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

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ABBREVIATIONS

ADAF	age-dependent adjustment factor
AhR	aryl hydrocarbon receptor
AIC	Akaike's information criterion
AKR	aldo-keto reductase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
B[a]P	benzo[a]pyrene
BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower confidence limit
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CI	confidence interval
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DHH	dihydrodiol dehydrogenase
DNA	deoxyribonucleic acid
DSF	dermal slope factor
EPA	Environmental Protection Agency
ETS	environmental tobacco smoke
FSH	follicle stimulating hormone
F_{TOT}	total fractional deposition
GD	gestation day
GGT	γ-glutamyl transferase
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HSC	hematopoietic stem cells
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.t.	intratracheal
IUR	inhalation unit risk
IVF	in vitro fertilization
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MMAD	mass median aerodynamic diameter
MN	micronuclei
MOA	mode of action
MPPP	Multi-Path Particle Deposition
NCEA	National Center for Environmental Assessment
NMDA	N-methyl-D-aspartate
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
OR	odds ratio
ORD	Office of Research and Development
OSF	oral slope factor
PAH	polycyclic aromatic hydrocarbon
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	post-natal day
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
RBC	red blood cells
$RDDR_{ER}$	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
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
s.c.	subcutaneous
SC SA	sperm chromatic structure assay
SD	standard deviation
SE	standard error
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SRBC	sheep red blood cells
SSB	single strand break
TWA	time-weighted average
UCL	upper confidence limit
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF^L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S.	United States of America
WBC	white blood cells
WHO-NCTB	World Health Organization Neurobehavioral Core Test Battery

AUTHORS | CONTRIBUTORS | REVIEWERS

Assessment Team

Christine Cai, MS Glinda Cooper, Ph.D. Louis D'Amico, Ph.D. Jason Fritz, Ph.D. Martin Gehlhaus, MHS Catherine Gibbons, Ph.D. Karen Hogan, MS Kathleen Newhouse, MS (Chemical Manager) Margaret Pratt, Ph.D. Keith Salazar, Ph.D. John Schaum (retired) Suryanarayana Vulimiri, DVM Gene Ching-Hung Hsu, Ph.D. (formerly with EPA)	U.S. EPA Office of Research and Development National Center for Environmental Assessment Washington, DC
John Cowden, Ph.D. John Stanek, Ph.D.	U.S. EPA Office of Research and Development National Center for Environmental Assessment Research Triangle Park, NC
Chris Brinkerhoff, Ph.D.	Oak Ridge Institute for Science and Education Fellow
Scott Glaberman, Ph.D.	American Association for the Advancement of Science Fellow

Scientific Support Team

Lynn Flowers, Ph.D., DABT John Fox Martin Gehlhaus, MHS Connie Kang-Sickel, Ph.D. Jamie B. Strong, Ph.D. Paul White	U.S. EPA Office of Research and Development National Center for Environmental Assessment Washington, DC
Reeder Sams, Ph.D. John Cowden, Ph.D.	U.S. EPA Office of Research and Development National Center for Environmental Assessment Research Triangle Park, NC

Production Team

Taukecha Cunningham Maureen Johnson Terri Konoza Vicki Soto	U.S. EPA Office of Research and Development National Center for Environmental Assessment Washington, DC

Contractor Support

Heather Carlson-Lynch, S.M. Peter McClure, Ph.D. Megan Riccardi Kelly Salinas Joe Santodonato Julie Stickney, Ph.D., DABT	SRC, Inc., Chemical, Biological, and Environmental Center, Syracuse, NY
George Holdsworth, Ph.D. Lutz W. Weber, Ph.D., DABT	Oak Ridge Institute for Science and Education, Oak Ridge, TN
Janusz Z. Byczkowski, Fairborn, OH, Ph.D., D.Sc., DABT	

1

Executive Direction

Rebecca Clark, MPH Vincent Cogliano, Ph.D. Lynn Flowers, Ph.D., DABT Samantha Jones, Ph.D. Jamie Strong, Ph.D.	U.S. EPA Office of Research and Development National Center for Environmental Assessment Washington, DC

2

Internal Review Team

Stephen Nesnow, Ph.D.	U.S. EPA National Health and Environmental Effects Research Laboratory Research Triangle Park, NC
Rita Schoeny, Ph.D.	U.S. EPA Office of Water Washington, DC

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Reviewers

This assessment was provided for review to scientists in EPA's Program and Regional Offices. Comments were submitted by:

Office of Children's Health Protection, Washington DC
Office of Policy, Washington, DC
Office of Solid Waste and Emergency Response, Washington DC
Office of Water, Washington DC
Region 2, New York, NY
Region 3, Philadelphia, PA
Region 8, Denver, CO

This assessment was provided for review to other federal agencies and Executive Offices of the President. Comments were submitted by:

Agency for Toxic Substances and Disease Registry, Centers for Disease Control, Department of Health and Human Services
Department of Defense
National Aeronautics and Space Administration
National Institute for Occupational Safety and Health, Centers for Disease Control, Department of Health and Human Services
Office of Management and Budget, Executive Office of the President
White House Council on Environmental Quality, Executive Office of the President

PREFACE

This Toxicological Review, prepared under the auspices of 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, it 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 often used as an indicator chemical to measure exposure to 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 9 out of 275 chemicals on the Priority List of Hazardous Substances for CERCLA (ATSDR, 2011). Benzo[a]pyrene is also listed as a drinking water contaminant under the Safe Drinking Water Act.

This assessment updates a previous 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 for skin cancer from dermal exposure (US 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 IRIS Toxicological Reviews. The findings of this assessment and related documents produced during its development are available on the IRIS website (http://www.epa.gov/iris). Appendices for chemical and physical properties, toxicokinetic information, and summaries of toxicity studies and other information 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.

[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.

Chemical Properties and Uses

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 dry deposition to land or water. In air, benzo[a]pyrene is predominantly adsorbed to particulates but may also exist as a vapor at high temperatures (NLM, 2010).

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 released to the environment via both natural sources (such as forest fires) and anthropogenic sources including stoves/furnaces burning fossil fuels (especially wood and coal), motor vehicle exhaust, cigarettes, and various industrial combustion processes (ATSDR, 1995). Benzo[a]pyrene is also found in soot and coal tars. Mahler et al. (2005) has reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for the majority of PAHs in many watersheds. Benzo[a]pyrene exposure can also occur to workers involved in the production of aluminum, coke, graphite, silicon carbide, and in coal tar distillation. The major sources of non-occupational exposure are cigarettes and food.

Assessments by Other National and International Health Agencies

Toxicity information on benzo[a]pyrene has been evaluated by California EPA (CalEPA), 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 A. It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after 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 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, EPA uses information on the adverse health effects 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 long-term exposure to chemicals and to characterize exposure-response relationships. An assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. 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; EPA's Integrated Science Assessments evaluate the effects from these pollutants in ambient air).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state government agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. These agents may be found in air, water, soil, or sediment. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. IRIS may assess other agents as an urgent public health need arises. IRIS also reassesses agents as significant new studies are published.

2. Process for Developing and Peer Reviewing IRIS Assessments

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

Step 1. Development of a draft Toxicological Review (usually about 11-1/2 months duration). The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate the studies, identify health effects, weigh the evidence of causation for each effect, identify mechanistic events and pathways, and derive toxicity values.

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

Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President (1-1/2 months). The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and EPA's response to major comments become part of the public record.

Step 4. External peer review, after public review and comment (3-1/2 months). EPA releases the draft assessment for

public review and comment, followed by external peer review. The peer review meeting is open to the public and includes time for oral public comments. The peer reviewers also receive the written public comments. 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, peer review report, and written public comments become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS Summary (2 months). 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 (1-1/2 months). The draft assessment and summary are revised to address EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

Step 7. Completion and posting (1 month). 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, 1986a, 1986b, 1991, 1996, 1998, 2000a, 2005a, 2005b) and other methods (U.S. EPA, 1994, 2000b, 2002, 2006a, 2006b, 2011). 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, 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 and other databases listed in EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. EPA posts the results of the literature search on the IRIS website and requests information from the public on additional studies and ongoing research.

EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted to EPA under the Toxic Substances Control 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, EPA will have it peer-reviewed.

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, 1986a, 2000a):

- 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 and case-control studies provide the strongest epidemiologic evidence, as they collect information about individual exposures and effect.
- Ecologic 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 ecologic studies and case reports but reports details only if they suggest effects not identified by other epidemiologic studies.

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental studies from the results of the literature search.

- 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 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 than studies of chronic exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information. Research involving human subjects is considered only if conducted according to ethical principles.

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, 1991, 1996, 1998, 2006b).

4. Evaluating the Quality of Individual Studies

4.1. Evaluating the quality of epidemiologic studies

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

- 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 under consideration.
- Ascertainment of disease or health effect.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during the 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 rate.
- 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.

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, 1991, 1996, 1998, 2005a).

4.2. Evaluating the quality of experimental studies

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

- Documentation of study design, animals and methods, basic data, and results.
- Relevance to humans of the animal model and the experimental methods.
- 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.
- Numbers of animals 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 group with 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).

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, 1991, 1996, 1998, 2005a). In multi-generation 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, 1991, 1998).

4.3. Reporting study results

The assessment uses evidence tables to summarize details of 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 characteristics in this section to identify the strongest studies or types of study. The tables report 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 material 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, EPA asks peer reviewers to identify studies that were not adequately considered.

5. Weighing the Overall Evidence of Each Effect

5.1. Weighing epidemiologic evidence

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole to determine the extent to which any observed associations may be causal. Positive, negative, and null results are given weight according to study quality. This evaluation considers aspects of an association that suggest causality, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998) (U.S. EPA, 1994, 2002, 2005a; DHHS, 2004).

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 causality 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 causality. 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 causality, 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 causality. A monotonic increase is not the only pattern consistent with causality. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causality is strengthened by data demonstrating plausible biologic mechanisms, if available.

Coherence: An inference of causality 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 of causality, for example, 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 causality.

These considerations are consistent with guidelines for systemic reviews that evaluate the quality and strength 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 reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al., 2008a,b).

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may choose a descriptor such as sufficient evidence, suggestive evidence, inadequate evidence, or evidence suggestive of no causal relationship to characterize the epidemiologic evidence of each effect (DHHS, 2004).

5.2. Weighing experimental animal evidence

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, 1994, 2002, 2005a).

In weighing evidence from multiple experiments, U.S. EPA (2005a) 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 inconclusive results do not invalidate positive results in a different experimental system. EPA regards both as valid observations and looks to methodological differences or, if available, mechanistic information to reconcile differing results.

It is well established that there are critical periods for some developmental and reproductive effects. Accordingly, the assessment determines whether critical periods have been adequately investigated (U.S. EPA, 1991, 1996, 1998, 2005a, 2005b, 2006b). 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, EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (EPA 1986b).

5.3. Characterizing modes 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. The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a). Information on mode of action is not required for a conclusion that an effect is causally related to an agent (EPA 2005a).

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 causality 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 but is not used to determine relevance. Similarly, anticipated levels of human exposure are not used to determine relevance.

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

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). 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., 2008b).

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 (EPA 2005a).

5.4. Characterizing the overall weight of the evidence

After weighing the epidemiologic and experimental studies pertinent to each effect, the assessment may select a standard descriptor to characterize the overall weight of the evidence. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a).

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 (EPA 2005a).

EPA is investigating and may on a trial basis propose 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 weigh-of-evidence descriptor. For example, EPA typically derives toxicity values for agents classified as *carcinogenic to humans* or as *likely to be carcinogenic* (U.S. EPA, 2005a).

Dose-response analysis requires different information than is needed to identify the occurrence of effects. Although dose-response relationships may contribute to a qualitative assessment quantitative measures of dose and response are essential to dose-response analysis. Then, other factors being equal (U.S. EPA, 1994, 2005a):

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and estimated 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 also be used to extrapolate across exposure routes.
- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects change with time.

- 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 non-monotonic 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, EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving Toxicity Values

7.1. General framework for dose-response analysis

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

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) (Sections 7.2 and 7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (Sections 7.4 and 7.5). When response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors that account for sources of uncertainty and variability to the point of departure (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, and 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 (EPA 2000a, 2005a).

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

7.2. Modeling dose

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, 1994, 2005a, 2006a).

Because toxicokinetic modeling can require many parameters and more data than are typically available, 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, 1991, 1996, 1998, 2005a).

- Doses are standardized to equivalent human terms to facilitate comparison of results from different species:
  - Oral doses can be 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, 2005a, 2011).
  - 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, 1994).

It can be informative to convert doses to target sites across different exposure routes. If this approach is followed, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a).

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

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, 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. EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2000b). Additional judgment or alternative analyses are used when 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).

Modeling is used to derive a point of departure (U.S. EPA, 2000b, 2005a). (See Section 7.6 for alternatives if a point of departure cannot be derived by modeling.)

- For dichotomous responses, the point of departure is often the 95% lower bound on the dose associated with a 10% response, but a lower response that falls within the observed range may be used instead. For example, reproductive or developmental studies often have power to detect a 5% response; epidemiologic studies, 1% or lower.
- For continuous responses, the point of departure is ideally the dose where the effect becomes biologically significant. In the absence of such definition, both statistical and biologic factors are considered.

7.4. Extrapolating to lower doses

The purpose of extrapolating to lower doses is to estimate risks from exposures below the observed data. Low-dose extrapolation is typically used for known and likely carcinogens. Low-dose extrapolation

considers what is known about modes of action (U.S. EPA, 2005a).

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 if the evidence is insufficient to establish a mode of action.

   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 extrapolation is used 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. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

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 calculates 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 life-stages

The assessment analyzes the available information on populations and life-stages that may be particularly susceptible to each effect. If adequate data are available, the assessment derives separate risk estimates for susceptible populations or life-stages. A tiered approach is used (U.S. EPA, 2005a).

1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or life-stage, these data are analyzed to derive separate risk estimates for susceptible individuals.

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

3) In the absence of chemical-specific data, EPA has developed *age-dependent adjustment factors* early-life exposure to suspected 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, EPA recommends (U.S. EPA, 2005b):

   – 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). 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, 1991, 1996, 1998).

To account for uncertainty and variability in the derivation of a lifetime human exposure where effects are not anticipated to occur, reference doses and reference concentrations are calculated by applying a series of *uncertainty factors* to the point of departure. If a modeled point of departure is not available, 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. A factor of 10 is applied to account 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. This factor is reduced only if the point of departure is derived specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 1991, 1994, 1996, 1998, 2002).

Animal-to-human extrapolation. A factor of 10 is applied if animal results are used to make inferences about humans. This factor is often regarded as comprising toxicokinetics and toxicodynamics in equal parts. Accordingly, if the point of departure is based on toxicokinetic modeling, dosimetry modeling, or allometric scaling across species, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicodynamic differences. An animal-to-human factor is not applied if a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species (U.S. EPA, 1991, 1994, 1996, 1998, 2002, 2011).

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, 1991, 1994, 1996, 1998, 2002).

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, 1994, 1998, 2002).

Incomplete database. If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or life-stage, the assessment may apply a database uncertainty factor (U.S. EPA, 1991, 1994, 1996, 1998, 2002). The size of the factor

depends on the nature of the database deficiency. For example, 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).

In this way, the assessment derives candidate reference 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). 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.

The assessment may also report reference values for each effect. This would facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (U.S. EPA, 2002).

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).

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., 2008a).

All assessments discuss the significant uncertainties encountered in the analysis. EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a). 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).

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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 foods grown in areas contaminated with benzo[a]pyrene (from the air and soil) or by eating certain food products, such as charred meats, where benzo[a]pyrene is formed during the cooking process. 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 adverse birth outcomes (including reduced birth weight, postnatal body weight, and head circumference) and decreased fertility with internal biomarkers of exposure to benzo[a]pyrene (benzo[a]pyrene diol epoxide-DNA adducts).

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 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 altered spatial learning and memory and cardiovascular effects following gestational exposures. Reproductive and immune effects include decreased sperm counts, ovarian 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-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 the hazards associated with benzo[a]pyrene exposure.

Developmental toxicity, represented by neurodevelopmental impairments, was chosen as the basis for the porposed RfD as the available data indicate that neurodevelopmental effects represent a sensitive hazard of benzo[a]pyrene exposure. The neurodevelopmental study by Chen et al. (2012) and the observed impaired spatial learning were used to derive the RfD. The endpoint of impaired spatial learning, as measured by the increase in latency time to find a hidden platform in the Morris water 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 0.06 mg/kg-day that was used as the point of departure (POD) for RfD derivation.

The RfD was calculated by dividing the POD by a composite 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) as shown in Table ES-1.

Table ES- 1. Summary of proposed reference dose (RfD) derivation

Critical effect	Point of departure	UF	Chronic RfD
Neurodevelopmental impairment PND 5-11 rat study, gavage (Chen et al., 2012)	$BMDL_{1SD}$: 0.06 mg/kg-day	300	2×10^{-4} mg/kg-day

Confidence in the Chronic 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 excellent and a wide variety of neurotoxicity endpoints were measured. Several subchronic and developmental studies covering a wide variety of endpoints are available; however, the lack of a multi-generation toxicity study with exposure throughout development is not available. Therefore, confidence in the database is medium.

Effects Other Than Cancer 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.

Developmental toxicity, represented by decreased fetal survival, was chosen as the basis for the proposed 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 GD 11-20 were used to derive the overall RfC. The 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, 1994b). 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 3000 to account for toxicodynamic differences between animals and humans (3), interindividual differences in human susceptibility (10), LOAEL-to-NOAEL extrapolation (10), and deficiencies in the toxicity database (10) as shown in Table ES-2.

Table ES- 2. Summary of proposed reference concentration (RfC) derivation

Critical effect	Point of departure*	UF	Chronic RfC
Decreased fetal survival GD 11-20 rat study (Archibong et al., 2002)	$LOAEL_{HEC}$: 4.6 μg/m^3 (4.6 x 10^{-3} mg/m^3)	3000	2 x 10^{-6} mg/m^3

* The POD was adjusted for continuous daily exposure: PODADJ= POD × hours exposed per day/24 hours, and was further adjusted to a human equivalent concentration by adjusting by the regional deposited dose ratio calculated using MPPD software (see Section 2.2.2. and Appendices)

Confidence in the Chronic Oral 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 dietary 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 diverse 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 specific 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).

EPA used the multistage-Weibull model for the derivation of the oral slope factor because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups. 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, esophagus, and forestomach, of male Syrian golden hamsters exposed to benzo[a]pyrene condensed onto NaCl particles (Thyssen et al., 1981). Supportive evidence for

the carcinogenicity of inhaled benzo[a]pyrene comes from additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation. The Thyssen et al. (1981) bioassay represents the only available data that exhibits a dose-response relationship for cancer from inhaled benzo[a]pyrene.

A time-to-tumor dose-response model was fit to the time-weighted average exposure concentrations and the individual animal occurrence data for tumors in the larynx, pharynx, trachea, esophagus, and forestomach. The inhalation unit risk of **5×10^{-4} per µg/m^3** was calculated by linear extrapolation (slope factor = 0.1/$BMCL_{10}$) from a $BMCL_{10}$ of 0.20 mg/m^3 for the occurrence of upper respiratory and upper digestive tract tumors in male hamsters chronically exposed by inhalation to benzo[a]pyrene (Thyssen et al., 1981).

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. The analysis in this assessment focuses on chronic carcinogenicity bioassays in several strains of mice demonstrating increasing incidence of benign and malignant skin tumors following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime.

The Poel (1959) and Sivak et al. (1997) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following dermal exposure to benzo[a]pyrene. Both studies included at least three exposure levels (including several low doses), group sizes of 30–50 mice, and reporting of intercurrent mortality. Following the modeling, the $BMDL_{10}$ was adjusted for interspecies differences by allometric scaling. The dermal slope factor of **0.005 per µg/day** was calculated by linear extrapolation (slope factor = 0.1/$BMDL_{10\text{-HED}}$) from the human equivalent POD for the occurrence of skin tumors in male mice chronically exposed dermally to benzo[a]pyrene. As this slope factor has been developed for a local effect, it is not intended to estimate systemic risk of cancer following dermal absorption of benzo[a]pyrene into the systemic circulation.

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 0.005 per µg/day, and dermal slope factor of 0.004 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 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) 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 dermal slope factor was developed based on data in animals, and because there is no established methodology for extrapolating dermal toxicity from animals to humans. As such, several alternative approaches were evaluated (See Appendix C 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 and keywords listed in Table LS-1. References that were evaluated in other Agency and international health assessments were also examined. A comprehensive literature search was last conducted in February 2012.

Figure LS-1 depicts the literature search, study selection strategy, and the 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).

1 **Table LS- 1. Details of the literature search strategy employed**

Database	Keywords[a]
Pubmed Toxcenter Toxline	Chemical name (CASRN): benzo[a]pyrene (50-32-8)[a] 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 Initial keyword search <u>Standard toxicology search</u> Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors <u>Secondary keyword search[b]</u> Chemical-specific keywords Cancer; genotoxicity, neurotoxicity, immunotoxicity, reproductive toxicity, developmental toxicity <u>Additional keywords</u> lung OR skin combined AND tumor, neoplasm, papilloma, OR carcinoma; leukemia; forestomach; tongue; auditory canal; esophagus; larynx; pharynx; fertility; sperm; epididymis; seminiferous; testosterone; cervical hyperplasia; corpus luteum; estrous; testicular, ovarian OR thymus with atrophy; weight AND testis, ovary, thymus, spleen, pup; spleen AND cells, lymphocytes; immunoglobulin; immunosuppression; motor; functional observational battery; neurobehavioral; rotarod; nerve conduction; locomotor; neuromuscular; neurodevelopment; cognitive; learning; memory; righting.
TSCATS ChemID Chemfinder CCRIS HSDB GENETOX RTECS	Searched by chemical names (including synonyms) and CASRNs

[a] Keywords and synonyms were applied to the Pubmed, Toxcenter, and Toxline databases.
[b] Secondary keywords were selected from an understanding of the targets of benzo[a]pyrene toxicity gained from review of papers identified in literature searches conducted at the start of document development and relevant review documents.

