workers (although not statistically significant); the trend in average benzo[a]pyrene exposure and association with IHD was statistically significant, with an approximately 60% increase in risk between the lowest and highest exposure groups (Burstyn et al., 2005). The two studies that associate benzo[a]pyrene exposure with cardiovascular effects (Friesen et al., 2010; Burstyn et al., 2005) rely on statistical models to create exposure groups rather than direct measurement of the cohort under examination. Additionally, while these studies used benzo[a]pyrene exposure groupings for analysis, they cannot address co-exposures that may have occurred in the occupational setting (asphalt or aluminum smelters) or exposures that occurred outside the workplace.

Increased systolic and diastolic blood pressure has been observed in the offspring of dams exposed to increasing concentrations of benzo[a]pyrene (Jules et al., 2012) (Table 1-1). At the highest dose tested (1.2 mg/kg body weight by gavage to the dams), systolic pressures were elevated approximately 50% and diastolic pressures were elevated approximately 80% above controls. An intranasal exposure of 0.01 mg/kg-day benzo[a]pyrene in adult male rats also produced an increase in blood pressure following a 7-day exposure (Gentner and Weber, 2011).

Reduced endothelial integrity and increased smooth muscle cell mass, both related to atherosclerosis, have been observed in Sprague-Dawley rats exposed to 10 mg/kg benzo[a]pyrene by i.p. injection (once/week for 8 weeks) (Zhang and Ramos, 1997). The molecular mechanisms underlying PAH-induced vascular injury and the development of atherosclerosis are not well established, but current hypotheses include cell proliferative responses to injury of endothelial cells from reactive metabolites (including reactive oxygen species [ROS]) and genomic alterations in smooth muscle cells from reactive metabolites leading to transformed vasculature cells and eventual plaque formation (Ramos and Moorthy, 2005). However, while the link between PAHs and atherosclerotic disease has been studied, experiments specifically looking at the relationship between levels of exposure to benzo[a]pyrene (via environmentally relevant routes) and the development of aortic wall lesions related to atherosclerosis have not generally been performed.

One exception to this observation comes from a series of experiments on Apolipoprotein E knock-out (ApoE−/−) mice exposed orally to benzo[a]pyrene. ApoE−/− mice develop spontaneous atherosclerosis, which is thought to be due to enhanced oxidative stress from the lack of ApoE (Godschalk et al., 2003). Overall, these studies suggest that benzo[a]pyrene exposure in ApoE−/− mice enhances the progression of atherosclerosis through a general local inflammatory process.

Nervous System Effects

Neurobehavioral function and mood state were evaluated in two studies of men occupationally exposed to PAH mixtures (Qiu et al., 2013; Niu et al., 2010). Alterations in neurobehavioral function was evaluated in coke oven workers using the Neurobehavioral Core Test Battery (self-reported symptoms by questionnaire). These studies also measured urinary levels of the PAH metabolite, 1-hydroxypyrene, as markers of PAH exposure. In both studies, exposure was associated with decrements in short-term memory and/or attention in digit span tests. In addition, Qiu et al. (2013) reported an association between benzo[a]pyrene exposure and decrements in tests related to sensorimotor coordination (i.e., reaction time, digit symbol, and pursuit aiming tests), as well as lower health ratings for the tension-anxiety mood category; Niu et al. (2010) performed the same test battery but did not detect these associations.

Alterations in neuromuscular, autonomic, sensorimotor, and electrophysiological endpoints have been reported in rats and mice following acute or short-term exposure to benzo[a]pyrene (Bouayed et al., 2009b; Grova et al., 2008; Grova et al., 2007; Saunders et al., 2006; Liu et al., 2002; Saunders et al., 2002; Saunders et al., 2001). Impaired learning and memory (as measured by Morris water maze performance or novel object recognition) was observed following subchronic

gavage in adult rats (Maciel et al., 2014; Chen et al., 2011; Chengzhi et al., 2011) and following subchronic or short-term i.p. exposure in adult mice (Qiu et al., 2011; Xia et al., 2011; Grova et al., 2007). Decreased anxiety-like behavior in hole board and elevated plus maze tests has been observed following short-term i.p. exposure (Grova et al., 2008), while decreased depressive-like activity was observed in the tail suspension test (but not the forced swim test) following short-term oral exposure (Bouayed et al., 2012). In addition, a 28-day gavage study in male mice observed an increase in aggressive behavior (as measured by the resident intruder test) and an increase in consummatory sexual behavior in mice treated with 0.02 mg/kg-day (Bouayed et al., 2009b). These data are consistent with the neurobehavioral effects observed following developmental exposure, and they suggest that benzo[a]pyrene exposure could be neurotoxic in adults; however, only limited data are available to inform the neurotoxic potential of repeated subchronic or chronic exposure to benzo[a]pyrene via the oral route (Table 1-9).

Table 1-9. Evidence pertaining to other toxicities of benzo[a]pyrene in animals

Reference and study design	Results[a]
Forestomach toxicity	
Kroese et al. (2001) Wistar (Riv:TOX) rats: male and female (52/sex/dose group) 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 104 wks (chronic) Wistar (Riv:TOX) rats: male and female (10/sex/dose group) 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d (subchronic) Wistar (specific pathogen-free Riv:TOX) rats (10/sex/dose group) 0, 1.5, 5, 15, or 50 mg/kg body weight by gavage 5 d/wk 5 wks (shorter-term)	Forestomach hyperplasia (basal cell hyperplasia) incidences[b]: M: 2/50; 8/52; 8/52; and 0/52 F: 1/52; 8/51; 13/51; and 2/52 Forestomach hyperplasia (slight basal cell hyperplasia) incidences: M: 2/10; 0/10; 6/10; and 7/10 F: 0/10; 2/10; 3/10; and 7/10 Forestomach hyperplasia (basal cell hyperplasia) incidences: M: 1/10; 1/10; 4/10; 3/10; and 7/10 F: 0/10; 1/10; 1/10; 3/10; and 7/10*
(Beland and Culp (1998); Culp et al. (1998)) B6C3F$_1$ mice: female (48/dose group) 0, 5, 25, or 100 ppm in the diet (average daily doses[b]: 0, 0.7, 3.3, and 16.5 mg/kg-d) 2 years	Forestomach hyperplasia Incidences: 13/48; 23/47; 33/46*; and 38/47* Forestomach hyperkeratosis Incidences: 13/48, 22/47, 33/46*, 38/47*
De Jong et al. (1999) Wistar rats: male (8/dose group) 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 5 wks	Forestomach hyperplasia (basal cell hyperplasia) statistically significantly increased incidences at 30 and 90 mg/kg-d were reported, but incidence data were not provided

Reference and study design	Results[a]
Hematological toxicity	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk for 35 d	RBC count and hemoglobin changes not statistically significant in males or females at any dose (numerical data not reported) RBC count: changes not statistically significant (numerical data not reported) Hemoglobin: changes not statistically significant (numerical data not reported)
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↓ RBC count Females (% change from control): statistically significant at 100 mg/kg-d (numerical data not reported) Males (% change from control): statistically significant at 50 and 100 mg/kg-d (numerical data not reported) ↓ hematocrit Females (% change from control): statistically significant at 100 mg/kg-d (numerical data not reported) Males (% change from control): statistically significant at 50 and 100 mg/kg-d (numerical data not reported) ↓ hemoglobin Females: statistically significant at 100 mg/kg-d (numerical data not reported) Males: statistically significant at 100 mg/kg-d (numerical data not reported)
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ RBC count % change from control: 0, −1, −5*, −10*, and −18* ↓ hemoglobin % change from control: 0, −1, −7*, −10*, and −18* ↓ hematocrit % change from control: 0, 0, −6*, −8*, and −14* ↓ WBC count % change from control: 0, −8, −9, −9, and −43* ↑ mean cell volume % change from control: 0, 0, −3, 0, and 3* ↓ mean corpuscular hemoglobin concentration % change from control: 0, −1, −1, −1, and −3*

Reference and study design	Results[a]
Liver toxicity	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk for 35 d	↑ liver weight Females (% change from control): 0, −2, 4, and 17* Males (% change from control): 0, 7, 15*, and 29* Liver histopathology: no effects reported ↑ liver weight Females (% change from control): 0, 3, 2, 9, and 18* Males (% change from control): 0, 2, 1, 3, and 18* Liver histopathology: no effects reported
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↑ liver:body weight ratio Females: no change (numerical data not reported) Males (% change from control): 23% change reported at 100 mg/kg-d (numerical data not reported)
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↑ liver weight % change from control: 0, −9, 7, 5, and 15* ↑ liver oval cell hyperplasia (numerical data not reported) reported as significant at 90 mg/kg-d;
Kidney effects	
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↑ abnormal tubular casts Females: not statistically significant (numerical data not reported) Males: apparent dose-dependent increase (numerical data not reported)
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ kidney weight % change from control: 0, −11*, −4, −10*, and −18*
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk 35 d	Kidney weight: no change (data not reported)
Nervous system effects	
Chengzhi et al. (2011) Sprague-Dawley rats, male, 32/dose 0 or 2 mg/kg-d by gavage 90 d	↑ time required for treated rats to locate platform in water maze (data reported graphically)

Reference and study design	Results[a]
Bouayed et al. (2009b) Swiss albino mice, male, 9/group 0, 0.02, or 0.2 mg/kg-d by gavage 28 d	Significant decrease in latency to attack and increase in the number of attacks in the resident-intruder test at 0.02 mg/kg-d (but not at high dose) Significant increase in mount number in the copulatory behavior test at 0.02 and 0.2 mg/kg-d

*Statistically significantly different from the control ($p < 0.05$).
[a]% change from control calculated as: (treated value − control value)/control value × 100.
[b]Reported incidences may not fully account for the occurrence of hyperplasias due to the scoring of only the highest-level lesion in an individual animal (e.g., animals with forestomach tumors that also showed hyperplasia would not have the observation of hyperplasia recorded).
[c]Based on the assumption that daily benzo[a]pyrene intake at 5 ppm was one-fifth of the 25-ppm intake (about 21 µg/d) and using TWA body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group.

1.1.5. Carcinogenicity

Evidence in Humans

Numerous epidemiologic studies indicate an association between PAH related occupations and lung, bladder, and skin cancer (Table 1-10). This discussion primarily focuses on epidemiologic studies that included a direct measure of benzo[a]pyrene exposure. All identified studies have co-exposures to other PAHs. The identified studies were separated into tiers according to the extent and quality of the exposure analysis and other study design features:

Tier 1: Detailed exposure assessment conducted (using a benzo(a)pyrene metric), large sample size (>~50 exposed cases), and adequate follow-up period to account for expected latency (e.g., >20 years for lung cancer).

Tier 2: Exposure assessment, sample size, or follow-up period did not meet the criteria for Tier 1, or only a single-estimate exposure analysis was conducted.

For lung cancer, each of the Tier 1 studies observed increasing risks of lung cancer with increasing cumulative exposure to benzo[a]pyrene (measured in µg/m^3-years), and each of these studies addressed in the analysis the potential for confounding by smoking (Armstrong and Gibbs, 2009; Spinelli et al., 2006; Xu et al., 1996) (Table 1-11). These three studies represent different geographic locations and two different industries. The pattern of results in the Tier 2 studies was mixed, as would be expected for studies with less precise exposure assessments or smaller sample sizes: one of the standardized mortality ratio (SMR) estimates was <1.0, with the other eight estimates ranging from 1.2 to 2.9 (Table 1-12). In considering all of the available studies, particularly those with the strongest methodology, there is considerable support for an association between benzo[a]pyrene exposure and lung cancer, although the relative contributions of benzo[a]pyrene and of other PAHs cannot be established.

For bladder cancer, the cohort and nested case-control studies observed a much smaller number of cases compared with lung cancer; this limits their ability to examine exposure-response relationships. Three cohort studies with detailed exposure data, however, identified 48–90 cases (Burstyn et al., 2007; Gibbs and Sevigny, 2007a; Gibbs et al., 2007; Gibbs and Sevigny, 2007b) (Spinelli et al., 2006) (Tier 1 studies, Table 1-13). Although cumulative exposure (up to approximately 2 μg/m^3-years) was not related to increasing risk in the study of asphalt workers by Burstyn et al. (2007), an exposure-response was seen with the wider exposure range (i.e., >80 μg/m^3-years) examined in two studies of aluminum smelter workers by (Gibbs and Sevigny (2007a); Gibbs et al. (2007); Gibbs and Sevigny (2007b)); and (Spinelli et al., 2006). This difference in response is not surprising, given that the highest exposure group in the asphalt worker studies corresponded to the exposures seen in the lowest exposure categories in the studies of aluminum smelter workers. The five studies with more limited exposure information or analyses each included between 2 and 16 bladder cancer cases, with relative risk (RR) estimates ranging from 0.6 to 2.9. None of these individual effect estimates was statistically significant (Tier 2 studies, Table 1-13).

Two of the identified occupational studies contained information on risk of mortality from melanoma. Neither of these studies observed increased risks of this type of cancer, with an SMR of 0.91 (95% confidence interval [CI] 0.26, 2.48) (22 cases) in Spinelli et al. (2006) and 0.58 (95% CI 0.12, 1.7) in Gibbs et al. (2007)(3 cases). These studies did not include information on non-melanoma skin cancers.

Non-melanoma skin cancer, specifically squamous cell carcinoma, is of particular interest with respect to dermal PAH exposures. The literature pertaining to this kind of cancer and PAH exposure goes back to the 18th century work of Sir Percivall Pott describing scrotal cancer, a squamous cell skin cancer, in English chimney sweeps (Brown and Thornton, 1957). Recent studies of chimney sweeps in several Nordic countries have not found increases in non-melanoma skin cancer incidence (Hogstedt et al., 2013; Pukkala et al., 2009; Evanoff et al., 1993), likely due to greatly reduced exposure associated with better occupational hygiene (IARC, 2012). A study among asphalt workers (roofers) reported an increased risk of mortality from non-melanoma skin cancer among asphalt workers (roofers), with an SMR of 4.0 (95% CI: 1.0, 10.9) among workers employed ≥20 years (Hammond et al., 1976). In addition to this study, two studies in Scandinavian countries examined non-melanoma skin cancer risk in relation to occupations with likely dermal exposure to creosote (i.e., timber workers and brick makers) using incidence data from population registries (Karlehagen et al., 1992; Törnqvist et al., 1986). The standardized incidence ratio (SIR) estimates were 1.5 (95% CI: 0.7, 2.6) based on five exposed cases and 2.37 (95% CI: 1.08, 4.50) based on nine cases in Törnqvist et al. (1986) and Karlehagen et al. (1992). Because non-melanoma skin cancers are rarely fatal if caught early, and the preventative excision of precancerous lesions is common, the available occupational studies and cancer registries likely underestimate the risk of

squamous cell carcinoma (Cargøe et al., 2013; Voelter-Mahlknecht et al., 2007; ONS, 2003; Letzel and Drexler, 1998).

In addition to cohorts of workers occupationally exposed to PAH mixtures, populations exposed to benzo[a]pyrene through topical coal tar formulations for the treatment of psoriasis, eczema, and dermatitis have also been studied (Roelofzen et al., 2010; Mitropoulos and Norman, 2005; Stern et al., 1998; Stern and Laird, 1994; Lindelöf and Sigurgeirsson, 1993; Torinuki and Tagami, 1988; Pittelkow et al., 1981; Maughan et al., 1980; Stern et al., 1980). Epidemiological studies examining skin cancer risk in relation to various types of topical tar exposure are summarized in the Supplemental Information, Table D-6. Case reports, reviews, and studies that did not include a measure of coal tar use are not included. The available studies examining therapeutic topical coal tar use and risk of skin cancer were limited by low quality exposure data with high potential of exposure misclassification (e.g., Roelofzen et al., 2010; Mitropoulos and Norman, 2005; Lindelöf and Sigurgeirsson, 1993), small size and short duration of follow-up up (e.g., Torinuki and Tagami, 1988), and choice of referent rates and differences in disease ascertainment between cases and the reference population (e.g., Pittelkow et al., 1981; Maughan et al., 1980). In addition, clinic-based studies focused on the regimen of coal tar in conjunction with ultraviolet-B (UVB) therapy, although some appear to indicate increased risk with coal tar exposure, cannot distinguish effects of coal tar from the effects of UVB (e.g., Stern et al., 1998; Stern and Laird, 1994; Lindelöf and Sigurgeirsson, 1993; Stern et al., 1980). Therefore, because of the limitations with respect to study design and analysis, EPA did not consider these studies further in the evaluation of the risk of skin cancer from exposure to benzo[a]pyrene. Although EPA does not consider the available studies sufficient to evaluate the risk of skin cancer, acute studies of coal tar treated patients provide in vivo evidence of benzo[a]pyrene-specific genotoxicity (increased BPDE-DNA adducts) in human skin (Godschalk et al., 2001; Rojas et al., 2001; Zhang et al., 1990), an early key event in the carcinogenic mode of action of benzo[a]pyrene (see Figure 1-6 of Section 1.1.5).

Lung, bladder, and skin cancers are the cancers that have been observed in occupational studies of PAH mixtures (Benbrahim-Tallaa et al., 2012; Baan et al., 2009; Secretan et al., 2009). The reproducibility of lung, bladder, and skin cancers in different populations and exposure settings after occupational exposure to PAH mixtures (see Table 1-10) adds plausibility to the hypothesis that common etiologic factors may be operating. The potential role that benzo[a]pyrene may play as a causal agent is further supported by the observation that these same sites are also increased in the studies that included a direct measure of benzo[a]pyrene.

Table 1-10. Cancer sites for PAH-related agents reviewed by IARC

PAH-related mixture or occupation	Sites with *sufficient evidence* in humans	Sites with *limited evidence* in humans	Reference
Aluminum production	Lung, urinary bladder		Baan et al. (2009)
Carbon electrode manufacture		Lung	IARC (2010)
Coal gasification	Lung		Baan et al. (2009)
Coal tar distillation	Skin		Baan et al. (2009)
Coal tar pitch (paving and roofing)	Lung	Urinary bladder	Baan et al. (2009)
Coke production	Lung		Baan et al. (2009)
Creosotes		Skin	IARC (2010)
Diesel exhaust	Lung	Urinary bladder	Benbrahim-Tallaa et al. (2012)
Indoor emissions from household combustion of biomass fuel (primarily wood)		Lung	Secretan et al. (2009)
Indoor emissions from household combustion of coal	Lung		Secretan et al. (2009)
Mineral oils, untreated or mildly treated	Skin		Baan et al. (2009)
Shale oils	Skin		Baan et al. (2009)
Soot (chimney sweeping)	Lung, skin	Urinary bladder	Baan et al. (2009)

Source: Adapted from IARC (2010).

Table 1-11. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to lung cancer risk: Tier 1 studies

Reference and study design	Results			
Armstrong and Gibbs (2009) (Quebec, Canada) Cohort, aluminum smelter workers, seven plants 16,431 (15,703 men; 728 women); duration minimum 1 yr, began work 1966–1990; follow-up through 1999 (mean ~30 yrs); smoking information collected from medical records Exposure: Job exposure matrix ~5,000 personal benzo[a]pyrene measures from the 1970s to 1999 Related references: Lavoué et al. (2007) (exposure data); (Gibbs and Sevigny (2007a); Gibbs et al. (2007); Gibbs and Sevigny (2007b); Armstrong et al.	SMR 1.32 (1.22, 1.42) [677 cases] Lung cancer risk by cumulative benzo[a]pyrene exposure			
	Median benzo[a]-pyrene $\mu g/m^3$-yrs	n cases	SMR (95% CI)	RR (95% CI)
	0	35	0.62 (0.44, 0.87)	1.0 (referent)
	10	266	1.09 (0.96, 1.23)	1.75 (1.23, 2.48)
	30	70	1.88 (1.47, 2.38)	3.02 (2.01, 4.52)
	60	53	1.21 (0.91, 1.59)	1.94 (1.27, 2.97)
	120	114	1.93 (1.59, 2.32)	3.09 (2.12, 4.51)

(1994))	240 116 1.79 (1.48, 2.15) 2.86 (1.96, 4.18) 480 23 2.36 (1.49, 3.54) 3.77 (2.23, 6.38) No evidence of confounding by smoking Additional modeling as continuous variable: RR 1.35 (95% CI 1.22, 1.51) at 100 µg/m^3-yrs (0.0035 per µg/m^3-yrs increase); other shapes of exposure-response curve examined.
Spinelli et al. (2006) (British Columbia, Canada) Cohort, aluminum smelter workers; 6,423 (all men); duration minimum ≥3 yrs; began work 1954–1997; follow-up through 1999 (14% loss to follow-up; mean ~24 yrs); smoking information from self-administered questionnaire Exposure: Job exposure matrix using 1,275 personal benzo[a]pyrene measures from 1977 to 2000 (69% for compliance monitoring) Related references: Friesen et al. (2006) (exposure data); Spinelli et al. (1991)	SMR: 1.07 (0.89, 1.28) [120 cases] SIR: 1.10 (0.93, 1.30) [147 cases] Lung cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]pyrene µg/m^3-yrs / n cases / RR (95% CI)[a] 0–0.5 / 25 / 1.0 (referent) 0.5–20 / 42 / 1.23 (0.74, 2.03) 20–40 / 23 / 1.35 (0.76, 2.40) 40–80 / 25 / 1.36 (0.78, 2.39) ≥80 / 32 / 1.79 (1.04, 3.01) [a]Adjusting for smoking category; trend $p < 0.001$.
Xu et al. (1996) (China) Nested case-control in iron-steel worker cohort 610 incident cases (96% participation); 959 controls (94% participation) (all men); duration data not reported; smoking information collected from interviews; next-of-kin interviews with 30% of lung cancer cases and 5% of controls Exposure: Job exposure matrix 82,867 historical monitoring records, 1956–1992	Lung cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene (µg/m^3-yrs) / n cases / RR (95% CI)[a] <0.84 / 72 / 1.1 (0.8, 1.7) 0.85–1.96 / 117 / 1.6 (1.2, 2.3) 1.97–3.2 / 96 / 1.6 (1.1, 2.3) ≥3.2[b] / 105 / 1.8 (1.2, 2.5) [a]Adjusting for birth year and smoking category; trend $p < 0.004$. Referent group is "nonexposed" (employed in administrative or low-exposure occupations). [b]Study table IV unclear; could be ≥3.0 for this category.

Table 1-12. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to lung cancer risk: Tier 2 studies

Reference and study design	Results
Limited follow-up period (≤20 yrs)	
Friesen et al. (2009) (Australia) Cohort, aluminum smelter workers; 4,316 (all men); duration minimum 90 d; began work after 1962; follow-up through 2002, mean 16 yrs (maximum 20 yrs); Smoking information from company records	RR 1.2 (0.7, 2.3) [19 cases in exposed; 20 in unexposed] Lung cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-yrs / n cases / RR (95% CI)[a] 0 / 20 / 1.0 (referent)

Reference and study design	Results		
if employed before 1995 and study interviews if employed after 1994 Exposure: Job/task exposure matrix using TWA benzo[a]pyrene measures (n = 655), 1977–2004 (79% from 1990 to 2004)	>0–0.41 0.41–10.9 >10.9 [a]Poisson regression, adjusting for smoking; trend p = 0.22.	6 6 7	0.7 (0.3, 1.8) 1.4 (0.6, 3.5) 1.7 (0.7, 4.2)
Proxy measure			
Olsson et al. (2010) (Denmark, Norway, Finland, Israel) Nested case-control, asphalt workers; 433 lung cancer cases (65% participation); 1,253 controls (58% participation), matched by year of birth, country (all men); duration: minimum ≥2 seasons, median 8 seasons; began work 1913–1999; follow-up: from 1980 to 2002–2005 (varied by country); smoking information from interviews Exposure: Compilation of coal tar exposure measures, production characteristics, and repeat measures in asphalt industry in each country used to develop exposure matrix Related references: (Boffetta et al. (2003); Burstyn et al. (2000))	Lung cancer risk by cumulative coal tar exposure[a] Coal tar unit-yrs[a] 0.39–4.29 4.30–9.42 9.43–16.88 16.89–196.48 (trend p-value) [a]Adjusting for set, age, country, tobacco pack-years.	n cases 43 32 30 54	RR 1.31 0.98 0.97 1.60	(95% CI) (0.87, 2.0) (0.62, 1.6) (0.61, 1.6) (1.09, 2.4) (0.07)
Costantino et al. (1995) (United States, Pennsylvania) Cohort, coke oven workers; 5,321 and 10,497 unexposed controls (non-oven steel workers; matched by age, race, date of first employment) (all men); duration data not reported; worked in 1953; follow-up through 1982 (length data not reported) Exposure: Average daily exposure coal tar pitch volatiles: 3.15 mg/m^3 top-side full-time jobs, 0.88 mg/m^3 side jobs; used to calculate weighted cumulative exposure index Related reference: Dong et al. (1988) (exposure data)	SMR 1.95 (1.59, 2.33) [255 cases] Lung cancer risk by cumulative exposure Coal tar pitch volatiles (mg/m^3-mo) 0 1–49 50–199 200–349 350–499 500–649 ≥650 [a]Adjusting for age, race, coke plant, period of follow-up; trend p < 0.001.	n cases 203 34 43 59 39 27 56	RR (95% CI)[a] 1.0 (referent) 1.2 (0.85, 1.8) 1.6 (1.1, 2.3) 2.0 (1.5, 2.8) 2.0 (1.6, 3.2) 2.7 (2.0, 4.6) 3.1 (2.4, 4.6)	

Reference and study design	Results
Limited exposure information	
Liu et al. (1997)(China) Cohort, various carbon plants and aluminum smelter workers; 6,635 (all men); duration minimum 15 yrs; began work before 1971; follow-up: through 1985 (mean ~14 yrs); smoking information from questionnaire Exposure: Area samples from one carbon plant, 1986–1987	SMR 2.2 (1.1, 2.8) [50 cases] Lung cancer risk by exposure category Exposure category / Mean benzo[a]-pyrene µg/m^3 / n cases / SMR (95% CI)[a] None / – / 13 / 1.49 (0.83, 2.5) Low / – / 6 / 1.19 (0.48, 2.5) Moderate / 0.30 / 5 / 1.52 (0.55, 3.4) High / 1.19 / 26 / 4.30 (2.9, 6.2) [a]Calculated by EPA from data in paper.
Berger and Manz (1992) (Germany) Cohort, coke oven workers; 789 (all men); duration minimum 10 yrs (mean 27 yrs); began work 1900–1989; follow-up through 1989 (length data not reported); smoking information from plant records and interviews Exposure: Mean benzo[a]pyrene: 28 µg/m^3 (range 0.9–89 µg/m^3)	SMR 2.88 (2.28, 3.59) [78 cases]
(Hansen (1991); Hansen, 1989) (Denmark) Cohort, asphalt workers; 679 workers (applicators) (all men); duration data not reported; employed 1959 to 1980; follow-up to 1986 (mean ~11 yrs); smoking information from 1982 surveys of industry and general population Exposure: Asphalt fume condensate, 35 personal samples during flooring: median 19.7 mg/m^3 (range 0.5–260 mg/m^3)	SMR 2.90 (1.88, 4.3) [25 cases] (ages 40–89) SMR 2.46 (1.59, 3.6) [25 cases] (with smoking adjustment)

Reference and study design	Results
Gustavsson et al. (1990) (Sweden) Cohort, gas production (coke oven) workers; 295 (all men); duration minimum 1 yr, median 15 yrs; employed 1965–1972; follow-up: 1966–1986 (mortality); 1966–1983 (incidence; mean ~15 yrs); smoking information from interviews with older workers Exposure: Area sampling - top of ovens; benzo[a]pyrene, 1,964 mean 4.3 µg/m^3 (range 0.007–33 µg/m^3); 1,965 mean 0.52 µg/m^3 (0.021–1.29 µg/m^3)	SMR 0.82 (0.22, 2.1) [4 cases] (referent group = employed men) SIR 1.35 (0.36, 3.5) [4 cases]
Moulin et al. (1989) (France) Cohort and nested case-control, two carbon electrode plants; 1,302 in Plant A (all men), employed in 1975; follow-up 1975–1985 (incidence); smoking information from plant records; 1,115 in Plant B (all men); employed in 1957; follow-up 1957–1984 (mortality); duration of employment and follow-up data not reported Exposure: Benzo[a]pyrene, 19 area samples and 16 personal samples in Plant A (personal sample mean 2.7 µg/m^3; range 0.59–6.2 µg/m^3); 10 area samples and 7 personal samples in Plant B; personal sample mean 0.17 µg/m^3, range 0.02–0.57 µg/m^3	Plant A: SMR 0.79 (0.32, 1.6) [7 cases] Plant B: SMR 1.18 (0.63, 2.0) [13 cases] Internal comparison (case-control), ≥1 yr duration: Plant A: OR 3.42 (0.35, 33.7) [7 cases, 21 controls] Plant B: OR 0.49 (0.12, 2.0) [13 cases, 33 controls]
Hammond et al. (1976) (United States) Cohort, asphalt roofers; 5,939 (all men); duration minimum 9 yrs, began before 1960; follow-up through 1971 Exposure: 52 personal samples (masks with filters) during specific jobs and tasks; mean benzo[a]pyrene 16.7 µg per 7-hr d	SMR 1.6 (1.3, 1.9) [99 cases] (≥20 yrs since joining union) (CIs calculated by EPA from data in paper)

Table 1-13. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to bladder cancer risk

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Tier 1 studies</td></tr>
<tr><td>Burstyn et al. (2007) (Denmark, Norway, Finland, Israel)

Cohort, asphalt workers; 7,298 (all men); duration minimum ≥2 seasons, median 8 seasons; began work 1913–1999; follow-up began around 1960, ended around 2000 (years varied by country); median 21 yrs; smoking information not collected

Exposure: Compilation of benzo[a]pyrene measures, production characteristics, and repeat measures in asphalt industry in each country used to develop exposure matrix

Related references: (Boffetta et al., 2003; Burstyn et al. (2000))</td><td>48 incident bladder cancer cases (39 cases in analyses with 15-yr lag)
Bladder cancer risk by cumulative benzo[a]pyrene exposure[a]
<table><tr><th>Benzo[a]-pyrene µg/m^3-yrs[a]</th><th>n cases</th><th>RR (95% CI) (no lag)[b]</th><th>RR (95% CI) (15-yr lag)[c]</th></tr><tr><td>0–0.253</td><td>12</td><td>1.0 (referent)</td><td>1.0 (referent)</td></tr><tr><td>0.253–0.895</td><td>12</td><td>0.69 (0.29, 1.6)</td><td>1.1 (0.44, 2.9)</td></tr><tr><td>0.895–1.665</td><td>12</td><td>1.21 (0.45, 3.3)</td><td>1.7 (0.62, 4.5)</td></tr><tr><td>≥1.665</td><td>12</td><td>0.84 (0.24, 2.9)</td><td>1.1 (0.30, 4.0)</td></tr></table>[a]Adjusting for age, calendar period, total duration of employment, country.
[b]Trend p = 0.9.
[c]Trend p = 0.63.
Stronger pattern seen with average exposure in 15-yr lag (RR 1.5, 2.7, 1.9 in second through fourth quartile; trend p = 0.15)</td></tr>
<tr><td>(Gibbs and Sevigny (2007a); Gibbs et al. (2007); Gibbs and Sevigny (2007b)) (Quebec, Canada)

Cohort, aluminum smelter workers, seven plants 16,431 (15,703 men; 728 women); duration minimum 1 yr, began work 1966–1990; follow-up: through 1999 (mean ~30 yrs); smoking information collected from medical records

Exposure: Job exposure matrix using ~5,000 personal benzo[a]pyrene measures from the 1970s to 1999

Related references: Lavoué et al. (2007) (exposure data); (Armstrong et al. (1994); Gibbs (1985); Gibbs and Horowitz (1979))</td><td>Hired before 1950: SMR 2.24 (1.77, 2.79) [78 cases]

Bladder cancer risk by cumulative benzo[a]pyrene exposure
<table><tr><th>Benzo[a]-pyrene µg/m^3-yrs[a]</th><th>n cases</th><th>SMR (95% CI)</th><th>Smoking-adjusted RR[b]</th></tr><tr><td>0</td><td>3</td><td>0.73 (0.15, 2.1)</td><td>1.0 (referent)</td></tr><tr><td>10</td><td>14</td><td>0.93 (0.45, 1.4)</td><td>1.11</td></tr><tr><td>30</td><td>3</td><td>1.37 (0.28, 4.0)</td><td>1.97</td></tr><tr><td>60</td><td>1</td><td>0.35 (0.9, 1.9)</td><td>0.49</td></tr><tr><td>120</td><td>15</td><td>4.2 (2.4, 6.9)</td><td>8.49</td></tr><tr><td>240</td><td>30</td><td>6.4 (4.3, 9.2)</td><td></td></tr><tr><td>480</td><td>12</td><td>23.9 (12.2, 41.7)</td><td></td></tr></table>[a]Category midpoint.
[b]CIs not reported; highest category is ≥80 µg/m^3-yrs (n observed = 57).

Mortality risk reduced in cohort hired in 1950–1959, SMR = 1.23.
Similar patterns seen in analysis of bladder cancer incidence.</td></tr>
</table>

Reference and study design	Results
Spinelli et al. (2006) (British Columbia, Canada) See Table 1-11 for study details; this study is considered a "Tier 2") study for bladder cancer because of the smaller number of bladder cancer cases (n = 12) compared with lung cancer cases (n = 120)	SMR 1.39 (0.72, 2.43) [12 cases] SIR 1.80; CI 1.45−2.21 [90 cases, including in situ] Bladder cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-years | n cases | RR (95% CI)[a] 0−0.5 | 17 | 1.0 (referent) 0.5−20 | 20 | 0.83 (0.43, 1.59) 20−40 | 13 | 1.16 (0.56, 2.39) 40−80 | 18 | 1.50 (0.77, 2.94) ≥80 | 22 | 1.92 (1.02, 3.65) [a]Adjusting for smoking category; trend $p < 0.001$.
Tier 2 studies	
Friesen et al. (2009) (Australia) See Table 1-12 for study details	RR 0.6 (0.2, 2.0) [five cases in exposed; eight in unexposed] Bladder cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-yrs | n cases | RR (95% CI)[a] 0 | 8 | 1.0 (referent) >0−0.41 | 1 | 0.2 (0.03, 1.9) 0.41−10.9 | 2 | 0.7 (0.2, 3.7) >10.9 | 2 | 1.2 (0.2, 5.6) [a]Poisson regression, adjusting for smoking category; trend $p = 0.22$.
Costantino et al. (1995) (United States, Pennsylvania) See Table 1-12 for study details	SMR 1.14 (0.61, 2.12) (16 cases)
Hammond et al. (1976) (United States) See Table 1-12 for study details	SMR 1.7 (0.94, 2.8) (13 cases) (≥20 yrs since joining union) (CIs calculated by EPA from data in paper)
Moulin et al. (1989) (France) See Table 1-12 for study details	Plant A: 0 observed cases; expected <1.0 Plant B: SMR 1.94 (0.40, 5.0) (3 cases)
Gustavsson et al. (1990) (Sweden) See Table 1-12 for study details	SMR 2.85 (0.30, 10.3) (2 cases) (referent group = employed men)

Evidence in Animals

Oral exposure

Evidence of tumorigenicity following oral exposure to benzo[a]pyrene has been demonstrated in rats and mice. As summarized in Table 1-14, oral exposure to benzo[a]pyrene has resulted in an increased incidence of tumors in the alimentary tract in male and female rats (Kroese et al., 2001; Brune et al., 1981) and female mice (Beland and Culp, 1998; Culp et al., 1998), liver carcinomas in male and female rats, kidney adenomas in male rats (Kroese et al., 2001), and auditory canal tumors in both sexes (Kroese et al., 2001).

Forestomach tumors have been observed in several lifetime cancer bioassays in rats and mice following both gavage and dietary exposure to benzo[a]pyrene at doses ranging from 0.016 mg/kg-day in Sprague-Dawley rats to 3.3 and 10 mg/kg-day in $B6C3F_1$ mice and Wistar rats, respectively (Kroese et al., 2001; Beland and Culp, 1998; Culp et al., 1998; Brune et al., 1981). In addition, multiple less-than-lifetime oral exposure cancer bioassays in mice provide supporting evidence that oral exposure to benzo[a]pyrene is associated with an increased incidence of forestomach tumors (Weyand et al., 1995; Benjamin et al., 1988; Robinson et al., 1987; El-Bayoumy, 1985; Triolo et al., 1977; Wattenberg, 1974; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955). Although humans do not have a forestomach, similar squamous epithelial tissue is present in the oral cavity (IARC, 2003; Wester and Kroes, 1988); therefore, EPA concluded that forestomach tumors observed in rodents following benzo[a]pyrene exposure are relevant in the assessment of carcinogenicity (Beland and Culp, 1998). For further discussion, see Sections 1.2 and 2.3.4.

Elsewhere in the alimentary tract, dose-related increases of benign and malignant tumors were observed. In rats, oral cavity tumors were induced in both sexes and adenocarcinomas of the jejunum were induced in males (Kroese et al., 2001). In mice, tumors were induced in the tongue, esophagus, and larynx of females (males were not tested) (Beland and Culp, 1998; Culp et al., 1998).

Chronic oral exposure to benzo[a]pyrene resulted in a dose-dependent increased incidence of liver carcinomas in both sexes of Wistar rats, with the first liver tumors 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 (Kroese et al., 2001). Treatment-related hepatocellular tumors were not observed in mice (Beland and Culp, 1998; Culp et al., 1998).

A statistically significantly increased incidence of kidney tumors (cortical adenomas) was observed in male Wistar rats following chronic gavage exposure (Kroese et al., 2001) (Table 1-14). The kidney tumors were observed at the mid- and high-dose groups. Treatment-related kidney tumors were not observed in two other chronic studies (Beland and Culp, 1998; Brune et al., 1981).

Lung tumors were also observed following almost nine months of dietary exposure to approximately 10 mg/kg-day in female AJ mice (Weyand et al., 1995). Other lifetime exposure studies did not report treatment-related increases in lung tumors (Kroese et al., 2001; Beland and Culp, 1998; Culp et al., 1998).

1 **Table 1-14. Tumors observed in chronic oral animal bioassays**

Study design and reference	Results
Kroese et al. (2001) Wistar (Riv:TOX) rats (52/sex/dose group) 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 2 yrs	Forestomach incidences: M: 0/52; 7/52*; 18/52*; and 17/52* (papilloma) M: 0/52; 1/52; 25/52*; and 35/52* (squamous cell carcinoma) F: 1/52; 3/51; 20/51*; and 25/52* (papilloma) F: 0/52; 3/51; 10/51*; and 25/52* (squamous cell carcinoma) Oral cavity incidences: M: 0/24; 0/24; 2/37; and 10/38* (papilloma) M: 1/24; 0/24; 5/37; and 11/38* (squamous cell carcinoma) F: 0/19; 0/21; 0/9; and 9/31*(papilloma) F: 1/19; 0/21; 0/9; and 9/31* (squamous cell carcinoma) Jejunum (adenocarcinomas) incidences: M: 0/51; 0/50; 1/51; and 8/49* F: 0/50; 0/48; 0/50; and 2/51 Duodenum (adenocarcinomas) incidences: M: 0/51; 0/50; 0/51; and 1/49 F: 0/49; 0/48; 0/50; and 2/51 Liver (adenomas and carcinomas) incidences: M: 0/52; 3/52; 15/52*; and 4/52 (adenoma) M: 0/52; 1/52; 23/52*; and 45/52* (carcinoma) F: 0/52; 2/52; 7/52*; and 1/52 (adenoma) F: 0/52; 0/52; 32/52*; and 50/52* (carcinoma) Kidney (cortical adenoma) incidences: M: 0/52; 0/52; 7/52*; and 8/52* F: increase not observed Auditory canal[b] (Zymbal gland) (carcinomas) incidences: M: 0/1; 0/0; 2/7; and 19/33* F: 0/0; 0/1; 0/0; and 13/20*

2

(Beland and Culp (1998); Culp et al. (1998)) B6C3F$_1$ mice: female (48/dose group) 0, 5, 25, or 100 ppm (average daily doses[a]: 0, 0.7, 3.3, and 16.5 mg/kg-d) in the diet 2 yrs	Forestomach (papillomas and squamous cell carcinomas) incidences: 1/48; 3/47; 36/46*; and 46/47* Esophagus (papillomas and carcinomas) incidences: 0/48; 0/48; 2/45; and 27/46* Tongue (papillomas and carcinomas) incidences: 0/49; 0/48; 2/46; and 23/48* Larynx (papillomas and carcinomas) incidences: 0/35; 0/35; 3/34; and 5/38
Brune et al. (1981) Sprague-Dawley rats: male and female (32/sex/dose) Gavage: 0, 6, 18, 39 mg/kg-yr (0, 0.016, 0.049, 0.107 mg/kg-d) Diet: 0, 6, 39 mg/kg-yr (0, 0.016, 0.107 mg/kg-d) Treated until moribund or dead 2 yrs	Forestomach (papillomas and carcinomas[c]); gavage incidences: 3/64; 12/64*; 26/64*; and 14/64* Forestomach (papillomas); diet incidences: 2/64; 1/64; and 9/64* Larynx and esophagus (papillomas); gavage incidences: 3/64; 1/64; 0/64; and 0/64 Larynx and esophagus (papillomas); diet incidences: 1/64; 2/64; and 1/64

*Indicates statistical significance as identified in study.
[a]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) and using TWA body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group.
[b]Incidences are for number of rats with tumors compared with number of tissues examined histologically. Auditory canal tissue was examined histologically when abnormalities were observed on macroscopic examination.
[c]Two malignant forestomach tumors were observed (one each in the mid- and high-dose groups).