2

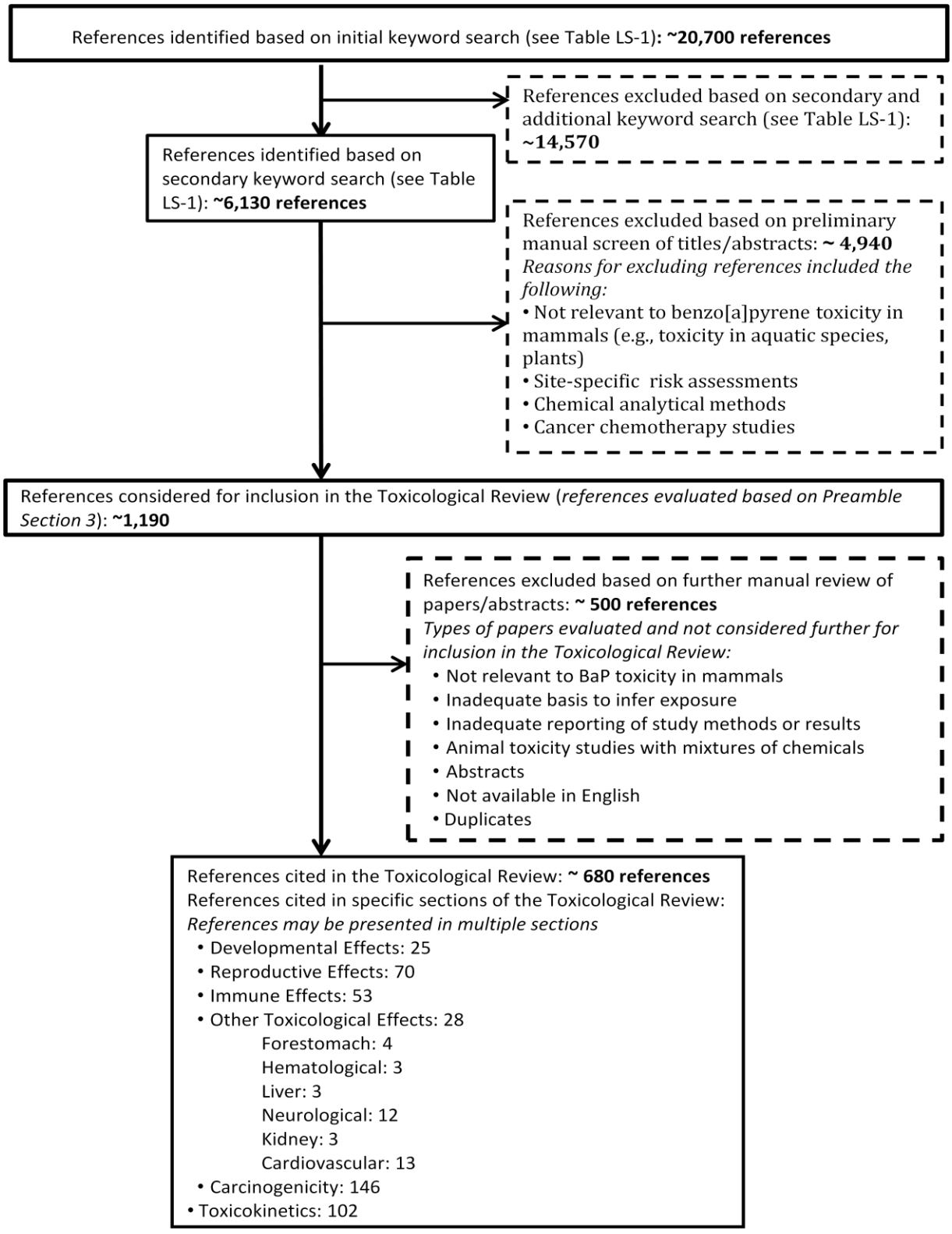


Figure LS- 1. Study selection strategy.

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 approximately 1,190 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]pyene-DNA adducts or urinary biomarkers) and potential adverse health outcomes were evaluated. Pertinent mechanistic studies in humans (e.g., identification of benzo[a]pyene-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. In addition, animal toxicity studies involving short-term duration and other routes of exposure were evaluated to inform conclusions about health hazards.

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] (Insert link when available).

[2]HERO 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. Synthesis of Evidence

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 no 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 often used as an indicator chemical to measure exposure to PAH mixtures.

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 pup weight, blood pressure, fertility, reproductive organ weight and histology, and neurological function in gestationally 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 early childhood. Two cohort studies in pregnant women in China and the United States examined cord blood levels of benzo[a]pyrene-DNA adducts in relation to measures of child growth following exposure to PAH mixtures (Tang et al., 2006; Perera et al., 2005a; 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]pyene-adducts or environmental tobacco smoke (ETS); 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., 2005a; 2004). Environmental tobacco smoke, also called secondhand smoke, is the smoke given off by a burning tobacco product and the smoke exhaled by a smoker that contains over 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.

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

In animals (Table 1-2), reduced bodyweight in offspring has been noted in some developmental studies. Decreases in body weight (up to 13%) were observed in mice following prenatal gavage exposure (GD 7-16), and as time from exposure increased (PND 20 to 42) the dose at which effects were observed decreased (40 to 10 mg/kg-day, respectively) (MacKenzie and Angevine, 1981). In addition, decreases in body weight (approximately 10 to 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 GD14-17 (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).

Decreased fetal survival has also been noted in gestationally treated animals at relatively high doses by the oral and inhalation route. An approximate 40% decrease in fetal survival was noted in mouse dams treated by gavage on GD7-16 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 GD14-17 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 $\mu g/m^3$ benzo[a]pyrene on GD 11-20 (Archibong et al., 2002) and 65% following exposure to 100 $\mu g/m^3$ benzo[a]pyrene on GD11-21 in (Wormley et al., 2004) F344 rats. Wu et al. (2003) also evaluated fetal survival as part of a study analyzing metabolites of benzo[a]pyrene and activation of the aryl hydrocarbon receptor (AhR) and cytochrome P450 (CYP) 1A1. The study authors reported decreased fetal survival at 75 and 100 $\mu g/m^3$, but not at 25 $\mu g/m^3$ following exposure to benzo[a]pyrene on GD 11-20. This study did not report number of dams or litters and no numerical data were reported.

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 been demonstrated (Kristensen et al., 1995; MacKenzie and Angevine 1981) (Table 1-2). 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 (10 mg/kg-day). 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 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). Testicular weight was decreased and atrophic seminiferous tubules and vacuolization was increased at ≥ 10 mg/kg-day in male mice exposed to benzo[a]pyrene gestationally from GD 7-16; severe atrophic seminiferous tubules were observed at 40 mg/kg-day (MacKenzie and Angevine, 1981).

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 30% 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 (MacKenzenie 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 (MacKenzenie and Angevine 1981; Kristensen et al., 1995).

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). Approximate elevations in systolic and diastolic blood pressure of 20- 30% and 50-80% were noted in the 0.6 mg/kg-day 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.

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

Reference and Study Design: Study Type/Period/Study Size/Location/Exposure Estimate	Results
Tang et al., 2006 Pregnancy cohort 150 non-smoking women that delivered babies between March 2002 – June 2002 Tongliang, China Exposure: mean hours per day exposed to environmental tobacco smoke 0.42 (SD 1.19); lived within 2.5 km of power plant that operated from December 2001 – May 2002; B[a]P-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 B[a]P-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 months: –0.048 (0.03) / –0.005 (0.48) 24 months: –0.041 (0.027) / –0.007 (0.28) 30 months: –0.040 (0.049) / –0.006 (0.44) Adjusted for environmental tobacco smoke, sex of child, maternal height, maternal weight, cesarean section delivery, maternal head circumference, and gestational age (for measures at birth)
Perera et al. (2005a; 2004) Pregnancy cohort 214 African-American and Dominican non-smoking women that delivered babies between April 1998 – October 2002; approximately 40% with smoker in the home New York, United States Exposure: B[a]P-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 B[a]P-DNA adducts and log-transformed birth weight and length: Weight Beta (p-value) / Length Beta (p-value): Interaction term: –0.088 (0.05) / –0.014 (0.39) B[a]P-DNA adducts: –0.012 (0.49) / –0.048 (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, gestational age. ETS = environmental tobacco smoke

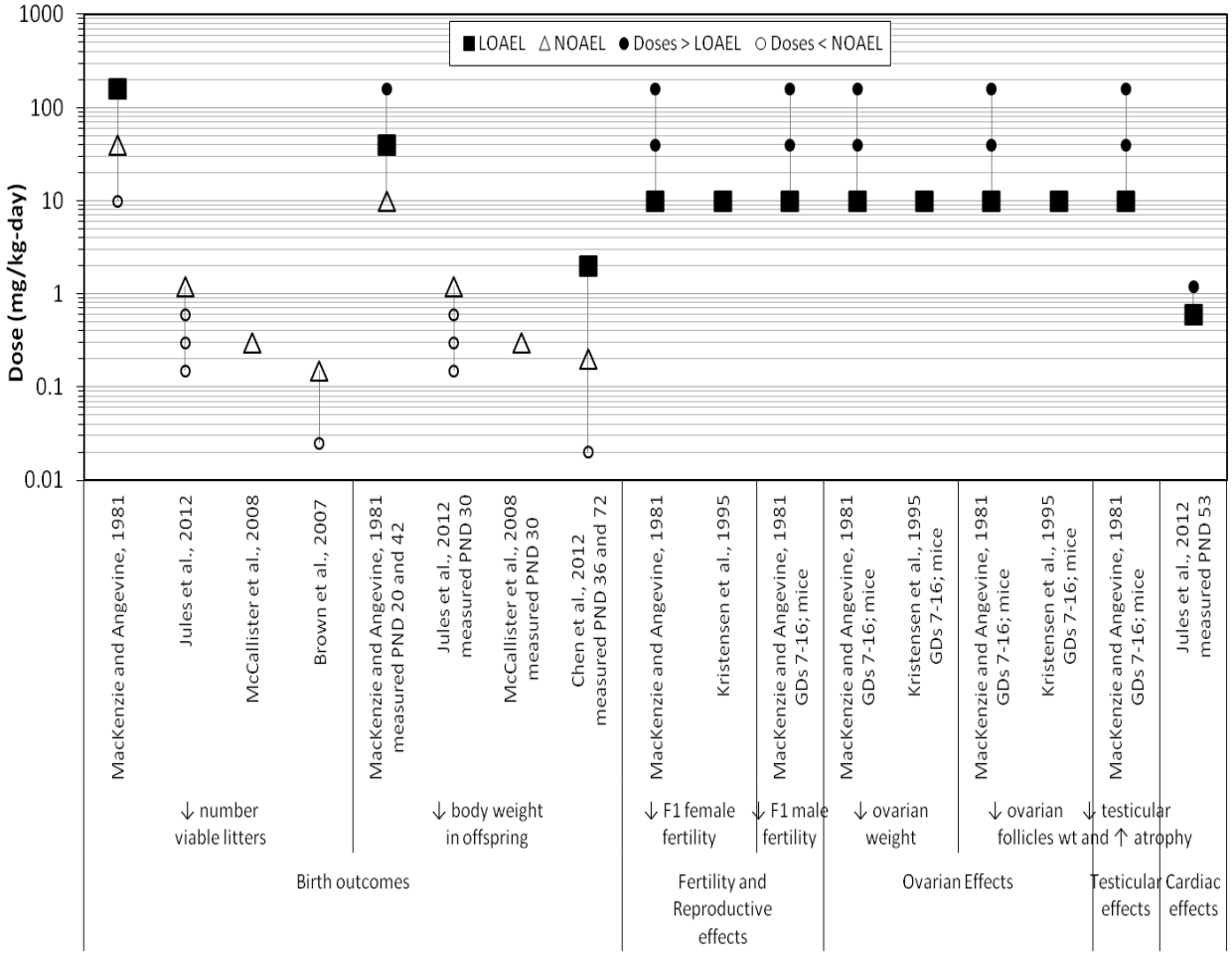
Table 1-2. Evidence pertaining to the developmental effects of benzo[a]pyrene in animals

Study Design and Reference	Results
Birth outcomes	
MacKenzie and Angevine, 1981 CD-1 mice, 30 or 60 F0 females/ dose 0, 10, 40, or 160 mg/kg-d by gavage GD 7–16	↓ Number of F0 females with viable litters: 46/60, 21/30, 44/60, 13/30* ↓ F1 body weight at PND 20 Response relative to control: 0, 4, -5*, -13* ↓ F1 body weight at PND 42 Response relative to control: 0, -6*, -6*, -10*
Kristensen et al., 1995 NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GD 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-day by gavage GD 14-17	No overt signs of toxicity in dams or offspring, differences in pup body weight, or number of pups per litter
McCallister et al. (2008) Long Evans Hooded rats, 5-6/group 0 or 0.3 mg/kg-day by gavage GD14- 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 PND15 and 30
Brown et al. (2007) Long Evans Hooded rats, 6/group 0, 0.025 or 0.15 mg/kg-day by gavage GD 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-day by gavage PND5-PND11	Statistically significant decrease in pup bodyweight (approximate 10-15% decrease) at 2 mg/kg-day measured on PND36 and 71 No differences among treatment groups in developmental milestones: incisor eruption, eye opening, development of fur, testis decent or vaginal opening
Fertility 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 GD 7-16	↓ Number of F1 females with viable litters: 35/35, 23/35*, 0/55*, 0/20* ↓F1 female fertility index (females pregnant/females exposed to males x 100): 100 , 66*, 0*, 0* ↓ F1 male fertility index (females pregnant/females exposed to males x 100): 80, 52*, 5*, 0*

	↓F1 litter size (20%) at 10 mg/kg-day (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 % change from control: 0, -42, -82, ND (statistical significance not reported) ↑ atrophic seminiferous tubules and vacuolization at ≥ 10 mg/kg-day; severe atrophic seminiferous tubules at 40 mg/kg-day (numerical data not reported)
Kristensen et al., 1995 NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GD 7–16	↓ Number of F1 litters (63%) ↓F1 litter size (30%) ↓ ovary weight (31%) in F1 females Few or no small, medium, or large follicles and corpora lutea (numerical data not reported)
Archibong et al., 2002 F344 rats, 10 females/group 0, 25, 75, or 100 µg/m^3 nose-only inhalation for 4 hrs/day GD 11–20	↓ Fetal survival ([pups/litter]/[implantation sites/litter] x 100) % fetal survival 97, 78*, 38*, and 34*% ↓ pup weight 0, 0, 14*, and 16*%
Wormley et al., 2004 F344 rats, 10 females/group 0 or 100 µg/m^3 nose-only inhalation for 4 hrs/day GD 11–21	↓ 65% decrease in pups/litter
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 GD 7-16	↓ 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 % change from control: 0, -42, -82, ND (statistical significance not reported) ↑ atrophic seminiferous tubules and vacuolization at ≥ 10 mg/kg-day; severe atrophic seminiferous tubules at 40 mg/kg-day (numerical data not reported)
Kristensen et al., 1995 NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GD 7–16	↓ ovary weight (31%) in F1 females Few or no small, medium, or large follicles and corpora lutea (numerical data not reported)
Cardiac Effects in Offspring	
Jules et al., 2012	↑ systolic blood pressure (measured at PND53)

Long-Evans rats, 6-17 F0 females/dose 0, 0.15, 0.3, 0.6, or 1.2 mg/kg-day by gavage GD 14-17	~20%* increase at 0.6 mg/kg-day ~50% *increase at 1.2 mg/kg-day (other dose groups not reported) ↑ diastolic blood pressure (measured at PND53) 33%* increase at 0.6 mg/kg-day 83% *increase at 1.2 mg/kg-day (other dose groups not reported) Statistically significant increase in heart rate at 0.6 mg/kg-day and statistically significant decrease at 1.2 mg/kg-day

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



Neurodevelopment 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 and anxiety, and electrophysiological changes have also been observed in animals following oral and inhalation exposure to benzo[a]pyrene.

The mammalian brain undergoes a period of rapid brain growth during the last 3 months of pregnancy through the first 2 years of life in humans (Dobbing and Sands, 1973, 1979) and the first 1–2 weeks of life in the rat and mouse neonate (Chen et al., 2011a). This period is characterized by the maturation of axonal and dendritic outgrowth and the establishment of 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 lactation exposure to benzo[a]pyrene (Bouayed et al., 2009; McCallister et al., 2008; Wormley et al., 2004).

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 pregnancy 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 environmental tobacco smoke (Tang et al., 2006; Perera et al., 2005a; 2004) (Table 1-3). One of these studies also examined aspects of neurodevelopment in two-year old children in China (Tang et al., 2008). In this follow-up of 2-year old children in an area surrounding a coal power plant, average scores on the Gesell Development Scale decreased with increased exposure (measured by benzo[a]pyrene-DNA adducts in cord blood) with similar effects seen in the motor, adaptive, and language domains (approximately 15 point decrement per unit increase in exposure).

Animal studies have also provided evidence of altered learning and memory behaviors following lactational or postnatal exposure to benzo[a]pyrene (Bouayed et al., 2009b, Chen et al., 2012) (Table 1-4). In mice, working memory was measured using the Y-maze spontaneous alternation test (Bouayed et al, 2009b). 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. This test may also be used to measure exploratory behavior in rodents and thus may be reflective of changes in anxiety-like behavior. A decrease 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 PND 14 (Bouayed et al., 2009b). The total number of arm entries in the Y-maze was unaffected by lactational exposure. 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 (Chen et al., 2012). Increased escape latency, decreased time in the target quadrant, and decreased number of platform crossings were observed in PND 39 – PND 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 PND 74 –PND 75. No difference in swim speed was observed between treatment groups, suggesting the difference observed is not attributable to general motor impairment.

Negative geotaxis and surface righting are discrete endpoints routinely used as part of a neurobehavioral test battery to assess acquisition of developmental milestones. In typical protocols, animals are tested on successive days (usually PND 3-7+ and PND 6-9+, respectively) and successful acquisition of these phenotypes is indicated when righting occurs within ~2 seconds or orienting 180° occurs within ~60 seconds (although latency for these events is often reported), respectively; in rats, both phenotypes are nearly always established (aka fully matured) before PND 10. Chen et al. (2012) performed these tests in a slightly atypical manner as quantitative measures of sensorimotor function at PND 12 and beyond, with control animals already to right within 0.8-1.8 seconds and able to orient 180° within 5-9 seconds. Although informative in terms of possible developmental delays, the sensitivity of these measures at these later timepoints has not been well established in the literature. Specifically, statistically significant differences observed by Chen et al. (2012) in the surface righting test were on the order of ~0.2-0.3 seconds and in the negative geotaxis test, ~3-4 seconds, with no automated recording of latency (such as use of video recordings). Additionally, male and female rats (which often show differences in the maturation of these developmental landmarks) were pooled for these measures. Due to these uncertainties, EPA considered the elevated plus maze and Morris water maze tests to be the most informative and appropriate measures of neurobehavioral function performed by Chen et al. (2012). The open field tests were considered less informative as male and female data were pooled and the test paradigm could not separate effects on motor activity from anxiety responses.

Neuromuscular function, coordination, and motor activity

Motor behavior, assessed by locomotion, reaching, balance, comprehension, drawing and hand control was one of the specific domains assessed in the Chinese pregnancy 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 ($p = 0.004$), and an approximate two-fold increased risk of development delay per unit increase in adducts (Table 1-3).

In laboratory animals (Table 1-4), impaired neuromuscular function and coordination have been consistently observed in mice lactationally exposed to ≥2mg/kg-day benzo[a]pyrene from PND 0 to PND 14 (Bouayed et al., 2009b) and in rat pups postnatally exposed to ≥0.02 mg/kg-day benzo[a]pyrene from PND5 to PND11 (Chen et al., 2012). In the righting reflex test, significant increases in righting time were observed in PND 3 – PND 5 mice and in PND 12 – PND 16 rats. In rats, increased righting time did not show a monotonic dose response on PND 12 or PND 14. In another test of neuromuscular function and coordination, dose-dependent increases in latency in the negative geotaxis test were observed in PND 5 – PND 9 mice and in PND 12 - PND 14 rats. The forelimb grip strength test was also evaluated in both mice and rats, but the results differed

between the species. In mice, a dose-dependent increase in duration of forelimb grip was observed on PND 9 and PND 11 during lactational exposure to benzo[a]pyrene, but not on PND 12. The Water Escape Pole Climbing test was also used to evaluate neuromuscular function and coordination in mice (Bouayed et al., 2009). No effect on climbing time was observed, whereas increased latency in pole grasping and pole escape in PND 20 male pups was observed. Increased locomotor activity on PND 69, measured using the open field test, has been reported in rats postnatally exposed to ≥0.2 mg/kg-day benzo[a]pyrene on PNDs 5-11 (Chen et al., 2012). This increase in locomotor activity was observed at doses that did not cause maternal toxicity and at several weeks post-exposure, suggesting that the developmental neurotoxic effects of benzo[a]pyrene persist through neurodevelopment and may become exacerbated over time. One issue in interpreting these results, however, is that it is difficult to separate an anxiety response from effects solely on motor function with this test.

Anxiety

Anxiety, attention, and hyperactivity in children ages 6 – 7 years were examined in relation to benzo[a]pyrene-DNA adducts measured at birth in a follow-up of a pregnancy cohort study conducted in New York City (Perera et al., 2012). The associations were stronger using the measures in cord blood compared with maternal samples, with indications of a 4-fold increased risk (p=0.051) of attention problems (Table 1-3). Exposure was treated as a dichotomy (i.e., detectable compared with non-detectable levels) in these analyses.

Decreased anxiety was reported in both rat and mouse pups following postnatal oral exposure to benzo[a]pyrene (Bouayed et al., 2009b, Chen et al., 2012) (Table 1-4). Anxiety-related 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 entries and time spent in open arms of the maze, as well as significantly decreased entries into closed arms of the maze, were observed on PND 32 following lactational exposure to ≥2 mg/kg-day benzo[a]pyrene (Bouayed et al., 2009b). The mice exhibited decreased latency in the elevated plus maze following lactational exposure to 20 mg/kg-day benzo[a]pyrene, while there was no exposure-related effect on the total number of times the mice entered arms of the maze. Decreased anxiety-like behaviors were also reported in rats following oral benzo[a]pyrene exposure from PND 5 to PND 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 significant decreases 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 of benzo[a]pyrene were higher than females. A significant increase in the number of open arm entries and significant decreases in the number of closed arm entries were observed in male rat pups exposed to 2 mg/kg-day, while a significant increase in time in the open arms of the maze was reported for males

exposed to ≥0.2 mg/kg-day. 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. These data indicate that oral postnatal exposure to benzo[a]pyrene resulted in decreased anxiety-like behavior in both mice and rats, with rats more sensitive than mice and female rats more sensitive than male rats.

Electrophysiological changes

Electrophysiological effects of gestational exposure to benzo[a]pyrene have been examined in animal studies through implanted electrodes in the rat cortex (Table 1-4). Inhalation exposure to 0.1 mg/m^3 benzo[a]pyrene resulted in reduced long-term potentiation in the dentate gyrus of male rats between PND 60 to PND 70 (Wormley et al., 2004). Oral exposure to 0.3 mg/kg-day benzo[a]pyrene resulted in decreased evoked neuronal activity in male rats following mechanical whisker stimulation between PND 90 to PND 120 (McCallister et al., 2008). The authors also noted reduced spike numbers in both the short and long latency responses to whisker stimulation, although no quantitative data were presented by the study authors. These effects were observed several months post-exposure, suggesting that gestational benzo[a]pyrene exposure has long-lasting functional effects on neuronal activity.

Table 1-3. Evidence pertaining to the neurodevelopmental effects of benzo[a]pyrene in humans

Reference, study design	Results
Tang et al. (2008, 2006) Tongliang, China Pregnancy cohort 150 non-smoking women (110 for Developmental Quotient analysis), delivered March 2002 – June 2002; lived within 2.5 km of power plant that operated from December 2001 – May 2002 Outcomes: head circumference at birth; Gesell Developmental Schedule, administered by physicians at 2 years of age (4 domains[a]: motor, adaptive, language, and social); standardized mean score = 100 ± SD 15 (score < 85 = developmental delay) Exposure: B[a]P-DNA adducts from maternal and cord blood samples; cord blood mean 0.32 (SD 0.14), range 0.125 – 0.812 adducts/10^{-8} nucleotides	Relation between cord blood B[a]P-DNA adducts and log-transformed head circumference Beta (p-value) Birth: –0.011 (0.057) 18 months: –0.012 (0.085) 24 months: –0.006 (0.19) 30 months: –0.005 (0.31) High versus low, dichotomized at median, adjusted for environmental tobacco smoke, sex of child, maternal height, maternal weight, cesarean section delivery, maternal head circumference, and gestational age (for measures at birth) Association between B[a]P adducts and development Beta (95% CI)[a] / OR (95% CI)[b] Motor: –16.0 (–31.3, –0.72)* / 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 B[a]P 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, environmental tobacco smoke, and cord lead levels.

Reference, study design	Results
Perera et al. (2012; 2005a; 2004) United States, New York Pregnancy cohort 214, non-smoking women (253 for behavior analysis), African-American and Dominican, delivered April 1998 – October 2002 Outcomes: head circumference at birth; Child Behavior Checklist (118 items), completed by mothers for children ages 6 – 7 years. 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 Exposure: B[a]P-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 B[a]P-DNA adducts and log-transformed head circumference Beta (p-value) Interaction term –0.088 (0.05) B[a]P-DNA adducts –0.012 (0.49) ETS in home –0.003 (0.90) High versus low, dichotomized at than 0.36 adducts/10^{-8} nucleotides, adjusted for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, gestational age. ETS = environmental tobacco smoke Logistic regression of risk of borderline or clinical status in relation to detectable levels of B[a]P adducts Prevalence; Maternal OR (95% CI); Cord Blood OR (95% CI) Anxious/depressed 6.32 % 1.4 (0.38, 5.4) 2.6 (0.69, 9.4) Attention problems 6.72% 2.2 (0.74, 6.8) 4.1 (0.99, 16.6) Anxiety (DSM) 9.48% 2.2 (0.79, 6.1) 2.5 (0.84, 7.7) Attention deficit – hyperactivity (DSM) 7.91% 1.8 (0.66, 5.1) 2.6 (0.68, 10.3) Exposure dichotomized as detectable (n=87 maternal, 56 cord blood samples) versus non-detectable; adjusted for sex, gestational age, maternal education, maternal IQ, prenatal environmental tobacco smoke, ethnicity, age, heating season, prenatal demoralization, and HOME inventory

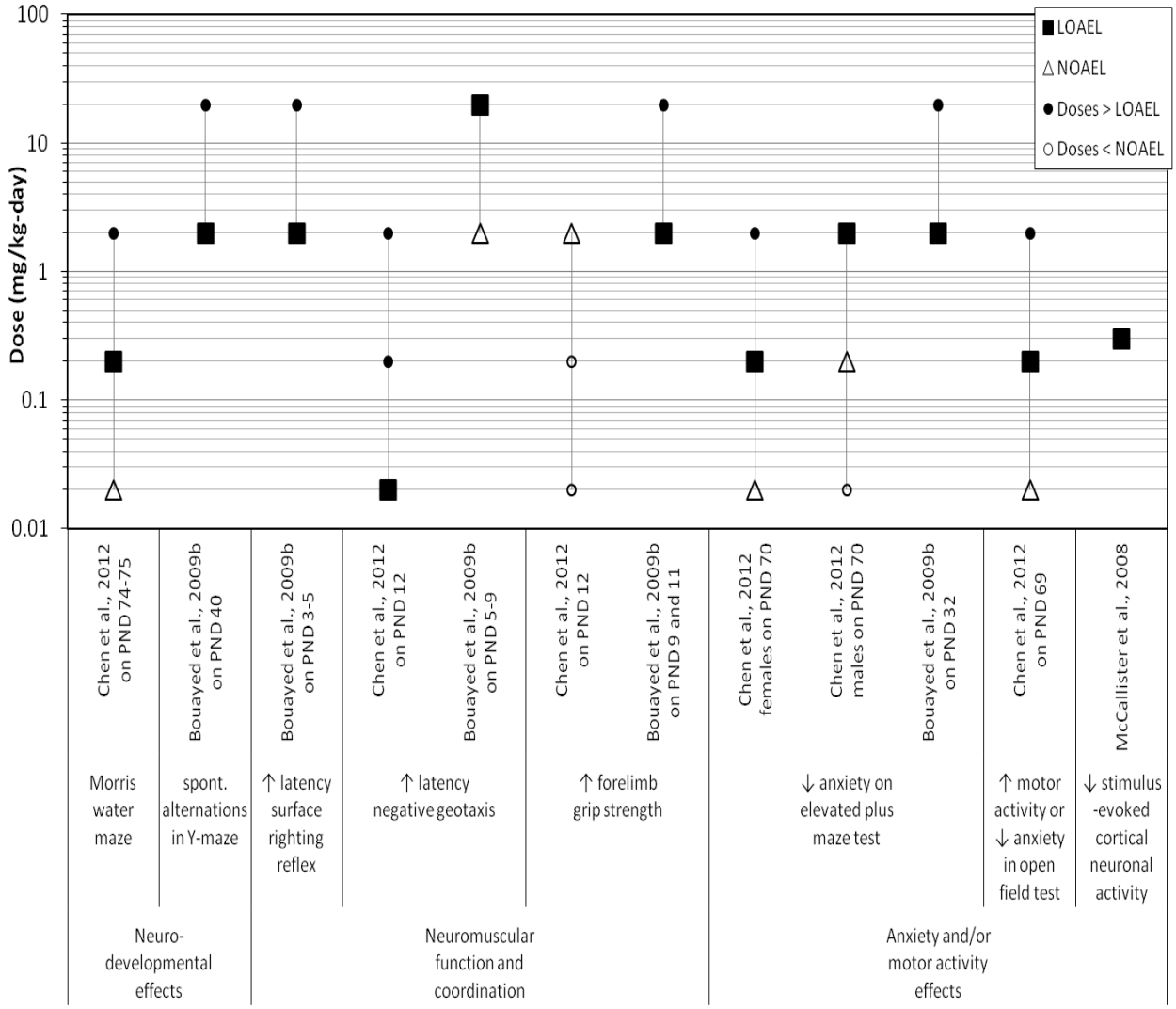
Table 1-4. Evidence pertaining to the neurodevelopmental effects of benzo[a]pyrene in animals

Study Design and Reference	Results
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-day by gavage PND5-PND11	Escape latency in the Morris water maze test: PND39: significant increase in escape latency at 2 mg/kg-day only PND74: significant increase in escape latency at ≥0.2 mg/kg-day Similar differences were seen at PND 36-38 or PND 71-73 time points; not statistically significantly different from control Time spent in the target quadrant in the Morris water maze test: PND40: significant decrease at 2 mg/kg-day only PND75: significant decrease at ≥ 0.2 mg/kg-day Number of platform crossings in the Morris water maze test: PND40: significant decrease at 2 mg/kg-day only PND75: significant decrease at ≥ 0.2 mg/kg-day (in females) and 2 mg/kg-day (in males)
Bouayed et al. (2009b) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-day maternal gavage PND 0 – 14 (lactational exposure)	Significant increase in the percent of spontaneous alternations in the Y-maze alternation test at 2 mg/kg-day but not at 20 mg/kg-day No effect on the total number of arm entries in the Y-maze alternation test

Neuromuscular function and coordination	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-day by gavage PND5-PND11	Latency in the surface righting reflex test PND12: significant increase at 0.2 mg/kg-day only PND14: Significant increase at 0.02 and 2 mg/kg-day only PND16 Significant difference at 2 mg/kg-day only PND 18 No significant difference Latency in the negative geotaxis test PND12: significant increase at all doses PND14: Significant increase at 2 mg/kg-day only PND16 and 18: No significant difference No effect on duration of forelimb grip in forelimb grip strength test
Bouayed et al. (2009b) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-day maternal gavage PND 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-day 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-day • 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-day
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-day by gavage PND5-PND11	• Significant increase in the number of entries into open arms in the elevated plus maze at PND 70 at ≥ 0.2 mg/kg-day (in females) and ≥ 2 mg/kg-day (in males) (no difference at PND 35) • Significant decrease in the number of entries into closed arms in the elevated plus maze at PND 70 at ≥ 0.2 mg/kg-day (in females) and ≥ 2 mg/kg-day (in males) (no difference at PND 35) • Significant increase in the time spent in open arms in the elevated plus maze at PND35 at ≥ 2 mg/kg-day in females and at PND70 at doses ≥ 0.02 mg/kg-day in females and ≥ 0.2 mg/kg-day in males • Significant decrease in latency time to first entry an open arm in the elevated plus maze on PND 70 at ≥ 0.2 mg/kg-day (no difference at PND 35) • Significant increase in the number of squares crossed in the open-field activity test: • PND34- significant increase at 2 mg/kg-day; PND69- significant increase at ≥ 0.02 mg/kg-day (no difference at PND18 and 20) • Significant increase in rearing activity in open-field activity at 0.2 mg/kg-day on PND 69 (no difference at PNDs 18, 20, and 34)
Bouayed et al. (2009b) Female Swiss albino mice, 5/group 0, 2 or 20 mg/kg-day maternal gavage PND 0 – 14 (lactational exposure)	• Significantly increased time in open arms in the elevated plus maze at ≥ 2 mg/kg-day • Significantly increased percentage of entries into open arms in the elevated plus maze at ≥ 2 mg/kg-day • Significantly decreased entries into closed arms in the elevated plus maze at 2 mg/kg-day, but not at 20 mg/kg-day • Significantly decreased latency time in the elevated plus maze at 20

	mg/kg-day • No effect on the total number of arm entries in the elevated plus maze • No effect on the latency to retract from the edge in the cliff aversion test
Electrophysiological changes	
McCallister et al. (2008) Long Evans Hooded rats, 5-6/group 0 or 0.3 mg/kg-day by gavage GD14- 17	Statistically significant decreases in stimulus-evoked cortical neuronal activity on PND90 – PND120 Reduction in the number of spikes in both the short and long latency periods on PND90 – PND120 (no quantitative data presented by authors)
Wormley et al., 2004 F344 rats, 10 females/group 0 or 100 µg/m^3 nose-only inhalation for 4 hrs/day GD 11–21	Electrophysiological changes in the hippocampus: Consistently lower LTP following gestational exposure (statistical analysis not reported) Response relative to control: -26%

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



Summary of Developmental Effects

Developmental effects following in utero exposure to benzo[a]pyrene have been reported in human and in animal models. In human populations, decreased head circumference, decreased birth weight, and decreased postnatal weight have been reported (Tang et al., 2006; Perera et al., 2005a, b). 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 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., 2005a; 2004), impaired cognitive ability (Tang et al., 2008, Perera et al., 2009), impaired neuromuscular function (Tang et al., 2008), and increased attention problems following prenatal exposure (Perera et al., 2012). These effects were seen in pregnancy 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) (Tang et al., 2008; 2006; Perera et al., 2012; 2005a; 2004). This type of measure covers a relevant time window of exposure with respect to gestational development. The analytical method was similar in the two studies (with a common set of investigators). The coefficient of variation of the exposure measures was relatively small (12%), but a high proportion of samples were below the detection limit and thus these studies were limited in terms of ability to examine a broad range of exposure. 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 and impaired neuromuscular function were consistently observed in multiple neurobehavioral tests in two separate species at comparable doses in the absence of maternal or fetal toxicity (Bouayed et al., 2009b; Chen et al., 2012).

In conclusion, the available human and animal data suggest that developmental toxicity and developmental neurotoxicity is a hazard 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 (Tang et al., 2008, 2006; Perera et al., 2005a, b). The occurrence of BPDE-DNA adducts in maternal and umbilical cord blood in conjunction with exposure to ETS was associated with reduced birth

weight and head circumference in pregnant women living in the vicinity of fires from the 09/11/2001 disaster site in New York City (Perera et al., 2005a). In other studies, elevated levels of BPDE-DNA adducts in umbilical cord blood were associated with: (1) reduced birth weights or reduced head circumference in the offspring of 529 Dominican or African-American nonsmoking women (Perera et al., 2005b); and (2) decreased body weight at 18, 24, and 30 months and deficits in several areas of development as assessed by the Gesell Developmental Schedules at 24 months in the offspring of nonsmoking Chinese women living in the vicinity of a coal-fired power plant (Tang et al., 2008, 2006).

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). An observational study of a Chinese population living in close proximity to a coal fired power plant found increased levels of benzo[a]pyrene-DNA adducts in cord blood were associated with decreased developmental quotients in offspring (Tang et al., 2008).

Evidence in animals of the effects of benzo[a]pyrene on neurological development includes: 1) decreased electrophysiological response to electrical stimulation of the dentate gyrus of the hippocampus and increased brain concentrations of benzo[a]pyrene metabolites in offspring of F344 rats exposed by inhalation to benzo[a]pyrene:carbon black aerosols on GD 11-21 (Wormley et al., 2004; Wu et al., 2003); 2) decreased evoked response in the field cortex and decreased cerebrocortical levels of mRNA for the NMDA receptor subunit in offspring of Long Evans rats exposed to 300 µg/kg on GD 14-17 (McCallister et al., 2008); and 3) decreased righting reflex and altered disinhibition behavior in offspring of lactating rats exposed to oral doses of 2 or 20 mg/kg-day on PND 1-14 (Bouayed et al., 2009b).

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 cigarette smoking reduces fertility; however, few studies have specifically examined levels of benzo[a]pyrene exposure and female reproductive outcomes (see Table 1-6). 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 which 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 vs. 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 IVF based on motility and morphology in patients of fertility clinics (Perrin et al., 2011a, b). An association between benzo[a]pyrene exposure levels and increased sperm DNA fragmentation using the sperm chromatin structure assay (SCSA) was observed by Rubes et al. (2012). However, it is currently unclear whether the SCSA, which measures sperm fragmentation following denaturation, is predictive of fertility (Sakkas et al., 2009; American Society of Reproductive Medicine 2008).

In several studies in rats and mice, decreased sperm count, motility, and production, and an increase in morphologically abnormal sperm have been observed (Table 1-5). 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 (25 to 50% compared to controls) counts have been observed in Sprague-Dawley rats and C57BL6 mice treated with 1- 5 mg/kg-day benzo[a]pyrene following oral exposure 42 or 90 days (Chen et al., 2011a; Mohamed et al., 2010). Additionally, a 15% decrease in epididymal sperm count was observed at a dose 100-fold lower in Sprague-Dawley rats exposed to benzo[a]pyrene for 90 days (Chung et al., 2011). However, confidence in this study is limited as the authors dosed 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. A short term study in mice and a subchronic inhalation study in rats 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 (~40% decrease from control in each parameter) were observed following 10 days of gavage exposure to 50 mg/kg-day benzo[a]pyrene in mice (Arafa et al., 2009). In addition, decrements in sperm count were observed following inhalation exposure to benzo[a]pyrene in rats for 60 days to 75 μg/m^3 (Archibong et al., 2008; Ramesh et al., 2008).

Both oral and inhalation exposure to benzo[a]pyrene have been shown to lead to decreased epididymal sperm motility and altered morphology in rodents. Decreased motility of 20 to 30% compared to controls was observed in C57BL6 mice (≥ 1mg/kg-day) and Sprague-Dawley rats (at 0.01 mg/kg-day) (Chung et al. 2011; Mohamed et al., 2010). However, the effective doses spanned two degrees of magnitude and as noted above, confidence in the study observing effects at 0.01 mg/kg-day benzo[a]pyrene (Chung et al., 2011) is limited by poor reporting. A short term oral study in mice 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 in rats (Arafa et al., 2009). In addition, decreased sperm motility was observed following inhalation exposure to benzo[a]pyrene in rats for 60 days to 75 μg/m^3 (Archibong et al., 2008; Ramesh et al., 2008) and for 10 days at ≥ 75 μg/m^3 (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., 2011a) 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). Decreases in testicular weight of approximately 35% have been observed in a 10 day gavage study in adult rats exposed to 50 mg/kg-day benzo(a)pyrene (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 36 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 the lack of 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 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 60d (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). 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%. No changes in epididymis weights were observed following an 84 day treatment in Sprague-Dawley rats of 5 mg/kg-day benzo[a]pyrene (Chen et al., 2011a).

Hormone changes

Several animal models have reported decreases in testosterone following both oral and inhalation exposure to benzo[a]pyrene (Table 1-5). 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 40% in intratesticular testosterone and 70% in serum testosterone (70%) at 0.1 mg/kg-day (Chung et al., 2011). 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 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 animals

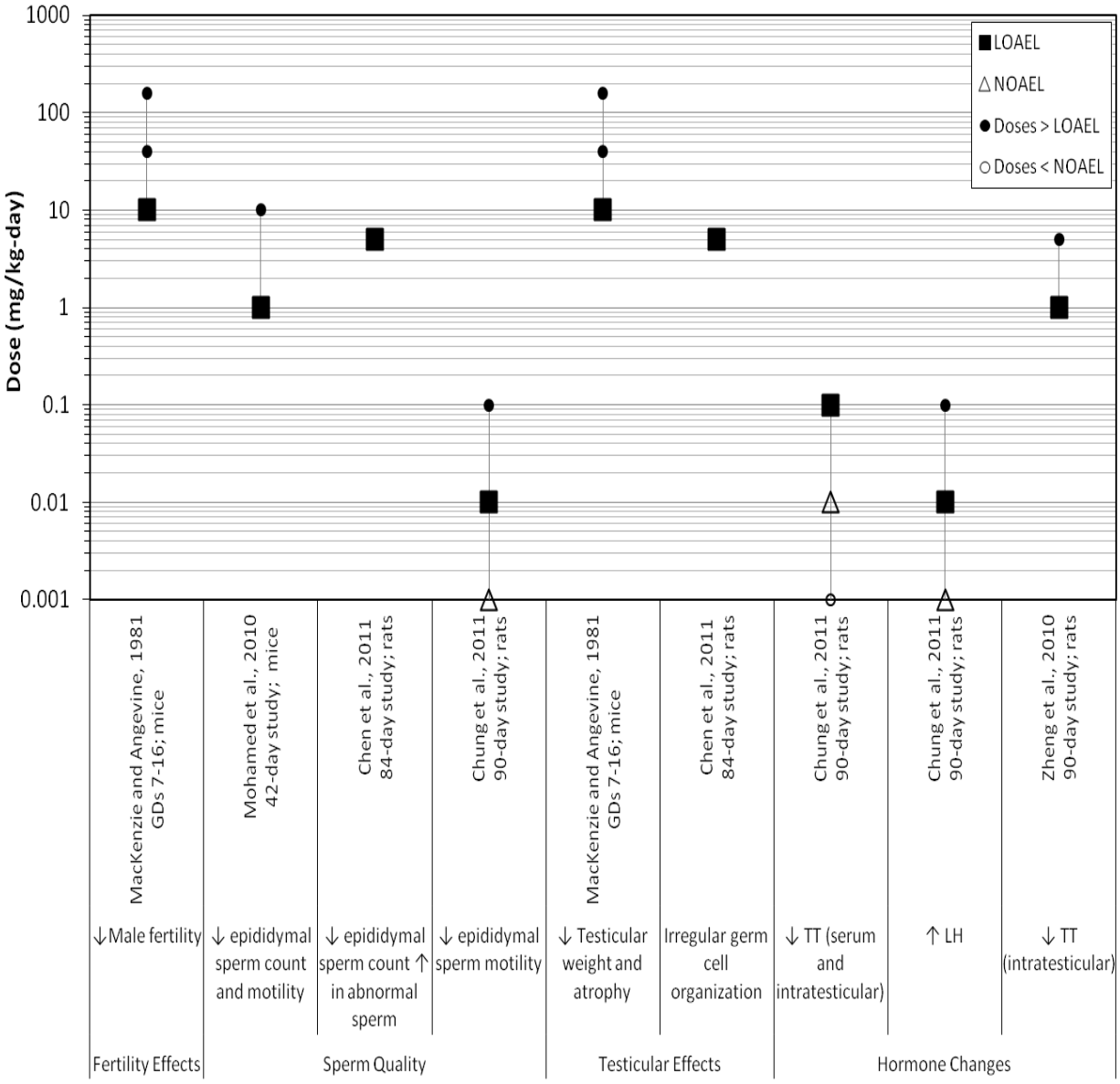
Study Design and Reference	Results
Sperm quality	
Mohamed et al., 2010 C57BL/6 mice, 10 males/dose (treated before mating with unexposed females) 0, 1, or 10 mg/kg-day, daily gavage	↓ epididymal sperm count in F0 mice Approximate % change from control: 0, -50*, -70* (numerical data not reported) ↓ epididymal sperm motility in F0 mice

Study Design and Reference	Results
(F0 males only) 42 days	Approximate % change from control: 0, -20*, -50* (numerical data not reported) ↓ epididymal sperm count in untreated F1 and F2 generations (numerical data not reported) No effects were observed in the F3 generation
Chen et al., 2011a Sprague-Dawley rats , 10 males/dose 0 or 5 mg/kg-day by gavage 84d	↓ epididymal sperm count(% change from control) 0, - 25%* ↑ % abnormal epididymal sperm 5, 8*
Chung et al., 2011 Sprague-Dawley rats, 20-25 males/dose 0, 0.001, 0.01, 0.1 mg/kg-day by gavage 90 days	↓ epididymal sperm motility (% response relative to control; reported only for 0.01 mg/kg-day) 0, -30* No statistically significant decrease in epididymal sperm count (reported only for 0.01 mg/kg-day)
Archibong et al., 2008; Ramesh et al., 2008 F344 rats, 10 males/group 0 or 75 µg/m^3, 4 hrs/day by inhalation 60 days	↓ epididymal sperm motility (% change from control) 0, -73* ↓ epididymal sperm count (% change from control) 0, -69* ↑ % abnormal epididymal sperm 33, 87* ↓ spermatids/g testis (approximate % change from control; numerical data not reported) 0, -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-day, daily gavage (F0 males only) 42 days	↓ seminiferous tubules with elongated spermatids (approximate % change from control; numerical data not reported)) 0, -20*, -35* No statistically significant change in area of seminiferous epithelium of testis (approximate % change from control; numerical data not reported) 0, 5, 20
Chung et al., 2011 Sprague-Dawley rats, 20-25 males/dose 0, 0.001, 0.01, 0.1 mg/kg-day by gavage 90 days	↑ number of apoptotic germ cells per tubule (TUNEL or caspase 3 positive) No change in testis weight or histology
Chen et al., 2011a Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-day by gavage 84 days	↑ testicular lesions characterized as irregular arrangement of germ cells and absence of spermatocytes (numerical data not reported) No change in testis weight

Study Design and Reference	Results
Archibong et al., 2008; Ramesh et al., 2008 F344 rats, 10 adult males/group 0 or 75 µg/m^3, 4 hrs/day by inhalation 60 days	↓ decreased testis weight (% change from control) 0, 34* ↓ 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-day by gavage 35 days	No change in testis weight
Knuckles et al., 2001 F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-day in diet 90 days	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, 0.1 mg/kg-day by gavage 90 days	↓ 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., 2011a Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-day by gavage 84 days	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-day by gavage 90 days	↓ Intratesticular testosterone (approximate % change from control; numerical data not reported) 0, -12, -25, -40* ↓ Serum testosterone (approximate % change from control ; numerical data not reported) 0, 0, -35, -70* ↑ serum LH (approximate % change from control; numerical data not reported) 0, 33, 67*, 87* ↓ hCG or dbcAMP-stimulated testosterone production in Leydig cells
Zheng et al., 2010 Sprague-Dawley rats, 8 males/dose 0, 1 or 5 mg/kg-day by gavage 90 days	↓ Intratesticular testosterone (pproximate % change from control ; numerical data not reported) 0, -15, -15*
Archibong et al., 2008; Ramesh et al., 2008 F344 rats, 10 adult males/group 0 or 75 µg/m^3, 4 hrs/day by inhalation 60 days	↓ intratesticular testosterone (approximate % change from control; numerical data not reported) 0, -80* ↓ serum testosterone (approximate % change from control) 0, -60*

Study Design and Reference	Results
	↑ serum LH (approximate % change from control) 0, 50*

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



Mode of Action Analysis—Male reproductive effects

Data regarding the potential mode of action for male reproductive effects associated with benzo[a]pyrene exposure are limited. Hypothesized modes of action include benzo[a]pyrene mediated DNA damage to male germ cells leading to genotoxicity, cytotoxicity, and apoptosis

(Chung et al., 2011; Olsen et al., 2010; Perrin et al., 2011 a,b; Revel et al., 2001), compromised function of Leydig and Sertoli cells (Chung et al., 2011; Raychoudhury and Kubinski, 2003), altered androgen hormone regulation (Inyang et al., 2003; Vinggaard et al., 2000), 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; Zenzes et al., 1998; Mancini et al., 1999; Melikian et al., 1999; 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). Although no association was seen with serum levels of benzo[a]pyrene, follicular fluid benzo[a]pyrene levels were significantly higher among the women who did not conceive with women who did get pregnant. The other study examined risk of what was termed a "missed abortion" (i.e., fetal death before 14 weeks gestation), using a case-control design with controls selected from women undergoing elective abortion (Wu et al., 2010). Benzo[a]pyrene-DNA adduct levels were similar in the aborted tissue of cases compared with controls, but a strong association was seen between maternal blood benzo[a]pyrene-DNA adduct levels and risk of missed abortion, with a 4-fold increased risk for levels above compared with below the median.