Inhalation exposure

The inhalation database of benzo[a]pyrene carcinogenicity studies consists of one lifetime inhalation bioassay in male hamsters (Thyssen et al., 1981). Intratracheal instillation studies in hamsters are also available (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972).

Several long term intratracheal installation studies in hamsters evaluated the carcinogenicity of benzo[a]pyrene (Feron and Kruysse, 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). These studies treated animals with benzo[a]pyrene once a week in a saline solution (0.5−0.9%) for ≥8 months and observed animals for 1−2 years following cessation of exposure. Tumors in the larynx, trachea, bronchi, bronchioles, and alveoli were observed. Individual studies also reported tumors in the nasal cavity and forestomach. These intratracheal instillation studies support the carcinogenicity of benzo[a]pyrene in the respiratory tract; however, direct extrapolation from a dose delivered by intratracheal instillation to an inhalation concentration expected to result in similar responses is not recommended (Driscoll et al., 2000).

Lifetime inhalation exposure to benzo[a]pyrene resulted in the development of tumors in the respiratory tract and pharynx in Syrian golden hamsters (Thyssen et al., 1981). The authors stated that the rates of tumors of other organs generally corresponded to the rates in controls. U.S. EPA (1990) obtained individual animal data (including individual animal pathology reports for the respiratory and upper digestive tracts, time-to-death data, and exposure chamber monitoring data) from the study authors (Clement Associates, 1990); this information is summarized in Table 1-15. Concentration-dependent increased incidences of tumors in the upper respiratory tract, including the larynx and trachea, were seen at measured exposure concentrations of ≥9.5 mg/m^3. In addition, a decrease in mean tumor latency was observed in the larynx and trachea. Nasal cavity tumors were observed at the mid- and high-concentration, but the incidences were not dose-dependent. A concentration-related increase in tumors in the upper digestive tract (pharynx and esophagus) was also reported. In addition, a single forestomach tumor was observed in each of the mid- and high-concentration groups. Also, in animals with a tumor in either the larynx or pharynx, forestomach tumors were not observed in control animals. The study authors suggested that the upper digestive tract tumors were a consequence of mucociliary particle clearance. All nasal, forestomach, esophageal, and tracheal tumors occurred in hamsters that also had tumors in the larynx or pharynx, except in the mid-concentration group, where two animals with nasal tumors had no tumors in the pharynx or larynx.

A re-analysis of the individual animal pathology reports and the exposure chamber monitoring data provided by the study authors yielded estimates of 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 (U.S. EPA, 1990).

Table 1-15. Tumors observed in chronic inhalation animal bioassays

Reference and study design	Results[b]
Thyssen et al. (1981) Syrian golden hamsters: male (26–34 animals/group placed on study) 0, 2.2, 9.5, or 46.5 mg/m^3 on NaCl particles by nose only inhalation for 3–4.5 hrs, 5–7 d/wk (TWA exposure concentrations[a]: 0, 0.25, 1.01, and 4.29 mg/m^3) Treated until moribund or dead (up to 130 wks) MMAD: not reported	Larynx incidences: 0/26; 0/21; 11/26; and 11/25 mean tumor latency[c]: 107 and 68 wks Pharynx incidences: 0/23; 0/19; 9/22; and 18/23 mean tumor latency: 97 and 68 wks Trachea incidences: 0/27; 0/21; 2/26; and 3/25 mean tumor latency: 115 and 63 wks Nasal cavity incidences: 0/26; 0/22; 4/26; and 1/34 mean tumor latency: 116 and 79 wks Esophagus incidences: 0/27; 0/22; 0/26; and 2/34 mean tumor latency: 71 wks Forestomach incidences: 0/27; 0/22; 1/26; and 2/34 mean tumor latency: 119 and 72 wks

[a]Duration-adjusted inhalation concentrations calculated from exposure chamber monitoring data and exposure treatment times. Daily exposure times: 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 reminder of the experiment.

[b]Thyssen et al. (1981) reported only the incidences of malignant tumors, confirmed by comparison with the original study pathology data (Clement Associates, 1990). The incidences summarized here include relevant benign tumors (papillomas, polyps, and papillary polyps). The malignant tumors were squamous cell carcinomas, with the exception of one in situ carcinoma of the larynx and one adenocarcinoma of the nasal cavity, both in the 9.5 mg/m^3 group. Denominators reflect the number of animals examined for histopathology for each tissue. See Section D.4.2 and Table E-17 in the Supplemental Material for study details and a complete listing of individual data, respectively.

[c]Mean time of observation of tumor, 9.5 and 46.5 mg/m^3 concentration groups.

[d]Thyssen et al. (1981) did not report statistical significance testing. See Section D.4.2.

Dermal exposure

Repeated application of benzo[a]pyrene to skin (in the absence of exogenous promoters) has been demonstrated to induce skin tumors in mice, rats, rabbits, and guinea pigs. These studies have been reviewed by multiple national and international health agencies (IARC, 2010; IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973). Mice have been the most extensively studied species in dermal carcinogenesis studies of benzo[a]pyrene because of evidence that they may be more sensitive than other animal species; however, comprehensive comparisons of species differences in sensitivity to

lifetime dermal exposure are not available. Systemic tumors in benzo[a]pyrene-treated mice were not increased compared to controls in lifetime dermal bioassays in which macroscopic examination of internal organs was included (Higginbotham et al., 1993; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959).

The analysis in this document focuses on lifetime carcinogenicity bioassays in several strains of mice following repeated dermal exposure to benzo[a]pyrene (Table 1-16). These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959).

Numerous studies in mice observed skin tumors following benzo[a]pyrene exposure, but were not considered further in this assessment because of the availability of the lifetime studies identified above. These studies included several "skin painting" studies in mouse skin that did not report the doses applied (e.g., Wynder and Hoffmann, 1959; Wynder et al., 1957); several shorter-term studies (Albert et al., 1991; Nesnow et al., 1983; Emmett et al., 1981; Levin et al., 1977); initiation-promotion studies utilizing acute dosing of benzo[a]pyrene followed by repeated exposure to a potent tumor promoter; and studies involving vehicles expected to interact with or enhance benzo[a]pyrene carcinogenicity (e.g., Bingham and Falk, 1969).

One study applied benzo[a]pyrene (topically once a week for 6 months) to immuno-compromised mice with human skin grafts (n = 10) and did not observe tumors, whereas all three control mice (mice with no skin grafts) developed skin tumors (Urano et al., 1995). The authors concluded this result indicates that human skin is much less susceptible to benzo[a]pyrene than mouse skin. Though some studies indicate that the skin grafts maintain some metabolic function (Das et al., 1986), it is unclear whether the human skin grafts maintain the same viability, vascularization, and full metabolic capacity as human skin in vivo (Kappes et al., 2004). Another concern is the short amount of time allowed for tumor development. All of the mice with human skin grafts treated with benzo[a]pyrene died within 6 months of the start of treatment (Urano et al., 1995). While 6 months is generally sufficient for the development of tumors in mouse skin, human latency for squamous cell carcinoma in PAH-exposed occupational cohorts is thought to be >20 years (Young et al., 2012; Voelter-Mahlknecht et al., 2007; Everall and Dowd, 1978). Potent mutagenic carcinogens such as 7,12-dimethylbenz[a]anthracene, methylcholanthrene, and methylnitronitrosoguanidine also fail to produce skin tumors in this model system (Soballe et al., 1996; Urano et al., 1995; Graem, 1986). Therefore, the ability of this model system to predict hazard for human skin cancer risk (particularly from metabolically active carcinogens) is unclear.

Table 1-16. Tumors observed in chronic dermal animal bioassays

Reference and study design	Results[a]
Poel (1959) C57L mice: male (13–56/dose) 0, 0.15, 0.38, 0.75, 3.8, 19, 94, 188, 376, or 752 µg Dermal; 3 times/wk for up to 103 wks or until the appearance of a tumor by gross examination	Skin tumors (gross skin tumors and epidermoid carcinoma); dose-dependent decreased time of tumor appearance incidences: Gross skin tumors: 0/33; 5/55; 11/55; 7/56; 41/49; 38/38; 35/35; 12/14; 14/14; and 13/13 Epidermoid carcinoma: 0/33; 0/55; 2/55; 4/56; 32/49; 37/38; 35/35; 10/14; 12/14; and 13/13 Cytotoxicity: information not provided
Poel (1963) SWR, C3HeB, or A/He mice: male (14–25/dose) 0, 0.15, 0.38, 0.75, 3.8, 19.0, 94.0, or 470 µg Dermal; 3 times/wk until mice died or a skin tumor was observed	Skin tumors and dose-dependent decreased time of first tumor appearance incidences: SWR: 0/20; 0/25; 2/22; 15/18; 12/17; 16/16; 16/17; and 14/14 C3HeB: 0/17; 0/19; 3/17; 4/17; 11/18; 17/17; 18/18; and 17/17 A/He mice: 0/17; 0/18; 0/19; 0/17; 0/17; 21/23; 11/16; and 17/17 Cytotoxicity: information not provided
Roe et al. (1970) Swiss mice: female (50/dose) 0, vehicle, 0.1, 0.3, 1, 3, or 9 µg Dermal; 3 times/wk for up to 93 wks	Skin tumors; malignant skin tumors were observed in 4/41 and 31/40 mice in the two high-dose groups, respectively incidences: 0/43; 0/47; 1/42; 0/42; 1/43; 8/41; and 34/46 Cytotoxicity: information not provided
Schmidt et al. (1973) NMRI mice: female (100/group) Swiss mice: female (100/group) 0, 0.05, 0.2, 0.8, or 2 µg Dermal; 2 times/wk until spontaneous death occurred or until an advanced carcinoma was observed	Skin tumors (carcinomas) incidences: NMRI: 2/100 at 2 µg (papillomas); 2/100 at 0.8 µg and 30/100 at 2 µg (carcinomas) Swiss: 3/80 at 2 µg (papillomas); 5/80 at 0.8 µg and 45/80 at 2 µg (carcinomas) Cytotoxicity: information not provided
Schmähl et al. (1977) NMRI mice: female (100/group) 0, 1, 1.7, or 3 µg Dermal; 2 times/wk until natural death or until they developed a carcinoma at the site of application	Skin tumors (papillomas and carcinomas) incidences: 0/81; 1/77; 0/88; and 2/81 (papillomas) 0/81; 10/77; 25/88; and 43/81 (carcinomas) Cytotoxicity: information not provided
Habs et al. (1980) NMRI mice: female (40/group) 0, 1.7, 2.8, or 4.6 µg Dermal; 2 times/wk until natural death or gross observation of infiltrative tumor growth	Skin tumors and dose-dependent increase in age-standardized tumor incidence incidences: 0/35; 8/34; 24/35; and 22/36 age-standardized tumor incidence: 0, 24.8, 89.3, and 91.7% Cytotoxicity: information not provided

Reference and study design	Results[a]
(Grimmer et al. (1984); Grimmer et al. (1983)) CFLP mice: female (65–80/group) 0, 3.9, 7.7, or 15.4 µg (1983 study) 0, 3.4, 6.7, or 13.5 µg (1984 study) Dermal; 2 times/wk for 104 wks	Skin tumors (papillomas and carcinomas) with a decrease in tumor latency incidences: 1983: 0/80; 7/65; 5/64; and 2/64 (papillomas) 0/80; 15/65; 34/64; and 54/64 (carcinomas) 1984: 0/65; 6/64; 8/65; and 4/65 (papillomas) 0/65; 37/64; 45/65; and 53/65 (carcinomas) Cytotoxicity: information not provided
Habs et al. (1984) NMRI mice: female (20/group) 0, 2, or 4 µg Dermal; 2 times/wk for life	Skin tumors (papillomas and carcinomas) with a decrease in mean survival time incidences: 0/20; 2/20; and 0/20 (papillomas) 0/20; 7/20; and 17/20 (carcinomas) Cytotoxicity: information not provided
(Sivak et al. (1997); Arthur D Little, 1989; NIOSH (1989)) C3H/HeJ mice: male (30/group) 0, 0.05, 0.5, or 5 µg Dermal; 2 times/wk for up to 104 wks	Skin tumors (papillomas and carcinomas) incidences: 0/30; 0/30; 5/30 (1 papilloma, 1 keratoacanthoma, 3 carcinomas); and 27/30 (1 papilloma, 28 carcinomas) Cytotoxicity: 80% incidence of scabs and sores in highest dose group; no cytotoxicity noted at lower doses

[a]Statistical significance not reported by study authors.

Mode-of-Action Analysis—Carcinogenicity

The carcinogenicity of benzo[a]pyrene, the most studied PAH, is well documented (IARC, 2010; Xu et al., 2009; Jiang et al., 2007; Jiang et al., 2005; Xue and Warshawsky, 2005; Ramesh et al., 2004; Boström et al., 2002; Penning et al., 1999; IPCS, 1998; Harvey, 1996; ATSDR, 1995; Cavalieri and Rogan, 1995; U.S. EPA, 1991b). The primary mode of action by which benzo[a]pyrene induces carcinogenicity is via a mutagenic mode of action. This mode of action is presumed to apply to all tumor types and is relevant for all routes of exposure. The general sequence of key events associated with a mutagenic mode of action for benzo[a]pyrene is: (1) bioactivation of benzo[a]pyrene to DNA-reactive metabolites via three possible metabolic activation pathways: a diol epoxide pathway, a radical cation pathway, and an *o*-quinone and ROS pathway; (2) direct DNA damage by reactive metabolites, including the formation of DNA adducts and ROS-mediated damage; (3) formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation; and (4) clonal expansion of mutated cells during the promotion and progression phases of cancer development. These events are depicted as stages of benzo[a]pyrene-induced carcinogenesis in Figure 1-6.

Benzo[a]pyrene is a complete carcinogen, in that it can act as both an initiator and a promoter of carcinogenesis. Initiation via direct DNA damage (key event 2) can occur via all three metabolites of benzo[a]pyrene. DNA damage that is not adequately repaired leads to mutation (key

event 3), and these mutations can undergo clonal expansion (key event 4) enabled by multiple mechanisms also induced by benzo[a]pyrene, including AhR binding leading to an upregulation of genes related to biotransformation, growth, and differentiation, and regenerative cell proliferation resulting from cytotoxicity and a sustained inflammatory response. However, there is not sufficient evidence that these mechanisms, which contribute to the promotion and progression phases of cancer development, act independently of DNA damage and mutation to produce benzo[a]pyrene-induced tumors (please see *Other possible modes of action*, below). The available human, animal, and in vitro evidence supports a mutagenic mode of action as the primary mode by which benzo[a]pyrene induces carcinogenesis.

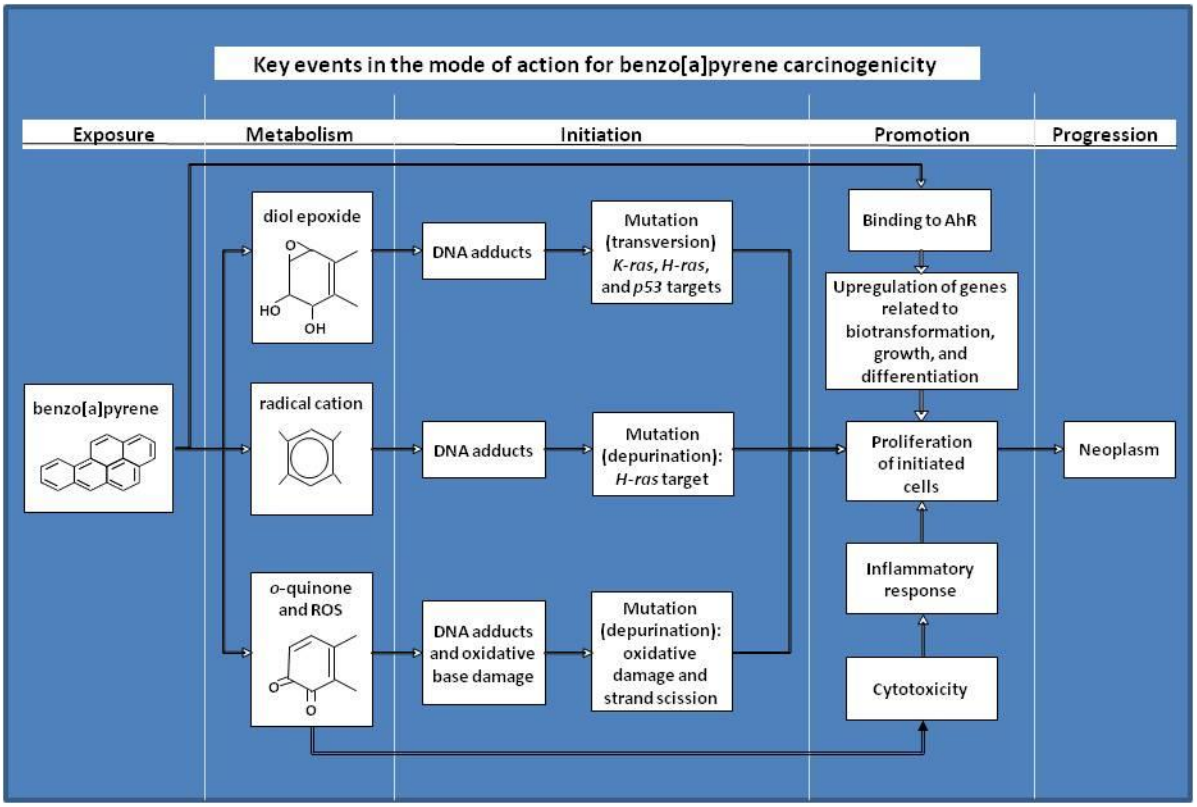

Figure 1-6. Proposed metabolic activation pathways and key events in the carcinogenic mode of action for benzo[a]pyrene.

Data in Support of the Mode of Action

Summary of metabolic activation pathways

Diol epoxide pathway. Benzo[a]pyrene diol epoxide metabolites, believed to be the most potent DNA-binding metabolites of benzo[a]pyrene, are formed through a series of Phase I metabolic reactions (see Appendix D of the Supplemental Information). The initial metabolism is carried out primarily by the inducible activities of CYP enzymes including CYP1A1, CYP1B1, and CYP1A2. Further metabolism by epoxide hydrolase and the mixed function oxidase system yields

(+)-anti-BPDE, one of the most potent DNA-binding metabolites of benzo[a]pyrene. 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- versus 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 (Boström et al., 2002).

Radical cation pathway. Radical cation formation involves a one-electron oxidation by CYP or peroxidase enzymes (i.e., horseradish peroxidase, prostaglandin H synthetase) that produce electrophilic radical cation intermediates (Cavalieri and Rogan, 1995, 1992). Radical cations can be further metabolized to phenols and quinones (Cavalieri et al., 1988e; Cavalieri et al., 1988d), or they can form unstable adducts with DNA that ultimately result in depurination. 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).

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) (see Appendix D of the Supplemental Information). Dihydrodiol dehydrogenase 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 *o*-quinone/ROS pathway also can produce depurinated DNA adducts from benzo[a]pyrene metabolites. In this pathway, and in the presence of $NAD(P)^+$, aldo-keto reductase 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 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 aldo-keto reductase (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-7,8-dihydro-2'-deoxyguanosine adducts) (Park et al., 2006), and strand scission (Flowers et al., 1997; Flowers et al., 1996).

Summary of genotoxicity and mutagenicity

The ability of metabolites 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 genotoxicity tables in Appendix D in Supplemental Information). With metabolic activation (e.g., the inclusion of S9), benzo[a]pyrene is consistently mutagenic in the prokaryotic *Salmonella*/Ames and *Escherichia coli* assays. In mammalian in vitro studies, benzo[a]pyrene is consistently mutagenic and clastogenic,

and induces cell transformation both with and without metabolic activation. Cytogenetic damage in the form of chromosomal aberrations (CAs), micronuclei (MN), sister chromatid exchanges (SCEs), and aneuploidy are commonplace following benzo[a]pyrene exposure as are DNA adduct formation, single-strand breaks (SSB), and induction of DNA repair and unscheduled DNA synthesis (UDS). In vitro mammalian cell assays have been conducted in various test systems, including human cell lines.

In the majority of in vivo studies, benzo[a]pyrene has tested positive in multiple species and strains and under various test conditions for cell transformation, CAs, DNA adducts, DNA strand breaks, MN formation, germline mutations, somatic mutations (*H-ras*, *K-ras*, *p53*, *lacZ*, *hprt*), and SCEs. Human studies are available following exposures to PAH mixtures through cigarette smoke or occupational exposure in which 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.

Experimental support for the hypothesized mode of action

EPA's *Guidelines for Carcinogen Risk Assessment* [Section 2.4; (2005a)] describe a procedure for evaluating mode-of-action data for cancer. A framework for analysis of mode of action information is provided and followed below.

Strength, consistency, and specificity of association. Strong evidence links the benzo[a]pyrene diol epoxide metabolic activation pathway with key mutational events in genes that are associated with tumor initiation (mutations in the *p53* tumor suppressor gene and *H-ras* or *K-ras* oncogenes) (Table 1-17). Results in support of a mutagenic mode of action via benzo[a]pyrene diol epoxide include observations of frequent G→T transversion mutations in *p53* and ras genes in lung tumors of human cancer patients exposed to coal smoke (Keohavong et al., 2003; DeMarini et al., 2001). These results are consistent with evidence that benzo[a]pyrene diol epoxide is reactive with guanine bases in DNA; that G→T transversions, displaying strand bias, are the predominant type of mutations caused by benzo[a]pyrene in several biological systems (Liu et al., 2005; Hainaut and Pfeifer, 2001; Marshall et al., 1984); and that sites of DNA adduction at guanine positions in cultured human HeLa or bronchial epithelial cells exposed to benzo[a]pyrene diol epoxide correspond to *p53* mutational hotspots observed in human lung cancers (Denissenko et al., 1996; Puisieux et al., 1991). In addition, mice exposed to benzo[a]pyrene in the diet (Culp et al., 2000) or by i.p. injection (Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993) had forestomach or lung tumors, respectively, showing frequent G→T or C transversions in the *K-ras* gene. Supporting evidence includes observations that benzo[a]pyrene diol epoxide (specifically (+)-anti-BPDE) is more potent than benzo[a]pyrene itself, benzo[a]pyrene phenols, or benzo[a]pyrene diols in mutagenicity assays in bacterial and in vitro mammalian systems (Malaveille et al., 1977; Newbold and Brookes, 1976) and in producing lung tumors in newborn mice following i.p. administration. Other supporting evidence includes observations of elevated BPDE-DNA adduct levels in WBCs of groups of coke oven workers and chimney sweeps,

occupations with known elevated risks of cancer (Vineis et al., 2007; Pavanello et al., 1999), and in lung tissue from tobacco smokers with lung cancer (Rojas et al., 2004; Godschalk et al., 2002; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992). Several epidemiological studies have indicated that PAH-exposed individuals who are homozygous for a CYP1A1 polymorphism, which increases the inducibility of this enzyme (thus increasing the capacity to produce benzo[a]pyrene diol epoxide), have increased levels of PAH or BPDE-DNA adducts (Aklillu et al., 2005; Alexandrov et al., 2002; Bartsch et al., 2000; Perera and Weinstein, 2000).

Additional supporting evidence of a mutagenic mode of action for benzo[a]pyrene carcinogenicity is the extensive database of in vitro and in vivo studies demonstrating the genotoxicity and mutagenicity of benzo[a]pyrene following metabolic activation (Table 1-17). 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.

Support for the radical cation activation pathway contributing to tumor initiation through mutagenic events includes observations that depurinated DNA adducts (expected products from reactions of benzo[a]pyrene radical cations with DNA) accounted for 74% of identified DNA adducts in mouse skin exposed to benzo[a]pyrene (Rogan et al., 1993) and that 9 of 13 tumors examined from mice exposed to dermal applications of benzo[a]pyrene had *H-ras* oncogene mutations attributed to depurinated DNA adducts from benzo[a]pyrene radical cations (Chakravarti et al., 1995).

Support for the *o*-quinone/ROS pathway contributing to tumor initiation via mutagenic events includes in vitro demonstration that several types of DNA damage can occur from *o*-quinones and ROS (Park et al., 2006; Balu et al., 2004; Mccoull et al., 1999; Flowers et al., 1997; Flowers et al., 1996). In addition, benzo[a]pyrene-7,8-dione can 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), and dominant *p53* mutations induced by benzo[a]pyrene-7,8-dione in this system corresponded to *p53* mutational hotspots observed in human lung cancer tissue (Park et al., 2008).

Dose-response concordance and temporal relationship. Studies in humans demonstrating that benzo[a]pyrene-induced mutational events in *p53* or *ras* oncogenes precede tumor formation are not available, but there is evidence linking benzo[a]pyrene exposure to signature mutational events in humans. In vitro exposure of human *p53* knock-in murine fibroblasts to 1 µM benzo[a]pyrene for 4–6 days induced *p53* mutations with similar features to those identified in *p53* mutations in human lung cancer; i.e., predominance of G→T transversions with strand bias and mutational hotspots at codons 157–158 (Liu et al., 2005).

Bennett et al. (1999) demonstrated a dose-response relationship between smoking history/intensity and the types of *p53* mutations associated with benzo[a]pyrene (G→T transversions) in human lung cancer patients (Table 1-17). In lung tumors of nonsmokers, 10% of

p53 mutations were G→T transversions, versus 40% in lung tumors from smokers with >60 pack-years of exposure.

In mice, dose-response and temporal relationships have been described between the formation of BPDE-DNA adducts and skin and forestomach tumors (Table 1-17). In a study using mice treated dermally with benzo[a]pyrene once or twice per week for up to 15 weeks (10, 25, or 50 nmol benzo[a]pyrene per application), levels of benzo[a]pyrene-DNA adducts in the skin, lung, and liver increased with increasing time of exposure and increasing dose levels (Talaska et al., 2006). Levels at the end of the exposure period were highest in the skin; levels in the lung and liver at the same time were 10- and 20-fold lower, respectively. Levels of benzo[a]pyrene-DNA adducts in skin and lung increased in an apparent biphasic manner showing a lower linear slope between the two lowest dose levels, compared with the slope from the middle to the highest dose.

Another study examined the dose-response relationship and the time course of benzo[a]pyrene-induced skin damage (Table 1-17), 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. A follow-up to this study by the same authors (Albert et al., 1996) measured DNA adducts, necrosis, and inflammation (marked by an increase in leukocytes) in the skin of treated mice after 5 weeks of dermal exposure. In the 64 µg/week dose group, statistically elevated levels of DNA adducts, inflammation, and necrosis were reported; however, in the lower dose group (16 µg/week), DNA adducts were statistically significantly elevated without increases in inflammation and necrosis.

Culp et al. (1996) 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) (Table 1-17). 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., 1996).

Biological plausibility and coherence. The evidence for a mutagenic mode of action for benzo[a]pyrene is consistent with the current understanding that mutations in *p53* and *ras* oncogenes are associated with increased risk of tumor initiation (Table 1-17). The benzo[a]pyrene database is internally consistent in providing evidence for BPDE-induced mutations associated with tumor initiation in cancer tissue from humans exposed to complex mixtures containing benzo[a]pyrene (Keohavong et al., 2003; Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; DeMarini et al., 2001; Hainaut and Pfeifer, 2001; Bennett et al., 1999), in animals exposed to benzo[a]pyrene (Culp et al., 2000; Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993), and in in vitro systems (Denissenko et al., 1996; Puisieux et al., 1991). Consistent supporting evidence includes: (1) elevated BPDE-DNA adduct levels in tobacco smokers with lung cancer (Rojas et al., 2004; Godschalk et al., 2002; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992); (2) demonstration of dose-response relationships between G→T transversions in *p53* mutations in lung tumors and smoking intensity (Bennett et al., 1999); (3) the extensive database of in vitro and in vivo studies demonstrating the genotoxicity and mutagenicity of benzo[a]pyrene following metabolic activation; and (4) general concordance between temporal and dose-response relationships for BPDE-DNA adduct levels and tumor incidence in studies of animals exposed to benzo[a]pyrene (Culp et al., 1996; Albert et al., 1991). There is also supporting evidence that contributions to tumor initiation through mutagenic events can be made by the radical cation (Chakravarti et al., 1995; Rogan et al., 1993) and *o*-quinone/ROS metabolic activation pathways (Park et al., 2008; Park et al., 2006; Shen et al., 2006; Balu et al., 2004; Yu et al., 2002; Mccoull et al., 1999; Flowers et al., 1997; Flowers et al., 1996).

Table 1-17. Experimental support for the postulated key events for mutagenic mode of action

1. Bioactivation of benzo[a]pyrene to DNA-reactive metabolites via three possible metabolic activation pathways: a diol epoxide pathway, a radical cation pathway, and an *o*-quinone and ROS pathway
Evidence that benzo[a]pyrene metabolites induce key events: • Metabolism of benzo[a]pyrene via all three pathways has been demonstrated in multiple in vitro studies, and the diol epoxide and radical cation metabolic activation pathways have been demonstrated in in vivo studies in humans and animals (see *Metabolic Activation Pathways* section) • Multiple in vivo studies in humans and animals have demonstrated distribution of reactive metabolites to target tissues *Human evidence that key events are necessary for carcinogenesis:* • Humans with CYP polymorphisms or lacking a functional GSTM1 gene form higher levels of benzo[a]pyrene diol epoxides, leading to increased BPDE-DNA adduct formation and increased risk of cancer (Vineis et al., 2007; Pavanello et al., 2005; Pavanello et al., 2004; Alexandrov et al., 2002; Perera and Weinstein, 2000)

2. Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage

Evidence that benzo[a]pyrene metabolites induce key events:

- Reactive benzo[a]pyrene metabolites have demonstrated genotoxicity in most in vivo and in vitro systems in which they have been tested, including the bacterial mutation assay, transgenic mouse assay, dominant lethal mutations in mice, BPDE-DNA adduct detection in humans and animals, and DNA damage, CAs, MN formation, and SCE in animals (Appendix D in Supplemental Information)
- Multiple in vivo benzo[a]pyrene animal exposure studies have demonstrated DNA adduct formation in target tissues that precede tumor formation and increase in frequency with dose (Culp et al., 1996; Talaska et al., 1996; Albert et al., 1991)
- Benzo[a]pyrene diol epoxide metabolites interact preferentially with the exocyclic amino groups of deoxyguanine and deoxyadenine in DNA (Geacintov et al., 1997; Jerina et al., 1991; Koreeda et al., 1978; Jeffrey et al., 1976)
- Benzo[a]pyrene *o*-quinone metabolites are capable of activating redox cycles and producing ROS that cause oxidative base damage (Park et al., 2006; Balu et al., 2004; Mccoull et al., 1999; Flowers et al., 1997; Flowers et al., 1996)

Human evidence that key events are necessary for carcinogenesis:

- Detection of benzo[a]pyrene diol epoxide-specific DNA adducts is associated with increased cancer risk in humans that are occupationally exposed (see *Evidence in Humans* section)
- These benzo[a]pyrene diol epoxides formed BPDE-DNA adducts preferentially at guanine residues that have been detected in tissues of humans with cancer who were exposed to PAHs (Vineis and Perera, 2007; Rojas et al., 2004; Godschalk et al., 2002; Li et al., 2001; Pavanello et al., 1999; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992)

3. Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation

Evidence that benzo[a]pyrene metabolites induce key events:

- Several in vivo exposure studies have observed benzo[a]pyrene diol epoxide-specific mutational spectra (e.g., G→T transversion mutations) in *K-ras*, *H-ras*, and *p53* in forestomach or lung tumors (Culp et al., 2000; Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993)
- Multiple animal exposure studies have identified benzo[a]pyrene-specific mutations in *H-ras*, *K-ras*, and *p53* in target tissues preceding tumor formation (Liu et al., 2005; Wei et al., 1999; Culp et al., 1996) (Chakravarti et al., 1995; Ruggeri et al., 1993)

Human evidence that key events are necessary for carcinogenesis:

- DNA adducts formed by the benzo[a]pyrene diol epoxide reacting with guanine bases lead predominantly to G→T transversion mutations; these specific mutational spectra have been identified in PAH-associated tumors in humans at mutational hotspots, including oncogenes (*K-ras*) and tumor suppressor genes (*p53*) (Liu et al., 2005; Keohavong et al., 2003; Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; DeMarini et al., 2001; Hainaut and Pfeifer, 2001; Bennett et al., 1999; Denissenko et al., 1996; Puisieux et al., 1991; Marshall et al., 1984; Koreeda et al., 1978; Jeffrey et al., 1976)

4. Clonal expansion of mutated cells during the promotion and progression phases of cancer development
Evidence that benzo[a]pyrene metabolites induce key events: • Benzo[a]pyrene has been shown to be a complete carcinogen, in that skin tumors in mice, rats, rabbits, and guinea pigs have been associated with repeated application of benzo[a]pyrene to skin in the absence of exogenous promoters (IPCS, 1998; Sivak et al., 1997; ATSDR, 1995; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; IARC, 1983, 1973; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959(IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973) • Mice exposed dermally to benzo[a]pyrene for 26 weeks were found to have increased frequencies of *H-ras* mutations in exposure-induced hyperplastic lesions that were further increased in tumors (Wei et al., 1999) • AhR activation by PAHs (including benzo[a]pyrene) upregulates genes responsible for tumor promotion and increases tumor incidence in mice (Ma and Lu, 2007; Talaska et al., 2006; Shimizu et al., 2000)

Other possible modes of action

The carcinogenic process for benzo[a]pyrene is likely to be related to some combination of molecular events resulting from the formation of several reactive metabolites that interact with DNA to form adducts and produce DNA damage resulting in mutations in cancer-related genes, such as tumor suppressor genes or oncogenes. These events may reflect the initiation potency of benzo[a]pyrene. However, benzo[a]pyrene possesses promotional capabilities that may be related to AhR affinity, immune suppression, cytotoxicity and inflammation (including the formation of ROS), as well as the inhibition of gap junctional intercellular communication (GJIC).

The ability of certain PAHs to act as initiators and promoters may increase their carcinogenic potency. The promotional effects of PAHs appear to be related to AhR affinity and the upregulation of genes related to growth and differentiation (Boström et al., 2002). The genes regulated by this receptor belong 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. The ligand-bound receptor is then transported to the nucleus in complex with the AhR nuclear translocator protein. The AhR complex interacts with AhR elements of DNA to increase the transcription of proteins associated with induction of metabolism and regulation of cell differentiation and proliferation. Following benzo[a]pyrene exposure, disparities have been observed in the tumor pattern and toxicity of Ah-responsive and Ah-nonresponsive mice, as Ah-responsive mice were more susceptible to tumorigenicity in target tissues such as liver, lung, and skin (Ma and Lu, 2007; Talaska et al., 2006; Shimizu et al., 2000).

Benzo[a]pyrene has both inflammatory and immunosuppressive effects that may function to promote tumorigenesis. Inflammatory responses to cytotoxicity may contribute to the tumor promotion process; for example, benzo[a]pyrene quinones (1,6-, 3,6-, and 6,12-benzo[a]pyrene-quinone) generated ROS and increased cell proliferation by enhancing the epidermal growth factor receptor pathway in cultured breast epithelial cells (Burdick et al., 2003). In addition, several studies have demonstrated that exposure to benzo[a]pyrene increases the production of

inflammatory cytokines, which may contribute to cancer progression (N'Diaye et al., 2006; Tamaki et al., 2004; Garçon et al., 2001b; Garçon et al., 2001a; Albert et al., 1996; Albert et al., 1991). One of these studies, Albert et al. (1996), measured DNA adducts, necrosis, and inflammation (marked by an increase in leukocytes) in the skin of benzo[a]pyrene-treated mice after 5 weeks of dermal exposure. In the highest dose group, statistically elevated levels of DNA adducts, inflammation, and necrosis were reported; however, in the lower dose group, DNA adducts were statistically significantly elevated without increases in inflammation and necrosis. It is likely that at high doses of benzo[a]pyrene, inflammation promotes the formation of tumors.

In addition to inflammation, immunosuppressive effects of benzo[a]pyrene have been noted (as reviewed in, Zaccaria and McClure, 2013). Immune effects of benzo[a]pyrene exposure (see Section 1.1.3) may provide an environment where tumor cells can evade detection by immune surveillance mechanisms normally responsible for recognizing and eliminating nascent cancer cells (Hanahan and Weinberg, 2011). In addition, the developing fetus may be even more sensitive to these effects; Urso and Gengozian (1980) found that mice exposed to benzo[a]pyrene in utero not only had a significantly increased tumor incidence as adults but also a persistently suppressed immune system.

Gap junctions are channels between cells that are crucial for differentiation, proliferation, apoptosis, and cell death. Interruption of GJIC is associated with a loss of cellular control of growth and differentiation, and consequently with the two epigenetic steps of tumor formation, promotion and progression. Thus, the inhibition of gap junctional intercellular communication by benzo[a]pyrene, observed in vitro (Sharovskaya et al., 2006; Bláha et al., 2002), provides another mechanism of tumor promotion.

In summary, there are tumor-promoting effects of PAH exposures that are not mutagenic. Although these effects are observed following benzo[a]pyrene-specific exposures, the occurrence of BPDE-DNA adducts and associated mutations that precede both cytotoxicity and tumor formation and increase with dose provides evidence that mutagenicity is the primary event that initiates tumorigenesis following benzo[a]pyrene exposures. A biologically plausible mode of action may involve a combination of effects induced by benzo[a]pyrene, with mutagenicity as the initiating tumorigenic event. Subsequent AhR activation and cytotoxicity could then lead to increased ROS formation, regenerative cell proliferation, and inflammatory responses, which, along with evasion of immune surveillance and GJIC, would provide an environment where the selection for mutated cells increases the rate of mutation, allowing clonal expansion and progression of these tumor cells to occur. However, it was determined that, in comparison to the large database on the mutagenicity of benzo[a]pyrene, there were insufficient data to develop a separate mode of action analysis for these promotional effects.

Conclusions about the hypothesized mode of action

There is sufficient evidence to conclude that the major mode of action for benzo[a]pyrene carcinogenicity involves mutagenicity mediated by DNA reactive metabolites. The evidence for a

mutagenic mode of action for benzo[a]pyrene is consistent with the current understanding that mutations in *p53* and *ras* oncogenes are associated with increased risk of tumor initiation. The benzo[a]pyrene database provides strong and consistent evidence for BPDE-induced mutations associated with tumor initiation in cancer tissue from humans exposed to complex mixtures containing benzo[a]pyrene, in animals exposed to benzo[a]pyrene, and in in vitro systems. Supporting evidence suggests that contributions to tumor initiation through potential mutagenic events can be made by the radical cation and *o*-quinone/ROS metabolic activation pathways. Other processes may contribute to the carcinogenicity of benzo[a]pyrene via the promotion and progression phases of cancer development (e.g., inflammation, cytotoxicity, sustained regenerative cell proliferation).