Experimental studies in animals also provide evidence that benzo[a]pyrene exposure affects fertility (Table 1-7). 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 GD 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 which 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 has 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 ovarian 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 36 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). 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; MacKenzenie and Angevine 1981). Additional support for the alteration of female reproductive endpoints comes from IP 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 intraperitoneal (i.p.) injection (Mattison et al., 1980; Swartz and Mattison, 1985; Miller et al., 1992; Borman et al., 2000). Further evidence is available from in vitro studies showing inhibition of antral follicle development and survival, as well as decreased production of estradiol, was demonstrated in mouse ovarian follicles cultured with benzo[a]pyrene for 13 days (Sadeu and Foster, 2011). Likewise, 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). 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 which investigated effects in the cervix following oral exposure to benzo[a]pyrene (Table 1-7). 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., 2011a, 2010). 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 (Beland and Culp, 1998; Kroese et al., 2001). 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

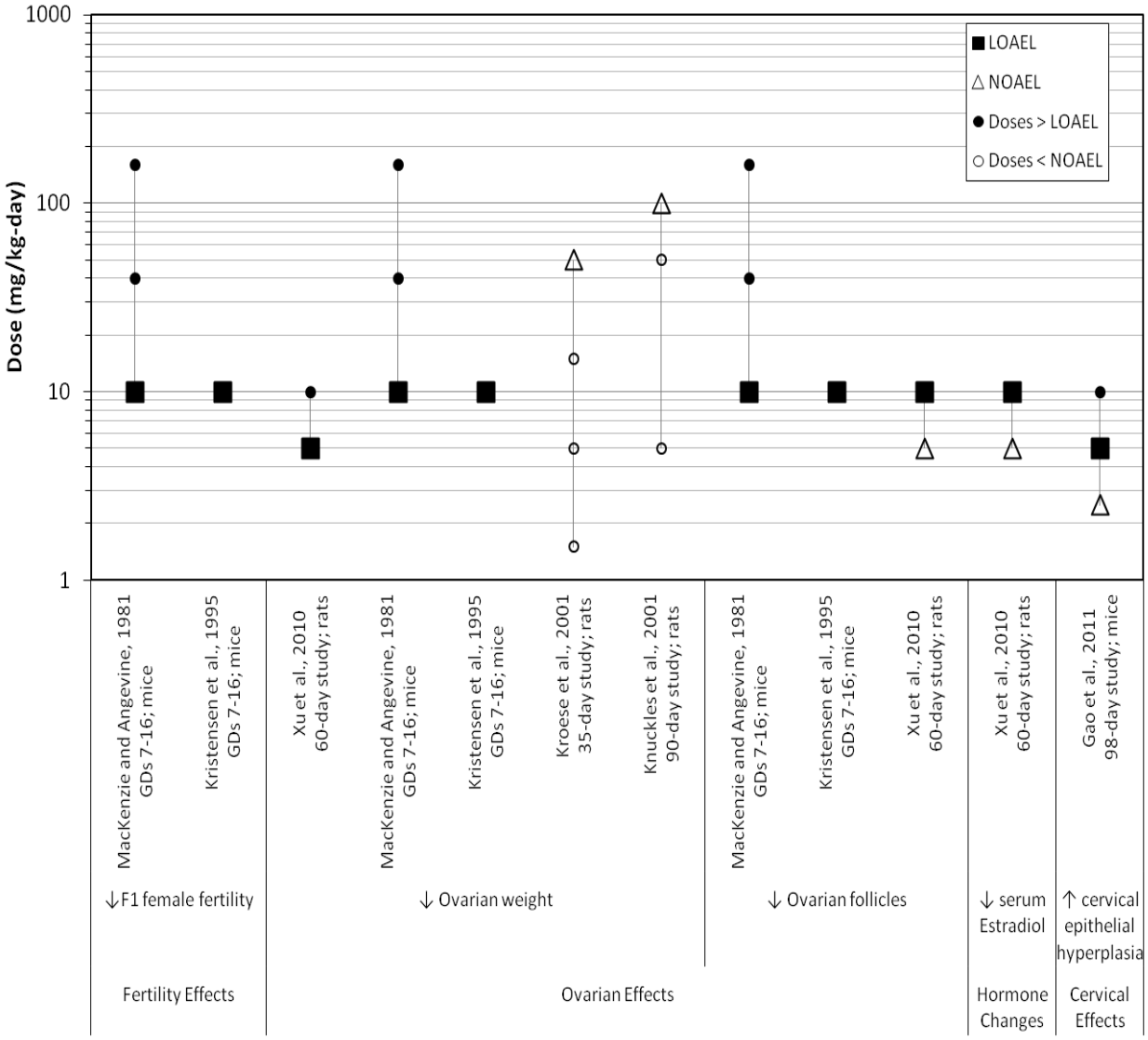
Reference and Study Design: Study Type/Period/Study Size/Location/Exposure Estimate	Results
Probability of Conception	
Neal et al., 2008 36 women undergoing in vitro fertilization (19 smokers, 7 passive smokers, and 10 non smokers) Exposure: B[a]P in serum and follicular fluid	B[a]P levels (ng/ml)[a] Conceived: Follicular fluid 0.1; Serum 0.01 Did not Conceive: Follicular fluid 1.7; Serum 0.05 (p-value): Follicular fluid (< 0.001); Serum (not reported) [a] estimated from Figure 3 of Neal et al., 2008
Fetal Death	
Wu et al., 2010 Case control study: 81 cases (96% participation rate) - fetal death confirmed by ultrasound before 14 weeks gestation; 81 controls (91% participation rate) - elective abortions); matched by age, gestational age, and gravidity; excluded smokers and occupational PAH exposure Tianjin, China Exposure: B[a]P in aborted tissue and maternal blood samples (51 cases and controls, 2 of 4 hospitals)	B[a]P 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 B[a]P adducts and missed abortion[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 animals

Study Design and Reference	Results
Fertility	
MacKenzie and Angevine, 1981 CD-1 mice, 30 or 60 F0 females/ dose 0, 10, 40, or 160 mg/kg-d by gavage GD 7–16	↓ Number of F0 females with viable litters 46/60, 21/30, 44/60, 13/30*
Kristensen et al., 1995 NMRI mice, 9 females/dose 0 or 10 mg/kg-d by gavage GD 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-day, adjusted) 60 days	↓ Ovary weight (% change from control) 0, 11*, 15* ↓ number of primordial follicles (20%* decrease at high dose) ↑ increased apoptosis of ovarian granulosa cells (approximate % apoptosis) 2, 24*, 14*
Knuckles et al., 2001 F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d in diet 90 days	No changes in ovary weight
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-day by gavage 5x/week 35 days	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-day, adjusted) 60 days	↓ Serum estradiol (approximate change from control) 0, 16%, 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/day GD 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
Cervical effects	

Study Design and Reference	Results
Gao et al, 2011 ICR mice, 26 females/dose 0, 2.5, 5, or 10 mg/kg by gavage 2 days/week 98 days	↑ cervical epithelial hyperplasia: 0/26, 4/26, 6/25*, 7/24* ↑ cervical atypical hyperplasia: 0/26, 0/26, 2/25, 4/24* ↑ inflammatory cells in cervical epithelium: 3/26, 10/26, 12/26*, 18/24* ↑ mortality: 0/26, 0/26, 1/26, 2/26

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



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; Matkainen et al., 2002, 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; 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; Vondracek 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., 2011a; 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. The evidence for male reproductive toxicity seen across multiple human and animal studies identifies the male reproductive system effects as a potential hazard associated with exposure to benzo[a]pyrene.

Female Reproductive Effects

A large body of mechanistic data, both in vivo and in vitro, suggests that benzo[a]pyene 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, 2007) and increased risk of early fetal death (i.e., before 14 weeks 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 consideration of the evidence from human, animal, and mechanistic studies, female reproductive effects are identified as a potential hazard associated with exposure to benzo(a)pyrene.

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., 2005; 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 missed abortion (Wu et al., 2010).

Inhalation exposure of pregnant female rats to benzo[a]pyrene:carbon black aerosols on GD 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 to 160 mg/kg-day on GD 7-16 (Kristensen et al., 1995; Mackenzie and Angevine, 1981).

Reductions in female fertility associated with decreased ovary weight following gestational exposure 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); 3) stimulation of oocyte apoptosis (Matkainen et al., 2002, 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 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 oral exposure of the F0 to benzo[a]pyrene (Mohammed 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, 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). 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, intraperitoneal (i.p.), intratracheal (i.t.), 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 3-90 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 13-41% 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 counts were attributed by the study authors to the decreased B cells, and suggest a possible selective toxicity of benzo[a]pyrene to B cell precursors in the bone marrow. The spleen effects observed in the single subchronic study 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 and Johnson, 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 (Szczeklik et al., 1994; Wu et al., 2003). Conversely, immunostimulation of immunoglobulin levels has also been observed in humans, specifically elevated IgG (Karakaya et al., 1999) and elevated IgE (Wu et al., 2003) following occupational PAH exposure.

Decreases in serum IgM (13 to 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 (Winkler et al., 1996) 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; 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 ≥5mg/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 NK 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 NK 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 (Galvan et al., 2006).

In contrast to studies that have shown immunosuppression, benzo[a]pyrene may also induce sensitization responses. Epicutaneous application of benzo[a]pyrene (100 μg benzo[a]pyrene to C3H/HeN mice followed by ear challenge with 20 μg benzo[a]pyrene 5 days later) produced a contact hypersensitivity (a significant ear swelling) response (Klemme et al., 1987).

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 (GD 11-13), late (GD 16-18), or both (GD 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 13-17, when examined on GD18 (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 gavage exposures of 50 or 100 mg/kg benzo[a]pyrene to the dams on GD 13-17, respectively (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 ConA and a weak response compared to controls in an allogeneic mixed lymphocyte reaction assay (Urso et al., 2008).

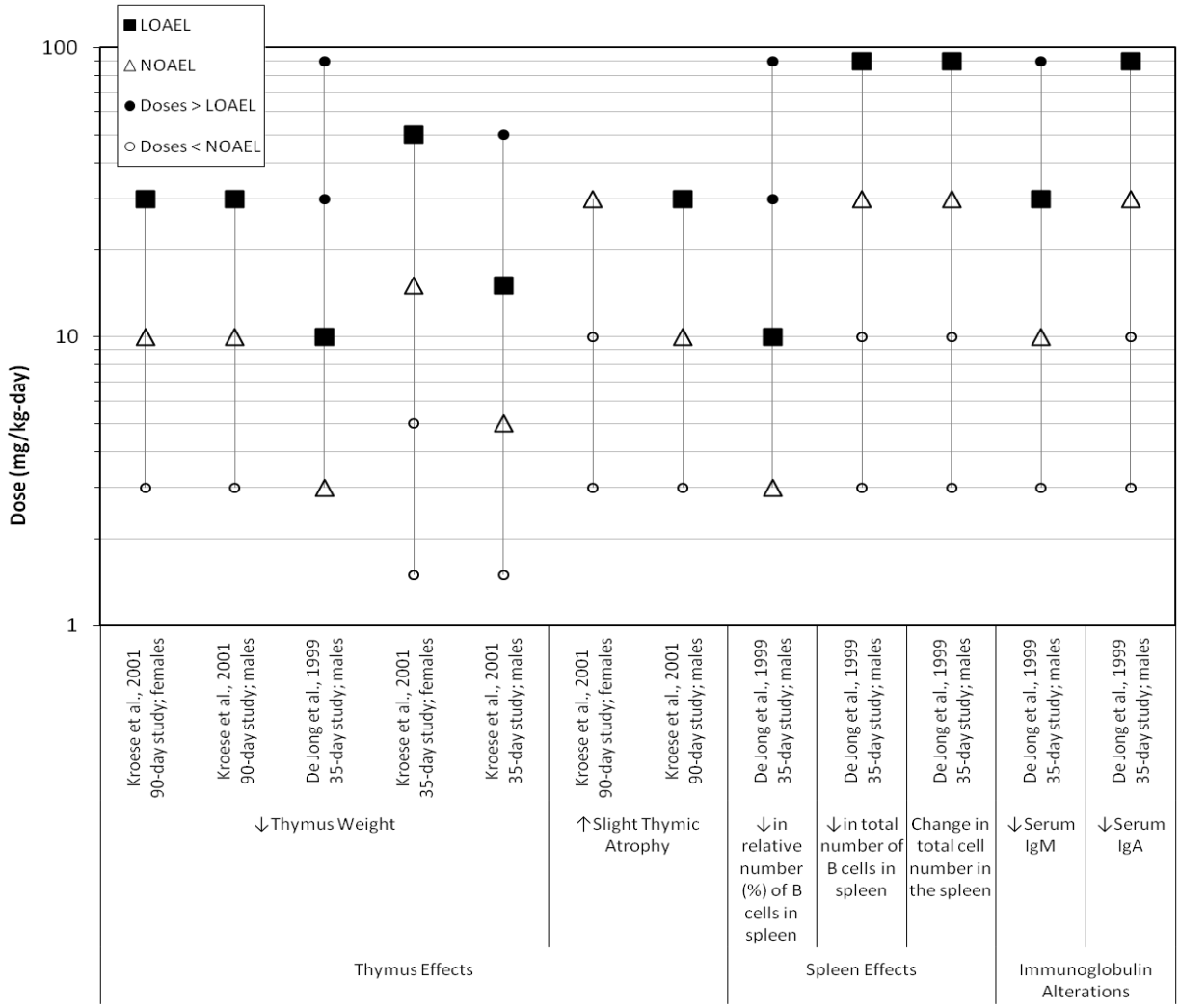
Table 1-8. Evidence pertaining to the immune effects of benzo[a]pyrene in animals

Study Design and Reference	Results
Thymus Effects	
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 3, 10, 30 mg/kg-d by gavage 5 days/week	↓ thymus weight Females (% change from controls): 0, -3, -6, -28* Males (% change from controls): 0, 0, -13, -29*

90 days	↑ slight thymic atrophy Females (incidence): 0/10, 0/10, 0/10, 3/10 Males (incidence): 0/10, 2/10, 1/10, 6/10*
De Jong et al., 1999 Wistar rats, 8 males/dose 0, 3, 10, 30, 90 mg/kg-d by gavage 5 days/week 35 days	↓ thymus weight % change from control: 0, -9, -15*, -25*, -62*
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 1.5, 5, 15, 50 mg/kg-d by gavage 5 days/week 35 days	↓ thymus weight Females (% change from controls): 0, +13, +8, -3, -17* Males (% change from controls): 0, -8, -11, -27*, -33*
Spleen Effects	
De Jong et al., 1999 Wistar rats, 8 males/dose 0, 3, 10, 30, 90 mg/kg-d by gavage 5 days/week 35 days	↓ in relative number (%) of B cells in spleen % change from control: 0, -8, -13*, -18*, -41* ↓ in total number of B cells in spleen % change from control: 0, +13, -13, -13, -61* Change in total cell number in the spleen % change from control: 0, +20, 0, +7, -31*
Immunoglobulin Alterations	
De Jong et al., 1999 Wistar rats, 8 males/dose 0, 3, 10, 30, 90 mg/kg-d by gavage 5 days/week 35 days	↓ Serum IgM % change from control: 0, -13, -14, -33*, -19 ↓ Serum IgA % change from control: 0, -27, -22, -28, -61*

1 *Statistically significant ($p<0.05$) as reported by study authors.

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 i.t. 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 aryl hydrocarbon receptor (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 (HSC) in the bone marrow, a major site of B cell proliferation and antibody production (Esser et al 2009). Benzo[a]pyrene induces greater reductions in bone marrow cultures from Ah-responsive (C57BL/6) mice compared to Ah-nonresponsive (DBA/2) mice (Hardin et al. 1992). Furthermore, B cell lymphopoiesis in Ah-responsive mice was dose

dependently suppressed but not in Ah-nonresponsive mice (Hardin et al., 1992). Addition of the AhR antagonist and cytochrome P450 (CYP450) inhibitor, α-NF, prevented the benzo[a]pyrene-induced inhibition 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 through the metabolite, BPDE (Allen 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

Immunotoxic effects of benzo[a]pyrene exposure are based on data 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.

Each of the immune effects reported in animal bioassays for benzo[a]pyrene provide equivocal evidence of immunotoxicity; functional assays provide greater support for immunotoxicity than observational findings such as organ weight, hematological, or histopathological measures (WHO, 2012). Although the overall database for benzo[a]pyrene immunotoxicity in experimental animals is limited and the key events underlying the mode of action are not 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. Overall, the weight of evidence in animals indicates that immunotoxicity may be a potential hazard associated with benzo[a]pyrene exposure, but the immune system does not appear to be a sensitive target for benzo[a]pyrene-induced toxicity.

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

dose sensitivity and disease persistence from developmental versus adult chemical exposure (reviewed in Luebke et al., 2006).

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

1.1.4. Other Toxicological Effects

There is some evidence that benzo[a]pyrene can produce systemic effects in several organ systems including 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 Section 1.1.

Forestomach effects

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., 200; De Jong et al., 1999) and in B6C3F1 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, limited hyperplasia was reported (Kroese et al., 2001). 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 largely uncharacterized 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).

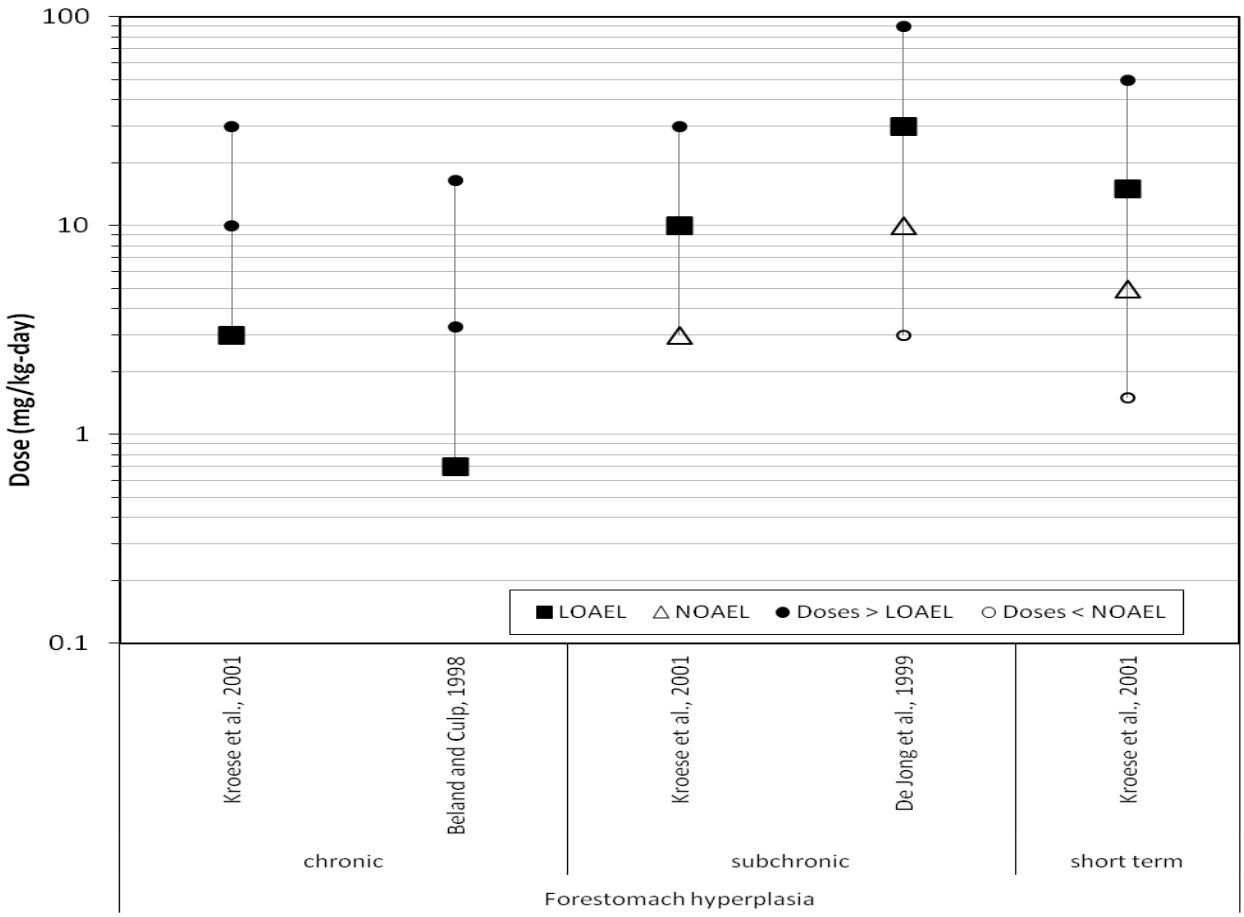
Table 1-9. Evidence pertaining to the forestomach effects of benzo[a]pyrene in animals

Study design and reference	Results
Kroese et al. (2001) Wistar (Riv:TOX) rats: male and female (52/sex/dose group) 0, 3, 10, or 30 mg/kg-day by gavage 5 days/week 104 weeks (chronic) Wistar (Riv:TOX) rats: male and female (10/sex/dose group) 0, 3, 10, or 30 mg/kg-day by gavage 5 days/week 90 days (subchronic) Wistar (specific pathogen-free Riv:TOX) rats (10/sex/dose group) 0, 1.5, 5, 15, or 50 mg/kg bw by gavage 5 days/week 5 weeks (shorter-term)	**Chronic:** **forestomach hyperplasia** – (basal cell hyperplasia) Incidences: M: 2/50; 8/52; 8/52; 0/52 F: 1/52; 8/51; 13/51; 2/52 **Subchronic:** **forestomach hyperplasia** – (slight basal cell hyperplasia) Incidences: M: 2/10; 0/10; 6/10; 7/10 F: 0/10; 2/10; 3/10; 7/10 **Short term:** **forestomach hyperplasia** – (basal cell hyperplasia) Incidences: M: 1/10; 1/10; 4/10; 3/10; 7/10 F: 0/10; 1/10; 1/10; 3/10; 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[a]: 0, 0.7, 3.3, and 16.5 mg/kg-day) 2 years	**forestomach hyperplasia** Incidences: 13/48; 23/47; 33/46*; 38/47*
De Jong et al. (1999) Wistar rats: male (8/ dose group) 0, 3, 10, 30, or 90 mg/kg-day by gavage 5 days/week 5 weeks	**forestomach hyperplasia** – (basal cell hyperplasia) statistically significantly increased incidences at 30 and 90 mg/kg-day were reported, but incidence data were not provided

* 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 time-weighted average (TWA) body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group

Figure 1-6. Exposure-response array for forestomach hyperplasia following oral exposure



Mode of Action Analysis—Forestomach Effects

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; DeJong 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]pyene 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, and 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.

Summary of Forestomach Effects

A dose-dependent increased incidence of forestomach hyperplasia was observed in both rats and mice and in multiple studies. Evidence for forestomach hyperplasia after both gavage and dietary administration demonstrates concordance of the effect across different modes of administration and illustrates that the hyperplasia is not dependent on bolus dosing or mechanical irritation associated with gavage administration. The increase in forestomach hyperplasia was also consistent across multiple exposure regimens, including chronic, subchronic, short-term, and acute exposures.

Hematological Effects

Altered hematological parameters, including decreases in RBC count, hemoglobin, and hematocrit have been observed in laboratory animals following benzo[a]pyrene exposure (Table 1-10). Statistically significant decreases in RBC count, hemoglobin, and hematocrit were observed in male Wistar rats at doses greater than 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 RBC's and/or RBC precursors in the bone marrow (DeJong et al., 1999). Similarly, male and female F344 rats also showed statistically significant decreases in RBC counts and hematocrit level, along with decreased hemoglobin levels in a 90 day dietary study (Knuckles et al. 2001). Statistically significant decreases were observed in the two highest doses (50 and 100 mg/kg-day) in male rats, while a maximum decrease of 12% was only observed at the highest dose in female rats at 100mg/kg-day. Small, but not statistically significant decreases in RBC counts and hemoglobin were observed in both a 35-day and 90-day study in Wistar rats (Kroese et al. 2001). It should be noted that when observed, the magnitudes of the decreases in RBC, 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 WBC's, attributed to reduced numbers of lymphocytes and eosinophils, was also observed at 90 mg/kg-day following gavage exposure for 35 days (DeJong et al., 1999). The mode of action by which benzo[a]pyrene exposure may lead to altered hematological parameters is undetermined.

Liver Effects

Liver effects other than cancer associated with benzo[a]pyrene exposure primarily include changes in liver weight and abnormal histopathology (Table 1-10). 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 1000 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 (ALT) or serum aspartate transaminase (AST) levels were observed, and a small dose-related decrease in γ-glutamyl transferase (GGT) 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 reported following exposure to 90 mg/kg-day benzo[a]pyrene for 35 days, however incidence data were not reported (De Jong et al., 1999). Other histopathological changes observed in the liver include an increased incidence of liver clear cell foci of alteration in males and females during a 2-year carcinogenicity study (Kroese et al., 2001), however organs with tumors were not evaluated. Since many of the animals in the top two doses developed liver tumors, the dose responsiveness of the increased incidence of clear cell foci is unclear.

A dose-dependent increase in liver microsomal EROD activity, indicative of CYP1A1 induction, was observed in both sexes at doses ≥1.5 mg/kg-day (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 Effects

There is minimal evidence of kidney toxicity following exposure to benzo[a]pyrene (Table 1-10). A statistically significant decrease in kidney weight was observed in the highest dose tested (90 mg/kg-day) in a 35-day gavage study in male Wistar rats (De Jong et al. 1999). Decreases in kidney weight at 3 and 30 mg/kg-day were also observed, but these changes were not dose-dependent. Additionally, kidney weights were not affected by following exposure to benzo[a]pyrene for 35 days (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. While there are some findings to suggest that the kidneys may be affected by benzo[a]pyrene exposure, overall there is insufficient data to suggest that the kidneys may be a primary target of benzo[a]pyrene-induced toxicity.

Cardiovascular Effects

Atherosclerotic vascular disease and increased risk of cardiovascular mortality has 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, 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 as well (Chau et al., 1993). Elevated risks of ischemic heart disease (IHD) were associated with past cumulative benzo[a]pyrene exposure (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 which associate benzo[a]pyrene exposure with cardiovascular effects (Friesen et al., 2010; Burstyn et al., 2005) rely on statistical models to create exposure groups based on previously gathered benzo[a]pyrene air samples that may or may not have included members 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 1-10). At the highest dose tested (1200 μg/kg BW by gavage to the dams), systolic pressures were elevated approximately 50% and diastolic pressures were elevated approximately 80% above controls.

Reduced endothelial integrity and increased smooth muscle cell mass, both related to atherosclerosis, have been observed in Sprague-Dawley rats exposed to 10mg/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 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 have 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 (Knaapen et al., 2007; Curfs et al., 2005, 2004; Godschalk et al., 2003). ApoE -/- mice develop spontaneous atherosclerosis, which is thought to be due to enhanced oxidative stress from the lack of ApoE (Godschalk et al., 2003). Overall, these studies suggest that benzo[a]pyrene exposure in ApoE-/- mice enhances the progression of atherosclerosis through a general local inflammatory process.

Neurological Effects

Impaired learning and memory, as well as neurochemical alterations, have been observed in humans following occupational exposure to PAH mixtures (Niu et al, 2009). Male coke oven workers were analyzed for alterations in neurobehavioral function using the World Health Organization Neurobehavioral Core Test Battery (WHO-NCTB), as well as changes in neurotransmitter concentrations in blood. Urinary levels of the PAH metabolite 1-hydroxypyrene were used as markers of PAH exposure. In the WHO-NCTB, coke workers had lower scores in the digit span and forward digit span tests than matched control subjects, suggesting that short-term memory was impaired. The authors also reported that the digit span and forward digit span scores cores significantly decreased with increasing 1-hydroxypyrene levels in urine. PAH exposure also altered the blood levels of several neurotransmitters. As in the functional assays, the authors reported that alterations in neurochemical measures were associated with urinary levels of 1-hydroxypyrene.

Reductions in neuromuscular, autonomic, sensorimotor, and electrophysiological endpoints have been reported in rats and mice following acute or short-term exposure to benzo[a]pyrene (Saunders et al., 2006, 2002, 2001; Liu et al., 2002; Grova et al., 2007, 2008; Bouayed et al., 2009a). Impaired Morris water maze performance was observed following subchronic oral gavage in adult rats (Chen et al. 2011b); however, the study was conducted with one dose group (Table 1-10). These data suggest that longer durations of benzo[a]pyrene exposure could be neurotoxic; however, only limited data are available to inform the neurotoxic potential of repeated sub-chronic

or chronic exposure to benzo[a]pyrene. The available data from the human and chronic and subchronic animal studies (Kroese et al., 2001; Beland and Culp 1998) provide limited support for benzo[a]pyrene as a neurotoxicant in adults.

Table 1-10. Evidence pertaining to other systemic effects of benzo[a]pyrene in animals

Study Design and Reference	Results
Hematological Parameters	
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 3, 10, 30 mg/kg-d by gavage 5 days/week 90 days	RBC count and hemoglobin changes not statistically significant in males or females at any dose (numerical data not reported)
Knuckles et al., 2001 F344 rats, 20/sex/dose 0, 5, 50, 100 mg/kg-d by diet 90 days	↓ RBC count Females (% change from controls): statistically significant at 100mg/kg-d (numerical data not reported) Males (% change from controls): statistically significant at 50 and 100mg/kg-d (numerical data not reported) ↓ hematocrit Females (% change from controls): statistically significant at 100mg/kg-d (numerical data not reported) Males (% change from controls): statistically significant at 50 and 100mg/kg-d (numerical data not reported) ↓ hemoglobin Females: statistically significant at 100mg/kg-d (numerical data not reported)Males: statistically significant at 100mg/kg-d (numerical data not reported)
De Jong et al., 1999 Wistar rats, 8 males/dose 0, 3, 10, 30, 90 mg/kg-d by gavage 5 days/week 35 days	↓ RBC count % change from controls: 0, -1,- 5*, -10*, -18* ↓ hemoglobin % change from controls: 0, -1, -7*, -10*, -18* ↓ hematocrit % change from controls: 0, 0, -6*, -8*, -14* ↓ WBC count % change from controls: 0, -8, -9, -9, -43* ↑ mean cell volume % change from controls: 0, 0, -3, 0, +3* ↓ mean corpuscular hemoglobin concentration % change from controls: 0, -1, -1, -1, -3*

Study Design and Reference	Results
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 1.5, 5, 15, 50 mg/kg-d by gavage 5 days/week 35 days	RBC count: not statistically significant (numerical data not reported) hemoglobin: not statistically significant (numerical data not reported)
Liver Effects	
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 3, 10, 30 mg/kg-d by gavage 5 days/week 90 days	↑ liver weight Females (% change from controls): 0, -2, +4, +17* Males (% change from controls): 0, +7, +15*, +29* Liver histopathology: no effects reported
Knuckles et al., 2001 F344 rats, 20/sex/dose 0, 5, 50, 100 mg/kg-d by diet 90 days	↑ liver:body weight ratio Females: no change (numerical data not reported) Males (% change from controls): 23% change reported at 100mg/kg-d (numerical data not reported)
De Jong et al., 1999 Wistar rats, 8 males/dose 0, 3, 10, 30, 90 mg/kg-d by gavage 5 days/week 35 days	↑ liver weight % change from controls: 0, -9, +7, +5, +15* ↑ liver oval cell hyperplasia (numerical data not reported)
Kroese et al., 2001 0, 1.5, 5, 15, 50 mg/kg-d by gavage 5d/wk for 35d Wistar rats, 10/sex/dose	↑ liver weight Females (% change from controls): 0, +3, +2, +9, +18* Males (% change from controls): 0, +2, +1, +3, +18* Liver histopathology: no effects reported
Kidney Effects	
Knuckles et al., 2001 F344 rats, 20/sex/dose 0, 5, 50, 100 mg/kg-d by diet 90 days	↑ 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, 90 mg/kg-d by gavage 5 days/week 35 days	↓ kidney weight % change from controls: 0, -11*, -4, -10*, -18*
Kroese et al., 2001 Wistar rats, 10/sex/dose 0, 1.5, 5, 15, 50 mg/kg-d by gavage 5 days/week 35 days	kidney weight: no change (data not reported)
Neurological Effects	

Study Design and Reference	Results
Chen et al. (2011b) Sprague-Dawley rats, male, 32/dose 0 or 2 mg/kg-day by gavage 90 days	↑ time required for treated rats to locate platform in water maze (data reported graphically)
Bouayed et al. (2009a) Male Swiss albino mice, 9/group 0, 0.02, 0.2 mg/kg by gavage 28 days	Significant decrease in latency to attack and increase in the number of attacks in the resident-intruder test at 0.02 mg/kg-day (but not at high dose) Significant increase in mount number in the copulatory behavior test at 0.02 mg/kg-day (but not at high dose)

*Statistically significant.

1.1.5. Carcinogenicity

Evidence in Humans

There are many epidemiologic studies involving exposure to PAH mixtures that contain benzo[a]pyrene (e.g., studies of coke oven workers, asphalt workers). This discussion primarily focuses on epidemiologic studies that included a direct measure of benzo[a]pyrene exposure.[4] The identified studies were separated into tiers according to extent and quality of the exposure analysis and other study design features:

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

Tier 2: More limited exposure assessment, or 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 $\mu g/m^3$-years), and each of these studies addressed in the analysis the potential for confounding by smoking (Armstrong et al., 2009; Spinelli et al., 2006; Xu et al., 1996) (Table 1-12). 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 SMR estimates was < 1.0, with the other 8 estimates ranging from 1.2 to 2.9 (Table 1-13). 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 contribution 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

[4] These studies were identified from PubMed searches (last search conducted May 15, 2012 using terms benzo(a)pyrene AND (cohort OR case-control) AND cancer) and two recent meta-analyses (Armstrong et al, 2004; Bosetti et al, 2007).

relationships. Two cohort studies with detailed exposure data, however, had sample sizes of 48 and 78 cases (Burstyn et al., 2009; Gibbs et al. 2007a,b,c) (Tier 1 studies, Table 1-14). Although cumulative exposure (up to approximately 2 μg/m^3-years) was not related to increasing risk in the study of asphalt workers by Bursyn et al. (2010), an exposure-response was seen with the wider exposure range (i.e., above 100 μg/m^3) examined in a study in an aluminum smelter workers by Gibbs et al (2007a,b,c). This difference in response is not surprising, given that the highest exposure group in the asphalt worker study corresponded to the exposures seen in the lowest exposure category in the study of aluminum smelter workers. The 6 studies with more limited exposure information or analyses each included between 2 and 16 bladder cancer cases, with relative risk estimates ranging from 0.6 to 2.9. None of these individual effect estimates was statistically significant (Table 1-14).

Two of the identified 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% CI 0.26, 2.48) [22 cases) in Spinelli et al., 2006 and 0.58 (95% CI 0.12, 1.7) in Gibbs et a. (2007a) [3 cases].

Of additional interest is non-melanoma skin cancer, particularly with respect to dermal exposures. The literature pertaining to this kind of cancer and PAH exposure goes back to the 18th century work of Sir Percival Pott describing scrotal cancer, a squamous cell skin cancer, in chimney sweeps (Brown and Thornton, 1957). One of the identified studies 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 with 20 or more years. In addition to this study, 3 studies in Scandinavian countries examined non-melanoma skin cancer risk in relation to occupations with likely dermal exposure to creosote (i.e., timber workers, brickmakers, power linesmen) using incidence data from population registries (Tornqvist et al., 1986; Karlehagen et al., 1992; Pukkala et al., 1995). The SIR estimates were 1.5 (95% CI: 0.7, 2.6) based on 5 exposed cases, 2.37 (95% CI: 1.08, 4.50) [9 cases], and 4.64 (95% CI: 1.51, 10.8) [5 cases], respectively, in Tornqvist et al. (1986), Karlehagen et al. (1992), and Pukkala et al. (1995). These studies provide additional support for the association between dermal PAH exposure, including benzo[a]pyrene exposure, and skin cancer.

Lung, bladder, and skin cancers are the cancers that have been observed in occupational studies of PAH mixtures (Benbrahim-Tallaa et al., 2012; IARC, 2010a,b, Secretan et al., 2009; Baan et al., 2009) (see Table 1-11). The reproducibility of these three sites in different populations and exposure settings 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 three sites are also increased in the studies that included a direct measure of benzo[a]pyrene.

Table 1-11. 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)

Adapted from IARC, 2010.

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

Reference, design	Results
Armstrong et al., 2009 (Quebec, Canada) Cohort, aluminum smelter workers, 7 plants 16,431 (15,703 men; 728 women) Duration: minimum 1 year, began work 1966–1990 Follow-up: through 1999 (mean ~ 30 years) Smoking information collected from medical records Exposure: Job exposure matrix ~5,000 personal B[a]P measures from the 1970s to 1999 Related references: Lavoué et al., 2007 (exposure data); Gibbs et al., 2007a,b,c; Armstrong et al., 1994	SMR 1.32 (1.22, 1.42) [677 cases] Lung cancer risk by cumulative B[a]P exposure Median B[a]P µg/m^3-years | 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) 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-years (0.0035 per µg/m^3-years increase); other shapes of exposure-response curve examined.

Adapted from IARC, 2010.

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

Reference, design	Results
Spinelli et al., 2006 (British Columbia, Canada) Cohort, aluminum smelter workers 6,423 (all men) Duration: minimum ≥ 3 years; began work 1954–1997 Follow-up: through 1999 (14% loss to follow-up; mean ~ 24years) Smoking information from self-administered questionnaire Exposure: Job exposure matrix using 1,275 personal B[a]P 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 B[a]P exposure B[a]P µg/m^3-years | n cases | RR (95% CI)[a] 0 to 0.5 | 25 | 1.0 (referent) 0.5 to 20 | 42 | 1.23 (0.74, 2.03) 20 to 40 | 23 | 1.35 (0.76, 2.40) 40 to 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 to 1992 (not sure what proportion were B[a]P)	Lung cancer risk by cumulative B[a]P exposure B[a]P (µg/m^3-years) | n cases | RR (95% CI)[a] < 0.84 | 72 | 1.1 (0.8, 1.7) 0.85 to 1.96 | 117 | 1.6 (1.2, 2.3) 1.97 to 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] Table IV of Xu et al. (1996) report unclear; could be >= 3.0 for this category

Table 1-13. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to lung cancer risk: Tier 2 studies

<table>
<tr><th>Reference, design</th><th>Results</th></tr>
<tr><td colspan="2">Limited Follow-up Period (≤ 20 years)</td></tr>
<tr><td>Friesen et al., 2009 (Australia)

Cohort, aluminum smelter workers
4,316 (all men)
Duration: minimum 90 days; began work after 1962
Follow-up: through 2002, mean 16 years (maximum 20)
Smoking information from company records if employed before 1995 and study interviews if employed after 1994
Exposure: Job / task exposure matrix using TWA B[a]P measures (n=655), 1977–2004 (79% from 1990 – 2004)</td><td>RR 1.2 (0.7, 2.3) [19 cases in exposed; 20 in unexposed]
Lung cancer risk by cumulative B[a]P exposure
<table><tr><th>B[a]P µg/m^3-years</th><th>n cases</th><th>RR</th><th>(95% CI)[a]</th></tr><tr><td>0</td><td>20</td><td>1.0</td><td>(referent)</td></tr><tr><td>> 0 to 0.41</td><td>6</td><td>0.7</td><td>(0.3, 1.8)</td></tr><tr><td>0.41 to 10.9</td><td>6</td><td>1.4</td><td>(0.6, 3.5)</td></tr><tr><td>> 10.9</td><td>7</td><td>1.7</td><td>(0.7, 4.2)</td></tr></table>[a] Poisson regression, adjusting for smoking; trend p = 0.22</td></tr>
<tr><td colspan="2">Proxy Measure</td></tr>
<tr><td>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: 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: Burstyn et al., 2000; Boffetta et al., 2003a</td><td>Lung cancer risk by cumulative coal tar exposure [a]
<table><tr><th>Coal tar unit-years[a]</th><th>n cases</th><th>RR</th><th>(95% CI)</th></tr><tr><td>0.39 – 4.29</td><td>43</td><td>1.31</td><td>(0.87, 2.0)</td></tr><tr><td>4.30 – 9.42</td><td>32</td><td>0.98</td><td>(0.62, 1.6)</td></tr><tr><td>9.43 – 16.88</td><td>30</td><td>0.97</td><td>(0.61, 1.6)</td></tr><tr><td>16.89 – 196.48</td><td>54</td><td>1.60</td><td>(1.09, 2.4)</td></tr></table>[a] Adjusting for set, age, country, tobacco pack-years
[b] trend p = 0.07</td></tr>
<tr><td>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: Mazumdar et al., 1991 (exposure data)</td><td>SMR 1.95 (1.59, 2.33) [255 cases]
Lung cancer risk by cumulative exposure
<table><tr><th>Coal tar pitch volatiles (mg/m^3-months)</th><th>n cases</th><th>RR (95% CI)[a]</th></tr><tr><td>0</td><td>203</td><td>1.0 (referent)</td></tr><tr><td>1 – 49</td><td>34</td><td>1.2 (0.85, 1.8)</td></tr><tr><td>50 – 199</td><td>43</td><td>1.6 (1.1, 2.3)</td></tr><tr><td>200 – 349</td><td>59</td><td>2.0 (1.5, 2.8)</td></tr><tr><td>350 – 499</td><td>39</td><td>2.0 (1.6, 3.2)</td></tr><tr><td>500 – 649</td><td>27</td><td>2.7 (2.0, 4.6)</td></tr><tr><td>≥ 650</td><td>56</td><td>3.1 (2.4, 4.6)</td></tr></table>[a] adjusted for age, race, coke plant, period of follow-up; trend p < 0.001</td></tr>
<tr><td colspan="2">Limited Exposure Information</td></tr>
<tr><td>Liu et al., 1997 (China)

Cohort, various carbon plants and aluminum smelter workers
6,635 (all men)
Duration: minimum 15 years; began work before 1971
Follow-up: through 1985 (mean ~ 14 years)
Smoking information from questionnaire</td><td>SMR 2.2 (1.1, 2.8) [50 cases]
Lung cancer risk by exposure category
<table><tr><th>Exposure Category</th><th>Mean B[a]P µg/m^3</th><th>n cases</th><th>SMR (95% CI)[a]</th></tr><tr><td>None</td><td>--</td><td>13</td><td>1.49 (0.83, 2.5)</td></tr><tr><td>Low</td><td>--</td><td>6</td><td>1.19 (0.48, 2.5)</td></tr></table></td></tr>
</table>

Exposure: Area samples from one carbon plant, 1986 - 1987	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 et al., 1992 (Germany) Cohort, coke oven workers 789 (all men) Duration: minimum 10 years (mean 27 years); began work 1900 to 1989 Follow-up through 1989 (length data not reported) Smoking information from plant records and interviews Exposure: mean B[a]P: 28 (range 0.9 – 89) µg/m^3 Related reference: Manz et al., 1983 (exposure information)	SMR 2.88 (2.28–3.59) [78 cases]
Hansen et al., 1991; 1989 (Denmark) Cohort, asphalt workers 679 workers (applicators) (all men) Duration: data not reported; employed 1959 to 1980 Follow-up to 1986 (mean ~ 11 years) Smoking information from 1982 surveys of industry and general population Exposure: asphalt fume condensate, 35 personal samples during flooring: median 19.7 (range 0.5 – 260) mg/m^3	SMR 2.90 (1.88, 4.3) [25 cases] (ages 40 to 89) SMR 2.46 (1.59, 3.6) [25 cases] (with smoking adjustment)
Gustavsson et al., 1990 (Sweden) Cohort, gas production (coke oven) workers 295 (all men) Duration: minimum 1 year, median 15 years; employed 1965 to 1972 Follow-up 1966 to 1986 (mortality); 1966 to 1983 (incidence; mean ~ 15 years) Smoking information from interviews with older workers Exposure: area sampling - top of ovens. B[a]P - 1964 mean 4.3 (range 0.007 to 33); 1965 mean 0.52, (0.021 to 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: B[a]P - 19 area samples and 16 personal samples in Plant A (personal sample mean 2.7; range 0.59 – 6.2 µg/m^3); 10 area samples and 7 personal samples in Plant B; personal sample mean 0.17, 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 year duration: Plant A: OR 3.42 (0.35, 33.7) [7cases, 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 years, began before 1960 Follow-up: through 1971 Exposure: 52 personal samples (masks with filters) during specific jobs and tasks. Mean B[a]P 16.7 µg per 7-hour day	SMR 1.6 (1.3, 1.9)[a] [99 cases] (≥ 20 years since joining union) [a] confidence intervals calculated by EPA from data in paper

Table 1-14. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to bladder cancer risk