Support for the Hypothesized Mode of Action 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 by all routes of exposure (see Appendix D in Supplemental Information). Strong, consistent evidence in animal models supports the postulated key events: the metabolism of benzo[a]pyrene to DNA-reactive intermediates, the formation of DNA adducts, the subsequent occurrence of mutations in oncogenes and tumor suppressor genes, and the clonal expansion of mutated cells.

Relevance of the Hypothesized Mode of Action to Humans

A substantial database indicates that the postulated key events for a mutagenic mode of action all occur in human tissues. Evidence is available from studies of humans exposed to PAH mixtures (including coal smoke and tobacco smoke) indicating a contributing role for benzo[a]pyrene diol epoxide in inducing key mutational events in genes that are associated with tumor initiation (mutations in the *p53* tumor suppressor gene and *H-ras* or *K-ras* oncogenes). The evidence includes observations of a spectrum of mutations in *ras* oncogenes and the *p53* gene in lung tumors of human patients exposed to coal smoke or tobacco smoke) that are similar to the spectrum of mutations caused by benzo[a]pyrene diol epoxide in several biological systems, including tumors from mice exposed to benzo[a]pyrene. Additional supporting evidence includes correspondence between hotspots of *p53* mutations in human lung cancers and sites of DNA adduction by benzo[a]pyrene diol epoxide in experimental systems, and elevated BPDE-DNA adduct levels in respiratory tissue of lung cancer patients or tobacco smokers with lung cancer.

Populations or Lifestages Particularly Susceptible to the Hypothesized Mode of Action

A mutagenic mode of action for benzo[a]pyrene-induced carcinogenicity 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 exposure versus later life exposure (U.S. EPA, 2005b; Vesselinovitch et al., 1979). The EPA's *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 that a determination benzo[a]pyrene acts through a mutagenic mode of carcinogenic action has been made, ADAFs should be applied along with exposure information to estimate cancer risks for early-life exposure.

Toxicokinetic information suggest early lifestages may have lower levels of some CYP enzymes than adults (Ginsberg et al., 2004; Cresteil, 1998), suggesting that lower levels of mutagenic metabolites may be formed in early lifestages. Though expression of bioactivating enzymes is believed to be lower in the developing fetus and children, metabolism of benzo[a]pyrene still occurs, as indicated by the detection of benzo[a]pyrene-DNA or protein adducts or urinary metabolites (Naufal et al., 2010; Ruchirawat et al., 2010; Suter et al., 2010; Mielzyńska et al., 2006; Perera et al., 2005a; Tang et al., 1999; Whyatt et al., 1998). While expression of CYP enzymes is lower in fetuses and infants, the greater liver to body mass ratio and increased blood flow to liver in fetuses and infants may compensate for the decreased expression of CYP enzymes (Ginsberg et al., 2004). Activity of Phase II detoxifying enzymes in neonates and children is adequate for sulfation but decreased for glucuronidation and glutathione conjugation (Ginsberg et al., 2004). The conjugation of benzo[a]pyrene-4,5-oxide with glutathione was approximately one-third less in human fetal than adult liver cytosol (Pacifici et al.,1988).

In addition, newborn or infant mice develop liver and lung tumors more readily than young adult mice following acute i.p. exposures to benzo[a]pyrene (Vesselinovitch et al., 1975). These results indicate that exposure to benzo[a]pyrene during early life stages presents additional risk for cancer, compared with exposure during adulthood, despite lower metabolic activity in early lifestages. 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 CYP enzymes have been implicated as determinants of increased individual cancer risk in some studies (Ickstadt et al., 2008; Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). Some evidence suggests that humans lacking a functional GSTM1 gene have higher BPDE-DNA adduct levels and are thus at greater risk for cancer (Binkova et al., 2007; Vineis et al., 2007; Pavanello et al., 2005; Pavanello et al., 2004; Alexandrov et al., 2002; Perera and Weinstein, 2000). 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 (Wang et al., 2010; Ickstadt et al., 2008; Binkova et al., 2007; 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 from benzo[a]pyrene or other PAHs is relatively weak or inconsistent. Combinations of polymorphisms, on the other hand, may be critical determinants of a cumulative DNA-damaging dose, and thus indicate greater susceptibility to cancer from benzo[a]pyrene exposure (Vineis et al., 2007).

Analysis of Toxicogenomics Data

An analysis of pathway-based transcriptomic data was conducted to help inform the cancer mode of action for benzo[a]pyrene (see the Supplemental Information for details of this analysis). These data support a mutagenic and cellular proliferation mode of action that follows three candidate pathways: aryl hydrocarbon signaling; DNA damage regulation of the G1/S phase transition; and/or Nrf2 regulation of oxidative stress. Specifically, the analysis showed that benzo[a]pyrene may activate the AhR, leading to the formation of oxidative metabolites and radicals which may lead to oxidative damage and DNA damage. Subsequently, DNA damage can occur and activate p53 and p53 target genes, including p21 and MDM2. In addition, the data indicate that p53 signaling may be decreased under these conditions, as ubiquitin and MDM2 are both upregulated, and work together to degrade p53. Furthermore, the transcriptional upregulation of cyclin D may result in enough cyclin D protein to overcome the p21 inhibitory competition for CDK4, allowing for G1/S phase transition to occur. The data also support the hypothesis that an upregulation of proliferating cell nuclear antigen (PCNA) in combination with the upregulation of ubiquitin indicates that cells are moving towards the G1/S phase transition. Although the alterations to the Nrf2 pathway suggest cells are preparing for a pro-apoptotic environment, there is no transcriptional evidence that the apoptotic pathways are being activated.

There are uncertainties associated with the available transcriptomics data. For instance, the available studies only evaluate gene expression following benzo[a]pyrene exposure and do not monitor changes in protein or metabolite expression, which would be more indicative of an actual cellular state change. Further research is required at the molecular level to demonstrate that the cellular signaling events being inferred from such data are actually operative and result in phenotypic changes. In addition, this analysis relied upon two short term studies that evaluated mRNA expression levels in a single tissue (liver) and species (mouse) and were conducted at relatively high doses.

1.2. SUMMARY AND EVALUATION

1.2.1. Weight of Evidence for Effects Other than Cancer

The weight of the evidence from human and animal studies indicates that the strongest evidence for human hazards following benzo[a]pyrene exposure is for developmental toxicity, reproductive toxicity, and immunotoxicity. Most of the available human data on benzo[a]pyrene report associations between particular health endpoints and concentrations of benzo[a]pyrene-DNA adducts, with fewer noncancer studies correlating health effects with external measures of exposure. In general, the available human studies report effects that are analogous to the effects observed in animal toxicological studies, and provide qualitative, supportive evidence for the effect-specific hazards identified in Sections 1.1.1–1.1.4.

In animals, evidence of developmental toxicity, reproductive toxicity, and immunotoxicity has been observed across species and dosing regimens. The available evidence from mice and rats

treated by gavage during gestation or in the early postnatal period demonstrate developmental effects including decreased body weight, decreased fetal survival, decreased fertility, atrophy of reproductive organs, and altered neurobehavioral outcomes (Chen et al., 2012; Jules et al., 2012; Bouayed et al., 2009a; Kristensen et al., 1995; Mackenzie and Angevine, 1981). Several studies in animals have indicated that exposure to benzo[a]pyrene in early life may result in altered neurobehavioral outcomes and sensorimotor development (Chen et al., 2012; Bouayed et al., 2009a). Male and female reproductive toxicity, as evidenced by effects on sperm parameters, decreased reproductive organ weights, histological changes, and hormone alterations, have been observed after oral exposure in rats and mice (Chen et al., 2011; Chung et al., 2011; Mohamed et al., 2010; Zheng et al., 2010; Mackenzie and Angevine, 1981). Benzo[a]pyrene exposure has also been shown to lead to altered immune cell populations and histopathological changes in immune system organs (Kroese et al., 2001; De Jong et al., 1999), as well as thymic and splenic effects following subchronic oral exposure. Varying immunosuppressive responses are also observed in short-term oral and injection studies. Overall, the oral data support the conclusion that developmental toxicity and reproductive toxicity are human hazards following exposure to benzo[a]pyrene and that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure.

Following inhalation exposure to benzo[a]pyrene in animals, evidence of developmental and reproductive toxicity has been observed. Decreased fetal survival has been observed in rats exposed to benzo[a]pyrene via inhalation during gestation (Wormley et al., 2004; Archibong et al., 2002). Male reproductive toxicity, as evidenced by effects on sperm parameters, decreased testes weight, and hormone alterations, has also been observed in rats following subchronic inhalation exposure to benzo[a]pyrene (Archibong et al., 2008; Ramesh et al., 2008). Female reproductive toxicity, as evidenced by modified hormone levels in dams, has been observed following inhalation exposure to benzo[a]pyrene during gestation (Archibong et al., 2002). The inhalation data support the conclusion that developmental toxicity and reproductive toxicity are human hazards following exposure to benzo[a]pyrene.

Other types of effects were observed following benzo[a]pyrene exposure including forestomach hyperplasia, hematological, hepatic, renal, cardiovascular, and adult neurological toxicity (see Section 1.1.4). Forestomach hyperplasia was observed following oral and inhalation exposure; however, this endpoint most likely reflects early events in the neoplastic progression of forestomach tumors following benzo[a]pyrene exposure (see Section 1.1.4), and was not considered further for dose-response analysis and the derivation of reference values. For the remaining effects, EPA concluded that the available evidence does not support these noncancer effects as potential human hazards.

1.2.2. Weight of Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), benzo[a]pyrene is "carcinogenic to humans." This guidance emphasizes the importance of weighing all of the evidence in reaching conclusions about human carcinogenic potential. The descriptor of "carcinogenic to

humans" can be used when the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association; (b) there is extensive evidence of carcinogenicity in animals; (c) the mode or modes of carcinogenic action and associated key precursor events have been identified in animals; and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. The data supporting these four conditions for benzo[a]pyrene are presented below and in Table 1-18.

a) Strong Human Evidence of Cancer or its Precursors

There is a large body of evidence for human carcinogenicity for complex PAH mixtures containing benzo[a]pyrene, including soot, coal tars, coal-tar pitch, mineral oils, shale oils, and smoke from domestic coal burning (IARC, 2010; Baan et al., 2009). There is also evidence of carcinogenicity, primarily of the lung and skin, in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, aluminum production, 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 (Benbrahim-Tallaa et al., 2012; Bosetti et al., 2007). There is extensive evidence of the carcinogenicity of tobacco smoke, of which benzo[a]pyrene is a notable constituent. The methodologically strongest epidemiology studies (in terms of exposure assessment, sample size, and follow-up period) provide consistent evidence of a strong association between benzo[a]pyrene exposure and lung cancer. Three large epidemiology studies in different geographic areas, representing two different industries, observed increasing risks of lung cancer with increasing cumulative exposure to benzo[a]pyrene (measured in μg/m^3-years), with approximately a twofold increased risk at the higher exposures; each of these studies addressed potential confounding by smoking (Armstrong and Gibbs, 2009; Spinelli et al., 2006; Xu et al., 1996) (Table 1-11). Although the relative contributions of benzo[a]pyrene and of other PAHs cannot be established, the exposure-response patterns seen with the benzo[a]pyrene measures make it unlikely that these results represent confounding by other exposures. Similarly, for bladder cancer, two of the three cohort studies with detailed exposure data observed an increasing risk with exposures >80 μg/m^3-years(Gibbs and Sevigny, 2007a; Gibbs et al., 2007; Gibbs and Sevigny, 2007b; Spinelli et al., 2006) (Table 1-13). The exposure range was much lower in the third study (Burstyn et al., 2007; Gibbs and Sevigny, 2007a; Gibbs et al., 2007; Gibbs and Sevigny, 2007b), such that the highest exposure group only reached the level of exposure seen in the lowest exposure categories in the other studies. Data pertaining to non-melanoma skin cancer is limited to studies with more indirect exposure measures, e.g., based on occupations with likely dermal exposure to creosote (i.e., timber workers, brick makers, and power linesmen); the RR estimates seen in the four available studies that provide risk estimates for this type of cancer ranged from 1.5 to 4.6, with three of these four

estimates >2.5 and statistically significant (Pukkala, 1995; Karlehagen et al., 1992; Törnqvist et al., 1986; Hammond et al., 1976). These four studies provide support for the association between dermal PAH exposure, including benzo[a]pyrene exposure, and skin cancer. Although it is likely that multiple carcinogens present in PAH mixtures contribute to the carcinogenic responses, strong evidence is available from several studies of humans exposed to PAH mixtures supporting a contributing role for benzo[a]pyrene diol epoxide in inducing key mutagenic precursor cancer events in target tissues. Elevated BPDE-DNA adducts have been reported in smokers compared to nonsmokers, and the increased adduct levels in smokers are typically increased twofold compared with nonsmokers (Phillips, 2002). Elevated BPDE-DNA adduct levels have been observed in WBCs of groups of coke oven workers and chimney sweeps, occupations with known elevated risks of cancer (Rojas et al., 2000; Bartsch et al., 1999; Pavanello et al., 1999; Bartsch et al., 1998; Rojas et al., 1998), and in lung tissue from tobacco smokers with lung cancer (Rojas et al., 2004; Godschalk et al., 2002; Bartsch et al., 1999; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992).

Mutation spectra distinctive to diol epoxides have been observed in the tumor suppressor gene *p53* and the *K-ras* oncogene in tumor tissues taken from lung cancer patients who were chronically exposed to two significant sources of PAH mixtures: coal smoke and tobacco smoke. Hackman et al. (2000) reported an increase of GC→TA transversions and a decrease of GC→AT transitions at the hprt locus in T-lymphocytes of humans with lung cancer who were smokers compared to non-smokers. Lung tumors from cancer patients exposed to emissions from burning smoky coal showed mutations in *p53* and *K-ras* that were primarily G→T transversions (76 and 86%, respectively) (DeMarini et al., 2001). Keohavong et al. (2003) investigated the *K-ras* mutational spectra from nonsmoking women and smoking men chronically exposed to emissions from burning smoky coal, and smoking men who resided in homes using natural gas; among those with *K-ras* mutations, 67, 86, and 67%, respectively, were G→T transversions. Lung tumors from tobacco smokers showed a higher frequency of *p53* mutations that were G→T transversions compared with lung tumors in non-smokers (Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001), and the frequency of these types of *p53* mutations in lung tumors from smokers increased with increasing smoking intensity (Bennett et al., 1999).

Similarly, investigations of mutagenesis following specific exposures to benzo[a]pyrene (as opposed to PAH mixtures) have consistently observed that the benzo[a]pyrene diol epoxide is very reactive with guanine bases in DNA, and that G→T transversions are the predominant type of mutations caused by benzo[a]pyrene diol epoxide in several biological test (Pfeifer and Hainaut, 2003; Hainaut and Pfeifer, 2001). Following treatment of human HeLa cells with benzo[a]pyrene diol epoxide, Denissenko et al. (1996) reported that the distribution of BPDE-DNA adducts within *p53* corresponded to mutational hotspots observed in *p53* in human lung cancers. Benzo[a]pyrene exposure induced mutations in embryonic fibroblasts from human *p53* "knock-in" mice that were similar to those found in smoking related human cancers, with a predominance of G→T transversions that displayed strand bias and were also located in the same mutational hotspots

found in *p53* in human lung tumors (Liu et al., 2005). These results, combined with a mechanistic understanding that mutations in *p53* (which encodes a key transcription factor in DNA repair and regulation of cell cycle and apoptosis) may be involved in the initiation phase of many types of cancer, are consistent with a common mechanism for mutagenesis following exposures to PAH mixtures and provide evidence of a contributing role of benzo[a]pyrene diol epoxide in the carcinogenic response of humans to coal smoke and tobacco smoke.

Therefore, while the epidemiological evidence alone does not establish a causal association between human exposure and cancer, there is strong evidence that the key precursor events of benzo[a]pyrene's mode of action are likely to be associated with tumor formation in humans.

b) Extensive Animal Evidence

In laboratory animals (rats, mice, and hamsters), exposures 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. Three 2-year oral bioassays are available that associate lifetime benzo[a]pyrene exposure with carcinogenicity at multiple sites. These bioassays observed forestomach, liver, oral cavity, jejunum, kidney, auditory canal (Zymbal gland), 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 (Beland and Culp, 1998; Culp et al., 1998). Repeated or short-term oral exposure to benzo[a]pyrene was associated with forestomach tumors in additional bioassays with several strains of mice (Weyand et al., 1995; Benjamin et al., 1988; Robinson et al., 1987; El-Bayoumy, 1985; Triolo et al., 1977; Wattenberg, 1974; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955). EPA has considered the uncertainty associated with the relevance of forestomach tumors for estimating human risk from benzo[a]pyrene exposure. While humans do not have a forestomach, squamous epithelial tissue similar to that seen in the rodent forestomach exists in the oral cavity and upper two-thirds of the esophagus in humans (IARC, 2003). Human studies, specifically associating exposure to benzo[a]pyrene with the alimentary tract tumors are not currently available. However, benzo[a]pyrene-DNA adducts have been detected in oral and esophageal tissue obtained from smokers (reviewed by Phillips, 2002) and several epidemiological studies have identified increased exposure to PAHs as an independent risk factor for esophageal cancer (Abedi-Ardekani et al., 2010; Szymańska et al., 2010; Gustavsson et al., 1998; Liu et al., 1997). Thus, EPA concluded that forestomach tumors in rodents are relevant for assessing the carcinogenic risk to humans.

Lifetime inhalation exposure to benzo[a]pyrene was associated primarily with tumors in the larynx and pharynx of male Syrian golden hamsters exposed to benzo[a]pyrene:NaCl aerosols (Thyssen et al., 1981). Additionally, less-than-lifetime oral exposure cancer bioassays in mice provide supporting evidence that exposure to benzo[a]pyrene is associated with an increased incidence of lung tumors in mice (Weyand et al., 1995; Robinson et al., 1987; Wattenberg, 1974). In

additional studies with hamsters, intratracheal instillation of benzo[a]pyrene was associated with upper and lower respiratory tract tumors (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). Dermal application of benzo[a]pyrene (2–3 times/week) has been associated with mouse skin tumors in numerous lifetime bioassays (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959). 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 (IPCS, 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 (Weyand et al., 1992; Cavalieri et al., 1991; Rice et al., 1985; El-Bayoumy et al., 1982; Lavoie et al., 1982; Raveh et al., 1982; Cavalieri et al., 1981; Slaga et al., 1980; Wood et al., 1980; Slaga et al., 1978; Hoffmann et al., 1972).

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 (Lavoie et al., 1994; Busby et al., 1989; Weyand and Lavoie, 1988; Lavoie et al., 1987; Wislocki et al., 1986; Busby et al., 1984; Buening et al., 1978; Kapitulnik et al., 1978); (2) increased lung tumor multiplicity in A/J adult mice given single i.p. injections (Mass et al., 1993); (3) injection site tumors in mice following s.c. injection (Nikonova, 1977; Pfeiffer, 1977; Homburger et al., 1972; Roe and Waters, 1967; Grant and Roe, 1963; Steiner, 1955; Rask-Nielsen, 1950; Pfeiffer and Allen, 1948; Bryan and Shimkin, 1943; Barry et al., 1935); (4) injection site sarcomas in mice following intramuscular injection(Sugiyama, 1973); (5) mammary tumors in rats with intramammilary administration (Cavalieri et al., 1991; Cavalieri et al., 1988c; Cavalieri et al., 1988b; Cavalieri et al., 1988a); (6) cervical tumors in mice with intravaginal application (Näslund et al., 1987); and (7) tracheal tumors in rats with intratracheal implantation (Topping et al., 1981; Nettesheim et al., 1977).

Therefore, the animal database provides extensive evidence of carcinogenicity in animals.

c) Key Precursor Events have been Identified in Animals

There is sufficient evidence to conclude that benzo[a]pyrene carcinogenicity involves a mutagenic mode of action mediated by DNA-reactive metabolites. The benzo[a]pyrene database provides strong and consistent evidence for BPDE-induced mutations associated with tumor initiation in cancer tissue from humans exposed to complex mixtures containing benzo[a]pyrene, in animals exposed to benzo[a]pyrene, and in in vitro systems. Other processes may contribute to the carcinogenicity of benzo[a]pyrene via the promotion and progression phases of cancer development (e.g., inflammation, cytotoxicity, sustained regenerative cell proliferation, anti-apoptotic signaling), but the available evidence best supports a mutagenic mode of action as the primary mode by which benzo[a]pyrene acts.

d) Strong Evidence that the Key Precursor Events are Anticipated to Occur in Humans

Mutations in *p53* and *ras* oncogenes have been observed in tumors from mice exposed to benzo[a]pyrene in the diet (Culp et al., 2000) or by i.p. injection (Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993). Mutations in these same genes have also been reported in lung tumors of human cancer patients, bearing distinctive mutation spectra (G→T transversions) that correlate with exposures to coal smoke (Keohavong et al., 2003; DeMarini et al., 2001) or tobacco smoke (Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001; Bennett et al., 1999).

Table 1-18. Supporting evidence for the *carcinogenic to humans* cancer descriptor for benzo[a]pyrene

Evidence	Reference
a) Strong human evidence of cancer or its precursors	
• Increased risk of lung, bladder, and skin cancer in humans exposed to complex PAH mixtures containing benzo[a]pyrene	(IARC (2010), 2004)); Secretan et al. (2009);Baan et al. (2009); Benbrahim-Tallaa et al. (2012)
• Benzo[a]pyrene-specific biomarkers detected in humans exposed to PAH mixtures associated with increased risk of cancer	
– BPDE-DNA adducts in WBCs of coke oven workers and chimney sweeps	(Rojas et al. (2000); Bartsch et al. (1999); Pavanello et al. (1999); Bartsch et al. (1998); Rojas et al. (1998))
– BPDE-DNA adducts in smokers	Phillips (2002)
• Benzo[a]pyrene-specific DNA adducts have been detected in target tissues in humans exposed to PAH mixtures	
– BPDE-DNA adducts in non-tumor lung tissues of cigarette smokers with lung cancer and in skin of eczema patients treated with coal tar	Rojas et al. (2004); (Godschalk et al. (2002); Bartsch et al. (1999); Godschalk et al. (1998b); Rojas et al. (1998); Andreassen et al. (1996); Alexandrov et al. (1992))
– BPDE-DNA adduct formation in *p53* in human cells in vitro corresponds to mutational hotspots at guanine residues in human lung tumors	(Denissenko et al. (1996); Puisieux et al. (1991))
• Benzo[a]pyrene-specific mutational spectra identified in PAH-associated tumors in humans	

Evidence	Reference
– GC→TA transversions and GC→AT transitions at hprt locus in T-lymphocytes of humans with lung cancer	Hackman et al. (2000)
– G→T transversions in exposed human-*p53* knock-in mouse fibroblasts at the same mutational hotspot in *p53* from smoking-related lung tumors in humans	Liu et al. (2005)
– G→T transversions at the same mutational hotspot in *p53* and *K-ras* in human lung tumors associated with smoky coal exposures	(Keohavong et al. (2003); DeMarini et al. (2001))
– Increased percentage of G→T transversions in *p53* in smokers versus nonsmokers	(Pfeifer and Hainaut (2003); Pfeifer et al. (2002); Hainaut and Pfeifer (2001); Bennett et al. (1999))
b) Extensive animal evidence	
Oral exposures	
• Forestomach tumors in male and female rats and in female mice following lifetime exposure	(Kroese et al. (2001); Beland and Culp (1998); Culp et al. (1998); Brune et al. (1981))
• Forestomach tumors in mice following less-than-lifetime exposures	(Weyand et al. (1995); Benjamin et al. (1988); Robinson et al. (1987); El-Bayoumy (1985); Triolo et al. (1977); Wattenberg (1974); Roe et al. (1970); Biancifiori et al. (1967); Chouroulinkov et al. (1967); Fedorenko and Yansheva (1967); Neal and Rigdon (1967); Berenblum and Haran (1955))
• Alimentary tract and liver tumors in male and female rats following lifetime exposure	Kroese et al. (2001)
• Kidney tumors in male rats following lifetime exposure	Kroese et al. (2001)
• Auditory canal tumors in male and female rats following lifetime exposure	Kroese et al. (2001)
• Esophageal, tongue, and laryngeal tumors in female mice following lifetime exposure	(Beland and Culp (1998); Culp et al. (1998))
• Lung tumors in mice following less-than-lifetime exposure	(Weyand et al. (1995); Robinson et al. (1987); Wattenberg (1974))

Evidence	Reference
Inhalation exposures	
• Upper respiratory tract tumors in male hamsters following chronic exposure	Thyssen et al. (1981)
Dermal exposures	
• Skin tumors in mice following lifetime exposures without a promoter	(Sivak et al. (1997); Grimmer et al. (1984); Habs et al. (1984); Grimmer et al. (1983); Habs et al. (1980); Schmähl et al. (1977); Schmidt et al. (1973); Roe et al. (1970); Poel (1963), 1959))
• Skin tumors in rats, rabbits, and guinea pigs following subchronic exposures	(IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973)
Other routes of exposure	
• Respiratory tract tumors in hamsters following intratracheal instillation	(Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972)
• Liver or lung tumors in newborn mice given acute postnatal i.p. injections	(Lavoie et al., 1994; Busby et al., 1989; Weyand and Lavoie, 1988; Lavoie et al., 1987; Wislocki et al., 1986; Busby et al., 1984; Buening et al., 1978; Kapitulnik et al., 1978)
• Lung tumor multiplicity in A/J adult mice given single i.p. injections	Mass et al. (1993)
c) Identification of key precursor events have been identified in animals	
• Bioactivation of benzo[a]pyrene to DNA-reactive metabolites has been shown to occur in multiple species and tissues by all routes of exposure • Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage • Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation	See 'Experimental Support for Hypothesized Mode of Action' section
d) Strong evidence that the key precursor events are anticipated to occur in humans	
• Mutations in *p53* or *ras* oncogenes have been observed in forestomach or lung tumors from mice exposed to benzo[a]pyrene	(Culp et al. (2000); Nesnow et al. (1998a); Nesnow et al. (1998b); Nesnow et al. (1996), 1995); Mass et al. (1993))

Evidence	Reference
– G→T transversions in *ras* oncogenes or the *p53* gene have been observed in lung tumors of human cancer patients exposed to coal smoke	(Keohavong et al. (2003); DeMarini et al. (2001))
– Higher frequency of G→T transversions in lung tumors from smokers versus nonsmokers	(Pfeifer and Hainaut (2003); Pfeifer et al. (2002); Hainaut and Pfeifer (2001); Bennett et al. (1999))

2. DOSE-RESPONSE ANALYSIS

2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The oral reference dose (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. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

2.1.1. Identification of Studies and Effects for Dose-Response Analysis

In Section 1.2.1, developmental, reproductive, and immunological toxicities were highlighted as human hazards or potential human hazards of benzo[a]pyrene exposure by the oral route. Studies within each effect category were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationales for selecting the studies and effects to represent each of these hazards are summarized below.

Human studies are preferred over animal studies when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure. For benzo[a]pyrene, human studies of environmental polycyclic aromatic hydrocarbon (PAH) mixtures across multiple cohorts have observed effects following exposure to complex mixtures of PAHs. The available data suggest that benzo[a]pyrene exposure may pose health hazards other than cancer including reproductive and developmental effects such as infertility, miscarriage, and reduced birth weight (Wu et al., 2010; Neal et al., 2008; Tang et al., 2008; Perera et al., 2005b; Perera et al., 2005a), effects on the developing nervous system (Perera et al., 2012a; Perera et al., 2009), and cardiovascular effects (Friesen et al., 2010; Burstyn et al., 2005). However, the available human studies that utilized benzo[a]pyrene-deoxyribonucleic acid (DNA) adducts as the exposure metric do not provide external exposure levels of benzo[a]pyrene from which to derive a value, and exposure is likely to have occurred by multiple routes. In addition, uncertainty exists due to concurrent exposure to other PAHs and other components of the mixture (such as metals).

Animal studies were evaluated to determine which provided the most relevant routes and durations of exposure; multiple exposure levels to provide information about the shape of the dose-response curve; and power to detect effects at low exposure levels (U.S. EPA, 2002). The oral database for benzo[a]pyrene includes a variety of studies and datasets that are suitable for use in

deriving reference values. Specifically, chronic effects associated with benzo[a]pyrene exposure in animals include observations of organ weight and histological changes and hematological parameters observed in several oral cancer bioassays (Kroese et al., 2001; Beland and Culp, 1998). Multiple subchronic studies are available that characterize a variety of effects other than cancer. In addition, several developmental studies are available that help inform hazards of exposure during sensitive developmental windows.

Developmental Toxicity

Numerous animal studies observed endpoints of developmental toxicity following oral exposure during gestational or early postnatal development (Chen et al., 2012; Jules et al., 2012; Sheng et al., 2010; Bouayed et al., 2009a; Kristensen et al., 1995; Mackenzie and Angevine, 1981) and were considered for dose-response analysis based on the above criteria. Kristensen et al. (1995), with only one dose group, was not considered further given its concordance with Mackenzie and Angevine (1981), which had multiple groups. From the remaining studies demonstrating developmental toxicity, the studies conducted by Chen et al. (2012) and Jules et al. (2012) were identified as the most informative studies for dose-response analysis. The neurodevelopmental study by Chen et al. (2012) was a well-designed and well-conducted study that evaluated multiple developmental endpoints and measures of neurotoxicity in neonatal, adolescent, and adult rats after early postnatal exposure. The study randomly assigned a total of 10 male and 10 female pups per treatment group, with no more than one male and one female from each litter for behavioral testing. In addition, the pups were cross-fostered with dams being rotated among litters every 2–3 days to distribute any maternal caretaking differences randomly across litters and treatment groups. Importantly, all tests were conducted by investigators blinded to treatment, and test order was randomized each day.

In the neurobehavioral tests, Chen et al. (2012) observed increased locomotion in the open field test, increased latency in negative geotaxis and surface righting tests, decreased anxiety-like behaviors in the elevated plus maze test, and impaired performance in the Morris water maze test at various time points following neonatal benzo[a]pyrene treatment. Altered behaviors and locomotion in open field tests could be attributed to anxiety responses due to open spaces and bright light, as well as changes to motor system function. Chen et al. (2012) reported increased quadrants crossed, which could indicate either increased motor activity or decreased anxiety (less fear of the open spaces/bright lights); thus, this response does not reflect a clear effect of exposure on a discrete neurological function. In addition, the biological significance of the impairments observed in the negative geotaxis and surface righting tests were considered somewhat uncertain as these effects were not persistent as compared to the effects noted in the elevated plus maze and Morris water maze (which persisted into adulthood). As a result, EPA considered the elevated plus maze and Morris water maze tests to be the most informative measures of neurobehavioral function performed by Chen et al. (2012).

Significant, dose-related effects were reported in an established test of spatial learning and memory (Morris water maze). Specifically, increased escape latency in hidden platform trials and decreased time spent in the target quadrant during a probe trial were observed following benzo[a]pyrene exposure in rats tested as adolescents or as adults. Due to the altered baseline performance of treated animals on day 1 of the hidden platform trials these findings cannot be specifically attributed to impaired learning. In fact, the slopes of the lines across trial days are nearly identical for the treatment groups, suggesting the lack of a robust effect on learning. The impaired Morris water maze performance of treated animals could be due to effects on several other components of neurological function besides learning, including anxiety, vision, and locomotion. Similarly, as escape latencies were not comparable across groups after learning acquisition (i.e., the end of the hidden platform trials), differences in probe trial performance are difficult to attribute to impaired memory retention alone. As a result of this lack of specificity, although they identify significant effects of benzo[a]pyrene exposure, the Morris water maze data were considered less informative than the results from the elevated plus maze test. Chen et al. (2012) reported an increase in the number of open arm entries in the elevated plus maze test, an indicator of decreased anxiety-like behavior. These results indicate effects on a single, discrete neurological function that are unlikely to be complicated by changes in other processes such as motor activity (total activity, calculated by summing open and closed arm entries was unchanged with treatment). This neurobehavioral endpoint is supported by similar observations in developing (Bouayed et al., 2009a) and adult (Grova et al., 2008) mice, and may be indirectly related to observations of increased aggression in mice (Bouayed et al., 2009b) and is considered adverse (see discussion in Section 1.1.1).

Jules et al. (2012) was also identified for dose-response analysis. This study was of sufficient duration, utilized multiple doses, did not observe maternal toxicity, and evaluated multiple cardiovascular endpoints. The study authors reported increases in both systolic (approximately 20–50%) and diastolic (approximately 33–83%) pressure and heart rate in adult rats that were exposed gestationally to benzo[a]pyrene. A limitation of this study is that the authors only reported effects at the two highest doses. However, given the magnitude of the response and the appearance of these effects in adulthood following gestational exposure, these endpoints were selected for dose-response analysis because of their sensitivity and biological plausibility.

Bouayed et al. (2009a) and Mackenzie and Angevine (1981) were not selected for dose-response analysis. Bouayed et al. (2009a) used the same tests as Chen et al. (2012), but the doses evaluated were higher (2 and 20 mg/kg-day compared to 0.02, 0.2, and 2 mg/kg-day, respectively). Similarly, Mackenzie and Angevine (1981) demonstrated developmental effects in a multi-dose study with relevant routes and durations of exposure; however, the doses studied (10–160 mg/kg-day) were much higher than those evaluated in other developmental toxicity studies (Chen et al., 2012; Jules et al., 2012).

Reproductive Toxicity

Male reproductive toxicity was demonstrated in numerous subchronic studies (Chen et al., 2011; Chung et al., 2011; Mohamed et al., 2010; Zheng et al., 2010). Chung et al. (2011) was not included in the dose-response analysis because numerical data were not reported or were only reported for the mid-dose of three doses. Chen et al. (2011) is a subchronic study that applied only a single dose level. This study corroborated other available multi-dose studies and is considered supportive, but was not considered for dose-response analysis due to the limited reporting of numerical data. The studies conducted by Mohamed et al. (2010) and Zheng et al. (2010) were identified as the most informative male reproductive toxicity studies for dose-response analysis. Decreased sperm count and motility observed by Mohamed et al. (2010) and decreased intratesticular testosterone levels observed by Zheng et al. (2010) were selected for dose-response analysis as both represent sensitive endpoints of male reproductive toxicity and are indicators of potentially decreased fertility. These effects are also consistent with human studies in PAH exposed populations, as effects on male fertility and semen quality have been demonstrated in epidemiological studies of smokers (reviewed by Soares and Melo, 2008).

Female reproductive toxicity was demonstrated in two subchronic studies (Gao et al., 2011; Xu et al., 2010). Specifically, Xu et al., 2010 demonstrated altered ovary weights and follicle numbers, and Gao et al. (2011) demonstrated cervical epithelial cell hyperplasia following oral exposure to benzo[a]pyrene. These studies were identified as the most informative studies on female reproductive toxicity for dose-response analysis. Gao et al. (2011) identified statistically-significant, dose-related increases in the incidence of cervical inflammatory cells in mice exposed to low doses of benzo[a]pyrene for 98 days (Gao et al., 2011). Cervical effects of increasing severity (including epithelial hyperplasia, atypical hyperplasia, apoptosis, and necrosis) were also observed at higher doses (Gao et al., 2011). There are no data on cervical effects in other species or in other mouse strains. However, Gao et al. (2011) also evaluated cervical effects in separate groups of mice exposed via intraperitoneal (i.p.) injection, and observed similar responses in these groups of mice, providing support for the association between effects in this target organ and benzo[a]pyrene exposure. Epidemiological studies have demonstrated an association between cigarette smoking and increased risk of cervical cancer (Pate Capps et al., 2009). In addition, benzo[a]pyrene metabolites and benzo[a]pyrene-DNA adducts have been detected in human cervical mucus and cervical tissues obtained from smokers (Phillips, 2002; Melikian et al., 1999). However, data to support that cervical hyperplasia following oral benzo[a]pyrene exposure progresses to cervical tumors were not available (no cervical tumors were noted in the two available lifetime oral cancer bioassays). Thus, in the absence of these data, cervical hyperplasia is presented as a noncancer effect.

Xu et al. (2010) 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 (Borman et al., 2000; Kristensen et al., 1995; Miller et al., 1992; Swartz and Mattison, 1985; Mackenzie and Angevine, 1981; Mattison et al., 1980). Additionally, epidemiology studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (Neal et al., 2008; El-Nemr et al., 1998). 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).

Immunotoxicity

As described in Section 1.1.3, the immune system was identified as a potential human hazard of benzo[a]pyrene exposure based on findings of organ weight and immunoglobulin alterations, as well as effects on cellularity and functional changes in the immune system in animals. The only available studies to support development of an RfD were conducted by Kroese et al. (2001) and De Jong et al. (1999). These are subchronic studies with multiple exposure levels and adequate power to detect effects. In comparing these studies, the Kroese et al. (2001) study is preferred for dose-response analysis due to its longer duration (90 days).

Decreased thymus weight, observed in Kroese et al. (2001), decreased IgM and IgA levels, and decreased relative numbers of B-cells, observed in De Jong et al. (1999), were selected for dose-response analysis. It is recognized that thymus weight changes on their own have been noted to be less reliable indicators of immunotoxicity (Luster et al., 1992). However, there are converging lines of evidence that support the derivation of an organ/system-specific RfD for benzo[a]pyrene immunotoxicity. Alterations in immunoglobulin levels have been noted in humans after exposure to PAHs, as well as in animal studies after exposure to benzo[a]pyrene. Changes in B cell populations in the spleen provide additional evidence of immunotoxicity. Finally, functional effects on the immune system, including dose-related decreases in SRBC-specific IgM levels and dose-dependent decreases in resistance to pneumonia or Herpes simplex type 2 following short-term subcutaneous (s.c.) injection have been reported (Temple et al., 1993; Munson et al., 1985). The observed decreases in thymus weight, IgM and IgA levels, and number of B cells associated with exposure to benzo[a]pyrene were concluded to be representative of immunotoxicity following benzo[a]pyrene exposure and were selected for dose-response analysis.

2.1.2. Methods of Analysis

No biologically based dose-response models are available for benzo[a]pyrene. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012c), the benchmark dose (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 continuous 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, studies, and assessments. The estimated BMDLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best fit model for each endpoint can be found in Appendix E of the Supplemental Information.

Among the endpoints identified as representative of the hazards of benzo[a]pyrene exposure, the data for neurobehavioral changes in the elevated plus maze and Morris water maze tests (Chen et al., 2012), decreased ovary weight (Xu et al., 2010), increased cervical hyperplasia (Gao et al. (2011), and decreased thymus weight (Kroese et al., 2001) were amenable to dose-response modeling. Although the data for Morris water maze performance (Chen et al., 2012) was ultimately considered to be less informative than the elevated plus maze data (see Section 2.1.1), EPA performed dose-response modeling on this endpoint to ensure that the elevated plus maze data were an accurate representation of other sensitive, behavioral changes in this study. See Appendix E of the Supplemental Information for details of statistical analyses.

The data for the remaining endpoints identified in Section 2.1.1 were not modeled. Specifically, the data for cardiovascular effects observed in Jules et al. (2012) were limited due to the reporting of results at only the two highest dose groups. The data for epididymal sperm counts presented in the Mohamed et al. (2010) study were reported graphically only and requests for the raw data were unsuccessful. The observed decrease in IgM and IgA (De Jong et al., 1999) was inconsistent and not amenable to dose-response modeling. NOAELs or LOAELs were used as the POD for these endpoints.

Human equivalent doses (HEDs) for oral exposures were derived from the PODs estimated from the laboratory animal data as described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011). In this guidance, EPA advocates a hierarchy of approaches for deriving HEDs from data in laboratory animals, with the preferred approach being physiologically-based toxicokinetic modeling. Other approaches can include using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. As discussed in Appendix D of the Supplemental Information, several animal physiologically based pharmacokinetic (PBPK) models for benzo[a]pyrene have been developed and published, but a validated human PBPK model for benzo[a]pyrene for extrapolating doses from animals to humans is not available. In lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., BW$^{3/4}$) approach is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD. BW$^{3/4}$ scaling was not employed for deriving HEDs from studies in which doses were administered directly to early postnatal animals because of the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans

due to presumed toxicokinetic and/or toxicodynamic differences between lifestages (U.S. EPA, 2011; Hattis et al., 2004).