Reference, design	Results
Tier 1 Studies	
Burstyn et al. (2010) (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 years Smoking information not collected Exposure: compilation of B[a]P measures, production characteristics, and repeat measures in asphalt industry in each country used to develop exposure matrix Related references: Burstyn et al., 2000; Boffetta et al., 2003a	48 incident bladder cancer cases (39 cases in analyses with 15 year lag) Bladder cancer risk by cumulative B[a]P exposure [a] B[a]P µg/m^3-years[a] / n cases / RR (95% CI) (no lag)[b] / RR (95% CI) (15 year lag)[c] 0 to 0.253 — 12 — 1.0 (referent) — 1.0 (referent) 0.253 to 0.895 — 12 — 0.69 (0.29, 1.6) — 1.1 (0.44, 2.9) 0.895 to 1.665 — 12 — 1.21 (0.45, 3.3) — 1.7 (0.62, 4.5) ≥ 1.665 — 12 — 0.84 (0.24, 2.9) — 1.1 (0.30, 4.0) [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-year lag (RR 1.5, 2.7, 1.9 in second through fourth quartile; trend p = 0.15)
Gibbs et al. (2007a,b,c) (Quebec, Canada) Cohort, aluminum smelter workers, 7 plants 16,431 (15,703 men; 728 women) Duration: minimum 1 year, began work 1966 – 1990 Follow-up: through 1999 (mean ~ 30 years) Smoking information collected from medical records Exposure: Job exposure matrix using ~5,000 personal B[a]P measures from the 1970s to 1999 Related references: Lavoué et al., 2007 (exposure data); Armstrong et al., 1994 ; Gibbs et al. 1979; 1985	Hired before 1950: SMR 2.24 (1.77, 2.79) [78 cases] Bladder cancer risk by cumulative B[a]P exposure B[a]P µg/m^3-years[a] / n cases / SMR (95% CI) / Smoking-adjusted RR[b] 0 — 3 — 0.73 (0.15, 2.1) — 1.0 (referent) 10 — 14 — 0.93 (0.45, 1.4) — 1.11 30 — 3 — 1.37 (0.28, 4.0) — 1.97 60 — 1 — 0.35 (0.9, 1.9) — 0.49 120 — 15 — 4.2 (2.4, 6.9) — 8.49 240 — 30 — 6.4 (4.3, 9.2) 480 — 12 — 23.9 (12.2, 41.7) [a] Category midpoint [b] Confidence intervals not reported; highest category is ≥ 80 µg/m^3-years (n observed = 57). Mortality risk reduced in cohort hired in 1950 – 1959, SMR=1.23. Similar patterns seen in analysis of bladder cancer incidence.
Tier 2 Studies	
Friesen et al., 2009 (Australia) See Table 1-Y for study details	RR 0.6 (0.2, 2.0) [5 cases in exposed; 8 in unexposed] Bladder cancer risk by cumulative B[a]P exposure B[a]P µg/m^3-years / n cases / RR (95% CI)[a] 0 — 8 — 1.0 (referent) > 0 to 0.41 — 1 — 0.2 (0.03, 1.9) 0.41 to 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
Spinelli et al., 2006 (British Columbia, Canada) See Table 1-Y for study details	SMR 1.39 (0.72, 2.43) [12 cases]
Costantino et al., 1995 (United States – Pennsylvania) See Table 1-Y for study details	SMR 1.14 (1.59, 2.33) [16 cases]

Hammond et al., 1976 (United States) See Table 1-Y for study details	SMR 1.7 (0.94, 2.8)[a] [13cases] (≥ 20 years since joining union) [a] confidence intervals calculated by EPA from data in paper
Moulin et al., 1989 (France) See Table 1-Y 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-Y 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. 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), and liver carcinomas in male and female rats, and kidney adenomas in male rats (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; Field and Roe, 1970; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955).

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 (Table 1-15), 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). 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 1-15). The kidney tumors were observed at the mid- and high-dose groups. Kidney tumors were not observed at a lower dose in another study (Brune et al., 1981).

Lung tumors were also observed following oral exposure in female AJ mice (Weyand et al., 1995).

Table 1-15. 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-day by gavage 5 days/week 104 weeks	**forestomach** – Incidences: M: 0/52; 7/52*; 18/52*; 17/52* (papilloma) M: 0/52; 1/52; 25/52*; 35/52* (squamous cell carcinoma) F: 1/52; 3/51; 20/51*; 25/52* (papilloma) F: 0/52; 3/51; 10/51*; 25/52* (squamous cell carcinoma) **oral cavity** – Incidences: M: 0/24; 0/24; 2/37; 10/38 (papilloma) M: 1/24; 0/24; 5/37; 11/38 (squamous cell carcinoma) F: 0/19; 0/21; 0/9; 9/31(papilloma) F: 1/19; 0/21; 0/9; 9/31 (squamous cell carcinoma) **jejunum** – (adenocarcinomas) Incidences: M: 0/51; 0/50; 1/51; 8/49 F: 0/50; 0/48; 0/50; 2/51 **duodenum** – (adenocarcinomas) Incidences: M: 0/51; 0/50; 0/51; 1/49 F: 0/49; 0/48; 0/50; 2/51
	liver – (adenomas and carcinomas) Incidences: M: 0/52; 3/52; 15/52*; 4/52 (adenoma) M: 0/52; 1/52; 23/52*; 45/52* (carcinoma) F: 0/52; 2/52; 7/52*; 1/52 (adenoma) F: 0/52; 0/52; 32/52*; 50/52* (carcinoma)
	kidney – (cortical adenoma) Incidences: M: 0/52; 0/52; 7/52; 8/52 F: increase not observed
	auditory canal[b] (Zymbal's gland) – (carcinomas) Incidences: M: 0/1; 0/0; 2/7; 19/33* F: 0/0; 0/1; 0/0; 13/20*
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-day) in the diet 2 years	**forestomach** – (papillomas and squamous cell carcinomas) Incidences: 1/48; 3/47; 36/46*; 46/47* **esophagus** – (papillomas and carcinomas) Incidences: 0/48; 0/48; 2/45; 27/46* **tongue** – (papillomas and carcinomas) Incidences: 0/49; 0/48; 2/46; 23/46* **larynx** – (papillomas and carcinomas) Incidences: 0/35; 0/35; 3/34; 5/38

Study design and reference	Results
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-day) Diet: 0, 6, 39 mg/kg-yr (0, 0.016, 0.107 mg/kg-day) treated until moribund or dead	**forestomach** – (papillomas and carcinomas[b]) – gavage Incidences: 3/64; 12/64*; 26/64*; 14/64* **forestomach** – (papillomas) – diet Incidences: 2/64; 1/64; 9/64* **larynx** and **esophagus** – (papillomas) – gavage Incidences: 3/64; 1/64; 0/64; 0/64 **larynx** and **esophagus** – (papillomas) – diet Incidences: 1/64; 2/64; 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 time-weighted average (TWA) body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group

[b] Auditory canal tissue was not histologically examined in the lower dose groups and the controls

[c] Two malignant forestomach tumors were observed (one each in the mid- and high-dose groups)

Inhalation Exposure

Chronic inhalation exposure to benzo[a]pyrene resulted in the development of tumors in the respiratory tract and pharynx in Syrian golden hamsters (Table 1-16). A dose-dependent increased incidence of tumors in the upper respiratory tract, including the larynx and trachea, were reported by Thyssen et al. (1981) at ≥10 mg/kg-day. In addition, a decrease in tumor latency was observed in the larynx and trachea, and nasal cavity tumors were observed at the mid- and high-dose but the incidences were not dose-dependently increased. A dose-related increase in tumors in the upper digestive tract (pharynx and esophagus) was also reported. In addition, a single forestomach tumor was observed at both the mid- and high-doses, and forestomach tumors were not observed in control animals. The study authors presumed that the pharyngeal and esophageal tumors were a consequence of mucociliary particle clearance.

Under contract to the U.S. EPA, Clement International Corporation (1990) obtained the individual animal data (including individual animal pathology reports, time-to-death data, and exposure chamber monitoring data) collected by Thyssen et al. (1981). A re-analysis of the individual animal pathology reports from the original study supports the dose-dependent increased incidence of tumors in the larynx and pharynx (Clement International Corporation, 1990; EPA, 1990). The exposure measurements and individual animal data from Thyssen et al. (1981) were used to calculate average continuous lifetime exposures for each individual hamster. Group averages of individual average continuous lifetime exposure concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3 for the control through high-exposure groups, as described in Clement International Corporation (1990).

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

Study design and reference	Results
Thyssen et al. (1981) Syrian golden hamsters: male (20–30 animals/group) Target exposure concentrations: 0, 2, 10, or 50 mg/m^3 Average exposure concentrations [b]: 0, 0.25, 1.01, and 4.29 mg/m^3 Inhalation: for 3–4.5 hours/5-7 days per week until hamsters died or became moribund	**larynx** – Incidences: 0/27; 0/27; 8/26; 13/25 tumor latency [a]: 107and 67.6 weeks **trachea** – Incidences: 0/27; 0/27; 1/26; 3/25 tumor latency: 115 and 63 weeks **nasal cavity** – incidences: 0/27; 0/27; 3/26; 1/25 tumor latency: 116 and 79 weeks **Revised tumor incidence data [c]** **larynx** – Incidences: 0/27; 0/27; 11/26; 12/34 **pharynx** – Incidences: 0/27; 0/27; 9/26; 18/34 **larynx and pharynx** (combined)[d] – Incidences: 0/27; 0/27; 16/26; 18/34
	pharynx – Incidences: 0/27; 0/27; 6/26; 14/25 **esophagus** – (papillomas and squamous cell carcinomas) Incidences: 0/27; 0/27; 0/27; 2/25 **Forestomach**-(papillomas and squamous cell carcinomas) Incidences: 0/27; 0/27; 1/26; 1/25

[a] tumor latency provided for 10 and 50 mg/m3 dose groups
[b] Duration adjusted inhalation concentrations calculated from exposure chamber monitoring data and exposure treatment times obtained by Clement International Corporation (1990). 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.
[c] Revised tumor incidence data based on original study pathology reports obtained by Clement International Corporation (1990).
[d]Nasal, forestomach, esophageal, and tracheal tumors occurred in hamsters that also had tumors in the larynx or pharynx, except for two animals in the mid-dose group that displayed nasal tumors (one malignant and one benign) without displaying tumors in the pharynx or larynx.

Dermal Exposure

Repeated application of benzo[a]pyene 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, 1983, 1973; WHO, 1998; ATSDR, 1995). The analysis in this document focuses on chronic carcinogenicity bioassays in several strains of mice following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime (Table 1-17). 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, 1983; Habs et al., 1984, 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959).

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 observed in lifetime dermal bioassays in which macroscopic examination of internal organs was included (Higginbotham et al., 1993; Habs et al., 1980; Schmahl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959).

Table 1-17. Tumor observations in dermal animal bioassays.

Study design and reference	Results
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/week for up to 103 weeks 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; 13/13 Epidermoid carcinoma: 0/33; 0/55; 2/55; 4/56; 32/49; 37/38; 35/35; 10/14; 12/14; 13/13
Poel (1960) 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/week 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; 14/14 C3HeB: 0/17; 0/19; 3/17; 4/17; 11/18;/ 17/17; 18/18; 17/17 A/He mice: 0/17; 0/18; 0/19; 0/17; 0/17; 21/23; 11/16; 17/17
Roe et al. (1970) Swiss mice (50/dose) 0, vehicle, 0.1, 0.3, 1, 3, or 9 µg Dermal - 3 times/week for up to 93 weeks	**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; 34/46
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/week 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)
Schmähl et al., 1977 NMRI mice: female (100/group) 0, 1, 1.7, or 3 µg Dermal - 2 times/week	**skin** tumors – (papillomas and carcinomas) Incidences: 0/81; 1/77; 0/88; 2/81 (papillomas) 0/81; 10/77; 25/88; 43/81 (carcinomas)
Habs et al., 1980 NMRI mice: female (40/group) 0, 1.7, 2.8, or 4.6 µg Dermal - 2 times/week 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; 22/36 Age-standarized tumor incidence: 0, 24.8, 89.3, 91.7%
Grimmer et al., 1984, 1983 CFLP mice: female (65–80/group) 0, 3.9, 7.7, and 15.4 µg (1983 study) 0, 3.4, 6.7, and 13.5 µg (1984 study)	**skin** tumors – (papillomas and carcinomas) – with a decrease in tumor latency Incidences: 1983: 0/80; 7/65; 5/64; 2/64 (papillomas)

Study design and reference	Results
Dermal - 2 times/wk for 104 weeks	0/80; 15/65; 34/64; 54/64 (carcinomas) 1984: 0/65; 6/64; 8/65; 4/65 (papillomas) 0/65; 37/64; 45/65; 53/64 (carcinomas)
Habs et al., 1984 NMRI mice: female (20/group) 0, 2, or 4 µg Dermal - 2 times/week for life	**skin** tumors – (papillomas and carcinomas) – with a decrease in mean survival time Incidences: 0/20; 2/20; 0/20 (papillomas) 0/20; 7/20; 17/20 (carcinomas)
Higginbotham et al., 1993 Ah-receptor-responsive Swiss mice: female (23-23/group) 0, 0.25, 1 or 2 µg Dermal - 2 times/wk for 40 weeks	**Skin** tumors were not observed
Sivak et al., 1997 C3H/HeJ mice: male (30/group) 0, 0.05, 0.5, or 5 µg Dermal - 2 times/wk for 104 weeks	**skin** tumors – (papillomas and carcinomas) Incidences: 0/30; 0/30; 5/30 (2 papillomas, 3 carcinomas); 27/30 (1 papilloma, 28 carcinomas)

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, 2005; Xue and Warshawsky, 2005; Ramesh et al., 2004; Bostrom et al., 2002; Penning et al., 1999; WHO, 1998; Harvey, 1996; ATSDR, 1995; Cavalieri and Rogan, 1995; U.S. EPA, 1991b). 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 are: (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 in Figure 1-7.

Also included in the figure are other processes that may contribute to the carcinogenicity of benzo[a]pyrene via the promotion and progression phases of cancer development, including inflammation, cytotoxicity, and sustained regenerative cell proliferation. 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.

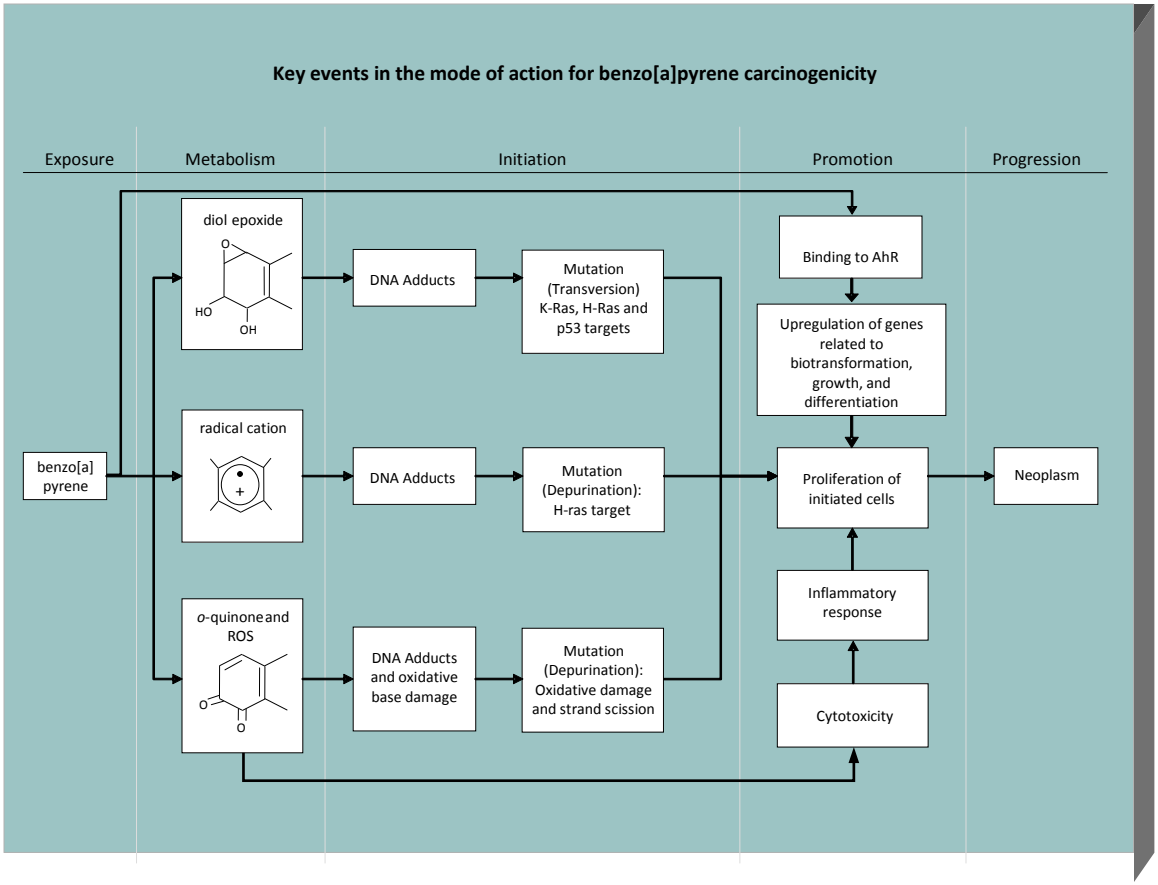


Figure 1-7. Proposed metabolic activation pathways and key events in the carcinogenic mode of action for benzo[a]pyrene

Data in Support of the MOA

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 B 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-benzo[a]pyrene-7,8-diol-9,10-epoxide (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 (Bostrom 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., 1988d, e), or they can form unstable adducts with DNA that ultimately result in depurination (Cavalieri et al., 2005, 1993; Rogan et al., 1993). The predominant depurinating adducts occur at the N-3 and N-7 positions of adenine and the C-8 and N-7 positions of guanine (Cavalieri and Rogan, 1995).

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 B of the Supplemental Information). DHH (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 (Jiang et al., 2007, 2005; McCoull et al., 1999). In this pathway, and in the presence of $NAD(P)^+$, AKR oxidizes benzo[a]pyrene-7,8-diol to a ketol, which subsequently forms benzo[a]pyrene-7-8-dione. This and other PAH o-quinones react with DNA to form unstable, depurinating DNA adducts. In the presence of cellular reducing equivalents, o-quinones can also activate redox cycles, which produce ROS (Penning et al., 1996). DNA damage in in vitro systems following exposure to benzo[a]pyrene-7,8-dione or other o-quinone PAH derivatives occurs through the AKR pathway and can involve the formation of stable DNA adducts (Balu et al., 2004), N-7 depurinated DNA adducts (McCoull et al., 1999), DNA damage from ROS (8-oxo-dG) (Park et al., 2006), and strand scission (Flowers et al., 1997, 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 B 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, clastogenic, and induces cell transformation both with and without metabolic activation. Cytogenetic damage in the form of chromosomal aberrations (CA), micronuclei (MN), sister chromatid exchanges (SCE), 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. 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 *Cancer Guidelines* (Section 2.4; 2005) 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 1-18). Results in support of a mutagenic MOA 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) or tobacco smoke (Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001, Bennett et al., 1999). These results are consistent with evidence that benzo[a]pyrene diol epoxide is reactive with guanine bases in DNA (Koreeda et al., 1978; Jeffrey et al., 1976); 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, b, 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 (Malveille et al., 1977; Newbold and Brookes, 1976) and in producing lung tumors in newborn mice following i.p. administration (Chang et al., 1987; Buening et al., 1978; Kapitulnik et al., 1978). Other supporting evidence includes observations of elevated BPDE-DNA adduct levels in respiratory tissue of lung cancer patients (Li et al., 2001; Xu et al., 1997) and white blood cells (WBC) of groups of coke oven

workers and chimney sweeps, occupations with known elevated risks of cancer (Vineis and Perera, 2007; Pavanello et al., 1999), and in lung tissue from tobacco smokers with lung cancer (Rojas et al., 2004; 1998; Andreassen et al., 2002; Godschalk et al., 2002; 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 (Bartsch et al., 2000; Aklillu et al., 2005; Alexandrov et al., 2002; 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 1-18). 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 9/13 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., 2006a; Balu et al., 2004; McCoull et al., 1999; Flowers et al., 1997, 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

In vivo demonstrations showing that benzo[a]pyrene-induced mutational events in *p53* or *ras* oncogenes precede tumor formation are not available. In vitro exposure of human *p53* knock-in murine fibroblasts to 1 μM benzo[a]pyrene for 4 to 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 1-18). 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. A dose-response relationship also has been demonstrated between BPDE-DNA adduct levels in lung tissue and odds ratios for cancer in a case control study of 221 lung cancer cases and 229 healthy controls (Li et al., 2001).

In mice, dose-response and temporal relationships have been described between the formation of BPDE-DNA adducts and skin and forestomach tumors (Table 1-18). 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., 1996). 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 1-18), DNA adduct formation, and tumor formation in female mice. Mice were treated dermally with 0, 16, 32, or 64 μg of benzo[a]pyrene once per week for 29 weeks (Albert et al., 1991). Indices of skin damage and levels of BPDE-DNA adducts in skin reached plateau levels in exposed groups by 2–4 weeks of exposure. With increasing dose level, levels of BPDE-DNA adducts (fmol/μg DNA) initially increased in a linear manner and began to plateau at doses ≥32 μg/week. Tumors began appearing after 12–14 weeks of exposure for the mid- and high-dose groups and at 18 weeks for the low-dose group. At study termination (35 weeks after start of exposure), the mean number of tumors per mouse was approximately one per mouse in the low- and mid-dose groups and eight per mouse in the high-dose group. The time-course data indicate that benzo[a]pyrene-induced increases in BPDE-DNA adducts preceded the appearance of skin tumors, consistent with the formation of DNA adducts as a precursor event in benzo[a]pyrene-induced skin tumors.

Culp et al. (1996a) compared dose-response relationships for BPDE-DNA adducts and tumors in female B6C3F_1 mice exposed to benzo[a]pyrene in the diet at 0, 18.5, 90, or 350 μg/day for 28 days (to examine adducts) or 2 years (to examine tumors) (Table 1-18). The benzo[a]pyrene dose-tumor response data showed a sharp increase in forestomach tumor incidence between the 18.5 μg/day group (6% incidence) and the 90 μg/day group (78% incidence). The BPDE-DNA adduct levels in forestomach showed a relatively linear dose-response throughout the benzo[a]pyrene dose range tested. The appearance of increased levels of BPDE-DNA adducts in the target tissue at 28 days is temporally consistent with the contribution of these adducts to the initiation of forestomach tumors. Furthermore, about 60% of the examined tumors had mutations in the *K-ras* oncogene at codons 12 and 13, which were G→T or G→C transversions indicative of BPDE reactions with DNA (Culp et al., 1996a).

Biological plausibility and coherence

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 1-18). 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, b, 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 respiratory tissue of lung cancer patients (Li et al., 2001; Xu et al., 1997) or tobacco smokers with lung cancer (Rojas et al., 2004; 1998; Andreassen et al., 2002; Godschalk et al., 2002; 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) and between odds ratios for lung cancer and BPDE-DNA adduct levels in lung tissue (Li et al., 2001); (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., 1996a; 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, 2006a; Shen et al., 2006; Balu et al., 2004; Yu et al., 2002; McCoull et al., 1999; Flowers et al., 1997, 1996).