Consistent with EPA guidance (U.S. EPA, 2011), the points of departure (PODs) estimated based on effects in adult animals are converted to HEDs employing a standard dosimetric adjustment factor (DAF) derived as follows:

$$DAF = (BW_a^{1/4} / BW_h^{1/4}),$$

where

BW_a = animal body weight; and

BW_h = human body weight.

Using BW_a of 0.25 kg for rats and 0.035 kg for mice and BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs for rats and mice are 0.24 and 0.15, respectively. Applying this DAF to the POD identified for effects in adult rats or mice yields a POD_{HED} as follows (see Table 2-1):

$$POD_{HED} = \text{Laboratory animal dose (mg/kg-day)} \times DAF.$$

Table 2-1 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each data set discussed above.

Table 2-1. Summary of derivation of PODs

Endpoint and reference	Species/ sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Developmental							
Neurobehavioral changes Chen et al. (2012)	Female Sprague-Dawley rats	Exponential (M4)[a]	1 SD	0.18	0.09	0.09	0.09
Cardiovascular effects Jules et al. (2012)	Long-Evans rats	LOAEL (0.6 mg/kg-d) (15% ↑ in systolic blood pressure; 33% ↑ in diastolic blood pressure)				0.6	0.15
Reproductive							
Decreased ovary weight Xu et al. (2010)	Female Sprague-Dawley rats	Linear[a]	1 SD	2.3	1.5	1.5	0.37
Decreased intratesticular testosterone Zheng et al. (2010)	Male Sprague-Dawley rats	NOAEL (1 mg/kg-d) (15% ↓ in testosterone)				1	0.24

Endpoint and reference	Species/ sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Decreased sperm count and motility Mohamed et al. (2010)	Male C57BL/6 mice	LOAEL (1 mg/kg-d) (50% ↓ in sperm count; 20% ↓ in sperm motility)				1	0.15
Cervical epithelial hyperplasia Gao et al. (2011)	Female ICR mice	Log-logistic[a]	10%	0.58	0.37	0.37	0.06
Immunological							
Decreased thymus weight Kroese et al. (2001)	Female Wistar rats	Linear[a]	1SD	10.5	7.6	7.6	1.9
Decreased IgM levels De Jong et al. (1999)	Male Wistar rats	NOAEL (10 mg/kg-d) (14% ↓ in IgM)				7.1	1.7
Decreased IgA levels De Jong et al. (1999)	Male Wistar rats	NOAEL (30 mg/kg-d) (28% ↓ in IgA)				21	5.2
Decreased number of B cells De Jong et al. (1999)	Male Wistar rats	NOAEL (30 mg/kg-d) (7% ↑ in B cells at NOAEL; 31% ↓ at LOAEL)				21	5.2

[a]For modeling details, see Appendix E in Supplemental Information.
[b]For studies in which animals were not dosed daily, administered doses were adjusted to calculate the TWA daily doses prior to BMD modeling.
[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011) for effects from dosing studies in adult animals (i.e., Gao et al., 2011; Mohamed et al., 2010; Xu et al., 2010; De Jong et al., 1999) or for developmental effects resulting from in utero exposures. $BW^{3/4}$ scaling was not employed for deriving HEDs from studies in which doses were administered directly to early postnatal animals (i.e., Chen et al., 2012) because of the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans due to presumed toxicokinetic and/or toxicodynamic differences between lifestages (U.S. EPA, 2011; Hattis et al., 2004).

2.1.3. Derivation of Candidate Values

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered. An explanation of the five possible areas of uncertainty and variability follows:

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for variability and uncertainty in toxicokinetic and toxicodynamic susceptibility within the subgroup of the human population most sensitive to the health hazards of benzo[a]pyrene (U.S. EPA, 2002). In the case of benzo[a]pyrene, the PODs were derived from studies in inbred animal strains and are not considered sufficiently representative of the exposure and dose-response of the most susceptible human subpopulations (in this case, the developing fetus). In certain cases, the toxicokinetic component of this factor may be replaced when a PBPK model is available that incorporates the

best available information on variability in toxicokinetic disposition in the human population (including sensitive subgroups). In the case of benzo[a]pyrene, insufficient information is available to quantitatively estimate variability in human susceptibility; therefore, the full value for the intraspecies UF was retained.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied, to all PODs in Table 2-2 except Chen et al. (2012), because $BW^{3/4}$ scaling is being used to extrapolate oral doses from laboratory animals to humans. Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of a UF of 3. $BW^{3/4}$ scaling was not employed for deriving HEDs from studies in which doses were administered directly to early postnatal animals (i.e. Chen et al., 2012) because of the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans due to presumed toxicokinetic and/or toxicodynamic differences between lifestages (U.S. EPA, 2011; Hattis et al., 2004). In this case, a value of 10 was applied because of the absence of quantitative information to characterize either the toxicokinetic or toxicodynamic differences between animals and humans at this lifestage. A subchronic to chronic uncertainty factor, UF_S, of 1 was applied when dosing occurred during gestation (Jules et al., 2012) or the early postnatal period (Chen et al., 2012) that is relevant to developmental effects. The developmental period is recognized as a susceptible lifestage and repeated exposure is not necessary for the manifestation of developmental toxicity (U.S. EPA, 1991c). A value of 10 was applied when the POD was based on a subchronic study (studies in Table 2-2, other than the two developmental toxicity studies, were 42–90 days in duration) to account for the possibility that longer exposure may induce effects at a lower dose.

A UF for extrapolation from a LOAEL to NOAEL, UF_L, of 1 was applied when the POD was based on a NOAEL (Zheng et al., 2010; De Jong et al., 1999). A value of 1 was applied for LOAEL-to-NOAEL extrapolation when a BMR of a 1 SD (Chen et al., 2012; Kroese et al., 2001) or 10% change (Gao et al., 2011) from the control was selected under an assumption that it represents a minimal biologically significant response level. A NOAEL was not determined for the most sensitive effects observed in Jules et al. (2012) and Mohamed et al. (2010). At the LOAEL, Jules et al. (2012) observed statistically significant increases in systolic (15%) and diastolic (33%) blood pressure when measured in adulthood following gestational exposure. Regarding the study by Mohamed et al. (2010), the authors observed a statistically significant decrease sperm count (50%) and motility (20%) in treated F0 males at the LOAEL and the observed decrements in sperm count persisted in untreated F1 male offspring. The data reported in these studies were not amenable to dose-response modeling, which would have allowed for extrapolation to a minimally biologically significant response level. Therefore, a full UF of 10 was applied to approximate a NOAEL for these studies, which observed a high magnitude of response at the LOAEL. A database uncertainty factor, UF_D, of 3 was applied to account for database deficiencies, including the lack of a standard

multigenerational study or extended 1-generation study that includes exposure from premating through lactation, considering that benzo[a]pyrene has been shown to affect fertility in adult male and female animals by multiple routes of exposure (see Section 1.1.2). Considering that decreased fertility in adult male and female mice is observed following gestational exposure, it is assumed that exposure occurring over this more comprehensive period of development could result in a lower POD. Also, the lack of a study examining functional neurological endpoints following a more comprehensive period of developmental exposure (i.e., gestation through lactation) is a data gap, considering human and animal evidence indicating altered neurological development (see Section 1.1.1).

Table 2-2 is a continuation of Table 2-1 and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for benzo[a]pyrene.

Table 2-2. Effects and corresponding derivation of candidate values

Endpoint and reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Developmental									
Neurobehavioral changes in rats Chen et al. (2012)	0.09	$BMDL_{1SD}$	10	10	1	1	3	300	3×10^{-4}
Cardiovascular effects in rats Jules et al. (2012)	0.15	LOAEL	3	10	10	1	3	1,000	2×10^{-4}
Reproductive									
Decreased ovary weight in rats Xu et al. (2010)	0.37	$BMDL_{1SD}$	3	10	1	10	3	1,000	4×10^{-4}
Decreased intratesticular testosterone in rats Zheng et al. (2010)	0.24	NOAEL	3	10	1	10	3	1,000	2×10^{-4}
Decreased sperm count and motility in mice Mohamed et al. (2010)	0.15	LOAEL	3	10	10	10	3	10,000	Not calculated due to UF >3,000[a]
Cervical epithelial hyperplasia in mice Gao et al. (2011)	0.06	$BMDL_{10}$	3	10	1	10	3	1,000	6×10^{-5}

Endpoint and reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Immunological									
Decreased thymus weight in rats Kroese et al. (2001)	1.9	$BMDL_{1SD}$	3	10	1	10	3	1,000	2×10^{-3}
Decreased serum IgM in rats De Jong et al. (1999)	1.7	NOAEL	3	10	1	10	3	1,000	2×10^{-3}
Decreased serum IgA in rats De Jong et al. (1999)	5.2	NOAEL	3	10	1	10	3	1,000	5×10^{-3}
Decreased number of B cells in rats De Jong et al. (1999)	5.2	NOAEL	3	10	1	10	3	1,000	5×10^{-3}

[a]As recommended in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation should be avoided.

Figure 2-1 presents graphically the candidate values, UFs, and PODs, with each bar corresponding to one data set described in Tables 2-1 and 2-2.

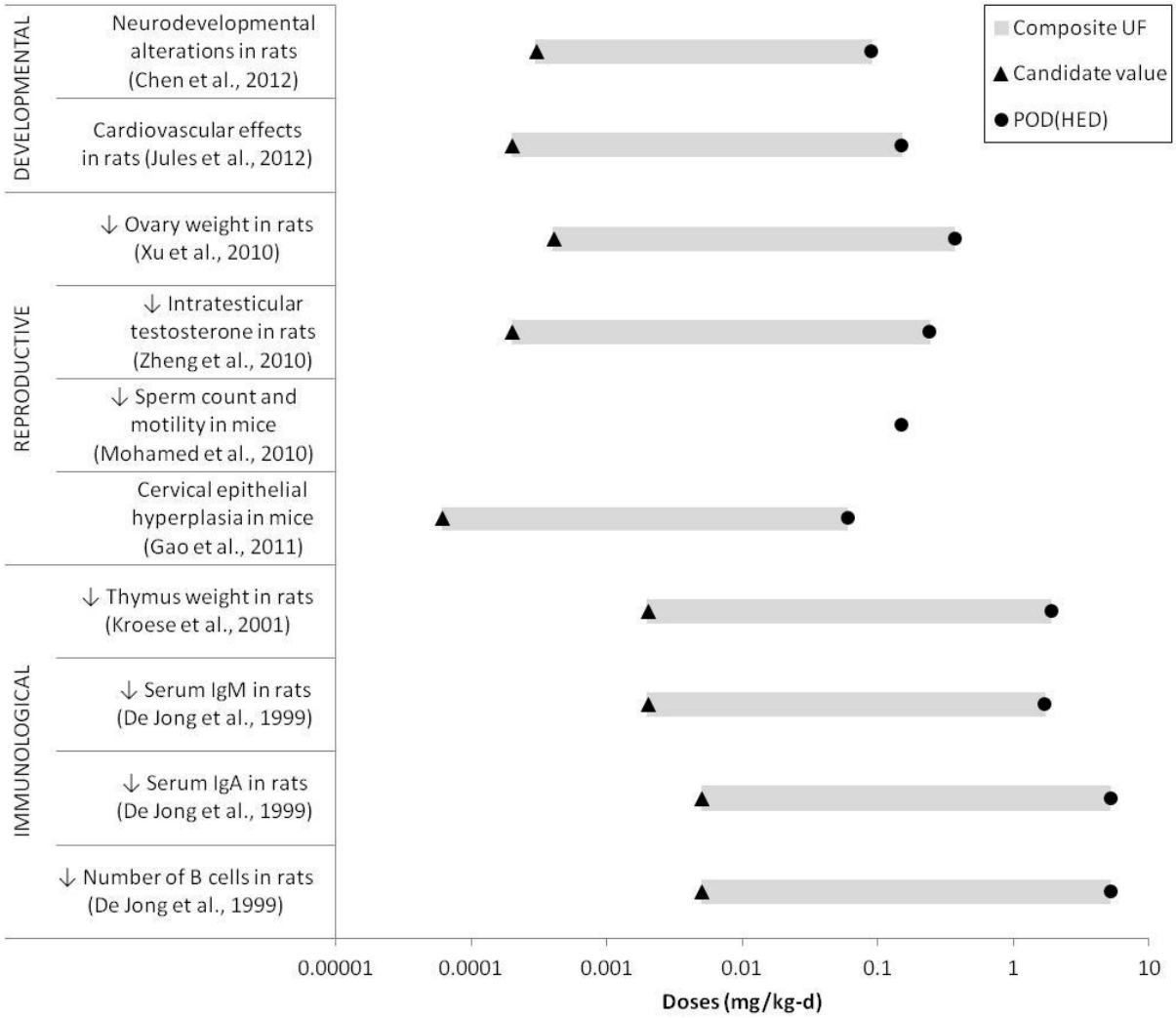

Figure 2-1. Candidate values with corresponding PODs and composite UFs.

2.1.4. Derivation of Organ/System-Specific Reference Doses

Table 2-3 distills the candidate values from Table 2-2 into a single value for each organ or system. These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Table 2-3. Organ/system-specific RfDs and proposed overall RfD for benzo[a]pyrene

Effect	Basis	RfD (mg/kg-d)	Study exposure description	Confidence
Developmental	Neurobehavioral changes	3×10^{-4}	Critical window of development (postnatal)	Medium
Reproductive	Decreased ovary weight	4×10^{-4}	Subchronic	Medium
Immunological	Decreased thymus weight and serum IgM	2×10^{-3}	Subchronic	Low
Proposed overall RfD	**Developmental toxicity**	**3×10^{-4}**	Critical window of development (postnatal)	**Medium**

Developmental Toxicity

The candidate value based on neurobehavioral changes in rats (Chen et al., 2012) was selected as the organ/system-specific RfD representing developmental toxicity. This candidate value was selected because it is associated with the application of the smaller composite UF and because similar effects were replicated across other studies (Maciel et al., 2014; Bouayed et al., 2009a; Bouayed et al., 2009b; Grova et al., 2008).

Reproductive Toxicity

Among the adverse reproductive effects associated with oral benzo[a]pyrene exposure, decrements in sperm parameters, decreases in testosterone, and effects in the ovary were supported by a large body of evidence. The data supporting cervical effects are limited to a single study, and were therefore given less weight compared to the other reproductive effects. The derivation of a candidate value based on decreased sperm count and motility (Mohamed et al., 2010) involved too much uncertainty (see Table 2-2) and the study used to derive a candidate value based on decreased testosterone (Zheng et al., 2010) did not observe a dose-response relationship (a 15% decrease in testosterone was seen at the low and high doses, with statistical significance at the high dose). The study by Xu et al. (2010) observed a dose-response relationship for decreased ovary weight (both doses were statistically significant). Additionally, statistically significant decreases in primordial follicles were observed at the high dose, supporting the ovaries as a target of toxicity. Therefore, the candidate value based on decreased ovary weight in rats from the Xu et al. (2010) study was selected as the organ/system-specific RfD representing reproductive toxicity. The ovarian effects are supported by a large database of animal studies and human studies of exposure to benzo[a]pyrene and PAH mixtures. While evidence in the benzo[a]pyrene database

supports a male and female reproductive hazard, there is more confidence in the POD from Xu et al. (2010) as the basis for an organ/system-specific RfD for reproductive effects.

Immunotoxicity

The candidate values based on decreased thymus weight (Kroese et al., 2001) and serum IgM levels in rats (De Jong et al., 1999) were selected as the organ/system-specific RfD representing immunotoxicity. The observed decreases in thymus weight, IgM and IgA levels, and number of B cells associated with exposure to benzo[a]pyrene were determined to be representative of immunotoxicity. In combination, these effects provide more robust evidence of immunotoxicity. The candidate values for decreased thymus weight (Kroese et al., 2001) and serum IgM levels in rats (De Jong et al., 1999) were equal and provided the most sensitive POD; thus, these candidate values were selected as the organ/system-specific RfD representing immunotoxicity.

2.1.5. Selection of the Proposed Overall Reference Dose

Multiple organ/system-specific reference doses were derived for effects identified as human hazards or potential hazards from benzo[a]pyrene including developmental toxicity, reproductive toxicity (representative of effects in both sexes), and immunotoxicity. To estimate an exposure level below which effects from benzo[a]pyrene exposure are not expected to occur, the lowest organ/system-specific RfD (3×10^{-4} mg/kg-day) is proposed as the overall RfD for benzo[a]pyrene. This value, based on induction of neurobehavioral changes in rats exposed to benzo[a]pyrene during a susceptible lifestage, is supported by several animal and human studies (see Section 1.1.1).

The overall RfD is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002). This value should be applied in general population risk assessments. However, decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. For example, fluctuations in exposure levels that result in elevated exposures during various lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. Alternatively, developmental toxicity may not be a concern due to exposure scenarios in which exposure is occurring outside of the critical window of development.

2.1.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

Confidence in the principal study (Chen et al., 2012) is medium-to-high. The study design included randomized experimental testing, blinded observations, culling of pups to account for

nutritional availability, treatment-randomization, and controls for litter and nursing bias. Some informative experimental details were, however, omitted including the sensitivity of some assays at the indicated developmental ages, gender-specific data for all outcomes, and individual animal data. Notably, these study limitations do not apply to the endpoint chosen to derive the RfD, and the overall methods and reporting are considered sufficient. Confidence in the database is medium, primarily due to the lack of a multigenerational reproductive toxicity study given the sensitivity to benzo[a]pyrene during development. Reflecting medium-to-high confidence in the principal study and medium confidence in the database, confidence in the RfD is medium.

2.1.7. Previous IRIS Assessment: Reference Dose

An RfD was not derived in the previous IRIS assessment.

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The inhalation reference concentration (RfC) (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95 percent lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

2.2.1. Identification of Studies and Effects for Dose-Response Analysis

In Section 1.2.1, developmental and reproductive toxicities were identified as hazards of benzo[a]pyrene exposure by the inhalation route. Studies within each effect category were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationales for selecting the studies and effects to represent each of these hazards are summarized below.

Human studies of environmental PAH mixtures across multiple cohorts have observed developmental and reproductive effects following prenatal exposure. However, these studies are limited by exposure to complex mixtures of PAHs; and, within individual studies, there may have been more than one route of exposure. In addition, the available human studies that utilized benzo[a]pyrene-DNA adducts as the exposure metric do not provide external exposure levels of benzo[a]pyrene from which to derive an RfC. Although preferred for derivation of reference values, human studies were not considered because of the contribution to the observed hazard of multiple PAHs across multiple routes of exposure and uncertainty due to concurrent exposure to other PAHs and other components of the mixtures (such as metals).

Animal studies were evaluated to determine which provided the most relevant routes and durations of exposure, multiple exposure levels to provide information about the shape of the dose response curve, and relative ability to detect effects at low exposure levels. The only chronic animal

inhalation study available for benzo[a]pyrene, Thyssen et al. (1981), was designed as a cancer bioassay and did not report other effects; however, the inhalation database for benzo[a]pyrene includes several shorter duration studies that are sufficient for use in deriving reference values (U.S. EPA, 2002). Specifically, several reproductive toxicity studies are available for the inhalation route, including one subchronic study Archibong et al. (2008). Furthermore, several developmental studies are available that help identify hazards of exposure during sensitive developmental windows (Wormley et al., 2004; Archibong et al., 2002). In addition, a 4-week inhalation study in rats is available that investigated, but did not detect, lung injury (Wolff et al., 1989). The inhalation database for benzo[a]pyrene is less extensive than the database of studies by the oral route; however, the types of noncancer effects observed are consistent between routes and are supported by studies in human populations (see Sections 1.1.1, 1.1.2, and 1.1.3).

Developmental Toxicity

Developmental toxicity, as represented by decreased fetal survival and developmental neurotoxicity, was observed in several inhalation studies (Wormley et al., 2004; Wu et al., 2003a; Archibong et al., 2002). Wu et al. (2003a) was not considered for dose-response analysis due to lack of study details related to number of dams and litters per group and lack of reporting of numerical data. Wormley et al. (2004) was not considered for dose-response analysis, as this study employed only a single exposure group at which overt toxicity was noted (a 66% reduction in fetal survival).

Of the studies demonstrating developmental toxicity, the study conducted by Archibong et al. (2002) was identified as the most informative study for dose-response analysis. Archibong et al. (2002) observed decreased fetal survival at the lowest dose tested by the inhalation route on GDs 11–20 (i.e., LOAEL of 25 μg/m^3). This study indicates that the developing fetus is a sensitive target following inhalation exposure to benzo[a]pyrene. The observed decrease in fetal survival is supported by the oral database for benzo[a]pyrene (e.g., decreased survival of litters in mice following in utero exposure to benzo[a]pyrene on GDs 7–16) (Mackenzie and Angevine, 1981).

Reproductive Toxicity

Reproductive toxicity, as represented by reductions in sperm quality, both count and motility, and testis weights in adults, was observed by Archibong et al. (2008), Ramesh et al. (2008) and Archibong et al. (2002). Archibong et al. (2008) and Ramesh et al. (2008) reported the results of a single exposure, subchronic inhalation exposure study in male rats. This subchronic study was of sufficient duration and possessed adequate power to detect effects, but utilized a single exposure concentration, which is less informative for dose-response analysis than a design using multiple exposure concentrations. However, this single-dose subchronic study is consistent with male reproductive effects observed across multiple studies by the oral route and with human studies in PAH exposed populations (see Section 1.1.2). The endpoints of decreased testes weight and sperm count and motility reported in Archibong et al. (2008) were selected for dose-response analysis as

both represent sensitive endpoints of male reproductive toxicity and are indicators of potentially decreased fertility. These effects are also consistent with human studies in PAH exposed populations as effects on male fertility and semen quality have been demonstrated in epidemiological studies of smokers (Soares and Melo, 2008).

2.2.2. Methods of Analysis

Data for decreased fetal survival from Archibong et al. (2002) were reported as litter means and SDs. These data were not amenable to BMD modeling due to the pattern of variability in the data set, and attempts to obtain the raw data from the study authors were unsuccessful. Therefore, the LOAEL from this study was used as the POD for dose-response analysis. The study by Archibong et al. (2008), using only one exposure level, was judged not to support dose-response modeling due to the lack of understanding of the underlying dose-response relationship. LOAELs were also used as the PODs for dose-response analysis.

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 LOAELs identified from Archibong et al. (2002) and Archibong et al. (2008) were adjusted to account for the discontinuous daily exposure as follows:

$$\begin{aligned} POD_{ADJ} &= POD \times \text{hours exposed per day/24 hours} \\ &= LOAEL \times (\text{duration of exposure/24 hours}) \\ &= POD_{ADJ} \end{aligned}$$

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

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

where:

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

The total fractional deposition includes particle deposition in the nasal-pharyngeal, tracheobronchial, and pulmonary regions. F_{TOT} for both animals and humans was calculated using

the Multi-Path Particle Dosimetry (MPPD) model, a computational model used for estimating human and rat airway particle deposition and clearance (MPPD; Version 2.0 © 2006, publicly available through the Hamner Institute). F_{TOT} was based on the average particle size of 1.7 ± 0.085 µm (mass median aerodynamic diameter [MMAD] ± geometric SD) as reported in Wu et al. (2003a) for the exposure range 25–100 μm^3. 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 E for MPPD model output). Both models were run under nasal breathing scenarios with the inhalability adjustment selected. A geometric SD of 1 was used as the default by the model because the reported geometric SD of 0.085 was ≤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 body weight, 70 kg; V_E, 13.8 L/minute; breathing frequency, 16 per minute; tidal volume, 860 mL; functional residual capacity, 3,300 mL; and upper respiratory tract volume, 50 mL. Although the most sensitive population in Archibong et al. (2002) is the developing fetus, the adult rat dams were directly exposed. Thus, adult rat parameters were used in the calculation of the HEC. The parameters used for the rat were body weight, 0.25 kg (a generic weight for male and female rats); V_E, 0.18 L/minute; breathing frequency, 102 per minute; tidal volume, 1.8 mL; functional residual capacity, 4 mL; and upper respiratory tract volume, 4.42 mL. All other parameters were set to default values (see Appendix E).

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 1.1.

From this, the POD_{HEC} was calculated as follows:

$$POD_{HEC} = POD_{ADJ} \times RDDR_{ER}$$

Table 2-4 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each data set discussed above.

Table 2-4. Summary of derivation of PODs

Endpoint and reference	Species/sex	Model	BMR	BMC ($\mu g/m^3$)	BMCL ($\mu g/m^3$)	POD_{ADJ}[a] ($\mu g/m^3$)	POD_{HEC}[b] ($\mu g/m^3$)
Developmental							
Decreased fetal survival Archibong et al. (2002)	Pregnant F344 rats	LOAEL (25 $\mu g/m^3$) 19% ↓				4.2	4.6
Reproductive							
Decreased testis weight Archibong et al. (2008)	Male F344 rats	LOAEL (75 $\mu g/m^3$) 34% ↓				12.5	13.8
Decreased sperm count and motility Archibong et al. (2008)	Male F344 rats	LOAEL (75 $\mu g/m^3$) 69% ↓sperm count 73% ↓ sperm motility 54% ↑ abnormal sperm				12.5	13.8

[a]PODs were adjusted for continuous daily exposure: POD_{ADJ}= POD × hours exposed per day/24 hours.
[b]POD_{HEC} calculated by adjusting the POD_{ADJ} by the RDDR calculated using particle size reported in Hood et al. (2000) using MPPD software as detailed in Section 2.2.2 and Appendix E in the Supplemental Information.

2.2.3. Derivation of Candidate Values

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered. An explanation of the five possible areas of uncertainty and variability follows:

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for variability and uncertainty in toxicokinetic and toxicodynamic susceptibility within the subgroup of the human population most sensitive to the health hazards of benzo[a]pyrene (U.S. EPA, 2002). In the case of benzo[a]pyrene, the PODs were derived from studies in inbred animal strains and are not considered sufficiently representative of the exposure and dose-response of the most susceptible human subpopulations (in this case, the developing fetus). In certain cases, the toxicokinetic component of this factor may be replaced when a PBPK model is available that incorporates the best available information on variability in toxicokinetic disposition in the human population (including sensitive subgroups). In the case of benzo[a]pyrene, insufficient information is available to quantitatively estimate variability in human susceptibility; therefore, the full value for the intraspecies UF was retained.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for residual uncertainty in the extrapolation from laboratory animals to humans in the absence of information to characterize toxicodynamic differences between rats and humans after inhalation exposure to benzo[a]pyrene. This value is adopted by convention where an adjustment from animal to a HEC has been performed as described in EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

A subchronic to chronic uncertainty factor, UF_S, of 1 was applied when dosing occurred during gestation (Archibong et al., 2002) or the early postnatal period that is relevant to developmental effects (U.S. EPA, 1991a). A value of 10 was applied when the POD is based on a subchronic study to account for the possibility that longer exposure may induce effects at a lower dose (Archibong et al., 2008) was 60 days in duration). A UF for extrapolation from a LOAEL to a NOAEL, UF_L, of 10 was applied when a LOAEL was used as the POD (Archibong et al., 2008; Archibong et al., 2002). The data reported in these studies were not amenable to dose-response modeling, which would have allowed for extrapolation to a minimally biologically significant response level. At the LOAEL, these studies observed a high magnitude of response (see Table 2-4). Therefore, a full UF of 10 was applied to approximate a NOAEL for studies that observed a high magnitude of response at the LOAEL. For example, the LOAEL used as the POD for the developmental effect observed in Archibong et al. (2002) was based on a 19% decrease in fetal survival.

A database uncertainty factor, UF_D, of 10 was applied to account for database deficiencies, including the lack of a standard multigenerational study or extended 1-generation study that includes exposure from premating through lactation, considering that benzo[a]pyrene has been shown to affect fertility in adult male and female animals by multiple routes of exposure and that decrements in fertility are greater following developmental exposure (see Section 1.1.2).

In addition, the lack of a study examining functional neurological endpoints following inhalation exposure during development is also a data gap, considering human and animal evidence indicating altered neurological development following exposure to benzo[a]pyrene alone or through PAH mixtures (see Section 1.1.1).

The most sensitive POD for the RfC candidate values in Table 2-5 is based on the endpoint of decreased fetal survival observed in Archibong et al. (2002). However, oral exposure studies have demonstrated neurotoxicity at doses lower than those where decreased fetal survival was observed. A statistically significant decrease in fetal survival was observed following treatment with 160 mg/kg-day benzo[a]pyrene, but not at lower doses (Mackenzie and Angevine, 1981); however, other oral studies observed statistically significant neurobehavioral effects at doses of benzo[a]pyrene around 0.2–2 mg/kg-day (Chen et al., 2012; Bouayed et al., 2009a). Considering the relative sensitivity of the systemic health effects observed in the oral database, it is likely that neurodevelopmental toxicity would be expected to occur at exposure concentrations below the POD for the RfC based on decreased fetal survival.

According to EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), the UF_D is intended to account for the potential for deriving an under-protective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity, but also including a review of existing data that may also suggest that a lower reference value might result if additional data were available. Therefore, a database UF of 10 for the benzo[a]pyrene

inhalation database was applied to account for the lack of a multigenerational study and the lack of a developmental neurotoxicity study.

Table 2-5 is a continuation of Table 2-4 and summarizes the application of UFs to each POD to derive a candidate values for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of an RfC for a specific hazard and subsequent overall RfC for benzo[a]pyrene.

Table 2-5. Effects and corresponding derivation of candidate values

Endpoint	POD_{HEC} ($\mu g/m^3$)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF[b]	Candidate value[a] (mg/m^3)
Developmental									
Decreased fetal survival in rats Archibong et al. (2002)	4.6	LOAEL	3	10	10	1	10	3,000	2×10^{-6}
Reproductive									
Decreased testis weight in rats Archibong et al. (2008)	13.8	LOAEL	3	10	10	10	10	30,000	Not calculated due to UF >3,000
Decreased sperm count and motility in rats Archibong et al. (2008)	13.8	LOAEL	3	10	10	10	10	30,000	Not calculated due to UF >3,000

[a]Candidate values were converted from $\mu g/m^3$ to mg/m^3.
[b]As recommended in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation should be avoided.

Figure 2-2 presents graphically these candidate values UFs, and PODs, with each bar corresponding to one data set described in Tables 2-4 and 2-5.

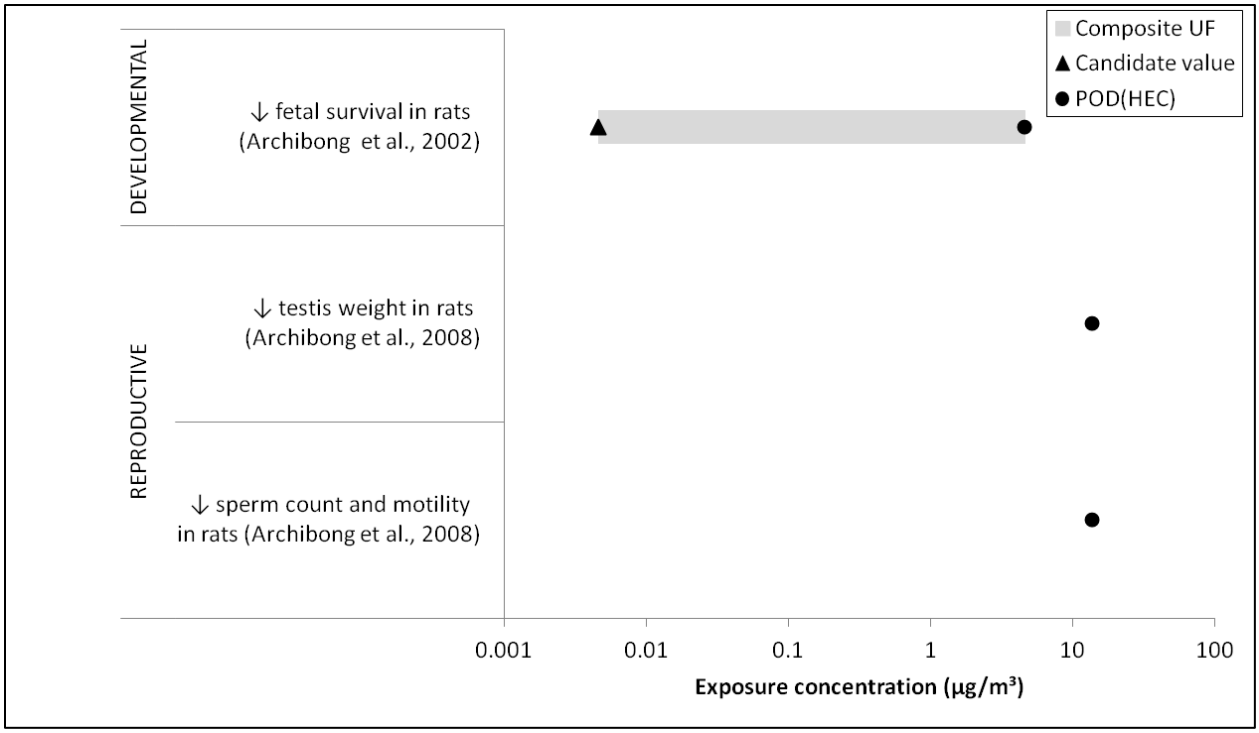

Figure 2-2. Candidate values with corresponding PODs and composite UFs.

2.2.4. Derivation of Organ/System-Specific Reference Concentrations

Table 2-6 distills the candidate values from Table 2-5 into a single value for each organ or system. These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. The candidate values for reproductive toxicity from Archibong et al. (2008) were not derived to represent reproductive toxicity because as recommended in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation should be avoided.

Table 2-6. Organ/system-specific RfCs and proposed overall RfC for benzo[a]pyrene

Effect	Basis	RfC (mg/m^3)	Study exposure description	Confidence
Developmental	Decreased fetal survival	2×10^{-6}	Critical window of development (prenatal)	Low-medium
Reproductive	Reductions in testes weight and sperm parameters	Not calculated	Subchronic	NA
Proposed Overall RfC	**Decreased fetal survival**	**2×10^{-6}**	Critical window of development (prenatal)	**Low-medium**

2.2.5. Selection of the Proposed Reference Concentration

The derivation of multiple organ/system-specific reference concentrations were considered for effects identified as human hazards of benzo[a]pyrene inhalation exposure, i.e., developmental and reproductive toxicity. However, an organ/system-specific RfC to represent reproductive toxicity could not be derived due to high uncertainty (i.e., a composite UF of >3,000).

An overall RfC of $\mathbf{2 \times 10^{-6}\ mg/m^3}$ was selected based on the hazard of developmental toxicity. The study by Archibong et al. (2002) was selected as the study used for the derivation of the proposed overall RfC, 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 and the observed decreased fetal survival/litter is the most sensitive noncancer effect observed following inhalation exposure to benzo[a]pyrene. Additional support for this endpoint of decreased fetal survival is provided by a developmental/reproductive study conducted via the oral route (Mackenzie and Angevine, 1981).

This overall RfC is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole, including potentially susceptible subgroups (U.S. EPA, 2002). This value should be applied in general population risk assessments. However, decisions concerning averaging exposures over time for comparison with the RfC should consider the types of toxicological effects and specific lifestages of concern. For example, fluctuations in exposure levels that result in elevated exposures during these lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfC. Alternatively, developmental toxicity may not be a concern due to exposure scenarios in which exposure is occurring outside of the critical window of development.

2.2.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

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 study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study, lack of studies on developmental neurotoxicity and immune endpoints, and lack of information regarding subchronic and chronic inhalation exposure. However, confidence in the RfC is bolstered by consistent systemic effects observed by the oral route (including reproductive and developmental effects) and similar effects observed in human populations exposed to PAH mixtures. Reflecting medium confidence in the principal study and low confidence in the database, confidence in the RfC is low-to-medium.

2.2.7. Previous IRIS Assessment: Reference Concentration

An RfC was not derived in the previous IRIS assessment.

2.2.8. Uncertainties in the Derivation of the RfD and RfC

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, 2000a, 1994) was applied to a POD based on neurobehavioral changes in rats treated developmentally. To derive the RfC, this same approach was applied to a POD from a developmental study for the effect of decreased fetal survival. UFs 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 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 within individual studies, 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 lifetime 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 including that the principal study for the RfD exposed animals during a relatively short period of brain development potentially underestimating the magnitude of resulting neurological effects. Also, the database lacks a comprehensive multi-generation reproductive/developmental toxicity studies and immune system endpoints were not evaluated in the available chronic-duration or developmental studies. Additionally, the only available chronic studies of oral or inhalational exposure to benzo[a]pyrene focused primarily on neoplastic effects leaving non-neoplastic effects mostly uncharacterized.

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). In addition, 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 stimuli 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.

The study selected as the basis of the RfC provided limited information regarding the inhalation exposures of the animals. Specifically, it is not clear whether the reported concentrations were target values or analytical concentrations and the method used to quantify benzo[a]pyrene in the generated aerosols was not provided. Requests to obtain additional study details from the authors were unsuccessful; therefore, the assumption was made that the reported concentrations were analytical concentrations.

One area of uncertainty in the database 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 ovotoxicity) (Borman et al., 2000), although the underlying mechanistic basis of this apparent difference is not understood. Most importantly, it is unknown which animal species may be more comparable to humans.

2.3. ORAL SLOPE FACTOR FOR CANCER

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. 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.

2.3.1. Analysis of Carcinogenicity Data

The database for benzo[a]pyrene contains numerous cancer bioassays that identify tumors, primarily of the alimentary tract including the forestomach, following oral exposure in rodents. Three 2-year oral bioassays are available that associate lifetime benzo[a]pyrene exposure with carcinogenicity at multiple sites: forestomach, liver, oral cavity, jejunum, kidney, auditory canal (Zymbal 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, esophageal, tongue, and larynx tumors in female B6C3F$_1$ mice (Beland and Culp, 1998; Culp et al., 1998).

In addition to these 2-year cancer bioassays, there are studies available that provide supporting evidence of carcinogenicity but are less suitable for dose-response analysis due to one or more limitations in study design: (1) no vehicle control group; (2) only one benzo[a]pyrene dose group; or (3) a 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; Berenblum and Haran, 1955). Of the controlled, multiple dose-group, repeat-dosing studies that remain, most treated animals for <1 year, which is less optimal for extrapolating to a lifetime exposure (Weyand et al., 1995; Triolo et al., 1977; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967).

Brune et al. (1981) dosed rats (32/sex/group) with benzo[a]pyrene in the diet or by gavage in a 1.5% caffeine solution, sometimes as infrequently as once every 9th day, for approximately 2 years and observed increased forestomach tumors. This study was not selected for quantitation due to the nonstandard treatment protocol in comparison to the Good Laboratory Practice (GLP) studies conducted by Kroese et al. (2001) and Beland and Culp (1998) and the limited reporting of study methods.

The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. The rat bioassay by Kroese et al. (2001) and the mouse bioassay by Beland and Culp (1998) were conducted in accordance with GLP as established by the Organisation for Economic Co-operation and Development (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 up to 2 years, and included detailed reporting of methods and results (including individual animal data).

Details of the rat (Kroese et al., 2001) and female mouse (Beland and Culp, 1998) study designs are provided in Appendix D of the Supplemental Information. Dose-related increasing trends in tumors were noted at the following sites:

- Squamous cell carcinomas (SCCs) or papillomas of the forestomach or oral cavity in male and female rats;
- SCCs 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/duodenum adenocarcinomas in female and male rats;
- Hepatocellular adenomas or carcinomas in male and female rats; and

- SCCs 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 Appendix D of the Supplemental Information. As recommended by the National Toxicology Program (NTP) (McConnell et al., 1986) and as outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 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. 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 that 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 analysis, for several reasons. First, the control and lower dose groups were not thoroughly examined, similar to the situation described above for oral cavity tumors. Unlike the oral cavity tumors, the auditory canal tumors were not clearly related to any other site or tumor type, as they were described as a mixture of squamous and sebaceous cells derived from pilosebaceous units. The tumors were observed mainly in the high-dose groups and were highly coincident with the oral cavity and forestomach tumors. Because the only mid-dose male with an auditory canal tumor did not also have a forestomach or oral cavity squamous cell tumor, and no auditory canal tumors were observed in low-dose male or female rats, the data are insufficient to conclude that the auditory canal tumors occur independently of other tumors. The investigators did not suggest that these tumors were metastases from other sites (in which case, the auditory canal tumors would be repetitions of other tumors, or statistically dependent). Therefore dose-response analysis was not pursued for this site, either separately or in combination with another tumor type.

The incidence data that were modeled are provided in Tables E-9, E-10, and E-11 (Kroese et al., 2001; Beland and Culp, 1998).

2.3.2. Dose-Response Analysis—Adjustments and Extrapolation Methods

EPA's *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 carcinogenicity involves a mutagenic mode of action (as discussed in Section 1.1.5). 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. In this case, EPA has used the multistage-Weibull model, which incorporates the time at which death-with-tumor occurred as well as the dose.

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 EPA's *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 HEDs 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)$^{1/4}$ (U.S. EPA, 1992), where the animal body weights were TWAs from each group, 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.

Details of the modeling and the model selection process can be found in Appendix E of the Supplemental Information. PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk.

2.3.3. Derivation of the Oral Slope Factor

The PODs estimated for each tumor site are summarized in Table 2-7. 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 represents a plausible upper bound on the true risk. 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 2-7.

Table 2-7. Summary of the oral slope factor derivations

Tumor	Species/ sex	Selected model	BMR	BMD (mg/kg-d)	POD = BMDL (mg/kg-d)	Slope factor[a] $(mg/kg\text{-}d)^{-1}$	
Forestomach, oral cavity: squamous cell tumors Kroese et al. (2001)	Male Wistar rats	Multistage Weibull	10%	0.453	0.281	0.4	0.5[b]
Hepatocellular adenomas or carcinomas Kroese et al. (2001)	Male Wistar rats	Multistage Weibull	10%	0.651	0.449	0.2	
Jejunum/duodenum adenocarcinomas Kroese et al. (2001)	Male Wistar rats	Multistage Weibull	10%	3.03	2.38	0.04	
Kidney: urothelial carcinomas Kroese et al. (2001)	Male Wistar rats	Multistage Weibull	10%	4.65	2.50	0.04	
Skin, mammary: Basal cell tumors Squamous cell tumors Kroese et al. (2001)	Male Wistar rats	Multistage Weibull	10%	 2.86 2.64	 2.35 1.77	 0.04 0.06	
Forestomach, oral cavity: squamous cell tumors Kroese et al. (2001)	Female Wistar rats	Multistage Weibull	10%	0.539	0.328	0.3	0.3[b]
Hepatocellular adenomas or carcinomas Kroese et al. (2001)	Female Wistar rats	Multistage Weibull	10%	0.575	0.507	0.2	
Jejunum/duodenum adenocarcinomas Kroese et al. (2001)	Female Wistar rats	Multistage Weibull	10%	3.43	1.95	0.05	
Forestomach, esophagus, tongue, larynx (alimentary tract): squamous cell tumors Beland and Culp (1998)	Female $B6C3F_1$ Mice	Multistage Weibull	10%	0.127	0.071	1	1

[a]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix E of the Supplemental Information for details of modeling results.
[b]Slope factor characterizing the 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 2-7). 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 (0.2 per mg/kg-day) were only slightly lower than slope factors based on alimentary tract tumors (0.2–0.3 per mg/kg-day). The oral slope factor for alimentary tract tumors in female mice was highest at 1 per mg/kg-day (Table 2-7), which was approximately twofold higher than the oral slope factor derived from the alimentary tract tumors in male rats.

Although the time-to-tumor modeling helps to account for competing risks associated with decreased survival times and other causes of death including 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. A method, for estimating overall risk, involving the assumption that the variability in the slope factors could be characterized by a normal distribution, is detailed in Appendix E of the Supplemental Information. The resulting composite slope factor for all tumor types for male rats was 0.5 per mg/kg-day, 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 2-7; see Appendix E of Supplemental Information for composite slope factor estimates).

The overall risk estimates from rats and mice spanned about a threefold 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 lifetime oral exposure to benzo[a]pyrene is **1 per mg/kg-day**, based on the alimentary tract tumor response in female $B6C3F_1$ mice. Note that the oral slope factor should only be used with lifetime human exposures <0.1 mg/kg-day, because above this level, the dose-response relationship is not expected to be proportional to benzo[a]pyrene exposure.

2.3.4. Uncertainties in the Derivation of the Oral Slope Factor

The oral slope factor for benzo[a]pyrene was based on the increased incidence of alimentary tract tumors, including forestomach tumors, observed in a lifetime dietary study in mice (Beland and Culp, 1998). EPA has considered the uncertainty associated with the relevance of forestomach tumors for estimating human risk from benzo[a]pyrene exposure. The rodent forestomach serves to store foods and liquids for several hours before contents continue to the stomach for further digestion (Clayson et al., 1990; Grice et al., 1986). Thus, tissue of the forestomach in rodents may be exposed to benzo[a]pyrene for longer durations than analogous human tissues in the oral cavity and esophagus. This suggests that the rodent forestomach may be quantitatively more sensitive to the development of squamous epithelial tumors in the forestomach compared to oral or esophageal tumors in humans.

Uncertainty in the magnitude of the recommended oral slope factor is reflected to some extent in the range of slope factors among tumors sites and species; the oral slope factor based on

the mouse alimentary tract data was about threefold higher than the overall oral slope factor based on male rat data (Table 2-8). These comparisons show that the selection of target organ, animal species, and interspecies 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 1.1.5).

Table 2-8. Summary of uncertainties in the derivation of cancer risk values for benzo[a]pyrene oral slope factor

Consideration and impact on cancer risk value	Decision	Justification and discussion
Selection of target organ ↓ oral slope factor, up to fivefold, if alimentary tract tumors not selected	Alimentary tract tumors (forestomach, esophagus, tongue, larynx)	Tumor site is concordant across rats and mice, increasing support for its relevance to humans. As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result for alimentary tract tumors was used to derive the oral slope factor.
Selection of data set ↓ oral slope factor ~threefold if rat bioassay were selected for oral slope factor derivation	Beland and Culp (1998)	Beland and Culp (1998) was a well-conducted study and had the lowest HEDs of the available cancer bioassays, reducing low-dose extrapolation uncertainty.
Selection of dose metric Alternatives could ↓ or ↑ slope factor	Administered dose	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites have not been identified.
Interspecies extrapolation Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by $BW^{2/3}$])	$BW^{3/4}$ scaling (default approach)	There are no data to support alternatives. Because the dose metric was not an area under the curve, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. While the true human correspondence is unknown, this overall approach is expected to neither over- nor underestimate human equivalent risks.
Dose-response modeling Alternatives could ↓ or ↑ slope factor	Multistage-Weibull model	No biologically based models for benzo[a]pyrene were available. Because the multistage-Weibull model could address additional available data (time of death with tumor, and whether a tumor caused the death of the animal), this model was superior to other available models.
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation	Linear extrapolation from POD (based on mutagenic mode of action)	Available mode-of-action data support linearity (mutagenicity is a primary mode of action of benzo[a]pyrene).

Consideration and impact on cancer risk value	Decision	Justification and discussion
Statistical uncertainty at POD ↓ oral slope factor 1.8-fold if BMD used as the POD rather than BMDL	BMDL (preferred approach for calculating plausible upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of alimentary tract tumors.
Sensitive subpopulations ↑ oral slope factor to unknown extent	ADAFs are recommended for early life exposures	No chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity.

2.3.5. Previous IRIS Assessment: Oral Slope Factor

The previous cancer assessment for benzo[a]pyrene was posted on the IRIS database in 1992. 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 of dietary benzo[a]pyrene administered in the diet for approximately 2 years in 10-week-old Sprague-Dawley rats (Brune et al., 1981) and administered for up 7 months in 2-week-old to 5-month-old CFW-Swiss mice (Neal and Rigdon, 1967). A single slope factor estimate of 11.7 per mg/kg-day, using a linearized multistage procedure applied to the combined incidence of forestomach, esophageal, and laryngeal tumors, was derived from the Brune et al. (1981) study (see Section 1.1.5 for study details). Three modeling procedures were used to derive risk estimates from the Neal and Rigdon (1967) bioassay (see Section 1.1.5). U.S. EPA (1991a) 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. U.S. EPA (1991b) derived a value of 9.0 per mg/kg-day by linear extrapolation from the 10% response point to the background response in a re-analysis of the 1990 model. Finally, using a Weibull-type model to reflect less-than-lifetime exposure to benzo[a]pyrene, the same assessment (U.S. EPA, 1991b) derived an upper-bound slope factor estimate of 4.5 per mg/kg-day. The four slope factor estimates, which reflected extrapolation to humans assuming surface area equivalence ($BW^{2/3}$ scaling) were within threefold of each other and were judged to be of equal merit. Consequently, the geometric mean of these four estimates, 7.3 per mg/kg-day, was recommended, in 1992, as the oral slope factor.

2.4. INHALATION UNIT RISK FOR CANCER

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. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

2.4.1. Analysis of Carcinogenicity Data

The inhalation database demonstrating carcinogenicity of benzo[a]pyrene consists of a lifetime inhalation bioassay in male hamsters (Thyssen et al., 1981) and intratracheal instillation studies, also in hamsters (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). The intratracheal instillation studies provide supporting evidence of carcinogenicity of inhaled benzo[a]pyrene; however, the use of this exposure method 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 bioassay by Thyssen et al. (1981) represents the only lifetime inhalation cancer bioassay available for describing exposure-response relationships for cancer from inhaled benzo[a]pyrene. As summarized in Section 1.1.5, increased incidences of benign and malignant tumors of the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach were seen with increasing exposure concentration. In addition, survival was decreased relative to control in the high-exposure group; mean survival times in the control, low-, and mid-concentration groups were 96.4, 95.2, and 96.4 weeks, respectively, compared to 59.5 weeks in the high-exposure group animals (Thyssen et al., 1981). Overall, tumors occurred earlier in the highest benzo[a]pyrene exposure group than in the mid-exposure group.

Strengths of the study included exposures until natural death, up to 2.5 years, multiple exposure groups; histological examination of multiple organ systems, and availability of individual animal pathology reports with time of death and tumor incidence data by site in the upper respiratory and digestive tracts. In addition, the availability of weekly chamber air monitoring data and individual times on study allowed the calculation of time-weighted average (TWA) lifetime continuous exposures for each hamster. Group averages of these TWA concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3 (U.S. EPA, 1990).

Several limitations concerning exposure conditions in the Thyssen et al. (1981) study were evaluated for their impact on the derivation of an inhalation unit risk for benzo[a]pyrene. These issues include minimal detail about the particle size distribution of the administered aerosols, variability of chamber concentrations, and the use of a sodium chloride aerosol as a carrier.

First, particle distribution analysis of aerosols, in particular the MMAD and geometric SD, was not reported, although the investigators did report that particles were within the respirable range for hamsters, with >99% of the particles having diameters 0.2–0.5 μm and >80% having diameters 0.2–0.3 μm.

Second, weekly averages of chamber concentration measurements varied two- to fivefold from the overall average for each group, which exceeds the limit for exposure variability of <20% for aerosols recommended by OECD (2009). For risk assessment purposes, EPA generally assumes that cancer risk is proportional to cumulative exposure, and therefore to lifetime average exposure as estimated here, when there is no information to the contrary. Under this assumption, the

variability of the chamber concentrations has little impact on the estimated exposure-response relationship. The impacts of alternative assumptions are considered in Section 2.4.4.

Lastly, exposure occurred through the inhalation of benzo[a]pyrene adsorbed onto sodium chloride aerosols, which might have irritant carrier effects, and may have a different deposition than benzo[a]pyrene adsorbed onto carbonaceous particles (as is more typical in the environment). The above study design and reporting issues concerning the particle size composition, exposure variability, and deposition do not negate the robust tumor response following benzo[a]pyrene inhalation exposure. Consequently, EPA concluded that the strengths of the study supported the use of the data to derive an inhalation unit risk for benzo[a]pyrene. See Section 2.4.4 for a discussion of uncertainties in the unit risk.

2.4.2. Dose-Response Analysis—Adjustments and Extrapolation Methods

Biologically based dose-response models for benzo[a]pyrene are not available. A simplified version of the two-stage carcinogenesis model proposed by Moolgavkar and Venzon (1979) and Moolgavkar and Knudson (1981) has been applied to the Thyssen et al. (1981) individual animal data (U.S. EPA, 1990). However, the simplifications necessary to fit the tumor incidence data reduced that model to an empirical model (i.e., there were no biological data to inform estimates of cell proliferation rates for background or initiated cells). Sufficient data were available to apply the multistage-Weibull model, as used for the oral slope factor (described in detail in Appendix E of the Supplemental Information), specifically the individual times of death for each animal. Unlike in the oral bioassays, Thyssen et al. (1981) did not determine cause of death for any of the animals. Since the investigators for the oral bioassays considered some of the same tumor types to be fatal at least some of the time, bounding estimates of the POD for these Thyssen et al. (1981) data were developed by treating the tumors alternately as either all incidental to the death of an affected animal or as causing the death of an affected animal.

The tumor incidence data used for dose-response modeling comprised the benign and malignant tumors in the pharynx and respiratory tract (see Table E-17). The tumors in these sites were judged to be sufficiently similar to combine in overall incidences, based on the assumption that the benign tumors could develop into malignancies, as outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (Section 2.2.2.1.2; U.S. EPA, 2005a). Specifically, while the pharynx and larynx are associated with the upper digestive tract and the upper respiratory tract, respectively, these sites are close anatomically and in some cases where both tissues were affected, the site of origin could not be distinguished (U.S. EPA, 1990). In addition, the benign tumors (e.g., papillomas, polyps, and papillary polyps) were considered early stages of the SCCs in these tissues (U.S. EPA, 1990). Consequently, the overall incidence of SCCs or benign tumors judged to originate from the same cell type (papillomas, polyps, or papillary polyps) were selected for dose-response modeling.

A toxicokinetic model to assist in cross-species scaling of benzo[a]pyrene inhalation exposure was not available. EPA's RfC default dosimetry adjustments (U.S. EPA, 1994) were utilized in the benzo[a]pyrene RfC calculation (see Section 2.2.2) but could not be applied to the

aerosols generated for the inhalation bioassay by Thyssen et al. (1981) as the approaches presented in the RfC methodology guidelines (U.S. EPA, 1994) were developed for insoluble and nonhygroscopic particles, not the sodium chloride particle used in Thyssen et al. (1981). Consequently, without data to inform a basis for extrapolation to humans, it was assumed that equal risk for all species would be associated with equal concentrations in air, at least at anticipated environmental concentrations. This is equivalent to assuming that any metabolism of benzo[a]pyrene is directly proportional to breathing rate and that the deposition rate is equal between species.

The multistage-Weibull model was fit to the TWA exposure concentrations and the individual animal tumor and survival data for tumors in the larynx, pharynx, trachea, or nasal cavity (tumors of the pharynx and upper respiratory tract), using the software program, MultiStage-Weibull (U.S. EPA, 2010c). Modeling results are provided in Appendix E of the Supplemental Information. Because benzo[a]pyrene carcinogenicity involves a mutagenic mode of action, linear low-exposure extrapolation from the $BMCL_{10}$ was used to derive the inhalation unit risk (U.S. EPA, 2005a).

2.4.3. Inhalation Unit Risk Derivation

The results from modeling the inhalation carcinogenicity data from Thyssen et al. (1981) are summarized in Table 2-9. Taking the tumors to have been the cause of death of the experimental animals with tumors, the BMC_{10} and $BMCL_{10}$ values were 0.468 and 0.256 mg/m^3, respectively. Then, taking all of the tumors to have been incidental to the cause of death for each animal with a tumor, the BMC_{10} and $BMCL_{10}$ values were 0.254 and 0.163 mg/m^3, respectively, about twofold lower than the first case. Because the tumors were unlikely to have all been fatal, the lower $BMCL_{10}$ from the incidental deaths analysis, 0.163 mg/m^3, is recommended for the calculation of the inhalation unit risk. Using linear extrapolation from the $BMCL_{10}$ (0.163 mg/m^3), an inhalation unit risk of **0.6 per mg/m^3**, or **6×10^{-4} per µg/m^3** (rounding to one significant digit), was calculated. Note that the inhalation unit risk should only be used with lifetime human exposures <0.3 mg/m^3, the human equivalent POD, because above this level, the dose-response relationship is not expected to be proportional to benzo[a]pyrene exposure.

Table 2-9. Summary of the inhalation unit risk derivation

Tumor site and context	Species/ sex	Selected model	BMR	BMC (mg/m^3)	POD = BMCL (mg/m^3)	Unit risk[a] $(mg/m^3)^{-1}$
Upper respiratory tract and pharynx; all treated as cause of death Thyssen et al. (1981)	Male hamsters	Multistage Weibull, 2°	10%	0.468	0.256	0.4
Upper respiratory tract and pharynx; all treated as incidental to death Thyssen et al. (1981)	Male hamsters	Multistage Weibull, 2°	10%	0.254	0.163	0.6

[a]Human equivalent unit risk = $0.10/BMCL_{10}$; see Appendix E for details of modeling results.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

Table 2-10 summarizes uncertainties in the derivation of the inhalation unit risk for benzo[a]pyrene; further detail is provided in the following discussion. Only one animal cancer bioassay, in one sex, by the inhalation route is available that describes the exposure-response relationship for respiratory tract tumors with lifetime inhalation exposure to benzo[a]pyrene (Thyssen et al., 1981). Although corroborative information on exposure-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 (upper and lower respiratory tract tumors), and with some of the portal-of-entry effects in oral exposure studies.

The hamster inhalation bioassay by Thyssen et al. (1981) observed upper respiratory tract tumors, but not lung tumors. The lack of a lung tumor response in hamsters, given the strong association of inhaled PAH mixtures with lung cancer in humans across many studies (see Section 1.1.5) suggests that this study may not be ideal for extrapolating to humans. Hamsters have an apparent lower sensitivity to lung carcinogenesis than rats and mice and a tendency to give false negatives for particles classified as carcinogenic to humans by IARC (Mauderly, 1997). However, hamster laryngeal tumors have been used as an indication of the carcinogenic hazard of cigarette smoke for more than 50 years (IARC, 2002). For example, a large study investigating the inhalation of cigarette smoke in hamsters (n = 4,400) indicated that the larynx was the most responsive tumor site, which the authors indicated was due to a large difference in particle deposition between the larynx and the lung (Dontenwill et al., 1973). EPA's *Guidelines for Carcinogen Assessment* (U.S. EPA, 2005a) stress that site concordance between animals and humans need not always be assumed. Therefore, the robust tumor response in the upper respiratory tract of Syrian golden hamsters was considered to be supportive of the use of the Thyssen et al. (1981) study for the derivation of an inhalation unit risk.

An additional uncertainty includes the inability to apply U.S. EPA (1994) dosimetry approaches to extrapolate inhaled concentrations from animals to humans, due to the use of a

soluble hygroscopic carrier particle (sodium chloride) 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-sodium chloride 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 and less deposition in the lung (Varghese and Gangamma, 2009; Asgharian, 2004; Ferron, 1994; Xu and Yu, 1985). In addition, sodium chloride can be irritating to the respiratory tract, depending on concentration. The Thyssen et al. (1981) study reported that vehicle controls were exposed to 240 μg/m^3, and it is unclear whether exposure to this concentration of sodium chloride could have potentiated the tumor response seen in the mid- and high-concentration benzo[a]pyrene groups. Exposure to benzo[a]pyrene in the environment predominantly occurs via non-soluble, non-hygroscopic, carbonaceous particles (such as soot and diesel exhaust particles). The potential impact of differences in carrier particle on the magnitude of the inhalation unit risk is unknown.

Regarding uncertainty associated with exposure characterization, the individual exposure chamber measurements varied from about an order of magnitude less than the target concentration to about twofold higher than the target concentration. Weekly average analytical concentrations were documented to vary by two- to fivefold in all exposed groups, with no particular trends over time. Continuous time-weighted group average concentrations were used for dose-response modeling under the assumption that equal cumulative exposures are expected to lead to similar outcomes. This assumption is generally expected to lead to an unbiased estimate of risk when there is incomplete information. However, it is possible that peak exposure above some concentration may be more associated with the observed effects, or that deposition of particles may have reached a maximum level or plateau, such as in the high-exposure group. Regarding the role of peak exposures, the higher exposures for each group were distributed evenly throughout the study for the most part, suggesting that any association of risk with peak exposures would also be proportional to cumulative exposure. If particle deposition reached a plateau with the high-exposure group, there is relatively less impact on the unit risk because the derivation relies on the dose-response at lower exposure. But the actual dynamics of particle deposition at these or other exposure levels are not well understood. There is not enough information available to estimate a more quantitative impact on the estimated unit risk due to these uncertainties.

Table 2-10. Summary of uncertainties in the derivation of cancer risk values for benzo[a]pyrene (inhalation unit risk)

Consideration and impact on cancer risk value	Decision	Justification and discussion
Selection of data set and target organ No inhalation unit risk if Thyssen et al. (1981) not used	Respiratory tract tumors from Thyssen et al. (1981)	The Thyssen et al. (1981) bioassay is the only lifetime inhalation cancer bioassay available for describing exposure-response relationships for cancer from inhaled benzo[a]pyrene. Intratracheal installation studies support the association of benzo[a]pyrene exposure with respiratory tract tumors.
Selection of dose metric Alternatives could ↓ or ↑ unit risk	Administered exposure as TWA	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified. The recommended unit risk is a reasonable estimate if the proportion of the carcinogenic moiety remains the same at lower exposures.
Interspecies extrapolation Alternatives could ↓ or ↑ slope factor	Cross-species scaling was not applied. The carrier particle used was soluble and hygroscopic, therefore the RfC methodology (U.S. EPA, 1994) dosimetric adjustments could not be applied.	There are no data to support alternatives. Equal risk per $\mu g/m^3$ is assumed.
Dose-response modeling Alternatives could ↓ or ↑ slope factor	Multistage-Weibull model	No biologically based models for benzo[a]pyrene were available. Because the multistage-Weibull model could address additional available data (time of death with tumor), this model was superior to other available empirical models.
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation	Linear extrapolation from the POD (based on mutagenic mode of action)	Available mode-of-action data support linearity (mutagenicity is a primary mode of action of benzo[a]pyrene).
Statistical uncertainty at POD ↓ inhalation unit risk 1.4-fold if BMC used as the POD rather than BMCL	BMCL (preferred approach for calculating plausible upper bound unit risk)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval (CI) on administered exposure at 10% extra risk of respiratory tract tumors.
Sensitive subpopulations ↑ inhalation unit risk to unknown extent	ADAFs are recommended for early life exposures	No chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity.

2.4.5. Previous IRIS Assessment: Inhalation Unit Risk

An inhalation unit risk for benzo[a]pyrene was not previously available on IRIS.

2.5. DERMAL SLOPE FACTOR FOR CANCER

Human and animal studies of exposure to PAH mixtures or benzo[a]pyrene alone demonstrate an increased incidence of skin tumors with increasing dermal exposure. 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 lifetime dermal exposure. This derivation provides the first dermal slope factor for the Integrated Risk Information System (IRIS) database.

2.5.1. Analysis of Carcinogenicity Data

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 pitches, non-refined mineral oils, shale oils, and soot (IARC, 2010; Baan et al., 2009; IPCS, 1998; Boffetta et al., 1997; ATSDR, 1995). Although studies of human exposure to benzo[a]pyrene alone are not available, repeated application of benzo[a]pyrene to skin (in the absence of exogenous promoters) has been demonstrated to induce skin tumors in guinea pigs, rabbits, rats, and mice. Given the availability of lifetime bioassays of dermal benzo[a]pyrene exposure in mice, this analysis focuses on carcinogenicity bioassays including repeated dermal exposure to benzo[a]pyrene for approximately 2 years. These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959). These studies, in several strains of mice, demonstrated primarily malignant skin tumors, as well as earlier occurrence of tumors with increasing exposure levels (see Tables D-15 to D-23 in the Supplemental Information for study details).

Other carcinogenicity studies in mice were judged to support the studies listed above, but were not considered in the dose-response analysis because of the availability of lifetime studies directly relevant to deriving a dermal slope factor. These other studies included: (1) early "skin painting" studies of benzo[a]pyrene carcinogenicity in mouse skin that did not report sufficient information to estimate the doses applied (e.g., Wynder and Hoffmann, 1959; Wynder et al., 1957); (2) bioassays with minimal dose-response information, either using just one benzo[a]pyrene dose level or with only dose levels inducing 90–100% incidence of mice with tumors, which provide relatively little information about the shape of the dose-response relationship especially for low exposures (e.g., Wilson and Holland, 1988); and (3) shorter studies (i.e., <1 year) (Higginbotham et al., 1993; Albert et al., 1991; Nesnow et al., 1983; Emmett et al., 1981; Levin et al., 1977), which would tend to underestimate lifetime risk by overlooking the potential for the development of tumors in later life.

The National Institute for Occupational Safety and Health (NIOSH) study (Sivak et al., 1997; NIOSH, 1989) is a well-conducted and documented study and was determined to be the best

available study for dose-response analysis. Specifically, mice were randomly assigned to treatment groups while maintaining comparable distributions of body weights, housed singly (minimizing grooming or other interference with application sites), and observed weekly for tumor status. Histopathology was evaluated by two pathologists, both without knowledge of treatment group, who reached a consensus diagnosis in each case. In addition, the availability of time-of-tumor appearance and time of death for all animals provided a clearer characterization of the number at risk of development of tumors and the extent of exposure associated with tumor development, supporting more accurate estimation of lifetime cancer risk.

The other lifetime studies provide support for the NIOSH study. Overall, study designs varied in terms of number of exposure levels used (two to nine, compared with three in typical NTP bioassays) and in number of mice per group (from ~17 to 100 mice/dose group, compared with 50 used in most NTP bioassays). While the largest studies would be expected to have greater ability to detect low responses at low doses (e.g., Schmidt et al., 1973), studies conducted at similar doses, but smaller group sizes, showed significant dose-response trends (e.g., Poel, 1959). For all of the supporting studies, individual animal data were not available).

Some aspects of study design and conduct of the supporting studies were not consistently reported. These study attributes included composition and purity of the test article, randomization of animals to treatment groups, frequency of tumor evaluations, exact length of treatment period, and blinding of treatment group from pathologists. As each study was affected by a similar number of omissions, possibly reflecting reporting practices at the time they were conducted, all but one study (Poel, 1963) were included in dose-response modeling for comparison (see Appendix E.2.4 of the Supplemental Information).

Poel (1963) exposed three strains of mice until natural death. The study authors did not report length of time that the control mice were on study, nor the duration of exposure for mice that did not develop tumors following treatment. Overall, while these three datasets support a finding of dermal carcinogenicity, they did not provide sufficient information to estimate the extent of exposure associated with the observed tumor incidence and were not used for dose-response modeling.

2.5.2. Dose-Response Analysis—Adjustments and Extrapolation Methods

Biologically based dose-response models for dermal exposure to benzo[a]pyrene are not available. As with the oral and inhalation benzo[a]pyrene carcinogenicity data, benzo[a]pyrene dermal exposure carcinogenicity data were generally characterized by earlier occurrence of tumors and increased mortality with increasing exposure level. Individual data were available to apply a time-to-tumor model (the multistage-Weibull model, described in detail in Appendix E.2.1 of the Supplemental Information) to the NIOSH study data, while each of the other lifetime dermal data sets (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959) was modeled using the multistage model. The following discussion describes the inputs for each model.

For both dose-response models, following EPA's *Guidelines for Carcinogen Risk Assessment* (Section 2.2.2.1.2; U.S. EPA, 2005a), incidence of malignant or benign skin tumors was preferred for dose-response modeling, based on evidence that skin papillomas can develop into malignant skin tumors. For some studies, it was not clear whether related benign tumors were included in the total tumors reported (e.g., Habs et al., 1980). In the NIOSH study, one tumor in the mid-dose group that had been reported as a papilloma by Sivak et al. (1997) was listed as a keratoacanthoma in the full report (NIOSH, 1989). Since it is not clear whether keratoacanthomas can develop into malignant skin tumors, two analyses were run for the NIOSH data set, one including the keratoacanthoma and one omitting it altogether (see Table E-23 of the Supplemental Information). For the other studies, the most inclusive tabulation available of mice with benign or malignant skin tumors, without double-counting, was used.

Concerning the appropriate dose measure, we have used continuous daily exposure (see Preamble, Section 7.2) unless there are data indicating that dose-rate effects are relevant. The exposure protocols for the selected bioassays varied between two or three applications per week. Although environmental dermal exposure may more likely occur intermittently than oral or inhalation exposures, due to interruption of exposure through bathing or washing of affected areas, the dermal slope factor was derived for use with estimates of constant daily lifetime exposure. Therefore, all administered doses were converted to TWA daily doses using the equation:

Average daily dose/day = (μg/application) × (number of applications/week ÷ 7 days/week)

A particular consideration for time-to-tumor modeling (affecting only the NIOSH study) is the time of tumor observation. The NIOSH data set provided the time of first appearance of skin tumors for each animal. Skin tumors were classified as incidental for the purposes of modeling because death generally occurred weeks later.

For the lifetime studies without individual animal data, some refinements of the dose-response information were made where possible before applying the multistage model, in order to approximate what would be accomplished had a time-to-tumor model been viable. These refinements involved adjustments for incidence (i.e., numbers of animals at risk were reduced if mortality occurred before tumors first appeared) and dose (i.e., in the event of shortened exposure duration due to 100% mortality in a dose group, an equivalent, lower lifetime exposure was estimated). These adjustments are described by study in Appendix E.2.3 of the Supplemental Information.

The multistage-Weibull and multistage-cancer models were then fit to the respective data sets and BMD_{10}s/$BMDL_{10}$s estimated (details in Appendix E.2.3 of the Supplemental Information). Because benzo[a]pyrene is expected to cause cancer via a mutagenic mode of action, a linear approach to low dose extrapolation from the PODs (i.e., $BMDL_{10}$) was used (U.S. EPA, 2005a) to derive the candidate dermal slope factors.

A toxicokinetic model to assist in cross-species scaling of benzo[a]pyrene dermal exposure was not available, nor was there any guidance to inform a basis for extrapolation of dermal dose-response in mice to humans. Several alternative approaches to interspecies extrapolation were developed that were linear, and that could be applied with equivalent results either before or after dose-response modeling. Consequently, these adjustments were evaluated separately from the dose-response modeling and applied after derivation of a dermal slope factor for mice (see Section 2.5.4).

2.5.3. Derivation of the Dermal Slope Factor

The results from modeling the dermal carcinogenicity data separated by sex are summarized in Table 2-11 (see Tables E-23 and E-24 in the Supplemental Information for more details). Adequate fits to the NIOSH data were obtained using time-to-tumor modeling, whether or not the keratoacanthoma in the mid-dose group was included. Excluding the keratoacanthoma, in view of its seemingly random occurrence and the uncertainty whether such tumors would progress to carcinomas, linear extrapolation from the $BMDL_{10}$ of 0.060 μg/day from the NIOSH study led to a candidate dermal slope factor for mice of 1.7 per μg/day.

Dermal slope factors calculated from the supporting studies (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959) using the multistage model and linear extrapolation from the $BMDL_{10}$ values ranged from 0.25 to 1.8 per μg/day, a roughly sevenfold range (Table 2-11). Values ranged from 0.9 to 1.7 per μg/day for male mice, and from 0.25 to 0.67 per μg/day for female mice. These results suggest that some female mouse strains may be as sensitive as some male mouse strains, but the associated uncertainties—e.g., increased extent of low-dose extrapolation and incomplete exposure information—provide less support for many of these values. Four female mice data sets were considered to be the most uncertain because of dose ranges covered and incomplete information regarding length of exposure (Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980). In particular, the data set reported by Habs et al. (1984) yielded the highest but most uncertain result, with only two dose-response points; the slope estimate is particularly affected by the characterization of the high exposure level. There was insufficient information to conclude that males were more sensitive because both sexes were not tested for any mouse strain.

Table 2-11. Summary of dermal slope factor derivations, unadjusted for interspecies differences

Reference	Mouse strain	Selected model[a]	BMR	BMD (µg/d)	POD = BMDL (µg/d)	Candidate dermal slope factors[b] $(µg/d)^{-1}$	Comments
Male mice							
Sivak et al. (1997); NIOSH (1989)	C3H/HeJ	Multistage-Weibull 2°	10%	0.11	0.060	1.7	Well-conducted and reported study, including individual times on study and individual tumor diagnoses
Poel (1959)[c]	C57L	Multistage 3°	10%	0.13	0.078	1.3	Grouped survival data reported
Female mice							
Roe et al. (1970)	Swiss	Multistage 2°	10%	0.69	0.39	0.25	Grouped survival data reported
Schmidt et al. (1973)	Swiss	Multistage 3°	10%	0.28	0.22	0.45	No characterization of exposure duration
Schmidt et al. (1973)	NMRI	Multistage 2°	10%	0.33	0.29	0.34	No characterization of exposure duration
Schmähl et al. (1977)	NMRI	Multistage 2°	10%	0.23	0.15	0.67	No characterization of exposure duration
Habs et al. (1980)	NMRI	Multistage 4°	10% 30%	0.36 0.49	0.24 0.44	0.42 0.69	Higher overall exposure range; unclear overall duration of exposure
(Habs et al., 1984)	NMRI	Multistage 1°	10% 50%	0.078 0.51	0.056 0.37	1.8 1.4	No characterization of exposure duration for high exposure; high response at lowest exposure limits usefulness of low-dose extrapolation
Grimmer et al. (1983)	CFLP	Multistage 1°	10% 40%	0.24 1.2	0.21 1.0	0.48 0.40	No characterization of exposure duration
Grimmer et al. (1984)	CFLP	Log-logistic	70%	1.07	0.48	1.5	No characterization of exposure duration; high response at lowest exposure limits usefulness of low-dose extrapolation

[a]See Appendix E.2.4 (Supplemental Information) for modeling details.
[b]Unadjusted for interspecies differences. Slope factor = $R/BMDL_R$, where R is the BMR expressed as a fraction.
[c]High exposure groups with 100% mortality omitted prior to dose-response modeling.

2.5.4. Dermal Slope Factor Cross-Species Scaling

Different methodologies have been established for interspecies scaling of PODs used to derive oral slope factors and inhalation unit risks. Cross-species adjustment of oral doses is based on allometric scaling using the ¾ power of body weight. This adjustment accounts for more rapid distribution, metabolism, and clearance in small animals (U.S. EPA, 2005a). Cross-species extrapolation of inhalation exposures is based on standard dosimetry models that consider factors such as solubility, reactivity, and persistence (U.S. EPA, 1994) in addition to species differences in physiology. Although no established methodology exists to adjust for interspecies differences in dermal toxicity at the point of contact, allometric scaling using body weight to the ¾ power was selected based on known species differences in dermal metabolism and penetration of benzo[a]pyrene. In vitro skin permeation was highest in the mouse, compared to rat, rabbit, and human, and was enhanced by induction of CYP enzymes (Kao et al., 1985). Using 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.

Alternative approaches were also evaluated, including: (1) assuming that 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 (e.g., no interspecies adjustment); (2) assuming that equal mass per day, if applied to equal fractions of total skin surface will have similar cancer risks; and (3) assuming that risk is directly proportional to dose expressed as mass per kg body weight per day. A comparison of these alternatives is provided in Appendix E of the Supplemental Information.

The POD_M derived from the NIOSH study (Sivak et al., 1997; NIOSH, 1989) is adjusted to a HED as follows:

$$POD_{HED}\ (\mu g/day) = POD_M\ (\mu g/day) \times (BW_H / BW_M)^{3/4}$$
$$= 0.060\ \mu g/day \times (70\ kg / 0.035\ kg)^{3/4}$$
$$= 17.9\ \mu g/day$$

The resulting POD_{HED} is used to calculate the dermal slope factor for benzo[a]pyrene:

Dermal slope factor = BMR/POD_{HED} = 0.1/(17.9 µg/day) = **0.006 per µg/day.**

Note that the dermal slope factor should only be used with lifetime human exposures <18 µg/day, the human equivalent of the POD_M, because above this level the dose-response relationship is not expected to be proportional to the mass of benzo[a]pyrene 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 similarly to interspecies differences in whole-body toxicokinetics. Secondly, it is assumed that the risk at low doses of benzo[a]pyrene is linear.

Although 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, 1969), this may be due to promotional effects, such as inflammation, that are observed at high doses of benzo[a]pyrene.

The dermal 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 in humans (Godschalk et al., 1998a), lifetime skin cancer bioassays that have included pathological examination of other organs have not found elevated incidences of tumors at distal sites (Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959). This may be because 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 metabolism of benzo[a]pyrene 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; Moody and Chu, 1995). Taken together, these data support the conclusion that the risk of skin cancer following dermal exposure likely outweighs cancer risks at distal organs.

2.5.5. Uncertainties in the Derivation of the Dermal Slope Factor

Table 2-12 summarizes uncertainties in the derivation of the dermal slope factor for benzo[a]pyrene; further detail is provided in the following discussion. Uncertainty in the recommended dermal slope factor is partly reflected in the range of POD values derived from the modeled mouse skin tumor data sets: the lowest and highest $BMDL_{10}$ values listed in Table 2-11 show a sevenfold difference (0.056–0.39 μg/day) in magnitude. However, many of the studies considered had incomplete information concerning time on study, which would tend to lead to underestimates of risk. Low-dose extrapolation uncertainty for several of these studies also decreases confidence in their results. Reliance on the NIOSH study, a relatively well-conducted study with the low exposure levels having low early mortality, exposures continuing for full lifetimes, and individual times on study minimizes 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). Consequently, 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. All of the dermal bioassays that were considered treated animals 2–3 times/week. This assessment makes the assumption that risk is proportional to total

cumulative exposure. However, this may overestimate risk if duration-adjusted doses are below doses that saturate or diminish detoxifying metabolic steps.

The relative impact of a particular vehicle on benzo[a]pyrene carcinogenicity and the relative sensitivity of male and female mice to benzo[a]pyrene exposure was difficult to ascertain in the supporting lifetime studies. Overall, the study designs for these studies included different mouse strains, sexes, and vehicles, but for any given mouse strain, even across multiple studies, only one sex and only one vehicle was tested. Thus, it was not possible to evaluate the relative impact of particular vehicles on benzo[a]pyrene carcinogenicity, or the relative sensitivity of male and female mice.

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 assumption is made that equal lifetime risks of cancer would follow from exposure to the same daily dose adjusted for $BW^{3/4}$. Qualitatively, the toxicokinetics and toxicodynamics in mouse and human skin appear to be similar (Knafla et al., 2011; 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 1.1.5).

The dermal slope factor for benzo[a]pyrene is based on skin cancer and does not 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 adducts have been detected in white blood cells (WBCs) following dermal exposure to benzo[a]pyrene, indicating that dermally applied benzo[a]pyrene enters systemic circulation (Godschalk et al., 1998a). 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 (Higginbotham et al., 1993; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959), most of these studies attempted to remove animals with grossly observed skin tumors 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 possibly competes with systemic risk of tumors. Currently, the potential contribution of dermally absorbed benzo[a]pyrene to systemic cancer risk is unclear.

Table 2-12. Summary of uncertainties in the derivation of cancer risk values for benzo[a]pyrene dermal slope factor

Consideration and impact on cancer risk value	Decision	Justification and discussion
Selection of data set ↓ dermal slope factor if alternative data set were selected	NIOSH (1989)	Study included lowest doses among available studies (where intercurrent mortality was less likely to impact the number at risk).
Selection of target organ No dermal slope factor if skin tumor studies not used	Selection of skin tumors	Skin tumors were replicated in numerous studies of male or female mice. No studies were available indicating that other tumors occur following dermal exposure.
Selection of dose metric Alternatives could ↓ or ↑ slope factor	Administered dose, as TWA in µg/d	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified.
Interspecies extrapolation Alternatives could ↓ or ↑ slope factor	Total daily dose scaled by $BW^{3/4}$	Alternatives discussed in Appendix E. An established methodology does not exist to adjust for interspecies differences in dermal toxicity at the point of contact. Benzo[a]pyrene metabolism is known to occur in the dermal layer. Viewing the skin as an organ, and without evidence to the contrary, metabolic processes were assumed to scale allometrically.
Dose-response modeling Alternatives could ↓ or ↑ slope factor	Multistage-Weibull model	No biologically based models for benzo[a]pyrene were available. The multistage-Weibull model is consistent with biological processes, incorporates timecourse information, and is preferred for IRIS cancer assessments when individual data are available (Gehlhaus et al., 2011).
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation	Linear extrapolation from POD (based on mutagenic mode of action)	Available mode of action data support linearity (mutagenicity is a primary mode of action of benzo[a]pyrene).
Sensitive subpopulations ↑ dermal slope factor to unknown extent	ADAFs are recommended for early life exposures	No chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity.