Table 1-18. Experimental support for the postulated key events for mutagenic MOA

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 and 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; 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, chromosomal aberrations, micronucleus formation, and sister chromatid exchange in animals (Appendix B 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., 1996a; 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., 2006a; Balu et al., 2004; McCoull et al., 1999; Flowers et al., 1997, 1996) *Human evidence that key events are necessary for carcinogenesis:* • Detection of benzo[a]pyrene diol epoxide-specific DNA adducts is strongly 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 that were exposed to PAHs (Li et al., 2001; Xu et al., 1997; Vineis and Perera, 2007; Pavanello et al., 1999; Rojas et al., 2004; 1998; Andreassen et al., 2002; Godschalk et al., 2002; 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, b, 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., 1996a)

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) (Denissenko et al., 1996; Puisieux et al., 1991; Liu et al., 2005; Marshall et al., 1984; Koreeda et al., 1978; Jeffrey et al., 1976; Keohavong et al., 2003; DeMarini et al., 2001; Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001; Bennett et al., 1999)

4. Clonal expansion of mutated cells during the promotion and progression phases of cancer development.

Evidence that benzo[a]pyrene metabolites induce key events:
- Numerous studies in animals have observed carcinogenesis following exposure to benzo[a]pyrene via all exposure routes (see *Evidence in Animals* section)
- 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)

Human evidence that key events are necessary for carcinogenesis:
- The frequency of G→T transversions in p53 was found to increase with increasing smoking duration and intensity in human lung tumors (Bennett et al., 1999)
- Benzo[a]pyrene-specific DNA adducts have been detected in preneoplastic tissues in humans exposed to PAH mixtures, e.g., in lung tissue of smokers with lung cancer, and in human skin treated with coal tar containing ointment (Godschalk et al., 2002; Bartsch et al., 1999; Alexandrov et al., 1992)
- Detection of benzo[a]pyrene-specific DNA adducts is strongly associated with increased cancer risk in coke oven workers and chimney sweeps (Pavanello et al., 1999)

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 which 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, cytotoxicity and the formation of reactive oxygen species, as well as the inhibition of gap junctional intercellular communication.

The ability of certain PAHs to act as initiators and promoters may increase their carcinogenic potency (Andrews et al., 1978). The promotional effects of PAHs appear to be related to AhR affinity and the upregulation of genes related to growth and differentiation (Bostrom 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 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).

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 reactive oxygen species 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., 2001a, b)

Gap junctions are channels between cells that are crucial for differentiation, proliferation, apoptosis, and cell death and consequently for the two epigenetic steps of tumor formation, promotion, and progression. Inhibition of gap junctional intercellular communication by benzo[a]pyrene has been observed in vitro (Sharovskaya et al., 2006; Blaha et al., 2002).

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 all routes of exposure (see Appendix B 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, and the subsequent occurrence of mutations in oncogenes and tumor suppressor genes.

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. Strong 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). Although the developing fetus and infants may have lower levels of some bioactivating enzymes than adults (e.g., CYP1A1/1B1), infants or children are expected to be more susceptible. Newborn or infant mice developed liver and lung tumors more readily than young adult mice following acute i.p. exposures to benzo[a]pyrene (Vesselinovitch et al., 1975). These results indicate that exposure to benzo[a]pyrene during early life stages presents additional risk for cancer, compared with exposure during adulthood. TheEPA'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.

Population variability in metabolism and detoxification of benzo[a]pyrene, in addition to DNA repair capability, may affect cancer risk. Polymorphic variations in the human population in CYP1A1, CYP1B1, and other CYPs have been implicated as determinants of increased individual lung cancer risk in some studies (Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). Some evidence suggests that humans lacking a functional GSTM1 gene have higher BPDE-DNA adduct levels and are thus at greater risk for cancer (Vineis et al., 2007; Pavanello et al., 2005; 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 (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 metabolic 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).

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 indicate that the strongest evidence for potential hazard following benzo[a]pyrene exposure are for developmental and reproductive toxicity and Immunotoxicity. In humans, exposure to PAH mixtures has been shown to result in developmental and reproductive toxicity and immunotoxicity. Most of the available human data report associations between particular health endpoints and concentrations of benzo[a]pyrene-DNA adducts, with fewer studies correlating health effects with external measures of exposure. The available human studies report effects that are generally analogous to the effects observed in animal toxicological studies, and provide qualitative, supportive evidence for the effect-specific hazards identified in Section 1.1..

In animals, evidence of developmental and reproductive toxicity and immunotoxicity has been observed across species and dosing regimens (Figure 1-8). 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., 2009b; Kristensen et al., 1995; MacKenzie and Angevine 1981). 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. The weight of the evidence indicates that developmental toxicity, reproductive toxicity and immunotoxicity are hazards following oral exposure to benzo[a]pyrene.

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 weight of the evidence indicates that developmental toxicity and reproductive toxicity are hazards following inhalation exposure to benzo[a]pyrene.

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 carried forward as an effect other than cancer for the derivation of reference values.

1.2.2. Weight of Evidence for Carcinogenicity

Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans." EPA's *Cancer Guidelines* (U.S. EPA, 2005a) emphasize 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 1-19.

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, 2010a,b; 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, 2010a; Baan et al., 2009; Straif et al., 2005). Increased cancer risks have been reported among other occupations involving exposure to PAH mixtures such as carbon black and diesel exhaust (Bosetti et al., 2007). Benzo[a]pyrene is also a notable carcinogenic constituent of tobacco smoke (IARC, 2004).

Evidence for a contributing role of benzo[a]pyrene to human carcinogenic responses to complex PAH mixtures is available. Elevated BPDE-DNA adducts have been reported in smokers

compared to nonsmokers, and the increased adduct levels in smokers are typically increased two-fold compared with nonsmokers (Philips, 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, 1998; Bartsch et al., 1999, 1998; Pavanello et al, 1999), and in lung tissue from tobacco smokers with lung cancer (Rojas et al., 2004; 1998; Godschalk et al., 2002; Bartsch et al., 1999; Andreassen et al., 1996; Alexandrov et al., 1992).

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. Distinctive mutation spectra have been observed in the tumor suppressor gene p53 and the *K-ras* oncogene in tumor tissues taken from lung cancer patients that 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 that were smokers compared to nonsmokers. 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 nonsmokers (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 systems (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), exposure to benzo[a]pyrene via the oral, inhalation, and dermal routes have been associated with carcinogenic responses both systemically and at the site of administration. 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's 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; Field and Roe, 1970; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955). Chronic inhalation exposure to benzo[a]pyrene was associated with tumors in the larynx and pharynx of male Syrian golden hamsters exposed to benzo[a]pyrene:NaCl aerosols (Thyssen et al., 1981). 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). Intratracheal instillation of benzo[a]pyrene was associated with respiratory tract tumors in additional studies with hamsters (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). Chronic dermal application of benzo[a]pyrene (2–3 times/week) has been associated with mouse skin tumors in numerous bioassays (Sivak et al., 1997; Habs et al., 1984, 1980; Grimmer et al., 1984, 1983; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 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 (WHO, 1998; ATSDR, 1995; IARC, 1983, 1973). When followed by repeated exposure to a potent tumor promoter, acute dermal exposure to benzo[a]pyrene induced skin tumors in numerous studies of mice, indicating that benzo[a]pyrene is a strong tumor-initiating agent in the mouse skin model (Weyand et al., 1992; Cavalieri et al., 1991, 1981; Rice et al., 1985; El-Bayoumy et al., 1982; LaVoie et al., 1982; Raveh et al., 1982; Slaga et al., 1980, 1978; Wood et al., 1980; Hoffmann et al., 1972).

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, 1987; Busby et al., 1989, 1984; Weyand and LaVoie, 1988; Wislocki et al., 1986; 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 Walters, 1967; Grant and Roe, 1963; Steiner, 1955; Rask-Nielson, 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, 1988a, b, c); (6) cervical tumors in mice with intravaginal application (Naslund 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) Identification of 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 intraperitoneal injection (Nesnow et al., 1998a,b, 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 1-19. 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 cancer risks in humans exposed to complex PAH mixtures containing benzo[a]pyrene	IARC, 2010a,b, 2004
• Benzo[a]pyrene-specific biomarkers detected in humans exposed to PAH mixtures	
– BPDE-DNA adducts in WBCs of coke oven workers and chimney sweeps	Rojas et al., 2000, 1998; Bartsch et al., 1999, 1998; Pavanello et al, 1999
– BPDE-DNA adducts in smokers	Philips, 2002
• Benzo[a]pyrene-specific DNA adducts have been detected in preneoplastic 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 eczema patients treated with coal tar	Rojas et al., 2004; 1998; Godschalk et al., 2002, 1998; Bartsch et al., 1999; 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	
– 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 at the same mutational hotspot in p53 from smoking-related lung tumors in humans	Liu et al., 2005; Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001
– 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 vs. nonsmokers	Bennett et al., 1999
b) Extensive animal evidence	
Oral exposures	
• Forestomach tumors in male and female rats and in female mice following chronic exposure	Kroese et al., 2001; Brune et al., 1981; Beland and Culp, 1998; Culp et al., 1998
• 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; Field and Roe, 1970; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955

Evidence	Reference
• Alimentary tract and Liver tumors in male and female rats following chronic exposure	Kroese et al., 2001
• Auditory canal tumors in male and female rats following chronic exposure	Kroese et al., 2001
• Esophageal, tongue, and laryngeal tumors in female mice following chronic 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
Inhalation exposures	
• Laryngeal and pharyngeal tumors in male hamsters following chronic exposure	Thyssen et al., 1981
Dermal exposures	
• Skin tumors in mice following chronic exposures without a promoter or acute exposures with a promoter	Sivak et al., 1997; Habs et al., 1984, 1980; Grimmer et al., 1984, 1983; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959
• Skin tumors in rats, rabbits, and guinea pigs following subchronic exposures	WHO, 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, 1987; Busby et al., 1989, 1984; Weyand and LaVoie, 1988; Wislocki et al., 1986; 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,b, 1996, 1995; Mass et al., 1993
– 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 vs. 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 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 percent 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 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 PAH mixtures across multiple cohorts have observed effects following exposure to complex mixtures of PAHs. 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 a value. Thus, these studies were not considered because of the contribution to the observed hazard of multiple PAHS across multiple routes of exposure. 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.

Developmental toxicity

Numerous animal studies observed endpoints of developmental toxicity following oral exposure during gestational or early post-natal development (Chen et al., 2012; Jules et al., 2012; Bouayed, 2009b; 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 conducted study that evaluated multiple neurobehavioral endpoints and measures of neurotoxicity in adolescent and adult animals. The study was designed with sufficient statistical power and randomized pups with a total of 10 males and 10 females per treatment group and no more than one male and one female from each litter (i.e., pups from 40 litters were used) 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.

Chen et al. (2012) observed increased latency in negative geotaxis and increased motor activity or anxiety in the open field test at 0.02 mg/kg-day, and decreased anxiety in the elevated plus maze test and impaired spatial learning as measured by an increase in latency time to find a hidden platform in the Morris water maze test at 0.2 mg/kg-day. Altered behaviors and locomotion in open field tests can 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). Similarly, this study reported the number of open arm entries in the elevated plus maze test and not time spent in the closed arms and open arms which would provide a more sensitive measurement of the effect. The number of open arm entries does serve as an indicator of decreased anxiety and is unlikely to be confounded by changes in motor activity (as total arm entries appears to be unchanged with treatment), but the magnitude of response in the elevated plus maze test was not as robust as the response observed in the Morris water maze response. Chen et al. (2012) also observed effects in the surface righting test that were on the order of ~0.2-0.3 seconds and in the negative geotaxis test of approximately 3-4 seconds with no automated recording of latency (such as use of video recordings). Additionally, male and female rats (which often show differences in the maturation of these developmental landmarks) were pooled for these measures. Due to these uncertainties altered behaviors and locomotion in open field tests, number of open arm entries in the elevated plus maze test, effects in the surface righting test, and effects in the negative geotaxis test were not considered.

Impaired spatial learning as measured by an increase in latency time to find a hidden platform in the Morris water maze test was selected for dose response analysis. This endpoint is supported by probe trials from the same study in which the platform was removed and the duration of time spent in the target quadrant and the number of times the animal crosses the location of the previous platform was measured. Similar impairments were observed in both the probe tests and water maze escape measures in rats treated with benzo[a]pyrene. Thus, these tests confirmed that the animals were using learned spatial cues to identify the location of the platform and not relying on random, non-spatial strategies such as circular swimming. In addition, there were no changes in measured swim speed indicating an effect on long term memory.

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. (2009b) and MacKenzie and Angevine (1981) were not selected for dose response analysis. Bouayed et al. (2009b) used the same tests as Chen et al. (2012), but at higher doses (2 and 20 mg/kg-day compared to 0.02, 0.2, and 2 mg/kg-day, respectively). Because Chen et al. (2012) reported adverse effects at doses lower than Bouayed (2009b), the later study was not selected for dose-response analysis. 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., 2011a; 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 of study reporting limitations (i.e., only reported significant observations at the mid-dose). Chen et al. (2011a) is a subchronic study that applied only a single dose level. Because the study corroborated available multi-dose studies it was not considered for dose-response analysis. 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 observed by Mohammed 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, these studies demonstrated altered ovarian weights and follicle numbers and cervical epithelial cell hyperplasia following oral exposure to benzo[a]pyrene. These studies were identified as the most informative studies 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,

2010). Cervical effects of increasing severity (including epithelial hyperplasia, atypical hyperplasia, apoptosis, and necrosis) were also observed at higher doses (Gao et al., 2011, 2010). 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 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 (Melikian et al., 1999; Phillips et al., 2002).

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 (Mattison et al., 1980; MacKenzie and Angevine 1981; Swartz and Mattison 1985; Miller et al., 1992; Kristensen et al., 1995; Borman et al, 2000). Additionally, epidemiology studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (El Nemr et al., 1998; Neal et al., 2005). Specific associations have also been made between infertility and increased levels of benzo[a]pyrene in follicular fluid in women undergoing in vitro fertilization (Neal et al., 2008).

Immunotoxicity

As described in Section 1.1.4, the immune system was identified as a target of benzo[a]pyrene-induced toxicity 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 studies conducted by Kroese et al. (2001) and De Jong et al. (1999) are subchronic studies with multiple exposure levels and adequate power to detect effects. In comparing these two 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 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 a candidate RfD for benzo[a]pyrene immunotoxicity, including: 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; and 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 s.c. injection (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 determined 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 to best empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available as part of EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), the BMD and the 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR of 1 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 Appendix C of the Supplemental Information.

Among the endpoints identified as representative of the hazards of benzo[a]pyrene exposure, the data for Morris water maze escape latency (Chen et al., 2012), decreased ovary weight (Xu et al., 2010), and decreased thymus weight (Kroese et al., 2000) were amenable to dose-response modeling. For the water maze escape latency data, the data for male and female rats were combined for dose-response analysis because of the strong similarity in responses and the lack of information available suggesting there would be sex-specific differences in the results of this test (see Appendix C of the Supplemental Information for details of statistical analyses).

The data for the remaining endpoints identified in Section 2.1 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 (i.e. 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, 2011b). 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 B of the Supplemental Information, several animal 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.

Consistent with EPA guidance (U.S. EPA, 2011b), the 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

BW_h = human body weight

Using a BW_a of 0.25 kg for rats and 0.035 kg for mice and a 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 Points of Departure

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMD (mg/kg-day)	BMDL (mg/kg-day)	POD_{ADJ}[b] (mg/kg-day)	POD_{HED}[c] (mg/kg-day)
Developmental							
Neurodevelopmental impairments Chen et al., 2012	Male and Female Sprague-Dawley Rats	Hill[a]	1SD	0.11	0.06	0.06	0.06
Cardiovascular effects Jules et al., 2012	Long-Evans rats	LOAEL (0.6 mg/kg-day)				0.6	0.15
Reproductive							
Decreased ovarian weight Xu et al., 2010	Female Sprague-Dawley rats	Linear[a]	1 SD	2.3	1.5	1.5	0.37
Decreased sperm count Mohamed et al., 2010	Male C57BL/6 mice	LOAEL (1 mg/kg-day)				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-day)				7.1	1.7
Decreased IgA levels De Jong et al., 1999	Male Wistar rats	NOAEL (30 mg/kg-day)				21	5.2
Decreased number of B cells DeJong et al., 1999	Male Wistar rats	NOAEL (30 mg/kg-day)				21	5.2

[a] For modeling details, see Appendix B in Supplemental Information

[b] For studies in which animals were not dosed daily, administered doses were adjusted to calculate the time-weighted average daily doses prior to BMD modeling.

[c] HED PODs were calculated using $BW^{3/4}$ scaling (US EPA, 2011b) for effects from dosing studies in adult animals (i.e., Mohamed et al., 2010; Xu et al., 2010; Gao et al., 2011; and 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, 2011b; Hattis et al., 2004).

2.1.3. Derivation of Candidate Reference Doses

Table 2-2 is a continuation of Table 2-1 and summarizes the application of uncertainty factors to each POD to derive a candidate RfD for each data set. The selection of uncertainty factors was based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and is described in Section 7.6 of the Preamble. Figure 2-1 presents graphically these candidate RfDs, uncertainty factors, and points of departure, with each bar corresponding to one data set described in Tables 2-1 and 2-2.

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

Endpoint and Reference	POD_{HED}[a]	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate RfD (mg/kg-d)
DEVELOPMENTAL									
Neurodevelopmental impairments in rats Chen et al., 2012	0.06	$BMDL_{1SD}$	10	10	1	1	3	300	2×10^{-4}
Cardiovascular effects in rats Jules et al., 2012	0.15	LOAEL	3	10	10	1	3	1000	2×10^{-4}
REPRODUCTIVE									
Decreased ovary weight and ovarian follicles in rats Xu et al., 2010	0.37	$BMDL_{1SD}$	3	10	1	10	3	1000	4×10^{-4}
Decreased sperm count in mice Mohamed et al., 2010	0.15	LOAEL	3	10	10	10	3	10000	Not calculated due to UF > 3000[a]
Cervical epithelial hyperplasia in mice Gao et al. (2011)	0.06	$BMDL_{10}$	3	10	1	10	3	1000	6×10^{-5}
IMMUNOLOGICAL									
Decreased thymus weight in rats Kroese et al., 2001	1.9	$BMDL_{1SD}$	3	10	1	10	3	1000	2×10^{-3}
Decreased serum IgM in rats De Jong et al., 1999	1.7	NOAEL	3	10	1	10	3	1000	2×10^{-3}
Decreased serum IgA in rats De Jong et al., 1999	5.2	NOAEL	3	10	1	10	3	1000	5×10^{-3}
Decreased number of B cells in rats De Jong et al., 1999	5.2	NOAEL	3	10	1	10	3	1000	5×10^{-3}

Endpoint and Reference	POD_{HED}[a]	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate RfD (mg/kg-d)

UF_A – A value of 3 ($10^{0.5}$ = 3.16, rounded to 3) was applied to account for uncertainty in characterizing toxicodynamic differences between rats and humans when an HED was calculated using $BW^{3/4}$ scaling as uncertainty in characterizing toxicokinetic differences was accounted for through calculation of an HED using a standard DAF consistent with EPA guidance (U.S. EPA 2011b). A value of 10 was applied when $BW^{3/4}$ scaling was not employed to account for uncertainty in extrapolating from laboratory animals to humans because of the absence of information to characterize either the toxicokinetic or toxicodynamic differences between animals and humans following oral exposure to BaP.

UF_H – A value of 10 was applied to account for potentially susceptible individuals because adequate information is not available to quantitatively estimate variability in human susceptibility. In the case of benzo[a]pyrene, Insufficient information is available to quantitatively estimate variability in human susceptibility.

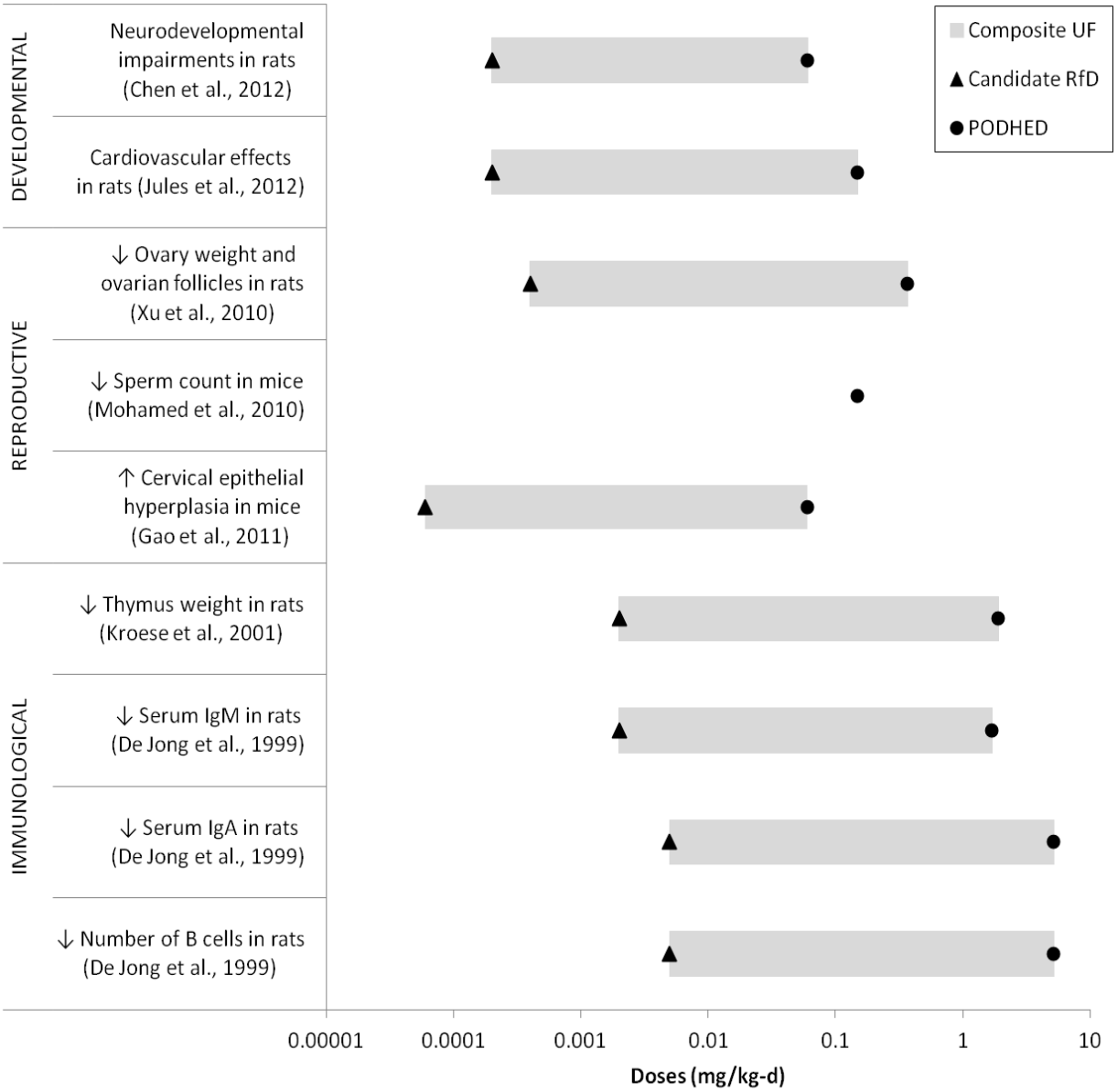
UF_L – A value of 1 was applied when the POD is based on dose-response modeling or a NOAEL; 10 when the POD is a LOAEL. In the case of benzo[a]pyrene, An UF_L of 1 was applied for LOAEL-to-NOAEL extrapolation because a BMR of a 1 SD change from the control mean in neurodevelopmental impairments was selected under an assumption that it represents a minimal biologically significant response level.