2.5.6. Previous IRIS Assessment: Dermal Slope Factor

A dermal slope factor for benzo[a]pyrene was not previously available on IRIS.

2.6. APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS (ADAFS)

Based on sufficient support in laboratory animals and relevance to humans, benzo[a]pyrene is determined to be carcinogenic by a mutagenic mode of action. 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 mode of action are assumed to have increased risk for cancer. The oral slope factor of 1 per mg/kg-day, inhalation unit risk of 0.6 per mg/m^3, and dermal slope factor of 0.006 per µg/day for benzo[a]pyrene, calculated from data applicable to adult exposures, do not reflect presumed early life susceptibility to this chemical. Although chemical-specific data exist for benzo[a]pyrene that quantitatively demonstrate increased early life susceptibility to cancer (Vesselinovitch et al., 1975), 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 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 at <2 years of age, 3 for exposed individuals at 2–<16 years of age, and 1 for exposed individuals ≥16 years of age. The 10- and 3-fold adjustments are combined with age-specific exposure estimates when estimating cancer risks from early life (<16 years of 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 2-13).

Table 2-13. Sample application of ADAFs for the estimation of benzo[a]pyrene cancer risk following lifetime (70-year) oral exposure

Age group	ADAF	Unit risk (per mg/kg-d)	Sample exposure concentration (mg/kg-d)	Duration adjustment	Cancer risk for age-specific exposure period
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	0.0008
Total risk					0.002

The example exposure duration scenarios include full lifetime exposure (assuming a 70-year lifespan). Table 2-13 lists the four factors (ADAFs, cancer risk estimate, assumed exposure, and duration adjustment) that are needed to calculate the age-specific 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–<2 years is the product of the values in columns 2–5 or $10 \times 1 \times 0.001 \times 2/70 = 3 \times 10^{-4}$. 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.

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 for ages 0–30 years, the duration adjustments would be 2/70, 14/70, and 14/70, and the age-specific 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 for ages 20–50 years, the duration adjustments would be 0/70, 0/70, and 30/70. The age-specific 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 (Table 2-13), 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 (presented in Tables 2-14 and 2-15).

Table 2-14. Sample application of ADAFs for the estimation of benzo[a]pyrene cancer risk following lifetime (70-year) inhalation exposure

Age group	ADAF	Unit risk (per µg/m^3)	Sample exposure concentration (µg/m^3)	Duration adjustment	Cancer risk for age-specific exposure period
0–<2 yrs	10	6×10^{-4}	0.1	2 yrs/70 yrs	0.00002
2–<16 yrs	3	6×10^{-4}	0.1	14 yrs/70 yrs	0.00004
≥16 yrs	1	6×10^{-4}	0.1	54 yrs/70 yrs	0.00005
Total risk					0.00010

Table 2-15. Sample application of ADAFs for the estimation of benzo[a]pyrene cancer risk following lifetime (70-year) dermal exposure

Age group	ADAF	Unit risk (per µg/d)	Sample exposure concentration (µg/d)	Duration adjustment	Cancer risk for age-specific exposure period
0–<2 yrs	10	0.006	0.001	2 yrs/70 yrs	2×10^{-6}
2–<16 yrs	3	0.006	0.001	14 yrs/70 yrs	4×10^{-6}
≥16 yrs	1	0.006	0.001	54 yrs/70 yrs	5×10^{-6}
Total risk					1×10^{-5}

REFERENCES

Abedi-Ardekani, B; Kamangar, F; Hewitt, SM; Hainaut, P; Sotoudeh, M; Abnet, CC; Taylor, PR; Boffetta, P; Malekzadeh, R; Dawsey, SM. (2010). Polycyclic aromatic hydrocarbon exposure in oesophageal tissue and risk of oesophageal squamous cell carcinoma in north-eastern Iran. Gut 59: 1178-1183. http://dx.doi.org/10.1136/gut.2010.210609

Aklillu, E; Øvrebø, S; Botnen, IV; Otter, C; Ingelman-Sundberg, M. (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: 5105-5111. http://dx.doi.org/10.1158/0008-5472.CAN-05-0113

Albert, RE; Miller, ML; Cody, T; Andringa, A; Shukla, R; Baxter, CS. (1991). Benzo[a]pyrene-induced skin damage and tumor promotion in the mouse. Carcinogenesis 12: 1273-1280. http://dx.doi.org/10.1093/carcin/12.7.1273

Albert, RE; Miller, ML; Cody, TE; Talaska, G; Underwood, P; Andringa, A. (1996). Epidermal cytokinetics, DNA adducts, and dermal inflammation in the mouse skin in response to repeated benzo[a]pyrene exposures. Toxicol Appl Pharmacol 136: 67-74.

Alexandrov, K; Cascorbi, I; Rojas, M; Bouvier, G; Kriek, E; Bartsch, H. (2002). CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation [Review]. Carcinogenesis 23: 1969-1977.

Alexandrov, K; Rojas, M; Geneste, O; Castegnaro, M; Camus, AM; Petruzzelli, S; Giuntini, C; Bartsch, H. (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: 6248-6253.

Allan, LL; Schlezinger, JJ; Shansab, M; Sherr, DH. (2006). CYP1A1 in polycyclic aromatic hydrocarbon-induced B lymphocyte growth suppression. Biochem Biophys Res Commun 342: 227-235. http://dx.doi.org/10.1016/j.bbrc.2006.01.131

Amos, CI; Xu, W; Spitz, MR. (1999). Is there a genetic basis for lung cancer susceptibility? [Review]. Recent Results Cancer Res 151: 3-12.

Andreassen, A; Kure, EH; Nielsen, PS; Autrup, H; Haugen, A. (1996). Comparative synchronous fluorescence spectrophotometry and 32P-postlabeling analysis of PAH-DNA adducts in human lung and the relationship to TP53 mutations. Mutat Res 368: 275-282.

Arafa, HM; Aly, HA; Abd-Ellah, MF; El-Refaey, HM. (2009). Hesperidin attenuates benzo[alpha] pyrene-induced testicular toxicity in rats via regulation of oxidant/antioxidant balance. Toxicol Ind Health 25: 417-427. http://dx.doi.org/10.1177/0748233709106624

Archibong, AE; Inyang, F; Ramesh, A; Greenwood, M; Nayyar, T; Kopsombut, P; Hood, DB; Nyanda, AM. (2002). Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene. Reprod Toxicol 16: 801-808.

Archibong, AE; Ramesh, A; Niaz, MS; Brooks, CM; Roberson, SI; Lunstra, DD. (2008). Effects of benzo(a)pyrene on intra-testicular function in F-344 rats. Int J Environ Res Public Health 5: 32-40. http://dx.doi.org/10.3390/ijerph5010032

Armstrong, B; Tremblay, C; Baris, D; Thériault, G. (1994). Lung cancer mortality and polynuclear aromatic hydrocarbons: A case-cohort study of aluminum production workers in Arvida, Quebec, Canada. Am J Epidemiol 139: 250-262.

Armstrong, BG; Gibbs, G. (2009). Exposure-response relationship between lung cancer and polycyclic aromatic hydrocarbons (PAHs). Occup Environ Med 66: 740-746. http://dx.doi.org/10.1136/oem.2008.043711

Arthur D Little (Arthur D Little Inc.). (1989). Assessment of the cocarcinogenic/promoting activity of asphalt fumes. National Institute for Occupational Safety and Health. http://www.ntis.gov/search/product.aspx?ABBR=PB91110213

Asgharian, B. (2004). A model of deposition of hygroscopic particles in the human lung. Aerosol Sci Technol 38: 938-947. http://dx.doi.org/10.1080/027868290511236

ASRM (American Society of Reproductive Medicine). (2008). The clinical utility of sperm DNA integrity testing [Review]. Fertil Steril 90: S178-S180. http://dx.doi.org/10.1016/j.fertnstert.2008.08.054

ATSDR (Agency for Toxic Substances and Disease Registry). (1995). Toxicological profile for polycyclic aromatic hydrocarbons [ATSDR Tox Profile]. Washington, DC: U.S. Department of Health and Human Services. http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

ATSDR (Agency for Toxic Substances and Disease Registry). (2011). The priority list of hazardous substances that will be the subject of toxicological profiles. http://www.atsdr.cdc.gov/SPL/index.html

Baan, R; Grosse, Y; Straif, K; Secretan, B; El Ghissassi, F; Bouvard, V; Benbrahim-Tallaa, L; Guha, N; Freeman, C; Galichet, L; Cogliano, V. (2009). A review of human carcinogens--part F: Chemical agents and related occupations. Lancet Oncol 10: 1143-1144. http://dx.doi.org/10.1016/S1470-2045(09)70358-4

Balu, N; Padgett, WT; Lambert, GR; Swank, AE; Richard, AM; Nesnow, S. (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: 827-838. http://dx.doi.org/10.1021/tx034207s

Barry, G; Cook, JW; Haslewood, GAD; Hewett, CL; Hieger, I; Kennaway, EL. (1935). The Production of Cancer by Pure Hydrocarbons.--Part III. Proc Biol Sci 117: 318-351. http://dx.doi.org/10.1098/rspb.1935.0032

Bartsch, H; Nair, U; Risch, A; Rojas, M; Wikman, H; Alexandrov, K. (2000). Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers [Review]. Cancer Epidemiol Biomarkers Prev 9: 3-28.

Bartsch, H; Rojas, M; Alexandrov, K; Risch, A. (1998). Impact of adduct determination on the assessment of cancer susceptibility. In M Schwab; HJ Senn; P Schlag; K Munk; H Rabes (Eds.), Recent results in cancer research, v154: Genes and environment in cancer (pp. 86-96). New York, NY: Springer.

Bartsch, H; Rojas, M; Nair, U; Nair, J; Alexandrov, K. (1999). Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. Cancer Detect Prev 23: 445-453.

Beland, F; Culp, S. (1998). Chronic bioassay of two composite samples from selected manufactured gas plant waste sites [unpublished report]. (Technical Report 6722.02). Jefferson, AK: National Center for Toxicological Research.

Benbrahim-Tallaa, L; Baan, RA; Grosse, Y; Lauby-Secretan, B; El ghissassi, F; Bouvard, V; Guha, N; Loomis, D; Straif, K. (2012). Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes. Lancet Oncol 13: 663-664. http://dx.doi.org/10.1016/S1470-2045(12)70280-2

Benjamin, H; Storkson, J; Tallas, PG; Pariza, MW. (1988). Reduction of benzo[a]pyrene-induced forestomach neoplasms in mice given nitrite and dietary soy sauce. Food Chem Toxicol 26: 671-678. http://dx.doi.org/10.1016/0278-6915(88)90066-X

Bennett, WP; Alavanja, MC; Blomeke, B; Vähäkangas, KH; Castrén, K; Welsh, JA; Bowman, ED; Khan, MA; Flieder, DB; Harris, CC. (1999). Environmental tobacco smoke, genetic susceptibility, and risk of lung cancer in never-smoking women. J Natl Cancer Inst 91: 2009-2014.

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: 510-516.

Berger, J; Manz, A. (1992). Cancer of the stomach and the colon-rectum among workers in a coke gas plant. Am J Ind Med 22: 825-834. http://dx.doi.org/10.1002/ajim.4700220605

Biancifiori, C; Caschera, F; Giornelli-Santulli, F; Bucciarelli, E. (1967). The action of oestrone and four chemical carcinogens in intact and ovariectomized BALB/c/Cb/Se mice. Br J Cancer 21: 452-459.

Bickers, DR; Mukhtar, H; Dutta-Choudhury, T; Marcelo, CL; Voorhees, JJ. (1984). Aryl hydrocarbon hydroxylase, epoxide hydrolase, and benzo[a]-pyrene metabolism in human epidermis: Comparative studies in normal subjects and patients with psoriasis. J Invest Dermatol 83: 51-56. http://dx.doi.org/10.1111/1523-1747.ep12261680

Bingham, E; Falk, HL. (1969). Environmental carcinogens. The modifying effect of cocarcinogens on the threshold response. Arch Environ Health 19: 779-783.

Binkova, B; Chvatalova, I; Lnenickova, Z; Milcova, A; Tulupova, E; Farmer, PB; Sram, RJ. (2007). PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. Mutat Res-Fundam Mol Mech Mutagen 620: 49-61. http://dx.doi.org/10.1016/j.mrfmmm.2007.02.022

Bláha, L; Kapplová, P; Vondrácek, J; Upham, B; Machala, M. (2002). Inhibition of gap-junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons. Toxicol Sci 65: 43-51. http://dx.doi.org/10.1093/toxsci/65.1.43

Blanton, RH; Lyte, M; Myers, MJ; Bick, PH. (1986). Immunomodulation by polyaromatic hydrocarbons in mice and murine cells. Cancer Res 46: 2735-2739.

Boffetta, P; Burstyn, I; Partanen, T; Kromhout, H; Svane, O; Langard, S; Jarvholm, B; Frentzel-Beyme, R; Kauppinen, T; Stucker, I; Shaham, J; Heederik, D; Ahrens, W; Bergdahl, IA; Cenee, S; Ferro, G; Heikkila, P; Hooiveld, M; Johansen, C; Randem, BG; Schill, W. (2003). Cancer mortality among European asphalt workers: an international epidemiological study II Exposure to bitumen fume and other agents. Am J Ind Med 43: 28-39. http://dx.doi.org/10.1002/ajim.10182

Boffetta, P; Jourenkova, N; Gustavsson, P. (1997). Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons [Review]. Cancer Causes Control 8: 444-472. http://dx.doi.org/10.1023/A:1018465507029

Bolton, JL; Trush, MA; Penning, TM; Dryhurst, G; Monks, TJ. (2000). Role of quinones in toxicology [Review]. Chem Res Toxicol 13: 135-160. http://dx.doi.org/10.1021/tx9902082

Borini, A; Tarozzi, N; Bizzaro, D; Bonu, MA; Fava, L; Flamigni, C; Coticchio, G. (2006). Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod 21: 2876-2881. http://dx.doi.org/10.1093/humrep/del251

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 Appl Pharmacol 167: 191-198. http://dx.doi.org/10.1006/taap.2000.9006

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 [Review]. Ann Oncol 18: 431-446. http://dx.doi.org/10.1093/annonc/mdl172

Boström, CE; Gerde, P; Hanberg, A; Jernström, B; Johansson, C; Kyrklund, T; Rannug, A; Törnqvist, M; Victorin, K; Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air [Review]. Environ Health Perspect 110 Suppl 3: 451-488.

Bouayed, J; Bohn, T; Tybl, E; Kiemer, AK; Soulimani, R. (2012). Benzo[α]pyrene-Induced Anti-Depressive-like Behaviour in Adult Female Mice: Role of Monoaminergic Systems. Basic & Clinical

Pharmacology & Toxicology Online Pharmacology Online 110: 544-550. http://dx.doi.org/10.1111/j.1742-7843.2011.00853.x

Bouayed, J; Desor, F; Rammal, H; Kiemer, AK; Tybl, E; Schroeder, H; Rychen, G; Soulimani, R. (2009a). Effects of lactational exposure to benzo[alpha]pyrene (B[alpha]P) on postnatal neurodevelopment, neuronal receptor gene expression and behaviour in mice. Toxicology 259: 97-106. http://dx.doi.org/10.1016/j.tox.2009.02.010

Bouayed, J; Desor, F; Soulimani, R. (2009b). Subacute oral exposure to benzo[alpha]pyrene (B[alpha]P) increases aggressiveness and affects consummatory aspects of sexual behaviour in male mice. J Hazard Mater 169: 581-585. http://dx.doi.org/10.1016/j.jhazmat.2009.03.131

Broekman, BF; Chan, YH; Chong, YS; Quek, SC; Fung, D; Low, YL; Ooi, YP; Gluckman, PD; Meaney, MJ; Wong, TY; Saw, SM. (2009). The influence of birth size on intelligence in healthy children. Pediatrics 123: e1011-e1016. http://dx.doi.org/10.1542/peds.2008-3344

Brown, J. R.; Thornton, JL. (1957). Percivall Pott (1714-1788) and chimney sweepers' cancer of the scrotum. Br J Ind Med 14: 68-70.

Brown, LA; Khousbouei, H; Goodwin, JS; Irvin-Wilson, CV; Ramesh, A; Sheng, L; Mccallister, MM; Jiang, GC; Aschner, M; Hood, DB. (2007). Down-regulation of early ionotrophic glutamate receptor subunit developmental expression as a mechanism for observed plasticity deficits following gestational exposure to benzo(a)pyrene. Neurotoxicology 28: 965-978. http://dx.doi.org/10.1016/j.neuro.2007.05.005

Brune, H; Deutsch-Wenzel, RP; Habs, M; Ivankovic, S; Schmähl, D. (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: 153-157. http://dx.doi.org/10.1007/BF00410666

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.

Buening, MK; Wislocki, PG; Levin, W; Yagi, H; Thakker, DR; Akagi, H; Koreeda, M; Jerina, DM; Conney, AH. (1978). Tumorigenicity of the optical enantiomers of the diastereomeric benzo[a]pyrene 7,8-diol-9,10-epoxides in newborn mice: exceptional activity of (+)-7"beta", 8"alpha"-dihydroxy-9"alpha",10"alpha"-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. PNAS 75: 5358-5361.

Burdick, AD; Davis, JW; Liu, KJ; Hudson, LG; Shi, H; Monske, ML; Burchiel, SW. (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: 7825-7833.

Burns-Naas, LA; Hastings, KL; Ladics, GS; Makris, SL; Parker, GA; Holsapple, MP. (2008). What's so special about the developing immune system? [Review]. Int J Toxicol 27: 223-254. http://dx.doi.org/10.1080/10915810801978110

Burstyn, I; Kromhout, H; Johansen, C; Langard, S; Kauppinen, T; Shaham, J; Ferro, G; Boffetta, P. (2007). Bladder cancer incidence and exposure to polycyclic aromatic hydrocarbons among asphalt pavers. Occup Environ Med 64: 520-526. http://dx.doi.org/10.1136/oem.2006.029801

Burstyn, I; Kromhout, H; Kauppinen, T; Heikkilä, P; Boffetta, P. (2000). Statistical modelling of the determinants of historical exposure to bitumen and polycyclic aromatic hydrocarbons among paving workers. Ann Occup Hyg 44: 43-56.

Burstyn, I; Kromhout, H; Partanen, T; Svane, O; Langård, S; Ahrens, W; Kauppinen, T; Stücker, I; Shaham, J; Heederik, D; Ferro, G; Heikkilä, P; Hooiveld, M; Johansen, C; Randem, BG; Boffetta, P. (2005). Polycyclic aromatic hydrocarbons and fatal ischemic heart disease. Epidemiology 16: 744-750. http://dx.doi.org/10.1097/01.ede.0000181310.65043.2f

Busby, WF, Jr; Goldman, ME; Newberne, PM; Wogan, GN. (1984). Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5: 1311-1316. http://dx.doi.org/10.1093/carcin/5.10.1311

Busby, WF, Jr; Stevens, EK; Martin, CN; Chow, FL; Garner, RC. (1989). Comparative lung tumorigenicity of parent and mononitro-polynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. Toxicol Appl Pharmacol 99: 555-563. http://dx.doi.org/10.1016/0041-008X(89)90162-2

Cal/EPA (California Environmental Protection Agency). (2010). Public health goals for chemicals in drinking water. Benzo(a)pyrene. http://oehha.ca.gov/water/phg/pdf/091610Benzopyrene.pdf

Cargøe, TK; Ebbehøj, NE; Wulf, HC; Agner, T. (2013). Occupational skin cancer may be underreported. 60: A4624.

Cavalieri, E; Rogan, E; Cremonesi, P; Higginbotham, S; Salmasi, S. (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. http://dx.doi.org/10.1007/BF00390479

Cavalieri, E; Rogan, E; Higginbotham, S; Cremonesi, P; Salmasi, S. (1988b). 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: 16-22.

Cavalieri, E; Rogan, E; Sinha, D. (1988c). Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. J Cancer Res Clin Oncol 114: 3-9. http://dx.doi.org/10.1007/BF00390478

Cavalieri, E; Rogan, E; Toth, B; Munhall, A. (1981). Carcinogenicity of the environmental pollutants cyclopenteno-[cd]pyrene and cyclopentano[cd]pyrene in mouse skin. Carcinogenesis 2: 277-281.

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: 2183-2187. http://dx.doi.org/10.1016/0006-2952(88)90579-5

Cavalieri, EL; Higginbotham, S; Ramakrishna, NV; Devanesan, PD; Todorovic, R; Rogan, EG; Salmasi, S. (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: 1939-1944. http://dx.doi.org/10.1093/carcin/12.10.1939

Cavalieri, EL; Rogan, EG. (1992). The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation [Review]. Pharmacol Ther 55: 183-199.

Cavalieri, EL; Rogan, EG. (1995). Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons [Review]. Xenobiotica 25: 677-688. http://dx.doi.org/10.3109/00498259509061885

Cavalieri, EL; Rogan, EG; Cremonesi, P; Devanesan, PD. (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: 2173-2182.

CDC (Centers for Disease Control and Prevention). (2004). The health consequences of smoking: A report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services. http://www.surgeongeneral.gov/library/smokingconsequences/

Chakravarti, D; Pelling, JC; Cavalieri, EL; Rogan, EG. (1995). Relating aromatic hydrocarbon-induced DNA adducts and c-H-ras mutations in mouse skin papillomas: The role of apurinic sites. PNAS 92: 10422-10426.

Charles, GD; Bartels, MJ; Zacharewski, TR; Gollapudi, BB; Freshour, NL; Carney, EW. (2000). Activity of benzo[a]pyrene and its hydroxylated metabolites in an estrogen receptor-alpha reporter gene assay. Toxicol Sci 55: 320-326. http://dx.doi.org/10.1093/toxsci/55.2.320

Chau, N; Bertrand, JP; Mur, JM; Figueredo, A; Patris, A; Moulin, JJ; Pham, QT. (1993). Mortality in retired coke oven plant workers. Br J Ind Med 50: 127-135.

Chen, C; Tang, Y; Jiang, X; Qi, Y; Cheng, S; Qiu, C; Peng, B; Tu, B. (2012). Early postnatal benzo(a)pyrene exposure in Sprague-Dawley rats causes persistent neurobehavioral impairments that emerge

postnatally and continue into adolescence and adulthood. Toxicol Sci 125: 248-261. http://dx.doi.org/10.1093/toxsci/kfr265

Chen, G; Gingerich, J; Soper, L; Douglas, GR; White, PA. (2010). Induction of lacZ mutations in MutaMouse primary hepatocytes. Environ Mol Mutagen 51: 330-337. http://dx.doi.org/10.1002/em.20540

Chen, X; An, H; Ao, L; Sun, L; Liu, W; Zhou, Z; Wang, Y; Cao, J. (2011). The combined toxicity of dibutyl phthalate and benzo(a)pyrene on the reproductive system of male Sprague Dawley rats in vivo. J Hazard Mater 186: 835-841. http://dx.doi.org/10.1016/j.jhazmat.2010.11.078

Cheng, L; Spitz, MR; Hong, WK; Wei, Q. (2000). Reduced expression levels of nucleotide excision repair genes in lung cancer: A case-control analysis. Carcinogenesis 21: 1527-1530.

Chengzhi, C; Yan, T; Shuqun, C; Xuejun, J; Youbin, Q; Yinyin, X; Qian, T; Baijie, T. (2011). New candidate proteins for benzo(a)pyrene-induced spatial learning and memory deficits. J Toxicol Sci 36: 163-171.

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: 67-78.

Chung, JY; Kim, YJ; Kim, JY; Lee, SG; Park, JE; Kim, WR; Yoon, YD; Yoo, KS; Yoo, YH; Kim, JM. (2011). Benzo[a]pyrene reduces testosterone production in rat Leydig cells via a direct disturbance of testicular steroidogenic machinery. Environ Health Perspect 119: 1569-1574. http://dx.doi.org/10.1289/ehp.1003391

Clayson, DB; Iverson, F; Nera, EA; Lok, E. (1990). The significance of induced forestomach tumors. Annu Rev Pharmacol Toxicol 30: 441-463.

Clement Associates, Inc, . (1990). Comparative potency approach for estimation of the total cancer risk associated with exposures to mixtures of polycyclic aromatic hydrocarbons in the environment: Appendices.

Cooper, AR; Moley, KH. (2008). Maternal tobacco use and its preimplantation effects on fertility: More reasons to stop smoking [Review]. Seminars in Reproductive Medicine 26: 204-212. http://dx.doi.org/10.1055/s-2008-1042959

Costantino, JP; Redmond, CK; Bearden, A. (1995). Occupationally related cancer risk among coke oven workers: 30 years of follow-up. J Occup Environ Med 37: 597-604.

Cresteil, T. (1998). Onset of xenobiotic metabolism in children: Toxicological implications. Food Addit Contam 15: 45-51. http://dx.doi.org/10.1080/02652039809374614

Culp, SJ; Gaylor, DW; Sheldon, WG; Goldstein, LS; Beland, FA. (1996). 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. Polycycl Aromat Compd 11: 161-168. http://dx.doi.org/10.1080/10406639608544662

Culp, SJ; Gaylor, DW; Sheldon, WG; Goldstein, LS; Beland, FA. (1998). A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis 19: 117-124.

Culp, SJ; Warbritton, AR; Smith, BA; Li, EE; Beland, FA. (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: 1433-1440. http://dx.doi.org/10.1093/carcin/21.7.1433

Das, M; Asokan, P; Don, PS; Krueger, GG; Bickers, DR; Mukhtar, H. (1986). Carcinogen metabolism in human skin grafted onto athymic nude mice: a model system for the study of human skin carcinogenesis. Biochem Biophys Res Commun 138: 33-39.

Davies, RF. (1969). Tumorigenicity of very fresh tobacco smoke condensate to mouse skin. Br J Cancer 23: 858-860.

De Jong, WH; Kroese, ED; Vos, JG; Van Loveren, H. (1999). Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci 50: 214-220. http://dx.doi.org/10.1093/toxsci/50.2.21

De Waal, EJ; Schuurman, HJ; Van Loveren, H; Vos, JG. (1997). Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, bis(tri-n-butyltin) oxide and cyclosporine on thymus histophysiology [Review]. Crit Rev Toxicol 27: 381-430. http://dx.doi.org/10.3109/10408449709089900

Dean, JH; Luster, MI; Boorman, GA; Lauer, LD; Leubke, RW; Lawson, L. (1983). Selective immunosuppression resulting from exposure to the carcinogenic congener of benzopyrene in B6C3F1 mice. Clin Exp Immunol 52: 199-206.

DeMarini, DM; Landi, S; Tian, D; Hanley, NM; Li, X; Hu, F; Roop, BC; Mass, MJ; Keohavong, P; Gao, W; Olivier, M; Hainaut, P; Mumford, JL. (2001). Lung tumor KRAS and TP53 mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. Cancer Res 61: 6679-6681.

Denissenko, MF; Pao, A; Tang, M; Pfeifer, GP. (1996). Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science 274: 430-432. http://dx.doi.org/10.1126/science.274.5286.430

Dietert, RR. (2008). Developmental immunotoxicology (DIT): windows of vulnerability, immune dysfunction and safety assessment. J Immunotoxicol 5: 401-412. http://dx.doi.org/10.1080/15476910802483324

Dietert, RR; Etzel, RA; Chen, D; Halonen, M; Holladay, SD; Jarabek, AM; Landreth, K; Peden, DB; Pinkerton, K; Smialowicz, RJ; Zoetis, T. (2000). Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary [Review]. Environ Health Perspect 108 Suppl 3: 483-490.

Dietert, RR; Piepenbrink, MS. (2006). Perinatal immunotoxicity: Why adult exposure assessment fails to predict risk [Review]. Environ Health Perspect 114: 477-483. http://dx.doi.org/10.1289/ehp.8566

Dobbing, J; Sands, J. (1973). Quantitative growth and development of human brain. Arch Dis Child 48: 757-767.

Dobbing, J; Sands, J. (1979). Comparative aspects of the brain growth spurt. Early Hum Dev 3: 79-83.

Dong, MH; Redmond, CK; Mazumdar, S; Costantino, JP. (1988). A multistage approach to the cohort analysis of lifetime lung cancer risk among steelworkers exposed to coke oven emissions. Am J Epidemiol 128: 860-873.

Dontenwill, W; Chevalier, HJ; Harke, HP; Lafrenz, U; Reckzeh, G; Schneider, B. (1973). Investigations on the effects of chronic cigarette-smoke inhalation in Syrian golden hamsters. J Natl Cancer Inst 51: 1781-1832. http://dx.doi.org/10.1093/jnci/51.6.1781

Driscoll, KE; Costa, DL; Hatch, G; Henderson, R; Oberdorster, G; Salem, H; Schlesinger, RB. (2000). Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: Uses and limitations. Toxicol Sci 55: 24-35.

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: 333-337. http://dx.doi.org/10.1016/0304-3835(82)90015-5

El-Nemr, A; Al-Shawaf, T; Sabatini, L; Wilson, C; Lower, AM; Grudzinskas, JG. (1998). Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. Hum Reprod 13: 2192-2198.

Emmett, EA; Bingham, EM; Barkley, W. (1981). A carcinogenic bioassay of certain roofing materials. Am J Ind Med 2: 59-64. http://dx.doi.org/10.1002/ajim.4700020110

Esser, C. (2009). The immune phenotype of AhR null mouse mutants: not a simple mirror of xenobiotic receptor over-activation [Review]. Biochem Pharmacol 77: 597-607. http://dx.doi.org/10.1016/j.bcp.2008.10.002

Evanoff, BA; Gustavsson, P; Hogstedt, C. (1993). Mortality and incidence of cancer in a cohort of Swedish chimney sweeps: an extended follow up study. Occup Environ Med 50: 450-459.

Everall, JD; Dowd, PM. (1978). Influence of environmental factors excluding ultra violet radiation on the incidence of skin cancer. Bull Cancer 65: 241-247.

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]. Gig Sanit 32: 168-173.

Fernandez-Salguero, P; Pineau, T; Hilbert, DM; McPhail, T; Lee, SS; Kimura, S; Nebert, DW; Rudikoff, S; Ward, JM; Gonzalez, FJ. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 268: 722-726.

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: 387-390.

Feron, VJ; Kruysse, A. (1978). Effects of exposure to furfural vapour in hamsters simultaneously treated with benzo[alpha] pyrene or diethylnitrosamine. Toxicology 11: 127-144.

Ferron, GA. (1994). Aerosol properties and lung deposition [Editorial]. Eur Respir J 7: 1392-1394.

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: 231-240. http://dx.doi.org/10.1093/toxsci/59.2.231

Fine, JS; Silverstone, AE; Gasiewicz, TA. (1990). Impairment of prothymocyte activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Immunol 144: 1169-1176.

Flowers, L; Bleczinski, WF; Burczynski, ME; Harvey, RG; Penning, TM. (1996). Disposition and biological activity of benzo[a]pyrene-7,8-dione. A genotoxic metabolite generated by dihydrodiol dehydrogenase. Biochemistry 35: 13664-13672. http://dx.doi.org/10.1021/bi961077w

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: 8640-8648. http://dx.doi.org/10.1021/bi970367p

Francis, EZ; Kimmel, CA; Rees, DC. (1990). Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity: Summary and implications. Neurotoxicol Teratol 12: 285-292. http://dx.doi.org/10.1016/0892-0362(90)90101-H

Friesen, MC; Benke, G; Del Monaco, A; Dennekamp, M; Fritschi, L; de Klerk, N; Hoving, JL; Macfarlane, E; Sim, MR. (2009). Relationship between cardiopulmonary mortality and cancer risk and quantitative exposure to polycyclic aromatic hydrocarbons, fluorides, and dust in two prebake aluminum smelters. Cancer Causes Control 20: 905-916. http://dx.doi.org/10.1007/s10552-009-9329-8

Friesen, MC; Demers, PA; Spinelli, JJ; Eisen, EA; Lorenzi, MF; Le, ND. (2010). Chronic and acute effects of coal tar pitch exposure and cardiopulmonary mortality among aluminum smelter workers. Am J Epidemiol 172: 790-799. http://dx.doi.org/10.1093/aje/kwq208

Friesen, MC; Macnab, YC; Marion, SA; Demers, PA; Davies, HW; Teschke, K. (2006). Mixed models and empirical bayes estimation for retrospective exposure assessment of dust exposures in Canadian sawmills. Ann Occup Hyg 50: 281-288. http://dx.doi.org/10.1093/annhyg/mei076

Gale, CR; O'Callaghan, FJ; Bredow, M; Martyn, CN; Team, ALSoPaCS. (2006). The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 118: 1486-1492. http://dx.doi.org/10.1542/peds.2005-2629

Galván, N; Page, TJ; Czuprynski, CJ; Jefcoate, CR. (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: 105-116. http://dx.doi.org/10.1016/j.taap.2005.09.018

Gao, M; Li, Y; Sun, Y; Shah, W; Yang, S; Wang, Y; Long, J. (2011). Benzo[a]pyrene exposure increases toxic biomarkers and morphological disorders in mouse cervix. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 109: 398-406. http://dx.doi.org/10.1111/j.1742-7843.2011.00755.x

Garçon, G; Gosset, P; Garry, S; Marez, T; Hannothiaux, MH; Shirali, P. (2001a). Pulmonary induction of proinflammatory mediators following the rat exposure to benzo(a)pyrene-coated onto Fe2O3 particles. Toxicol Lett 121: 107-117.

Garçon, G; Zerimech, F; Hannothiaux, M; Gosset, P; Martin, A; Marez, T; Shirali, P. (2001b). Antioxidant defense disruption by polycyclic aromatic hydrocarbons-coated onto Fe(2)O(3) particles in human lung cells (A549). Toxicology 166: 129-137.

Gaspari, L; Chang, SS; Santella, RM; Garte, S; Pedotti, P; Taioli, E. (2003). Polycyclic aromatic hydrocarbon-DNA adducts in human sperm as a marker of DNA damage and infertility. Mutat Res 535: 155-160.

Geacintov, NE; Cosman, M; Hingerty, BE; Amin, S; Broyde, S; Patel, DJ. (1997). NMR solution structures of stereoisometric covalent polycyclic aromatic carcinogen-DNA adduct: principles, patterns, and diversity [Review]. Chem Res Toxicol 10: 111-146. http://dx.doi.org/10.1021/tx9601418

Gehlhaus, MW; Gift, JS; Hogan, KA; Kopylev, L; Schlosser, PM; Kadry, AR. (2011). Approaches to cancer assessment in EPA's Integrated Risk Information System [Review]. Toxicol Appl Pharmacol 254: 170-180. http://dx.doi.org/10.1016/j.taap.2010.10.019

Gentner, NJ; Weber, LP. (2011). Intranasal benzo[a]pyrene alters circadian blood pressure patterns and causes lung inflammation in rats. Arch Toxicol 85: 337-346. http://dx.doi.org/10.1007/s00204-010-0589-6

Gibbs, GW. (1985). Mortality of aluminum reduction plant workers, 1950 through 1977. J Occup Environ Med 27: 761-770.

Gibbs, GW; Armstrong, B; Sevigny, M. (2007). Mortality and cancer experience of Quebec aluminum reduction plant workers. Part 2: mortality of three cohorts hired on or before january 1, 1951. J Occup Environ Med 49: 1105-1123. http://dx.doi.org/10.1097/JOM.0b013e318157d34a

Gibbs, GW; Horowitz, I. (1979). Lung cancer mortality in aluminum reduction plant workers. J Occup Med 21: 347-353.

Gibbs, GW; Sevigny, M. (2007a). Mortality and cancer experience of Quebec aluminum reduction plant workers, part 4: cancer incidence. J Occup Environ Med 49: 1351-1366. http://dx.doi.org/10.1097/JOM.0b013e318156ecbc

Gibbs, GW; Sevigny, M. (2007b). Mortality and cancer experience of Quebec aluminum reduction plant workers. Part 3: monitoring the mortality of workers first employed after January 1, 1950. J Occup Environ Med 49: 1269-1287. http://dx.doi.org/10.1097/JOM.0b013e3181593da8

Ginsberg, G; Hattis, D; Sonawane, B. (2004). Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants [Review]. Toxicol Appl Pharmacol 198: 164-183. http://dx.doi.org/10.1016/j.taap.2003.10.010

GLC (Great Lakes Commission). (2007). Assessment of benzo(a)pyrene air emissions in the Great Lakes region: A report of the Great Lakes Regional Toxic Air Emissions Inventory Steering Committee. Ann Arbor, MI.

Godschalk, R; Curfs, D; Bartsch, H; Van Schooten, FJ; Nair, J. (2003). Benzo[a]pyrene enhances lipid peroxidation induced DNA damage in aorta of apolipoprotein E knockout mice. Free Radic Res 37: 1299-1305.

Godschalk, R; Nair, J; van Schooten, FJ; Risch, A; Drings, P; Kayser, K; Dienemann, H; Bartsch, H. (2002). Comparison of multiple DNA adduct types in tumor adjacent human lung tissue: effect of cigarette smoking. Carcinogenesis 23: 2081-2086.

Godschalk, RW; Maas, LM; Kleinjans, JC; Van Schooten, FJ. (1998a). Influences of DNA isolation and RNA contamination on carcinogen-DNA adduct analysis by 32P-postlabeling. Environ Mol Mutagen 32: 344-350. http://dx.doi.org/10.1002/(SICI)1098-2280(1998)32:4<344::AID-EM8>3.0.CO;2-P

Godschalk, RW; Ostertag, JU; Moonen, EJ; Neumann, HA; Kleinjans, JC; van Schooten, FJ. (1998b). Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. Cancer Epidemiol Biomarkers Prev 7: 767-773.

Godschalk, RW; Ostertag, JU; Zandsteeg, AM; Van Agen, B; Neuman, HA; Van Straaten, H; Van Schooten, FJ. (2001). Impact of GSTM1 on aromatic-DNA adducts and p53 accumulation in human skin and lymphocytes. Pharmacogenetics 11: 537-543.

Graem, N. (1986). Epidermal changes following application of 7,12-dimethylbenz(a)anthracene and 12-O-tetradecanoylphorbol-13-acetate to human skin transplanted to nude mice studied with histological species markers. Cancer Res 46: 278-284.

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.

Grice, HC; Clayson, DB; Flamm, WG; Ito, N; Kroes, R; Newberne, PM; Scheuplein, R. (1986). Panel discussion: Possible mechanisms of BHA carcinogenicity from a consideration of its chemical and biological properties. Food Chem Toxicol 24: 1235-1242.

Grimmer, G; Brune, H; Deutsch-Wenzel, R; Dettbarn, G; Misfeld, J. (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: 733-739.

Grimmer, G; Brune, H; Deutsch-Wenzel, R; Naujack, KW; Misfeld, J; Timm, J. (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: 105-113.

Grova, N; Schroeder, H; Farinelle, S; Prodhomme, E; Valley, A; Muller, CP. (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: S295-S302. http://dx.doi.org/10.1016/j.chemosphere.2007.12.037

Grova, N; Valley, A; Turner, JD; Morel, A; Muller, CP; Schroeder, H. (2007). Modulation of behavior and NMDA-R1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. Neurotoxicology 28: 630-636. http://dx.doi.org/10.1016/j.neuro.2007.01.010

Gustavsson, P; Jakobsson, R; Johansson, H; Lewin, F; Norell, S; Rutkvist, LE. (1998). Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx, and oesophagus: A case-control study in Sweden. Occup Environ Med 55: 393-400.

Gustavsson, P; Plato, N; Lidstrom, EB; Hogstedt, C. (1990). Lung cancer and exposure to diesel exhaust among bus garage workers. Scand J Work Environ Health 16: 348-354.

Guyatt, GH; Oxman, AD; Kunz, R; Vist, GE; Falck-Ytter, Y; Schünemann, HJ. (2008a). GRADE: What is "quality of evidence" and why is it important to clinicians? [Review]. BMJ 336: 995-998. http://dx.doi.org/10.1136/bmj.39490.551019.BE

Guyatt, GH; Oxman, AD; Vist, GE; Kunz, R; Falck-Ytter, Y; Alonso-Coello, P; Schünemann, HJ. (2008b). GRADE: An emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336: 924-926. http://dx.doi.org/10.1136/bmj.39489.470347.AD

Habs, M; Jahn, SA; Schmähl, D. (1984). Carcinogenic activity of condensate from coloquint seeds (Citrullus colocynthis) after chronic epicutaneous administration to mice. J Cancer Res Clin Oncol 108: 154-156. http://dx.doi.org/10.1007/BF00390988

Habs, M; Schmähl, D; Misfeld, J. (1980). Local carcinogenicity of some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. Arch Geschwulstforsch 50: 266-274.

Hackman, P; Hou, SM; Nyberg, F; Pershagen, G; Lambert, B. (2000). Mutational spectra at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus in T-lymphocytes of nonsmoking and smoking lung cancer patients. Mutat Res 468: 45-61.

Hainaut, P; Pfeifer, GP. (2001). Patterns of p53 G-->T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. Carcinogenesis 22: 367-374.

Hakim, FT; Memon, SA; Cepeda, R; Jones, EC; Chow, CK; Kasten-Sportes, C; Odom, J; Vance, BA; Christensen, BL; Mackall, CL; Gress, RE. (2005). Age-dependent incidence, time course, and consequences of thymic renewal in adults. J Clin Invest 115: 930-939. http://dx.doi.org/10.1172/JCI22492

Hales, DB. (2002). Testicular macrophage modulation of Leydig cell steroidogenesis [Review]. J Reprod Immunol 57: 3-18.

Hammond, EC; Selikoff, IJ; Lawther, PL; Seidman, H. (1976). Inhalation of benzpyrene and cancer in man. Ann N Y Acad Sci 271: 116-124. http://dx.doi.org/10.1111/j.1749-6632.1976.tb23100.x

Hanahan, D; Weinberg, RA. (2011). Hallmarks of cancer: The next generation. Cell 144: 646-674. http://dx.doi.org/10.1016/j.cell.2011.02.013

Hansen, ES. (1989). Cancer incidence in an occupational cohort exposed to bitumen fumes. Scand J Work Environ Health 15: 101-105.

Hansen, ES. (1991). Mortality of mastic asphalt workers. Scand J Work Environ Health 17: 20-24. http://dx.doi.org/10.5271/sjweh.1739

Hardin, JA; Hinoshita, F; Sherr, DH. (1992). Mechanisms by which benzo[a]pyrene, an environmental carcinogen, suppresses B cell lymphopoiesis. Toxicol Appl Pharmacol 117: 155-164.

Harvey, RG. (1996). Mechanisms of carcinogenesis of polycyclic aromatic hydrocarbons. Polycycl Aromat Compd 9: 1-23. http://dx.doi.org/10.1080/10406639608031196

Hattis, D; Goble, R; Russ, A; Chu, M; Ericson, J. (2004). Age-related differences in susceptibility to carcinogenesis: A quantitative analysis of empirical animal bioassay data. Environ Health Perspect 112: 1152-1158. http://dx.doi.org/10.1289/ehp.6871

Henry, MC; Port, CD; Bates, RR; Kaufman, DG. (1973). Respiratory tract tumors in hamsters induced by benzo(a)pyrene. Cancer Res 33: 1585-1592.

HEW (U.S. Department of Health, Education and Welfare). (1964). Smoking and health: Report of the advisory committee to the surgeon general of the public health service. Washington, DC: U.S. Department of Health, Education, and Welfare. http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/NNBBMQ

Higginbotham, S; Ramakrishna, NVS; Johansson, SL; Rogan, EG; Cavalieri, EL. (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: 875-878. http://dx.doi.org/10.1093/carcin/14.5.875

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300.

Hoffmann, D; Rathkamp, G; Nesnow, S; Wynder, EL. (1972). Fluoranthenes: Quantitative determination in cigarette smoke, formation by pyrolysis, and tumor-initiating activity. J Natl Cancer Inst 49: 1165-1175.

Hogstedt, C; Jansson, C; Hugosson, M; Tinnerberg, H; Gustavsson, P. (2013). Cancer incidence in a cohort of Swedish chimney sweeps, 1958-2006. Am J Public Health 103: 1708-1714. http://dx.doi.org/10.2105/AJPH.2012.300860

Holladay, SD; Smialowicz, RJ. (2000). Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure [Review]. Environ Health Perspect 108: 463-473.

Holladay, SD; Smith, BJ. (1994). Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: A cytometric evaluation. J Toxicol Environ Health 42: 259-273. http://dx.doi.org/10.1080/15287399409531878

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: 99-104.

Homburger, F; Hsueh, SS; Kerr, CS; Russfield, AB. (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: 360-366.

Hood, DB; Nayyar, T; Ramesh, A; Greenwood, M; Inyang, F. (2000). Modulation in the developmental expression profile of Sp1 subsequent to transplacental exposure of fetal rats to desorbed benzo[a]pyrene following maternal inhalation. Inhal Toxicol 12: 511-535. http://dx.doi.org/10.1080/089583700402897

HSDB (Hazardous Substances Data Bank). (2012). Benzo(a)pyrene [Database]. Bethesda, MD: National Library of Medicine. Retrieved from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Hsu, PC; Chen, IY; Pan, CH; Wu, KY; Pan, MH; Chen, JR; Chen, CJ; Chang-Chien, GP; Hsu, CH; Liu, CS; Wu, MT. (2006). Sperm DNA damage correlates with polycyclic aromatic hydrocarbons biomarker in coke-oven workers. Int Arch Occup Environ Health 79: 349-356. http://dx.doi.org/10.1007/s00420-005-0066-3

IARC (International Agency for Research on Cancer). (1973). Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. (HEEP/74/11596). Lyon, France.

IARC (International Agency for Research on Cancer). (1983). Polynuclear aromatic compounds, part 1: Chemical, environmental and experimental data. Lyon, France: World Health Organization. http://monographs.iarc.fr/ENG/Monographs/vol32/volume32.pdf

IARC (International Agency for Research on Cancer). (2002). Section 3. Carcinogenicity studies in experimental animals [IARC Monograph] (pp. 973-1003). Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol83/mono83-6C.pdf

IARC (International Agency for Research on Cancer). (2003). Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans: Views and expert opinions of an IARC working group, Lyon, 29 November - 1 December 1999. (IARC Technical Publication No. 39). Lyon, France. http://monographs.iarc.fr/ENG/Publications/techrep39/IARCrep39.pdf

IARC (International Agency for Research on Cancer). (2004). Tobacco smoke and involuntary smoking [IARC Monograph] (pp. 1-1438). (RISKLINE/2004080004). Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol83/mono83.pdf

IARC (International Agency for Research on Cancer). (2006). Preamble to the IARC monographs. Lyon, France. http://monographs.iarc.fr/ENG/Preamble/

IARC (International Agency for Research on Cancer). (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures [IARC Monograph] (pp. 1-853). Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf

IARC (International Agency for Research on Cancer). (2012). Soot, as found in occupational exposure of chimney sweeps [IARC Monograph]. In Chemical agents and related occupations A review of human carcinogens (pp. 209-214). Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-21.pdf

Ickstadt, K; Schäfer, M; Fritsch, A; Schwender, H; Abel, J; Bolt, HM; Brüning, T; Ko, YD; Vetter, H; Harth, V. (2008). Statistical methods for detecting genetic interactions: a head and neck squamous-cell cancer study. J Toxicol Environ Health A 71: 803-815. http://dx.doi.org/10.1080/15287390801985745

Inyang, F; Ramesh, A; Kopsombut, P; Niaz, MS; Hood, DB; Nyanda, AM; Archibong, AE. (2003). Disruption of testicular steroidogenesis and epididymal function by inhaled benzo(a)pyrene. Reprod Toxicol 17: 527-537.

IOM (Institute of Medicine). (2008). Improving the presumptive disability decision-making process for veterans. In JM Samet; CC Bodurow (Eds.). Washington, DC: National Academies Press. http://www.nap.edu/openbook.php?record_id=11908

IPCS (International Programme on Chemical Safety). (1998). Selected non-heterocyclic polycyclic aromatic hydrocarbons [WHO EHC]. In Environmental Health Criteria (pp. CHF 174). (CIS/00/00657). Geneva, Switzerland: World Health Organization. http://www.inchem.org/documents/ehc/ehc/ehc202.htm

Jayasekara, S; Sharma, RP; Drown, DB. (1992). Effects of benzo[a]pyrene on steady-state levels of biogenic amines and metabolizing enzymes in mouse brain regions. Ecotoxicol Environ Saf 24: 1-12. http://dx.doi.org/10.1016/0147-6513(92)90029-3

Jeffrey, AM; Jennette, KW; Blobstein, SH; Weinstein, IB; Beland, FA; Harvey, RG; Kasal, H; Miura, I; Nakanishi, K. (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: 5714-5715.

Jeng, HA; Yordt, D; Davis, S; Swanson, JR. (2013). Assessment of alteration of reproductive system in vivo induced by subchronic exposure to benzo(a)pyrene via oral administration. Environ Toxicol. http://dx.doi.org/10.1002/tox.21889

Jensen, TK; Henriksen, TB; Hjollund, NH; Scheike, T; Kolstad, H; Giwercman, A; Ernst, E; Bonde, JP; Skakkebaek, NE; Olsen, J. (1998). Adult and prenatal exposures to tobacco smoke as risk indicators of fertility among 430 Danish couples. Am J Epidemiol 148: 992-997.

Jerina, DM; Chadha, A; Cheh, AM; Schurdak, ME; Wood, AW; Sayer, JM. (1991). Covalent bonding of bay-region diol epoxides to nucleic acids [Review]. Adv Exp Med Biol 283: 533-553.

Jiang, H; Gelhaus, SL; Mangal, D; Harvey, RG; Blair, IA; Penning, TM. (2007). Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. Chem Res Toxicol 20: 1331-1341. http://dx.doi.org/10.1021/tx700107z

Jiang, H; Shen, YM; Quinn, AM; Penning, TM. (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: 365-374. http://dx.doi.org/10.1021/tx0497245

Jules, GE; Pratap, S; Ramesh, A; Hood, DB. (2012). In utero exposure to benzo(a)pyrene predisposes offspring to cardiovascular dysfunction in later-life. Toxicology 295: 56-67. http://dx.doi.org/10.1016/j.tox.2012.01.017

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: 502-516.

Kapitulnik, J; Wislocki, PG; Levin, W; Yagi, H; Jerina, DM; Conney, AH. (1978). Tumorigenicity studies with diol-epoxides of benzo(a)pyrene which indicate that "(+/-)"-trans-7"beta",8"alpha"-dihydroxy-9"alpha" 10"alpha"-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an ultimate carcinogen in newborn mice. Cancer Res 38: 354-358.

Kappes, U; Schliemann-Willers, S; Bankova, L; Heinemann, C; Fischer, TW; Ziemer, M; Schubert, H; Norgauer, J; Fluhr, JW; Elsner, P. (2004). The quality of human skin xenografts on SCID mice: A

noninvasive bioengineering approach. Br J Dermatol 151: 971-976. http://dx.doi.org/10.1111/j.1365-2133.2004.06191.x

Karakaya, A; Ates, I; Yucesoy, B. (2004). Effects of occupational polycyclic aromatic hydrocarbon exposure on T-lymphocyte functions and natural killer cell activity in asphalt and coke oven workers. Hum Exp Toxicol 23: 317-322.

Karakaya, A; Yücesoy, B; Turhan, A; Erdem, O; Burgaz, S; Karakaya, AE. (1999). Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. Toxicology 135: 43-47.

Karlehagen, S; Andersen, A; Ohlson, CG. (1992). Cancer incidence among creosote-exposed workers. Scand J Work Environ Health 18: 26-29. http://dx.doi.org/10.5271/sjweh.1612

Kee, K; Flores, M; Cedars, MI; Reijo Pera, RA. (2010). Human primordial germ cell formation is diminished by exposure to environmental toxicants acting through the AHR signaling pathway. Toxicol Sci 117: 218-224. http://dx.doi.org/10.1093/toxsci/kfq179

Keohavong, P; Lan, Q; Gao, WM; DeMarini, DM; Mass, MJ; Li, XM; Roop, BC; Weissfeld, J; Tian, D; Mumford, JL. (2003). K-ras mutations in lung carcinomas from nonsmoking women exposed to unvented coal smoke in China. Lung Cancer 41: 21-27. http://dx.doi.org/10.1016/S0169-5002(03)00125-9

Ketkar, M; Reznik, G; Schneider, P; Mohr, U. (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: 235-239. http://dx.doi.org/10.1016/S0304-3835(78)94787-0

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: 6074-6078.

Knafla, A; Petrovic, S; Richardson, M; Campbell, J; Rowat, C. (2011). Development and application of a skin cancer slope factor for exposures to benzo[a]pyrene in soil. Regul Toxicol Pharmacol 59: 101-110. http://dx.doi.org/10.1016/j.yrtph.2010.09.011

Knuckles, ME; Inyang, F; Ramesh, A. (2001). Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. Toxicol Sci 61: 382-388.

Kolb, B; Whishaw, IQ. (1989). Plasticity in the neocortex: mechanisms underlying recovery from early brain damage. Prog Neurobiol 32: 235-276.

Kong, LY; Luster, MI; Dixon, D; O'Grady, J; Rosenthal, GJ. (1994). Inhibition of lung immunity after intratracheal instillation of benzo(a)pyrene. Am J Respir Crit Care Med 150: 1123-1129.

Koreeda, M; Moore, PD; Wislocki, PG; Levin, W; Yagi, H; Jerina, DM. (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: 778-781.

Kristensen, P; Eilertsen, E; Einarsdottir, E; Haugen, A; Skaug, V; Ovrebo, S. (1995). Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. Environ Health Perspect 103: 588-590. http://dx.doi.org/10.1289/ehp.95103588

Kroese, ED; Muller, JJA; Mohn, GR; Dortant, PM; Wester, PW. (2001). Tumorigenic effects in Wistar rats orally administered benzo[a]pyrene for two years (gavage studies): Implications for human cancer risks associated with oral exposure to polycyclic aromatic hydrocarbons. (658603 010). Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM). http://www.rivm.nl/bibliotheek/rapporten/658603010.pdf

Kummer, V; Masková, J; Zralý, Z; Matiasovic, J; Faldyna, M. (2007). Effect of postnatal exposure to benzo[a]pyrene on the uterus of immature rats. Exp Toxicol Pathol 59: 69-76. http://dx.doi.org/10.1016/j.etp.2007.02.007

Kummer, V; Masková, J; Zralý, Z; Neca, J; Simecková, P; Vondrácek, J; Machala, M. (2008). Estrogenic activity of environmental polycyclic aromatic hydrocarbons in uterus of immature Wistar rats. Toxicol Lett 180: 212-221. http://dx.doi.org/10.1016/j.toxlet.2008.06.862

Kuper, CF; Beems, RB; Bruijntjes, JP; Schuurman, HJ; Vos, JG. (1992). Normal development, growth, and aging of the thymus. In U Mohr; DL Dungworth; CC Capen (Eds.), Pathobiology of the aging rat, volume 1 (pp. 25-49). Washington, DC: ILSI press.

Kuper, CF; De Heer, E; Van Loveren, H; Vos, JG. (2002). Immune system. In MA Wallig (Ed.), Handbook of toxicologic pathology (2nd ed., pp. 585-644). San Diego, CA: Academic Press.

Landreth, KS. (2002). Critical windows in development of the rodent immune system [Review]. Hum Exp Toxicol 21: 493-498.

Landreth, KS; Dodson, SVM. (2005). Development of the rodent immune system. In SD Holladay (Ed.), Developmental immunotoxicology (pp. 3-19). Boca Raton, FL: CRC Press. http://dx.doi.org/10.1201/9781420024036.pt1

Lavoie, EJ; Amin, S; Hecht, SS; Furuya, K; Hoffmann, D. (1982). Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. Carcinogenesis 3: 49-52. http://dx.doi.org/10.1093/carcin/3.1.49

Lavoie, EJ; Braley, J; Rice, JE; Rivenson, A. (1987). Tumorigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. Cancer Lett 34: 15-20.

Lavoie, EJ; Cai, ZW; Meschter, CL; Weyand, EH. (1994). Tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn CD-1 mice. Carcinogenesis 15: 2131-2135. http://dx.doi.org/10.1093/carcin/15.10.2131

Lavoué, J; Gérin, M; Côté, J; Lapointe, R. (2007). Mortality and cancer experience of Quebec aluminum reduction plant workers. Part I: The reduction plants and coal tar pitch volatile (CTPV) exposure assessment. J Occup Environ Med 49: 997-1008. http://dx.doi.org/10.1097/JOM.0b013e3181484cf3

Letzel, S; Drexler, H. (1998). Occupationally related tumors in tar refinery workers. J Am Acad Dermatol 39: 712-720. http://dx.doi.org/10.1016/S0190-9622(98)70043-X

Levin, W; Wood, AW; Wislocki, PG; Kapitulnik, J; Yagi, H; Jerina, DM; Conney, AH. (1977). Carcinogenicity of benzo-ring derivatives of benzo(a)pyrene on mouse skin. Cancer Res 37: 3356-3361.

Li, D; Firozi, PF; Wang, LE; Bosken, CH; Spitz, MR; Hong, WK; Wei, Q. (2001). Sensitivity to DNA damage induced by benzo(a)pyrene diol epoxide and risk of lung cancer: a case-control analysis. Cancer Res 61: 1445-1450.

Li, Z; Chadalapaka, G; Ramesh, A; Khoshbouei, H; Maguire, M; Safe, S; Rhoades, RE; Clark, R; Jules, G; Mccallister, M; Aschner, M; Hood, DB. (2012). PAH particles perturb prenatal processes and phenotypes: protection from deficits in object discrimination afforded by dampening of brain oxidoreductase following in utero exposure to inhaled benzo(a)pyrene. Toxicol Sci 125: 233-247. http://dx.doi.org/10.1093/toxsci/kfr261

Liang, J; Zhu, H; Li, C; Ding, Y; Zhou, Z; Wu, Q. (2012). Neonatal exposure to benzo[a]pyrene decreases the levels of serum testosterone and histone H3K14 acetylation of the StAR promoter in the testes of SD rats. Toxicology 302: 285-291. http://dx.doi.org/10.1016/j.tox.2012.08.010

Lindelöf, B; Sigurgeirsson, B. (1993). PUVA and cancer: a case-control study. Br J Dermatol 129: 39-41. http://dx.doi.org/10.1111/j.1365-2133.1993.tb03308.x

Liu, M; Grant, SG; Macina, OT; Klopman, G; Rosenkranz, HS. (1997). Structural and mechanistic bases for the induction of mitotic chromosomal loss and duplication (malsegregation') in the yeast Saccharomyces cerevisiae: Relevance to human carcinogenesis and developmental toxicology. Mutat Res-Fundam Mol Mech Mutagen 374: 209-231. http://dx.doi.org/10.1016/S0027-5107(96)00236-9

Liu, S; Abdelrahim, M; Khan, S; Ariazi, E; Jordan, VC; Safe, S. (2006). Aryl hydrocarbon receptor agonists directly activate estrogen receptor alpha in MCF-7 breast cancer cells. Biol Chem 387: 1209-1213. http://dx.doi.org/10.1515/BC.2006.149

Liu, SH; Wang, JH; Chuu, JJ; Lin-Shiau, SY. (2002). Alterations of motor nerve functions in animals exposed to motorcycle exhaust. J Toxicol Environ Health A 65: 803-812. http://dx.doi.org/10.1080/00984100290071144

Liu, Z; Muehlbauer, KR; Schmeiser, HH; Hergenhahn, M; Belharazem, D; Hollstein, MC. (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: 2583-2587. http://dx.doi.org/10.1158/0008-5472.CAN-04-3675

Luebke, RW; Chen, DH; Dietert, R; Yang, Y; King, M; Luster, MI. (2006). The comparative immunotoxicity of five selected compounds following developmental or adult exposure [Review]. J Toxicol Environ Health B Crit Rev 9: 1-26. http://dx.doi.org/10.1080/15287390500194326

Lummus, ZL; Henningsen, G. (1995). Modulation of T-cell ontogeny by transplacental benzo(a)pyrene. International Journal of Immunopharmacology 17: 339-350.

Luster, MI; Portier, C; Pait, DG; White, KL, Jr; Gennings, C; Munson, AE; Rosenthal, GJ. (1992). Risk assessment in immunotoxicology: I: Sensitivity and predictability of immune tests. Fundam Appl Toxicol 18: 200-210. http://dx.doi.org/10.1016/0272-0590(92)90047-L

Lyte, M; Bick, PH. (1985). Differential immunotoxic effects of the environmental chemical benzo[a]pyrene in young and aged mice. Mech Ageing Dev 30: 333-341. http://dx.doi.org/10.1016/0047-6374(85)90122-8

Ma, Q; Lu, AY. (2007). CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies [Review]. Drug Metab Dispos 35: 1009-1016. http://dx.doi.org/10.1124/dmd.107.015826

Maciel, ES; Biasibetti, R; Costa, AP; Lunardi, P; Schunck, RV; Becker, GC; Arbo, MD; Dallegrave, E; Gonçalves, CA; Saldiva, PH; Garcia, SC; Leal, RB; Leal, MB. (2014). Subchronic oral administration of Benzo[a]pyrene impairs motor and cognitive behavior and modulates S100B levels and MAPKs in rats. Neurochem Res 39: 731-740. http://dx.doi.org/10.1007/s11064-014-1261-y

Mackall, CL; Fleisher, TA; Brown, MR; Andrich, MP; Chen, CC; Feuerstein, IM; Horowitz, ME; Magrath, IT; Shad, AT; Steinberg, SM. (1995). Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 332: 143-149. http://dx.doi.org/10.1056/NEJM199501193320303

Mackenzie, KM; Angevine, DM. (1981). Infertility in mice exposed in utero to benzo(a)pyrene. Biol Reprod 24: 183-192.

Mahler, BJ; Van Metre, PC; Bashara, TJ; Wilson, JT; Johns, DA. (2005). Parking lot sealcoat: an unrecognized source of urban polycyclic aromatic hydrocarbons. Environ Sci Technol 39: 5560-5566. http://dx.doi.org/10.1021/es0501565

Malaveille, C; Kuroki, T; Sims, P; Grover, PL; Bartsch, H. (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: 313-326.

Mancini, R; Romano, G; Sgambato, A; Flamini, G; Giovagnoli, MR; Boninsegna, A; Carraro, C; Vecchione, A; Cittadini, A. (1999). Polycyclic aromatic hydrocarbon-DNA adducts in cervical smears of smokers and nonsmokers. Gynecol Oncol 75: 68-71. http://dx.doi.org/10.1006/gyno.1999.5525

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: 586-589. http://dx.doi.org/10.1038/310586a0

Mass, MJ; Jeffers, AJ; Ross, JA; Nelson, G; Galati, AJ; Stoner, GD; Nesnow, S. (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: 186-192. http://dx.doi.org/10.1002/mc.2940080309

Matiasovic, J; Leva, L; Maskova, J; Kummer, V; Faldyna, M. (2008). Effects of postnatal exposure to benzo[a]pyrene on the immunity of immature rats. Veterinarni Medicina 53: 93-100.

Matikainen, T; Perez, GI; Jurisicova, A; Pru, JK; Schlezinger, JJ; Ryu, HY; Laine, J; Sakai, T; Korsmeyer, SJ; Casper, RF; Sherr, DH; Tilly, JL. (2001). Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet 28: 355-360. http://dx.doi.org/10.1038/ng575

Matikainen, TM; Moriyama, T; Morita, Y; Perez, GI; Korsmeyer, SJ; Sherr, DH; Tilly, JL. (2002). Ligand activation of the aromatic hydrocarbon receptor transcription factor drives Bax-dependent apoptosis in developing fetal ovarian germ cells. Endocrinology 143: 615-620.

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 oocyte toxicity of polycyclic aromatic hydrocarbons in mice. Cancer Res 39: 3471-3475.

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. Pediatr Pharmacol 1: 143-151.

Matullo, G; Peluso, M; Polidoro, S; Guarrera, S; Munnia, A; Krogh, V; Masala, G; Berrino, F; Panico, S; Tumino, R; Vineis, P; Palli, D. (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: 674-677.

Mauderly, JL. (1997). Relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk [Review]. Environ Health Perspect 105 (Suppl 5): 1337-1346.

Maughan, WZ; Muller, SA; Perry, HO; Pittelkow, MR; O'Brien, PC. (1980). Incidence of skin cancers in patients with atopic dermatitis treated with ocal tar: A 25-year follow-up study. J Am Acad Dermatol 3: 612-615.

McCallister, MM; Maguire, M; Ramesh, A; Qiao, AM; Sheng, L; Khoshbouei, H; Aschner, M; Ebner, FF; Hood, DB. (2008). Prenatal exposure to benzo(a)pyrene impairs later-life cortical neuronal function. Neurotoxicology 29: 846-854. http://dx.doi.org/10.1016/j.neuro.2008.07.008

McConnell, EE; Solleveld, HA; Swenberg, JA; Boorman, GA. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76: 283-289.

Mccoull, KD; Rindgen, D; Blair, IA; Penning, TM. (1999). Synthesis and characterization of polycyclic aromatic hydrocarbon o-quinone depurinating N7-guanine adducts. Chem Res Toxicol 12: 237-246. http://dx.doi.org/10.1021/tx980182z

Melikian, AA; Sun, P; Prokopczyk, B; El-Bayoumy, K; Hoffmann, D; Wang, X; Waggoner, S. (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: 127-134. http://dx.doi.org/10.1016/S0304-3835(99)00203-7

Midgette, AS; Baron, JA. (1990). Cigarette smoking and the risk of natural menopause [Review]. Epidemiology 1: 474-480.

Mielzyńska, D; Siwińska, E; Kapka, L; Szyfter, K; Knudsen, LE; Merlo, DF. (2006). The influence of environmental exposure to complex mixtures including PAHs and lead on genotoxic effects in children living in Upper Silesia, Poland. Mutagenesis 21: 295-304. http://dx.doi.org/10.1093/mutage/gel037

Miller, KP; Ramos, KS. (2001). Impact of cellular metabolism on the biological effects of benzo[a]pyrene and related hydrocarbons [Review]. Drug Metab Rev 33: 1-35. http://dx.doi.org/10.1081/DMR-100000138

Miller, MM; Plowchalk, DR; Weitzman, GA; London, SN; Mattison, DR. (1992). The effect of benzo(a)pyrene on murine ovarian and corpora lutea volumes. Am J Obstet Gynecol 166: 1535-1541.

Mitropoulos, P; Norman, R. (2005). Occupational nonsolar risk factors of squamous cell carcinoma of the skin: a population-based case-controlled study. Dermatol Online J 11: 5.

Mohamed, E; Song, WH; Oh, SA; Park, YJ; You, YA; Lee, S; Choi, JY; Kim, YJ; Jo, I; Pang, MG. (2010). The transgenerational impact of benzo(a)pyrene on murine male fertility. Hum Reprod 25: 2427-2433. http://dx.doi.org/10.1093/humrep/deq205

Moody, RP; Chu, I. (1995). Dermal exposure to environmental contaminants in the Great Lakes [Review]. Environ Health Perspect 103 Suppl 9: 103-114.

Moody, RP; Joncas, J; Richardson, M; Chu, I. (2007). Contaminated soils (I): In vitro dermal absorption of benzo[a]pyrene in human skin. J Toxicol Environ Health A 70: 1858-1865. http://dx.doi.org/10.1080/15287390701459296

Moolgavkar, SH; Knudson, AG, Jr. (1981). Mutation and cancer: A model for human carcinogenesis. J Natl Cancer Inst 66: 1037-1052.

Moolgavkar, SH; Venzon, DJ. (1979). Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math Biosci 47: 55-77. http://dx.doi.org/10.1016/0025-5564(79)90005-1

Moulin, JJ; Wild, P; Mur, JM; Lafontaine, M; Lefer, M; Mercier-Gallay, M; Villemot, P; Whebi, V; Coulon, JP. (1989). Risk of lung, larynx, pharynx and buccal cavity cancers among carbon electrode manufacturing workers. Scand J Work Environ Health 15: 30-37. http://dx.doi.org/10.5271/sjweh.1885

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.

Munson, AE; White, KLJ. (1983). Effects of subchronic 14-day exposure to benzopyrene in b6c3f1 male and female mice on specific immunological parameters. American Petroleum Institute.

N'Diaye, M; Le Ferrec, E; Lagadic-Gossmann, D; Corre, S; Gilot, D; Lecureur, V; Monteiro, P; Rauch, C; Galibert, MD; Fardel, O. (2006). Aryl hydrocarbon receptor- and calcium-dependent induction of the chemokine CCL1 by the environmental contaminant benzo[a]pyrene. J Biol Chem 281: 19906-19915. http://dx.doi.org/10.1074/jbc.M601192200

Nanez, A; Ramos, IN; Ramos, KS. (2011). A mutant Ahr allele protects the embryonic kidney from hydrocarbon-induced deficits in fetal programming. Environ Health Perspect 119: 1745-1753. http://dx.doi.org/10.1289/ehp.1103692

Näslund, 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: 411-418.

Naufal, Z; Zhiwen, L; Zhu, L; Zhou, GD; Mcdonald, T; He, LY; Mitchell, L; Ren, A; Zhu, H; Finnell, R; Donnelly, KC. (2010). Biomarkers of exposure to combustion by-products in a human population in Shanxi, China. J Expo Sci Environ Epidemiol 20: 310-319. http://dx.doi.org/10.1038/jes.2009.19

Neal, J; Rigdon, RH. (1967). Gastric tumors in mice fed benzo(a)pyrene: A quantitative study. Tex Rep Biol Med 25: 553-557.

Neal, MS; Mulligan Tuttle, AM; Casper, RF; Lagunov, A; Foster, WG. (2010). Aryl hydrocarbon receptor antagonists attenuate the deleterious effects of benzo[a]pyrene on isolated rat follicle development. Reprod Biomed Online 21: 100-108. http://dx.doi.org/10.1016/j.rbmo.2010.03.025

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. Reprod Toxicol 25: 100-106. http://dx.doi.org/10.1016/j.reprotox.2007.10.012

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. Hum Reprod 22: 961-967. http://dx.doi.org/10.1093/humrep/del487

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 [Review]. Ann N Y Acad Sci 685: 624-640. http://dx.doi.org/10.1111/j.1749-6632.1993.tb35928.x

Nesnow, S; Mass, MJ; Ross, JA; Galati, AJ; Lambert, GR; Gennings, C; Carter, WH; Stoner, GD. (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; Stoner, GD. (1998b). Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. Exp Lung Res 24: 395-405.

Nesnow, S; Ross, JA; Stoner, GD; Mass, MJ. (1995). Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. Toxicology 105: 403-413. http://dx.doi.org/10.1016/0300-483X(95)03238-B

Nesnow, S; Ross, JA; Stoner, GD; Mass, MJ. (1996). Tumorigenesis of Carcinogenic Environmental Polycyclic Aromatic Hydrocarbons in Strain A/J Mice: Linkage to DNA Adducts and Mutations in Oncogenes. Polycycl Aromat Compd 10: 259-266. http://dx.doi.org/10.1080/10406639608034705

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.

Nettesheim, P; Griesemer, RA; Martin, DH; Caton, JE, Jr. (1977). Induction of preneoplastic and neoplastic lesions in grafted rat tracheas continuously exposed to benzo(a)pyrene. Cancer Res 37: 1272-1278.

Newbold, RF; Brookes, P. (1976). Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature 261: 52-54. http://dx.doi.org/10.1038/261052a0

Nikonova, TV. (1977). [Transplacental effect of benz(a)pyrene and pyrene]. Biull Eksp Biol Med 84: 88-91.

NIOSH (National Institute for Occupational Safety and Health). (1989). Assessment of the cocarcinogenic/promoting activity of asphalt fumes. http://www.ntis.gov/search/product.aspx?ABBR=PB91110213

Niu, Q; Zhang, H; Li, X; Li, M. (2010). Benzo[a]pyrene-induced neurobehavioral function and neurotransmitter alterations in coke oven workers. Occup Environ Med 67: 444-448. http://dx.doi.org/10.1136/oem.2009.047969

NRC (National Research Council). (1983). Risk assessment in the federal government: Managing the process. Washington, DC: National Academies Press. http://www.nap.edu/openbook.php?record_id=366&page=R1

NRC (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: National Academies Press. http://www.nap.edu/catalog/12209.html

NRC (National Research Council). (2011). Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. Washington, DC: National Academies Press. http://www.nap.edu/catalog/13142.html

OECD (Organisation for Economic Co-operation and Development). (2009). Series on testing and assesment: Number 39. Guidance document on acute inhalation toxicity testing [OECD SIDS]. (ENV/JM/MONO(2009)28; JT03268144). Paris, Fr. http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282009%2928&doclanguage=en

Okey, AB; Riddick, DS; Harper, PA. (1994). Molecular biology of the aromatic hydrocarbon (dioxin) receptor [Review]. Trends Pharmacol Sci 15: 226-232. http://dx.doi.org/10.1016/0165-6147(94)90316-6

Olsen, AK; Andreassen, A; Singh, R; Wiger, R; Duale, N; Farmer, PB; Brunborg, G. (2010). Environmental exposure of the mouse germ line: DNA adducts in spermatozoa and formation of de novo mutations during spermatogenesis. PLoS ONE 5: e11349. http://dx.doi.org/10.1371/journal.pone.0011349

Olsson, A; Kromhout, H; Agostini, M; Hansen, J; Lassen, CF; Johansen, C; Kjaerheim, K; Langård, S; Stücker, I; Ahrens, W; Behrens, T; Lindbohm, ML; Heikkilä, P; Heederik, D; Portengen, L; Shaham, J; Ferro, G; de Vocht, F; Burstyn, I; Boffetta, P. (2010). A case-control study of lung cancer nested in a cohort of European asphalt workers. Environ Health Perspect 118: 1418-1424. http://dx.doi.org/10.1289/ehp.0901800

ONS (Office for National Statistics). (2003). Cancer statistics registrations: Registrations of cancer diagnosed in 2003, England. London, UK: Palgrave Macmillan. http://www.ons.gov.uk/ons/rel/vsob1/cancer-statistics-registrations--england--series-mb1-/no--34--2003/index.html

Park, JH; Gelhaus, S; Vedantam, S; Oliva, AL; Batra, A; Blair, IA; Troxel, AB; Field, J; Penning, TM. (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: 1039-1049. http://dx.doi.org/10.1021/tx700404a

Park, JH; Troxel, AB; Harvey, RG; Penning, TM. (2006). 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: 719-728. http://dx.doi.org/10.1021/tx0600245

Pate Capps, N; Stewart, A; Burns, C. (2009). The interplay between secondhand cigarette smoke, genetics, and cervical cancer: a review of the literature [Review]. Biol Res Nurs 10: 392-399. http://dx.doi.org/10.1177/1099800408330849

Pavanello, S; Favretto, D; Brugnone, F; Mastrangelo, G; Dal Pra, G; Clonfero, E. (1999). HPLC/fluorescence determination of anti-BPDE-DNA adducts in mononuclear white blood cells from PAH-exposed humans. Carcinogenesis 20: 431-435.

Pavanello, S; Pulliero, A; Siwinska, E; Mielzynska, D; Clonfero, E. (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: 169-175. http://dx.doi.org/10.1093/carcin/bgh303

Pavanello, S; Siwinska, E; Mielzynska, D; Clonfero, E. (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: 53-62. http://dx.doi.org/10.1016/j.mrgentox.2003.10.019

Penit, C; Vasseur, F. (1989). Cell proliferation and differentiation in the fetal and early postnatal mouse thymus. J Immunol 142: 3369-3377.

Penning, TM; Burczynski, ME; Hung, CF; Mccoull, KD; Palackal, NT; Tsuruda, LS. (1999). Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones [Review]. Chem Res Toxicol 12: 1-18. http://dx.doi.org/10.1021/tx980143n

Penning, TM; Ohnishi, ST; Ohnishi, T; Harvey, RG. (1996). Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. Chem Res Toxicol 9: 84-92. http://dx.doi.org/10.1021/tx950055s

Perera, F; Li, TY; Lin, C; Tang, D. (2012a). Effects of prenatal polycyclic aromatic hydrocarbon exposure and environmental tobacco smoke on child IQ in a Chinese cohort. Environ Res 114: 40-46. http://dx.doi.org/10.1016/j.envres.2011.12.011

Perera, FP; Li, Z; Whyatt, R; Hoepner, L; Wang, S; Camann, D; Rauh, V. (2009). Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics 124: e195-e202. http://dx.doi.org/10.1542/peds.2008-3506

Perera, FP; Rauh, V; Whyatt, RM; Tang, D; Tsai, WY; Bernert, JT; Tu, YH; Andrews, H; Barr, DB; Camann, DE; Diaz, D; Dietrich, J; Reyes, A; Kinney, PL. (2005a). A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures [Review]. Neurotoxicology 26: 573-587. http://dx.doi.org/10.1016/j.neuro.2004.07.007

Perera, FP; Rauh, V; Whyatt, RM; Tsai, WY; Bernert, JT; Tu, YH; Andrews, H; Ramirez, J; Qu, L; Tang, D. (2004). Molecular evidence of an interaction between prenatal environmental exposure and birth outcomes in a multiethnic population. Environ Health Perspect 112: 626-630.

Perera, FP; Tang, D; Rauh, V; Lester, K; Tsai, WY; Tu, YH; Weiss, L; Hoepner, L; King, J; Del Priore, G; Lederman, SA. (2005b). Relationships among polycyclic aromatic hydrocarbon-DNA adducts, proximity to the World Trade Center, and effects on fetal growth. Environ Health Perspect 113: 1062-1067.

Perera, FP; Tang, D; Wang, S; Vishnevetsky, J; Zhang, B; Diaz, D; Camann, D; Rauh, V. (2012b). Prenatal Polycyclic Aromatic Hydrocarbon (PAH) Exposure and Child Behavior at Age 6-7 Years. Environ Health Perspect 120: 921-926. http://dx.doi.org/10.1289/ehp.1104315

Perera, FP; Weinstein, IB. (2000). Molecular epidemiology: Recent advances and future directions [Review]. Carcinogenesis 21: 517-524. http://dx.doi.org/10.1093/carcin/21.3.517

Perrin, J; Tassistro, V; Mandon, M; Grillo, JM; Botta, A; Sari-Minodier, I. (2011a). Tobacco consumption and benzo(a)pyrene-diol-epoxide-DNA adducts in spermatozoa: in smokers, swim-up procedure selects spermatozoa with decreased DNA damage. Fertil Steril 95: 2013-2017. http://dx.doi.org/10.1016/j.fertnstert.2011.02.021

Perrin, J; Tassistro, V; Paulmyer-Lacroix, O; Courbière, B; Botta, A; Sari-Minodier, I. (2011b). In smokers, swim-up and discontinuous gradient centrifugation recover spermatozoa with equally lower amounts of DNA damage than spermatozoa obtained from neat semen. Fertil Steril 95: 2680-2682. http://dx.doi.org/10.1016/j.fertnstert.2011.04.092

Petrie, HT. (2002). Role of thymic organ structure and stromal composition in steady-state postnatal T-cell production [Review]. Immunol Rev 189: 8-19.

Pfeifer, GP; Denissenko, MF; Olivier, M; Tretyakova, N; Hecht, SS; Hainaut, P. (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers [Review]. Oncogene 21: 7435-7451. http://dx.doi.org/10.1038/sj.onc.1205803

Pfeifer, GP; Hainaut, P. (2003). On the origin of G --> T transversions in lung cancer [Review]. Mutat Res 526: 39-43. http://dx.doi.org/10.1016/S0027-5107(03)00013-7

Pfeiffer, CA; Allen, E. (1948). Attempts to produce cancer in rhesus monkeys with carcinogenic hydrocarbons and estrogens. Cancer Res 8: 97-127.

Pfeiffer, EH. (1977). Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons in mice. In Air pollution and cancer in man. Lyon, France: IARC.

Phillips, DH. (2002). Smoking-related DNA and protein adducts in human tissues [Review]. Carcinogenesis 23: 1979-2004.

Pittelkow, MR; Perry, HO; Muller, SA; Maughan, WZ; O'Brien, PC. (1981). Skin cancer in patients with psoriasis treated with coal tar: A 25-year follow-up study. Arch Dermatol 117: 465-468.

Poel, WE. (1959). Effect of carcinogenic dosage and duration of exposure on skin-tumor induction in mice. J Natl Cancer Inst 22: 19-43.

Poel, WE. (1963). Skin as a test site for the bioassay of carcinogens and carcinogen precursors [IARC Monograph]. In Biology of cutaneous cancers (pp. 611-631). Poel, WE.

Postlind, H; Vu, TP; Tukey, RH; Quattrochi, LC. (1993). Response of human CYP1-luciferase plasmids to 2,3,7,8-tetrachlorodibenzo-p-dioxin and polycyclic aromatic hydrocarbons. Toxicol Appl Pharmacol 118: 255-262. http://dx.doi.org/10.1006/taap.1993.1031

Pru, JK; Kaneko-Tarui, T; Jurisicova, A; Kashiwagi, A; Selesniemi, K; Tilly, JL. (2009). Induction of proapoptotic gene expression and recruitment of p53 herald ovarian follicle loss caused by polycyclic aromatic hydrocarbons. Reproductive Sciences 16: 347-356. http://dx.doi.org/10.1177/1933719108327596

Puisieux, A; Lim, S; Groopman, J; Ozturk, M. (1991). Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res 51: 6185-6189.

Pukkala, E. (1995). Cancer risk by social class and occupation : a survey of 109,000 cancer cases among Finns of working age. In J Wahrendorf (Ed.). Basel, Switzerland: Karger.

Pukkala, E; Martinsen, J; Lynge, E; Gunnarsdottir, H; Sparén, P; Tryggvadottir, L; Weiderpass, E; Kjaerheim, K. (2009). Occupation and cancer - follow-up of 15 million people in five Nordic countries. Acta Oncol 48: 646-790. http://dx.doi.org/10.1080/02841860902913546

Qiu, C; Cheng, S; Xia, Y; Peng, B; Tang, Q; Tu, B. (2011). Effects of subchronic benzo(a)pyrene exposure on neurotransmitter receptor gene expression in the rat hippocampus related with spatial learning and memory change. Toxicology 289: 83-90. http://dx.doi.org/10.1016/j.tox.2011.07.012

Qiu, C; Peng, B; Cheng, S; Xia, Y; Tu, B. (2013). The effect of occupational exposure to benzo[a]pyrene on neurobehavioral function in coke oven workers. Am J Ind Med 56: 347-355. http://dx.doi.org/10.1002/ajim.22119

Ramesh, A; Inyang, F; Lunstra, DD; Niaz, MS; Kopsombut, P; Jones, KM; Hood, DB; Hills, ER; Archibong, AE. (2008). Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene. Exp Toxicol Pathol 60: 269-280. http://dx.doi.org/10.1016/j.etp.2008.02.010

Ramesh, A; Walker, SA; Hood, DB; Guillén, MD; Schneider, K; Weyand, EH. (2004). Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons [Review]. Int J Toxicol 23: 301-333. http://dx.doi.org/10.1080/10915810490517063

Ramos, KS; Moorthy, B. (2005). Bioactivation of polycyclic aromatic hydrocarbon carcinogens within the vascular wall: implications for human atherogenesis [Review]. Drug Metab Rev 37: 595-610. http://dx.doi.org/10.1080/03602530500251253

Rask-Nielsen, R. (1950). On the susceptibility of the thymus, lung, subcutaneous and mammary tissues in strain street mice to direct application of small doses of four different carcinogenic 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: 763-766. http://dx.doi.org/10.1093/carcin/3.7.763

Raychoudhury, SS; Kubinski, D. (2003). Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. Environ Health Perspect 111: 33-38.

Revel, A; Raanani, H; Younglai, E; Xu, J; Han, R; Savouret, JF; Casper, RF. (2001). Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. Reprod Toxicol 15: 479-486.

Rice, JE; Makowski, GS; Hosted, TJ; Lavoie, EJ. (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: 199-206. http://dx.doi.org/10.1016/0304-3835(84)90119-8

Robinson, M; Laurie, RD; Bull, RJ; Stober, JA. (1987). Carcinogenic effects in A/J mice of particulate of a coal tar paint used in potable water systems. Cancer Lett 34: 49-54. http://dx.doi.org/10.1016/0304-3835(87)90072-3

Robles, R; Morita, Y; Mann, KK; Perez, GI; Yang, S; Matikainen, T; Sherr, DH; Tilly, JL. (2000). The aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse. Endocrinology 141: 450-453.

Rodriguez, JW; Kirlin, WG; Wirsiy, YG; Matheravidathu, S; Hodge, TW; Urso, P. (1999). Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. Immunopharmacol Immunotoxicol 21: 379-396. http://dx.doi.org/10.3109/08923979909052769

Roe, FJ; Peto, R; Kearns, F; Bishop, D. (1970). The mechanism of carcinogenesis by the neutral fraction of cigarette smoke condensate. Br J Cancer 24: 788-806.

Roe, FJ; Waters, MA. (1967). Induction of hepatoma in mice by carcinogens of the polycyclic hydrocarbon type. Nature 214: 299-300.

Roelofzen, JH; Aben, KK; Oldenhof, UT; Coenraads, PJ; Alkemade, HA; van de Kerkhof, PC; van der Valk, PG; Kiemeney, LA. (2010). No increased risk of cancer after coal tar treatment in patients with psoriasis or eczema. J Invest Dermatol 130: 953-961. http://dx.doi.org/10.1038/jid.2009.389

Rogan, EG; Devanesan, PD; Ramakrishna, NV; Higginbotham, S; Padmavathi, NS; Chapman, K; Cavalieri, EL; Jeong, H; Jankowiak, R; Small, GJ. (1993). Identification and quantitation of benzo[a]pyrene-DNA adducts formed in mouse skin. Chem Res Toxicol 6: 356-363. http://dx.doi.org/10.1021/tx00033a017

Rojas, M; Alexandrov, K; Cascorbi, I; Brockmöller, J; Likhachev, A; Pozharisski, K; Bouvier, G; Auburtin, G; Mayer, L; Kopp-Schneider, A; Roots, I; Bartsch, H. (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: 109-118.

Rojas, M; Cascorbi, I; Alexandrov, K; Kriek, E; Auburtin, G; Mayer, L; Kopp-Schneider, A; Roots, I; Bartsch, H. (2000). Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 21: 35-41. http://dx.doi.org/10.1093/carcin/21.1.35

Rojas, M; Godschalk, R; Alexandrov, K; Cascorbi, I; Kriek, E; Ostertag, J; Van Schooten, FJ; Bartsch, H. (2001). Myeloperoxidase--463A variant reduces benzo[a]pyrene diol epoxide DNA adducts in skin of coal tar treated patients. Carcinogenesis 22: 1015-1018. http://dx.doi.org/10.1093/carcin/22.7.1015

Rojas, M; Marie, B; Vignaud, JM; Martinet, N; Siat, J; Grosdidier, G; Cascorbi, I; Alexandrov, K. (2004). High DNA damage by benzo[a]pyrene 7,8-diol-9,10-epoxide in bronchial epithelial cells from patients with lung cancer: comparison with lung parenchyma. Cancer Lett 207: 157-163. http://dx.doi.org/10.1016/j.canlet.2003.11.016

Rothman, KJ; Greenland, S. (1998). Modern epidemiology (2nd ed.). Philadelphia, PA: Lippincott, Williams, & Wilkins.

Rowe, AA; O'Connor, TP. (2011). Assessment of water quality of runoff from sealed asphalt surfaces. (EPA/600/R-10/178). Cincinnati, OH: U.S. Environmental Protection Agency.

Rubes, J; Rybar, R; Prinosilova, P; Veznik, Z; Chvatalova, I; Solansky, I; Sram, RJ. (2010). Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res 683: 9-15. http://dx.doi.org/10.1016/j.mrfmmm.2009.09.010

Ruchirawat, M; Navasumrit, P; Settachan, D. (2010). Exposure to benzene in various susceptible populations: co-exposures to 1,3-butadiene and PAHs and implications for carcinogenic risk. Chem Biol Interact 184: 67-76. http://dx.doi.org/10.1016/j.cbi.2009.12.026

Ruggeri, B; Dirado, M; Zhang, SY; Bauer, B; Goodrow, T; Kleinszanto, AJP. (1993). Benzo[a]pyrene-induced murine skin tumors exhibit frequent and characteristic G to T mutations in the p53 gene. PNAS 90: 1013-1017. http://dx.doi.org/10.1073/pnas.90.3.1013

Sadeu, JC; Foster, WG. (2011). Cigarette smoke condensate exposure delays follicular development and function in a stage-dependent manner. Fertil Steril 95: 2410-2417. http://dx.doi.org/10.1016/j.fertnstert.2011.03.072

Saffiotti, U; Montesano, R; Sellakumar, AR; Kaufman, DG. (1972). Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[a]pyrene and ferric oxide. J Natl Cancer Inst 49: 1199-1204.

Sakkas, D; Alvarez, JG. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis [Review]. Fertil Steril 93: 1027-1036. http://dx.doi.org/10.1016/j.fertnstert.2009.10.046

Saunders, CR; Das, SK; Ramesh, A; Shockley, DC; Mukherjee, S. (2006). Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. J Appl Toxicol 26: 427-438. http://dx.doi.org/10.1002/jat.1157

Saunders, CR; Ramesh, A; Shockley, DC. (2002). Modulation of neurotoxic behavior in F-344 rats by temporal disposition of benzo(a)pyrene. Toxicol Lett 129: 33-45.

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. http://dx.doi.org/10.1007/BF03033211

Schmähl, D; Schmidt, KG; Habs, M. (1977). Syncarcinogenic action of polycyclic hydrocarbons in automobile exhaust gas condensates. In U Mohr; D Schmahl; L Tomatis; W Davis (Eds.), Air pollution and cancer in man (pp. 53-59). Lyon, France: IARC.

Schmidt, JV; Su, GH; Reddy, JK; Simon, MC; Bradfield, CA. (1996). Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. PNAS 93: 6731-6736.

Schmidt, KG; Schmähl, D; Misfeld, J. (1973). Investigations on the carcinogenic burden by air pollution in man. VI. Experimental investigations to determine a dose-response relationship and to estimate a threshold dose of benzo(a)pyrene in the skin of two different mouse strains. Zentralbl Bakteriol, Parasitenkd, Infektionskrankh Hyg, Abt 1: Orig, Reihe 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. International Journal of Immunopharmacology 9: 99-106.

Schönland, SO; Zimmer, JK; Lopez-Benitez, CM; Widmann, T; Ramin, KD; Goronzy, JJ; Weyand, CM. (2003). Homeostatic control of T-cell generation in neonates. Blood 102: 1428-1434. http://dx.doi.org/10.1182/blood-2002-11-3591

Secretan, B; Straif, K; Baan, R; Grosse, Y; El Ghissassi, F; Bouvard, V; Benbrahim-Tallaa, L; Guha, N; Freeman, C; Galichet, L; Cogliano, V. (2009). A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol 10: 1033-1034.

Seli, E; Gardner, DK; Schoolcraft, WB; Moffatt, O; Sakkas, D. (2004). Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 82: 378-383. http://dx.doi.org/10.1016/j.fertnstert.2003.12.039

Shamsuddin, AK; Gan, R. (1988). Immunocytochemical localization of benzo(a)pyrene-DNA adducts in human tissue. Hum Pathol 19: 309-315. http://dx.doi.org/10.1016/S0046-8177(88)80524-0

Sharovskaya, J; Kobliakova, I; Solomatina, N; Kobliakov, V. (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: 387-397. http://dx.doi.org/10.1016/j.ejcb.2005.11.006

Shen, H; Spitz, MR; Qiao, Y; Guo, Z; Wang, LE; Bosken, CH; Amos, CI; Wei, Q. (2003). Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. Int J Cancer 107: 84-88. http://dx.doi.org/10.1002/ijc.11346

Shen, YM; Troxel, AB; Vedantam, S; Penning, TM; Field, J. (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: 1441-1450. http://dx.doi.org/10.1021/tx0601206

Sheng, L, iu; Ding, X; Ferguson, M; Mccallister, M; Rhoades, R; Maguire, M; Ramesh, A; Aschner, M; Campbell, D; Levitt, P, at; Hood, DB. (2010). Prenatal Polycyclic Aromatic Hydrocarbon Exposure Leads to Behavioral Deficits and Downregulation of Receptor Tyrosine Kinase, MET. Toxicol Sci 118: 625-634. http://dx.doi.org/10.1093/toxsci/kfq304

Shimizu, Y; Nakatsuru, Y; Ichinose, M; Takahashi, Y; Kume, H; Mimura, J; Fujii-Kuriyama, Y; Ishikawa, T. (2000). Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. PNAS 97: 779-782. http://dx.doi.org/10.1073/pnas.97.2.779

Silverstone, AE; Cantor, H; Goldstein, G; Baltimore, D. (1976). Terminal deoxynucleotidyl transferase is found in prothymocytes. J Exp Med 144: 543-548.

Sivak, A; Niemeier, R; Lynch, D; Beltis, K; Simon, S; Salomon, R; Latta, R; Belinky, B; Menzies, K; Lunsford, A; Cooper, C; Ross, A; Bruner, R. (1997). Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. Cancer Lett 117: 113-123. http://dx.doi.org/10.1016/S0304-3835(97)00214-0

Slaga, TJ; Gleason, GL; Mills, G; Ewald, L; Fu, PP; Lee, HM; Harvey, RG. (1980). Comparison of the skin tumor-initiating activities of dihydrodiols and diol-epoxides of various polycyclic aromatic hydrocarbons. Cancer Res 40: 1981-1984.

Slaga, TJ; Huberman, E; Selkirk, JK; Harvey, RG; Bracken, WM. (1978). Carcinogenicity and mutagenicity of benz(a)anthracene diols and diol-epoxides. Cancer Res 38: 1699-1704.

Soares, SR; Melo, MA. (2008). Cigarette smoking and reproductive function [Review]. Curr Opin Obstet Gynecol 20: 281-291. http://dx.doi.org/10.1097/GCO.0b013e3282fc9c1e

Soballe, PW; Montone, KT; Satyamoorthy, K; Nesbit, M; Herlyn, M. (1996). Carcinogenesis in human skin grafted to SCID mice. Cancer Res 56: 757-764.

Spinelli, JJ; Band, PR; Svirchev, LM; Gallagher, RP. (1991). Mortality and cancer incidence in aluminum reduction plant workers. J Occup Med 33: 1150-1155.

Spinelli, JJ; Demers, PA; Le, ND; Friesen, MD; Lorenzi, MF; Fang, R; Gallagher, RP. (2006). Cancer risk in aluminum reduction plant workers (Canada). Cancer Causes Control 17: 939-948. http://dx.doi.org/10.1007/s10552-006-0031-9

Steiner, PE. (1955). Carcinogenicity of multiple chemicals simultaneously administered. Cancer Res 15: 632-635.

Stephanou, P; Konstandi, M; Pappas, P; Marselos, M. (1998). Alterations in central monoaminergic neurotransmission induced by polycyclic aromatic hydrocarbons in rats. European Journal of Drug Metabolism and Pharmacokinetics 23: 475-481. http://dx.doi.org/10.1007/BF03189998

Stern, RS; Laird, N. (1994). The carcinogenic risk of treatments for severe psoriasis. Photochemotherapy Follow-up Study. Cancer 73: 2759-2764.

Stern, RS; Liebman, EJ; Väkevä, L. (1998). Oral psoralen and ultraviolet-A light (PUVA) treatment of psoriasis and persistent risk of nonmelanoma skin cancer. PUVA Follow-up Study. J Natl Cancer Inst 90: 1278-1284.

Stern, RS; Zierler, S; Parrish, JA. (1980). Skin carcinoma in patients with psoriasis treated with topical tar and artificial ultraviolet radiation. Lancet 1: 732-735.

Straif, K; Baan, R; Grosse, Y; Secretan, B; El Ghissassi, F; Cogliano, V. (2005). Carcinogenicity of polycyclic aromatic hydrocarbons. Lancet Oncol 6: 931-932. http://dx.doi.org/10.1016/S1470-2045(05)70458-7

Sugiyama, T. (1973). Chromosomal aberrations and carcinogenesis by various benz[a]anthracene derivatives. Cancer Sci 64: 637-639.

Suter, M; Abramovici, A; Showalter, L; Hu, M; Shope, CD; Varner, M; Aagaard-Tillery, K. (2010). In utero tobacco exposure epigenetically modifies placental CYP1A1 expression. Metabolism 59: 1481-1490. http://dx.doi.org/10.1016/j.metabol.2010.01.013

Swartz, WJ; Mattison, DR. (1985). Benzo(a)pyrene inhibits ovulation in C57BL/6N mice. Anat Rec 212: 268-276. http://dx.doi.org/10.1002/ar.1092120308

Szczeklik, A; Szczeklik, J; Galuszka, Z; Musial, J; Kolarzyk, E; Targosz, D. (1994). Humoral immunosuppression in men exposed to polycyclic aromatic hydrocarbons and related carcinogens in polluted environments. Environ Health Perspect 102: 302-304.

Szymańska, K; Matos, E; Hung, RJ; Wünsch-Filho, V; Eluf-Neto, J; Menezes, A; Daudt, AW; Brennan, P; Boffetta, P. (2010). Drinking of maté and the risk of cancers of the upper aerodigestive tract in Latin America: a case-control study. Cancer Causes Control 21: 1799-1806. http://dx.doi.org/10.1007/s10552-010-9606-6

Takizawa, K; Yagi, H; Jerina, DM; Mattison, DR. (1984). Murine strain differences in ovotoxicity following intraovarian injection with benzo(a)pyrene, (+)-(7R,8S)-oxide, (-)-(7R,8R)-dihydrodiol, or (+)-(7R,8S)-diol-(9S,10R)-epoxide-2. Cancer Res 44: 2571-2576.

Talaska, G; Ginsburg, D; Ladow, K; Puga, A; Dalton, T; Warshawsky, D. (2006). Impact of Cyp1a2 or Ahr gene knockout in mice: implications for biomonitoring studies. Toxicol Lett 162: 246-249. http://dx.doi.org/10.1016/j.toxlet.2005.09.020

Talaska, G; Jaeger, M; Reilman, R; Collins, T; Warshawsky, D. (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. PNAS 93: 7789-7793.

Tamaki, A; Hayashi, H; Nakajima, H; Takii, T; Katagiri, D; Miyazawa, K; Hirose, K; Onozaki, K. (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: 407-410.

Tang, D; Li, TY; Liu, JJ; Chen, YH; Qu, L; Perera, F. (2006). PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. Environ Health Perspect 114: 1297-1300. http://dx.doi.org/10.1289/ehp.8939

Tang, D; Warburton, D; Tannenbaum, SR; Skipper, P; Santella, RM; Cereijido, GS; Crawford, FG; Perera, FP. (1999). Molecular and genetic damage from environmental tobacco smoke in young children. Cancer Epidemiol Biomarkers Prev 8: 427-431.

Tang, DL; Li, TY; Liu, JJ; Zhou, ZJ; Yuan, T; Chen, YH; Rauh, VA; Xie, J; Perera, F. (2008). Effects of prenatal exposure to coal-burning pollutants on children's development in China. Environ Health Perspect 116: 674-679. http://dx.doi.org/10.1289/ehp.10471

Tang, Q; Xia, Y; Cheng, S; Tu, B. (2011). Modulation of behavior and glutamate receptor mRNA expression in rats after sub-chronic administration of benzo(a)pyrene. Biomed Environ Sci 24: 408-414. http://dx.doi.org/10.3967/0895-3988.2011.04.012

Temple, L; Kawabata, TT; Munson, AE; White, KL. (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. Toxicol Sci 21: 412-419. http://dx.doi.org/10.1093/toxsci/21.4.412

Thirman, MJ; Albrecht, JH; Krueger, MA; Erickson, RR; Cherwitz, DL; Park, SS; Gelboin, HV; Holtzman, JL. (1994). Induction of cytochrome CYPIA1 and formation of toxic metabolites of benzo[a]pyrene by rat aorta: a possible role in atherogenesis. PNAS 91: 5397-5401.

Thyssen, J; Althoff, J; Kimmerle, G; Mohr, U. (1981). Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J Natl Cancer Inst 66: 575-577.

Topping, DC; Martin, DH; Nettesheim, P. (1981). Determination of cocarcinogenic activity of benzo[e]pyrene for respiratory tract mucosa. Cancer Lett 11: 315-321. http://dx.doi.org/10.1016/0304-3835(81)90097-5

Torinuki, W; Tagami, H. (1988). Incidence of skin cancer in Japanese psoriatic patients treated with either methoxsalen phototherapy, Goeckerman regimen, or both therapies: A 10-year follow-up study. J Am Acad Dermatol 18: 1278-1281.

Törnqvist, S; Norell, S; Ahlbom, A; Knave, B. (1986). Cancer in the electric power industry. Br J Ind Med 43: 212-213.

Triolo, AJ; Aponte, GE; Herr, DL. (1977). Induction of aryl hydrocarbon hydroxylase and forestomach tumors ben benzo(a)pyrene. Cancer Res 37: 3018-3021.

U.S. Congress. (2011). Consolidated Appropriations Act, 2012. (Pub. L. No. 112-74; 125 STAT. 786). 112th U.S. Congress. http://www.gpo.gov/fdsys/pkg/PLAW-112publ74/pdf/PLAW-112publ74.pdf

U.S. EPA (U.S. Environmental Protection Agency). (1986a). Guidelines for mutagenicity risk assessment. (EPA/630/R-98/003). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/iris/backgrd.html

U.S. EPA (U.S. Environmental Protection Agency). (1986b). Guidelines for the health risk assessment of chemical mixtures. Fed Reg 51: 34014-34025.

U.S. EPA (U.S. Environmental Protection Agency). (1986c). Guidelines for the health risk assessment of chemical mixtures. (EPA/630/R-98/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=22567

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

U.S. EPA (U.S. Environmental Protection Agency). (1990). Development of a dose-response model for inhaled B(a)P [EPA Report]. (EPA-600/R-92-135). http://www.ntis.gov/search/product.aspx?ABBR=PB93161016

U.S. EPA (U.S. Environmental Protection Agency). (1991a). Dose-response analysis of ingested Benzo(a)pyrene (CAS No. 50-32-8) [EPA Report]. (EPA600R92045). http://www.ntis.gov/search/product.aspx?ABBR=PB93167484

U.S. EPA (U.S. Environmental Protection Agency). (1991b). Drinking water criteria document for polycyclic aromatic hydrocarbons (PAHs). (ECAOCIND010). http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100CSXZ.txt

U.S. EPA (U.S. Environmental Protection Agency). (1991c). Guidelines for developmental toxicity risk assessment. (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/guidelines-dev-toxicity-risk-assessment.htm

U.S. EPA (U.S. Environmental Protection Agency). (1992). Draft report: A cross-species scaling factor for carcinogen risk assessment based on equivalence of mg/kg(3/4)/day. Fed Reg 57: 24151-24173.

U.S. EPA (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993

U.S. EPA (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment. (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/REPRO51.PDF

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment. (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF

U.S. EPA (U.S. Environmental Protection Agency). (2000a). Science policy council handbook: risk characterization. (EPA/100/B-00/002). Washington, D.C.: U.S. Environmental Protection Agency, Office of Science Policy. http://www.epa.gov/osa/spc/pdfs/rchandbk.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2000b). Supplementary guidance for conducting health risk assessment of chemical mixtures. (EPA/630/R-00/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=51717

U.S. EPA (U.S. Environmental Protection Agency). (2004). Risk Assessment Guidance for Superfund (RAGS), Volume I: Human health evaluation manual, (part E: Supplemental guidance for dermal risk assessment): Final. (EPA/540/R/99/005). Washington, DC. http://www.epa.gov/oswer/riskassessment/ragse/index.htm

U.S. EPA (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment. (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/cancerguidelines/

U.S. EPA (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. In US Environmental Protection Agency, Risk Assessment Forum (pp. 1125-1133). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/cancerguidelines/guidelines-carcinogen-supplement.htm

U.S. EPA (U.S. Environmental Protection Agency). (2006a). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (Final Report) [EPA Report]. (EPA/600/R-05/043F). Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668

U.S. EPA (U.S. Environmental Protection Agency). (2006b). A framework for assessing health risk of environmental exposures to children. (EPA/600/R-05/093F). Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363

U.S. EPA (U.S. Environmental Protection Agency). (2010a). Consumer factsheet on benzo(a)pyrene: Ground water and drinking water [Fact Sheet]. http://water.epa.gov/drink/contaminants/basicinformation/historical/upload/Archived-Consumer-Factsheet-on-Benzo.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2010b). Integrated science assessment for carbon monoxide [EPA Report]. (EPA/600/R-09/019F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=218686

U.S. EPA (U.S. Environmental Protection Agency). (2010c). Revised draft: Multistage Weibull time-to-tumor model in EPA's benchmark dose software (BMDS): Methodology description. Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (2011). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/interspecies-extrapolation.htm

U.S. EPA (U.S. Environmental Protection Agency). (2012a). Advances in inhalation gas dosimetry for derivation of a reference concentration (RFC) and use in risk assessment. (EPA/600/R-12/044). Washington, DC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=244650

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Benchmark Dose Software (BMDS). Version 2.2. Retrieved from http://www.epa.gov/NCEA/bmds/index.html

U.S. EPA (U.S. Environmental Protection Agency). (2012c). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2012d). EPA announces NAS' review of IRIS Assessment development process. Available online at http://yosemite.epa.gov/opa/admpress.nsf/0/1ce2a7875daf093485257a000054df54?OpenDocument

U.S. EPA (U.S. Environmental Protection Agency). (2014). EPAs Integrated Risk Information System: Assessment development process [EPA Report]. Washington, DC. http://epa.gov/iris/process.htm

Urano, K; Katakai, Y; Tokuda, Y; Ueyama, Y; Nomura, T; Yamamoto, S. (1995). Failure of genotoxic carcinogens to produce tumors in human skin xenografts transplanted to SCID mice. Carcinogenesis 16: 2223-2226. http://dx.doi.org/10.1093/carcin/16.9.2223

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: 569-576. http://dx.doi.org/10.1080/15287398009529874

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: 817-835. http://dx.doi.org/10.1080/15287398209530297

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: 569-584. http://dx.doi.org/10.1080/15287398409530606

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: 1-10.

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: 195-217. http://dx.doi.org/10.3109/08923978809014333

Urso, P; Kramer, MK. (2008). The role of adherent cells in the immunosuppressed state of mouse progeny transplacentally exposed to benzo(alpha)pyrene. In Vitro Cell Dev Biol Anim 44: 273-279. http://dx.doi.org/10.1007/s11626-008-9112-2

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: 179-194. http://dx.doi.org/10.3109/08923978809014332

Urso, P; Zhang, W; Cobb, JR. (1992). Immunological consequences from exposure to benzo(a)pyrene during pregnancy. Scand J Immunol Suppl 11: 203-206.

van den Berg, FA; Baken, KA; Vermeulen, JP; Gremmer, ER; van Steeg, H; van Loveren, H. (2005). Use of the local lymph node assay in assessment of immune function. Toxicology 211: 107-114. http://dx.doi.org/10.1016/j.tox.2005.03.003

van Lipzig, MM; Vermeulen, NP; Gusinu, R; Legler, J; Frank, H; Seidel, A; Meerman, JH. (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: 41-55. http://dx.doi.org/10.1016/j.etap.2004.03.010

Van Metre, PC; Mahler, BJ. (2010). Contribution of PAHs from coal-tar pavement sealcoat and other sources to 40 U.S. lakes. Sci Total Environ 409: 334-344. http://dx.doi.org/10.1016/j.scitotenv.2010.08.014

Varghese, SK; Gangamma, S. (2009). Particle deposition in human respiratory system: deposition of concentrated hygroscopic aerosols. Inhal Toxicol 21: 619-630. http://dx.doi.org/10.1080/08958370802380792

Vesselinovitch, SD; Kyriazis, AP; Mihailovich, N; Rao, KV. (1975). Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. Cancer Res 35: 2948-2953.

Vesselinovitch, SD; Rao, KV; Mihailovich, N. (1979). Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis [Review]. Natl Canc Inst Monogr239-250.

Vineis, P; Anttila, S; Benhamou, S; Spinola, M; Hirvonen, A; Kiyohara, C; Garte, SJ; Puntoni, R; Rannug, A; Strange, RC; Taioli, E. (2007). Evidence of gene gene interactions in lung carcinogenesis in a large pooled analysis [Review]. Carcinogenesis 28: 1902-1905. http://dx.doi.org/10.1093/carcin/bgm039

Vineis, P; Perera, F. (2007). Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old [Review]. Cancer Epidemiol Biomarkers Prev 16: 1954-1965. http://dx.doi.org/10.1158/1055-9965.EPI-07-0457

Voelter-Mahlknecht, S; Scheriau, R; Zwahr, G; Koch, B; Escobar Pinzon, LC; Drexler, H; Letzel, S. (2007). Skin tumors among employees of a tar refinery: the current data and their implications. Int Arch Occup Environ Health 80: 485-495. http://dx.doi.org/10.1007/s00420-006-0155-y

Vondrácek, J; Kozubík, 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: 193-201.

Wang, F; He, Y; Guo, H; Li, J; Yang, Y; Wu, Z; Zheng, H; Wu, T. (2010). Genetic variants of nucleotide excision repair genes are associated with DNA damage in coke oven workers. Cancer Epidemiol Biomarkers Prev 19: 211-218. http://dx.doi.org/10.1158/1055-9965.EPI-09-0270

Wattenberg, LW. (1974). Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulfur-containing compounds. J Natl Cancer Inst 52: 1583-1587.

Waylen, AL; Metwally, M; Jones, GL; Wilkinson, AJ; Ledger, WL. (2009). Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis [Review]. Hum Reprod Update 15: 31-44. http://dx.doi.org/10.1093/humupd/dmn046

Wei, Q; Cheng, L; Amos, CI; Wang, LE; Guo, Z; Hong, WK; Spitz, MR. (2000). Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. J Natl Cancer Inst 92: 1764-1772.

Wei, SJ; Chang, RL; Merkler, KA; Gwynne, M; Cui, XX; Murthy, B; Huang, MT; Xie, JG; Lu, YP; Lou, YR; Jerina, DM; Conney, AH. (1999). Dose-dependent mutation profile in the c-Ha-ras proto-oncogene of skin tumors in mice initiated with benzo[a]pyrene. Carcinogenesis 20: 1689-1696.

Weinberg, CR; Wilcox, AJ; Baird, DD. (1989). Reduced fecundability in women with prenatal exposure to cigarette smoking. Am J Epidemiol 129: 1072-1078.

Wester, PW; Kroes, R. (1988). Forestomach carcinogens: pathology and relevance to man [Review]. Toxicol Pathol 16: 165-171.

Wester, RC; Maibach, HI; Bucks, DA; Sedik, L; Melendres, J; Liao, C; Dizio, S. (1990). Percutaneous absorption of [14C]DDT and [14C]benzo[a]pyrene from soil. Fundam Appl Toxicol 15: 510-516.

Weyand, EH; Chen, YC; Wu, Y; Koganti, A; Dunsford, HA; Rodriguez, LV. (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: 949-954. http://dx.doi.org/10.1021/tx00049a008

Weyand, EH; He, ZM; Ghodrati, F; Wu, Y; Marshall, MV; Lavoie, EJ. (1992). Effect of fluorine substitution on benzo[j]fluoranthene genotoxicity. Chem Biol Interact 84: 37-53.

Weyand, EH; Lavoie, EJ. (1988). Comparison of PAH DNA adduct formation and tumor initiating activity in newborn mice. Proc Am Assoc Cancer Res 29: 98.

White, KL; Lysy, HH; Holsapple, MP. (1985). Immunosuppression by polycyclic aromatic hydrocarbons: a structure-activity relationship in B6C3F1 and DBA/2 mice. Immunopharmacology 9: 155-164.

WHO (World Health Organization). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Whyatt, RM; Santella, RM; Jedrychowski, W; Garte, SJ; Bell, DA; Ottman, R; Gladek-Yarborough, A; Cosma, G; Young, TL; Cooper, TB; Randall, MC; Manchester, DK; Perera, FP. (1998). Relationship between ambient air pollution and DNA damage in Polish mothers and newborns. Environ Health Perspect 106: 821-826.

Wilson, JS; Holland, LM. (1988). Periodic response difference in mouse epidermis chronically exposed to crude-oils or BaP: Males vs. females. Toxicology 50: 83-94. http://dx.doi.org/10.1016/0300-483X(88)90123-0

Winker, N; Tuschl, H; Kovac, R; Weber, E. (1997). Immunological investigations in a group of workers exposed to various levels of polycyclic aromatic hydrocarbons. J Appl Toxicol 17: 23-29.

Wislocki, PG; Bagan, ES; Lu, AYH; Dooley, KL; Fu, PP; Han-Hsu, H; Beland, FA; Kadlubar, FF. (1986). Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene, and benzo[a]pyrene in the newborn mouse assay. Carcinogenesis 7: 1317-1322. http://dx.doi.org/10.1093/carcin/7.8.1317

Wolff, RK; Griffith, WC; Henderson, RF; Hahn, FF; Harkema, J. R.; Rebar, AH; Eidson, AF; Mcclellan, RO. (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: 123-138. http://dx.doi.org/10.1080/15287398909531283

Wood, AW; Levin, W; Chang, RL; Huang, MT; Ryan, DE; Thomas, PE; Lehr, RE; Kumar, S; Koreeda, M; Akagi, H; Ittah, Y; Dansette, P; Yagi, H; Jerina, DM; Conney, AH. (1980). Mutagenicity and tumor-initiating activity of cyclopenta(c,d)pyrene and structurally related compounds. Cancer Res 40: 642-649.

Wormley, DD; Chirwa, S; Nayyar, T; Wu, J; Johnson, S; Brown, LA; Harris, E; Hood, DB. (2004). Inhaled benzo(a)pyrene impairs long-term potentiation in the F1 generation rat dentate gyrus. Cell Mol Biol (Noisy-le-grand) 50: 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. http://dx.doi.org/10.1016/j.scitotenv.2010.02.028

Wu, J; Ramesh, A; Nayyar, T; Hood, DB. (2003a). Assessment of metabolites and AhR and CYP1A1 mRNA expression subsequent to prenatal exposure to inhaled benzo(a)pyrene. Int J Dev Neurosci 21: 333-346.

Wu, MT; Pan, CH; Huang, YL; Tsai, PJ; Chen, CJ; Wu, TN. (2003b). Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. Environ Mol Mutagen 42: 98-105. http://dx.doi.org/10.1002/em.10176

Wynder, EL; Fritz, L; Furth, N. (1957). Effect of concentration of benzopyrene in skin carcinogenesis. J Natl Cancer Inst 19: 361-370.

Wynder, EL; Hoffmann, D. (1959). A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. Cancer 12: 1079-1086. http://dx.doi.org/10.1002/1097-0142(195911/12)12:6<1079::AID-CNCR2820120604>3.0.CO;2-I

Xia, Y; Cheng, S; He, J; Liu, X; Tang, Y; Yuan, H; He, L; Lu, T; Tu, B; Wang, Y. (2011). Effects of subchronic exposure to benzo[a]pyrene (B[a]P) on learning and memory, and neurotransmitters in male Sprague-Dawley rat. Neurotoxicology 32: 188-198. http://dx.doi.org/10.1016/j.neuro.2010.12.015

Xia, Y; Zhu, P; Han, Y; Lu, C; Wang, S; Gu, A; Fu, G; Zhao, R; Song, L; Wang, X. (2009). Urinary metabolites of polycyclic aromatic hydrocarbons in relation to idiopathic male infertility. Hum Reprod 24: 1067-1074. http://dx.doi.org/10.1093/humrep/dep006

Xu, C; Chen, J; Qiu, Z; Zhao, Q; Luo, J; Yang, L; Zeng, H; Huang, Y; Zhang, L; Cao, J; Shu, W. (2010). Ovotoxicity and PPAR-mediated aromatase downregulation in female Sprague-Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl) phthalate. Toxicol Lett 199: 323-332. http://dx.doi.org/10.1016/j.toxlet.2010.09.015

Xu, D; Penning, TM; Blair, IA; Harvey, RG. (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: 597-604. http://dx.doi.org/10.1021/jo801864m

Xu, GB; Yu, CP. (1985). Theoretical lung deposition of hygroscopic NaCl aerosols. Aerosol Sci Technol 4: 455-461. http://dx.doi.org/10.1080/02786828508959070

Xu, Z; Brown, LM; Pan, GW; Liu, TF; Gao, GS; Stone, BJ; Cao, RM; Guan, DX; Sheng, JH; Yan, ZS; Dosemeci, M; Fraumeni, JF; Blot, WJ. (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: 7-15. http://dx.doi.org/10.1002/(SICI)1097-0274(199607)30:1<7::AID-AJIM2>3.0.CO;2-#

Xue, W; Warshawsky, D. (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review [Review]. Toxicol Appl Pharmacol 206: 73-93. http://dx.doi.org/10.1016/j.taap.2004.11.006

Ye, X; Skjaerven, R; Basso, O; Baird, DD; Eggesbo, M; Cupul Uicab, LA; Haug, K; Longnecker, MP. (2010). In utero exposure to tobacco smoke and subsequent reduced fertility in females. Hum Reprod 25: 2901-2906. http://dx.doi.org/10.1093/humrep/deq235

Young, C; Cherrie, J; Van Tongeren, M; Fortunato, L; Hutchings, S; Rushton, L. (2012). The burden of occupational cancer in Great Britain: Non-melanoma skin cancer. Sudbury, Suffolk: Health and Safety Executive (HSE). http://www.hse.gov.uk/research/rrhtm/rr928.htm

Yu, D; Berlin, JA; Penning, TM; Field, J. (2002). Reactive oxygen species generated by PAH o-quinones cause change-in-function mutations in p53. Chem Res Toxicol 15: 832-842.

Zaccaria, KJ; McClure, PR. (2013). Using immunotoxicity information to improve cancer risk assessment for polycyclic aromatic hydrocarbon mixtures [Review]. Int J Toxicol 32: 236-250. http://dx.doi.org/10.1177/1091581813492829

Zandi-Nejad, K; Luyckx, VA; Brenner, BM. (2006). Adult hypertension and kidney disease - The role of fetal programming [Review]. Hypertension 47: 502-508. http://dx.doi.org/10.1161/01.HYP.0000198544.09909.1a

Zenzes, MT; Puy, LA; Bielecki, R. (1998). Immunodetection of benzo[a]pyrene adducts in ovarian cells of women exposed to cigarette smoke. Mol Hum Reprod 4: 159-165. http://dx.doi.org/10.1093/molehr/4.2.159

Zenzes, MT; Puy, LA; Bielecki, R; Reed, TE. (1999). Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol Hum Reprod 5: 125-131.

Zhang, HM; Nie, JS; Li, X; Niu, Q. (2012). Characteristic analysis of peripheral blood mononuclear cell apoptosis in coke oven workers. J Occup Health 54: 44-50. http://dx.doi.org/10.1539/joh.11-0155-OA

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: 285-292. http://dx.doi.org/10.1016/S0027-5107(96)00213-8

Zhang, YJ; Li, Y; Deleo, VA; Santella, RM. (1990). Detection of DNA adducts in skin biopsies of coal tar-treated psoriasis patients: immunofluorescence and 32P postlabeling. Skin Pharmacol Physiol 3: 171-179. http://dx.doi.org/10.1159/000210867

Zheng, SJ; Tian, HJ; Cao, J; Gao, YQ. (2010). Exposure to di(n-butyl)phthalate and benzo(a)pyrene alters IL-1β secretion and subset expression of testicular macrophages, resulting in decreased testosterone production in rats. Toxicol Appl Pharmacol 248: 28-37. http://dx.doi.org/10.1016/j.taap.2010.07.008