UF_S – A value of 1 was applied when dosing occurred during gestation or the early postnatal period that is relevant to developmental effects (U.S. EPA, 1991a); 10 when the POD is based on a subchronic study (studies in this table, other than the developmental toxicity studies, were 42-90 days in duration) to account for the possibility that longer exposure may induce effects at a lower dose.

UF_D – A value 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). 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).

[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 uncertainty factor in four or more areas of extrapolation should be avoided.

Figure 2-1. Candidate RfDs with corresponding POD and composite UF



2.1.4. Derivation of Organ/System-specific Reference Doses

Table 2-3 distills the candidate reference doses 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.

Developmental Toxicity

The candidate RfD based on neurodevelopmental impairment in rats (Chen et al., 2012) was selected as the organ/system-specific RfD representing developmental toxicity. This candidate RfD was selected because it is associated with the application of the smaller composite uncertainty factor and because similar effects were replicated across other studies.

Reproductive Toxicity

The candidate RfD based on decreased ovary weight and ovarian follicle numbers 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. The data supporting cervical effects associated with oral benzo[a]pyrene exposure are limited to a single study; however the finding is supported by corollary findings after i.p. exposure and by studies in humans.

Immunotoxicity

The candidate RfDs based on decreased thymus weight (Kroese et al., 2001) and serum IgM levels in rats (DeJong 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 RfDs for decreased thymus weight (Kroese et al., 2001) and serum IgM levels in rats (DeJong et al., 1999) were equal and provided the most senstitive candidate RfDs, thus these candidate RfDs were selected as the organ/system-specific RfDs representing immunotoxicity.

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

Effect	Basis	RfD (mg/kg-d)	Confidence
Developmental	Neurodevelopmental impairments	2×10^{-4}	MEDIUM
Reproductive	Decreased ovary weight and ovarian follicles	4×10^{-4}	MEDIUM
Immunological	Decreased thymus weight and serum IgM	2×10^{-3}	LOW
Proposed Overall RfD	**Developmental toxicity**	**2×10^{-4}**	**MEDIUM**

2.1.5. Selection of the Proposed Overall Reference Dose

To estimate an exposure level below which the effects identified as potential hazards from benzo[a]pyrene exposure are not expected to occur, the lowest organ/system-specific RfD (2×10^{-4} mg/kg-day) is proposed as the overall reference dose for benzo[a]pyrene. This value, based on

induction of neurodevelopmental impairments in rats exposed to benzo[a]pyrene during a susceptible lifestage is supported by a large number of animal and human studies.

2.1.6. 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).

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 and lack of reporting gender-specific data for all outcomes. 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 Reference Dose

An RfD was not derived in the previous IRIS assessment.

2.2. Inhalation Reference Concentration for Effects Other Than Cancer

The 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 highlighted 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 a reference concentration. 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.

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. The only chronic inhalation study available for benzo[a]pyrene, Thyssen et al. (1981), was designed as a cancer bioassay and did not report other effects. However, several reproductive and developmental toxicity studies are available in which effects on fetal survival and the male reproductive system have been observed.

Developmental toxicity

Developmental toxicity, as represented by decreased fetal survival and developmental neurotoxicity, was observed by Archibong et al. (2002), Wu et al. (2003), and Wormley et al. (2004). Wu et al. (2003) 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. From the remaining studies demonstrating developmental toxicity, the studies conducted by Archibong et al. (2002) and Wormley et al. (2004) were identified as the most informative studies for dose-response analysis. Archibong et al. (2002) observed biologically significant effects at the lowest dose tested by the inhalation route (i.e., LOAEL of 25 $\mu g/m^3$). 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 available oral database for benzo[a]pyrene (e.g., decreased survival of litters in mice following in utero exposure to benzo[a]pyrene on GD 7-16) (MacKenzie and Angevine, 1981). In addition, a single exposure inhalation study by Wormley et al. (2004) demonstrated developmental toxicity, represented by decreased pups/litter and electrophysiological changes in the hippocampus, as a result of gestational exposure. The single exposure concentration used by Wormley et al. study was the equivalent of the high-dose exposure concentration applied in the Archibong et al. (2002) and Wu et al. (2003) studies, thus the endpoint of fetal survival observed by Wormley et al. (2004) was not considered for dose-response analysis. However, similar to oral studies of benzo[a]pyrene exposure, Wormley et al. (2004) observed effects indicative of developmental neurotoxicity and therefore these effects were considered further for dose-response analysis.

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) and 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. 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 (reviewed by Soares and Melo, 2008).

2.2.2. Methods of Analysis

Data for decreased fetal survival (Archibong et al., 2002) were not amenable to BMD modeling due to the pattern of variability (heterogeneous variances) in the data set; the response at the lowest exposure showed the greatest variability. Therefore, the LOAEL from this study was used as the POD for dose-response analysis. Wormley et al. (2004) and Archibong et al. (2008), using only one exposure level, were judged not to support dose-response modeling due to the lack of understanding of the underlying dose-response relationship (i.e., limited database). LOAELs were also used as the PODs for dose-respopnse analysis.

By definition, the RfC is intended to apply to continuous lifetime exposures for humans (U.S. EPA, 1994b). 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), Archibong et al. (2008), and Wormley et al. (2004) were adjusted to account for the discontinuous daily exposure as follows:

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

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

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

where:

BW = body weight (kg)

V_E = ventilation rate (L/minute)

F_{TOT} = total fractional deposition

The total fractional deposition (F_{TOT}) includes particle deposition in the nasal-pharyngeal, tracheobronchial, and pulmonary regions. F_{TOT} for both animals and humans was calculated using the Multi-Path Particle Dosimetry model, a computational model used for estimating human and rat airway particle deposition and clearance [Multi-Path Particle Dosimetry (MPPD); Version 2.0 © 2006, publicly available through the Hamner Institute]. F_{TOT} was based on the average particle size of 1.7 ± 0.085 (MMAD ± geometric SD) as reported in Wu et al. (2003) for the exposure range 25 - 100 um^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 C 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) and Wormley et al. (2004) studies 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 C).

Under these conditions, the MPPD model calculated F_{TOT} values of 0.621 for the human and 0.181 for the rat. Using the above equation, the $RDDR_{ER}$ was calculated to be 1.1.

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 concentration for each data set discussed above.

Table 2-4. Summary of Derivation of Points of Departure

Endpoint and Reference	Study Design	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 rats GD 11-20, 4hrs/d 7d/wk	LOAEL (25 $\mu g/m^3$)				4.2	4.6
Decreased long term potentiation in hippocampus Wormley et al., 2004	Pregnant rats GD 11-21, 4 hrs/d, 7d/wk	LOAEL (100 $\mu g/m^3$)				16.7	18.3
REPRODUCTIVE							
Decreased testis weight Archibong et al., 2008	Male rats, 60d, 4hrs/d, 7d/wk	LOAEL (75 $\mu g/m^3$)				12.5	13.8
Decreased sperm count and motility Archibong et al., 2008	Male rats, 60d, 4hrs/d, 7d/wk	LOAEL (75 $\mu g/m^3$)				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 regional deposited dose ratio calculated using particle size reported in Hood et al. (2000) using MPPD software as detailed in Section 2.2.2. and Appendix C in the Supplemental Information.

2.2.3. Derivation of Candidate Reference Concentrations

Table 2-5 is a continuation of Table 2-4 and summarizes the application of uncertainty factors to each POD to derive a candidate reference concentration for each data set. The selection of uncertainty factors was based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and is described in the Section 7.6 of the Preamble. Figure 2-2 presents graphically these candidate reference doses, uncertainty factors, and points of departure, with each bar corresponding to one data set described in Tables 2-4 and 2-5.

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

Endpoint	POD_{HEC} ($\mu g/m^3$)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate RfC (mg/m^3)[a]
DEVELOPMENTAL									
Decreased fetal survival in rats Archibong et al., 2002	4.6	LOAEL	3	10	10	1	10	3000	2×10^{-6}
Decreased long term potentiation in the hippocampus of rats Wormley et al., 2004	18.3	LOAEL	3	10	10	1	10	3000	6×10^{-6}
REPRODUCTIVE									
Decreased testis weight in rats Archibong et al., 2008	13.8	LOAEL	3	10	10	10	10	30000	Not calculated due to UF > 3000[b]
Decreased sperm count and motility in rats Archibong et al., 2008	13.8	LOAEL	3	10	10	10	10	30000	Not calculated due to UF > 3000[b]

UF_A – 3 to account for residual toxicodynamic uncertainties when an HEC was calculated by inhalation particle dosimetry methods (U.S. EPA, 1994b), as is the case with benzo[a]pyrene.

UF_H – 10 to account for potentially susceptible individuals when adequate information is not available to quantitatively estimate variability in human susceptibility. In the case of benzo[a]pyrene, Insufficient information is available to quantitatively estimate variability in human susceptibility to benzo(a)pyrene.

UF_L – 1 when the POD is based on BMD modeling or a NOAEL; 10 or 3 when the POD is a LOAEL. In the case of benzo[a]pyrene, UF_L of 10 was applied to account for the use of a LOAEL. A NOAEL was not identified for decreased fetal survival observed by Archibong et al (2002). At the lowest dose, benzo[a]pyrene treated dams gave birth to 15% fewer pups compared to dams treated with vehicle alone (carbon black particles). Due to the lack of a NOAEL and the inability to model the data set for decreased fetal survival, a UF of 10 was applied to extrapolate to a NOAEL.

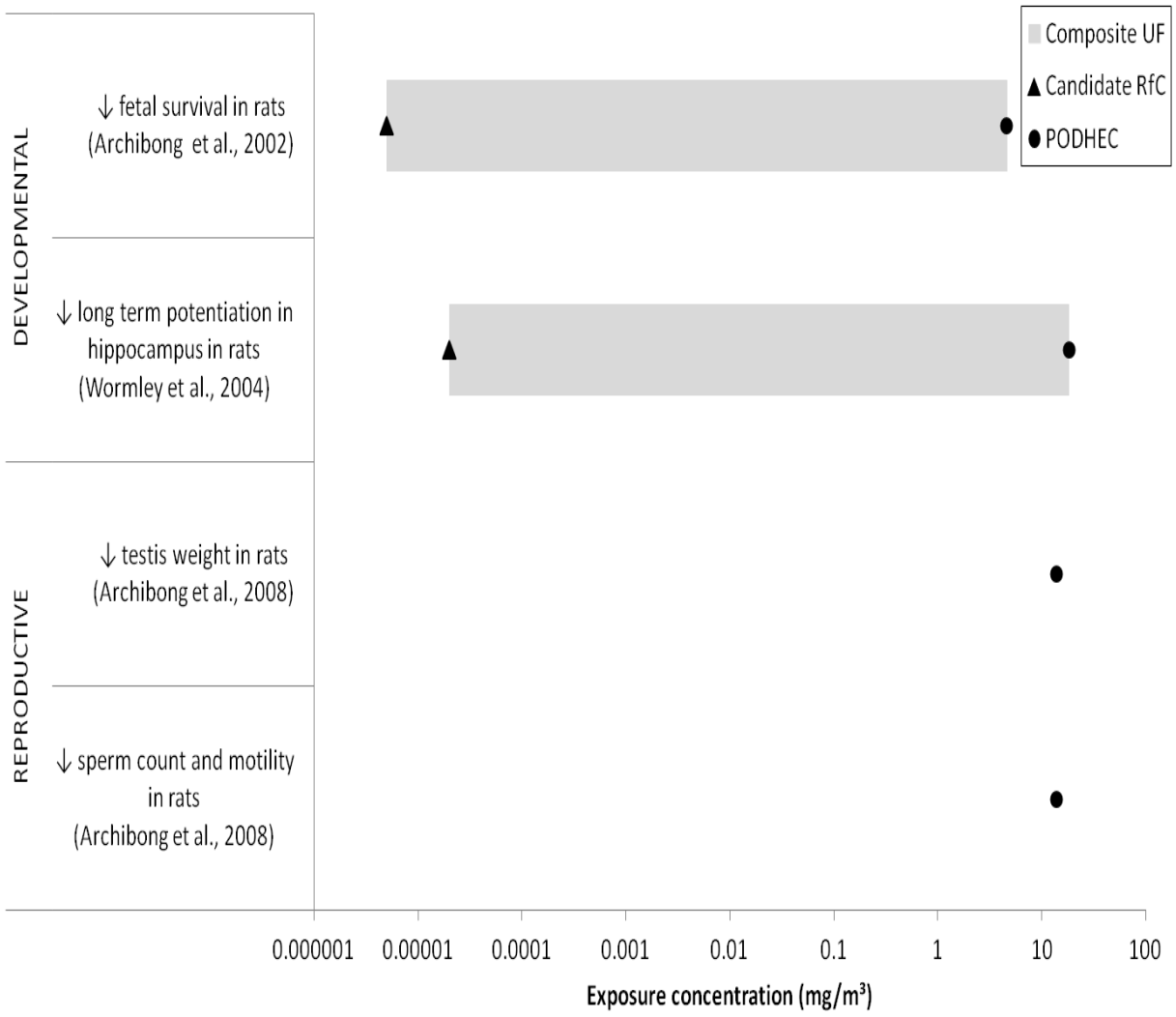
UF_S – 1 when dosing occurred during gestation or the early postnatal period that is relevant to developmental effects (U.S. EPA, 1991a); 10 when the POD is based on a subchronic study (studies in this table were 60 days in duration) to account for the possibility that longer exposure may induce effects at a lower dose

UF_D – 10 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). 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 underprotective 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. In the case of benzo[a]pyrene, oral exposure studies have demonstrated effects at doses lower than those where mortality was observed in the inhalation study by Archibong et al. (2002). The lack of a study examining functional neurological endpoints following a more comprehensive period of developmental exposure (i.e. gestation through lactation) is also a data gap, considering human and animal evidence indicating altered neurological development (see Section 1.1.1).

[a] Candidate RfCs 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 uncertainty factor in four or more areas of extrapolation should be avoided.

Figure 2-2. Candidate RfCs with corresponding POD and composite UF



2.2.4. Derivation of Organ/System-specific Reference Concentrations

Table 2-6 distills the candidate reference concentrations 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 RfCs for reproductive toxicity derived from Archibong et al. (2008) were not selected 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 uncertainty factor 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)	Confidence
Developmental	Decreased fetal survival	2×10^{-6}	low-to-medium
Reproductive	Reductions in sperm parameters	Not calculated	NA
Proposed Overall RfC	**Decreased fetal survival**	**2×10^{-6}**	**low-to-medium**

2.2.5. Selection of the Proposed Overall Reference Concentration

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 decreases in number of pups/litter and fetal survival/litter are the most sensitive noncancer effects observed following inhalation exposure to benzo[a]pyrene. Additional support for this endpoint is provided by the oral studies of benzo[a]pyrene. A developmental/reproductive study conducted via the oral route in mice observed decreased survival of litters, decreased pup weight, and decreased reproductive organ weight following in utero exposure to benzo[a]pyrene on GD 7–16 (MacKenzie and Angevine, 1981).

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 dietary study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study, the lack of studies on immune endpoints, and the 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 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, 2000, 1994b) was applied to a POD based on neurodevelopmental impairments 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 chronic bioassays in rats and mice, two developmental studies in mice, and several subchronic studies in rats.

Although the database is adequate for RfD derivation, there is uncertainty associated with the database 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). However, subchronic and short-term inhalation studies are available, which examine developmental and reproductive endpoints in rats. Developmental studies by the inhalation route identified biologically significant reductions in the number of pups/litter and percent fetal survival and possible neurodevelopmental effects (e.g., diminished electrophysiological responses to 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. One area of uncertainty pertains to the lack of information regarding fertility in animals exposed gestationally to benzo[a]pyrene, especially in light of developmental studies by the oral route indicating reduced fertility in the F1 generation and decreased reproductive organ weights. The database also lacks a multigenerational reproductive study via the

inhalation route. Areas of uncertainty include the lack of chronic inhalation studies focusing on noncancer effects, limited data on dose-response relationships for impaired male or female fertility with gestational exposure or across several generations, and limited data on immune system endpoints with chronic exposure to benzo[a]pyrene.

The toxicokinetic and toxicodynamic differences for benzo[a]pyrene between the animal species in which the POD was derived and humans are unknown. PBPK models can be useful for the evaluation of interspecies toxicokinetics; however, the benzo[a]pyrene database lacks an adequate model that would inform potential differences. There is some evidence from the oral toxicity data that mice may be more susceptible than rats to some benzo[a]pyrene effects (such as ovotocity [Borman et al., 2000]), 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

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's gland) tumors, and skin or mammary gland tumors in male and female Wistar rats (Kroese et al., 2001); forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981); and forestomach, esophageal, tongue, and larynx tumors in female $B6C3F_1$ mice (Beland and Culp, 1998; additional results reported by 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 limitation 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; Field and Roe, 1970; 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 several concentrations of benzo[a]pyrene dissolved in a 1.5% caffeine solution, sometimes as infrequently as once every ninth day, for up to 2 years and observed increased forestomach tumors. This study was not selected for quantitation due to the nonstandard treatment protocol in comparison to the studies conducted by Kroese et al. (2001) and Beland and Culp (1998).

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 Good Laboratory Practice principles as established by the Organization 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 B of the Supplemental Information. Dose-related, statistically significant 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 C of the Supplemental Information. As recommended by NTP (McConnell et al., 1986) and as outlined in EPA's *Cancer Guidelines* (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 only one mid-dose male had an auditory canal tumor which did not also have a forestomach or oral cavity squamous cell tumor, and none were observed in low-dose male or female rats, the data are insufficient to conclude whether 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.

2.3.2. Dose Response Analysis – Adjustments and Extrapolations Methods

EPA's *Cancer Guidelines* (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 *Cancer Guidelines* (U.S. EPA, 2005a), an adjustment for cross-species scaling was applied to address toxicological equivalence

across species. Following EPA's cross-species scaling methodology, the time-weighted daily average doses were converted to human equivalent doses (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)$^{0.25}$ (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 can be found in Appendix C 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. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = 0.1/$BMDL_{10}$). This slope, a 95% upper confidence limit (UCL) represents a plausible upper bound on the true risk.

2.3.3. Derivation of the Oral Slope Factor

The PODs estimated for each tumor site are summarized in Table 2-7. Details of the model selection process are provided in Appendix C of the Supplemental Information. 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-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: squamous cell tumors Beland and Culp 1998	Female B6C3F1 Mice	Multistage Weibull	10%	0.127	0.071	1	1

[a] Human equivalent slope factor = 0.1/BMDL10HED; see Appendix C of the Supplemental Information for details of modeling results.
[b] Estimates of risk of incurring at least one of the tumor types listed.

Oral slope factors derived from rat bioassay data varied by gender and tumor site (Table 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 fourfold 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, not just the risk of developing all simultaneously. A method involving the assumption that the variability in the slope factors could be characterized by a normal distribution is detailed in Appendix C 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 C 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 chronic oral exposure to benzo[a]pyrene is **1 per mg/kg-day**, based on the alimentary tract tumor response in female $B6C3F_1$ mice.

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 projecting 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). 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, 1999). However, due to the storage function of the forestomach, tissue of the forestomach 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 development of squamous epithelial tumors in the forestomach compared to oral or esophageal tumors in humans.

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; Szymanska et al., 2010; Wang et al., 2002; Gustavsson et al., 1998a; Liu et al., 1997).

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 lowest and highest slope

factors by individual sites, as listed in Table 2-8, show about a 35-fold difference. However, consideration of individual sites when multiple sites are affected perhaps overemphasizes an expectation of site concordance. While the highest risk estimates were derived from the incidence data for forestomach tumors in both rats and mice, the oral slope factor based on the mouse 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 benzo[a]pyrene oral cancer slope factor (OSF)

Consideration	Impact on unit risk	Decision	Justification
Target organ	↓ OSF, 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.
Data set	↓ OSF ~threefold if rat bioassay were selected for OSF derivation	Beland and Culp (1998)	Beland and Culp (1998) was a well conducted study and used the lowest HEDs of the available cancer bioassays, reducing low-dose extrapolation uncertainty.
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.
Cross-species scaling	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 neither to over- or 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	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	Impact on unit risk	Decision	Justification
	nonlinear low-dose extrapolation		
Statistical uncertainty at POD	↓ OSF 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% confidence interval on administered exposure at 10% extra risk of alimentary tract tumors.
Sensitive subpopulations	↑ OSF 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 Oral Slope Factor

The previous cancer assessment for benzo[a]pyrene was posted on the IRIS database in 1987. At that time, benzo[a]pyrene was classified as a probable human carcinogen (Group B2) based on inadequate data in humans and sufficient data in animals via several routes of exposure. An oral slope factor was derived from the geometric mean of four slope factor estimates based on studies in Sprague-Dawley rats (Brune et al., 1981) and CFW-Swiss mice (Neal and Rigdon, 1967). 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 1.1.5.2 for study details). Three modeling procedures were used to derive risk estimates from the Neal and Rigdon (1967) bioassay (see Section 1.1.5.2). In a report commissioned by EPA, Clement International Corporation (1990) fit a two-stage response model, based on exposure-dependent changes in both transition rates and growth rates of preneoplastic cells, to derive a value of 5.9 per mg/kg-day. 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 Clement 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 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 as the oral slope factor.

2.4. Inhalation Unit Risk for Cancer

2.4.1. Analysis of Carcinogenicity Data

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

The bioassay by Thyssen et al. (1981) represents the only lifetime inhalation cancer bioassay available for describing dose-response relationships for cancer from inhaled

benzo[a]pyrene. Strengths of the study include the following: 1) exposures was conductd for 2 years; 2) histological examination of organs was extensive and adequate; 3) multiple exposure groups were used; and 4) individual animal pathology reports with time of death and tumor detection data were available. Increased incidences of benign and malignant tumors of the larynx and pharynx were seen with increasing exposure concentration. Survival was decreased relative to control only in the high-dose exposure group; mean survival times in the 0, 2, and 10 mg/m^3 concentration groups were 96.4, 95.2, and 96.4 weeks, respectively, and 59.5 weeks in the 50 mg/m^3 group animals. Overall, tumors occurred earlier in the highest benzo[a]pyrene exposure group than in the mid-exposure group.

Limitations of the study include the following: (1) only male animals were tested; (2) particle analysis of aerosols was not reported (i.e., mass median aerodynamic diameter and geometric SD were not reported); and (3) benzo[a]pyrene exposure occurred through the inhalation of hygroscopic particles (benzo[a]pyrene was adsorbed onto sodium chloride aerosols), which may have a different deposition than benzo[a]pyrene adsorbed onto carbonaceous non-hygroscopic particles as is more typical in the environment. In addition, reported information regarding the actual exposure levels achieved during the study was incomplete. Analytic measurements varied more than 20% from the average achieved for each dose group during the first 79 weeks of the study, but were not available for the remaining 52 weeks that it took to complete the experiment. Despite the limitations, the strengths of the study were judged to support use of the data to derive an inhalation unit risk for benzo[a]pyrene.

The intratracheal instillation studies provide supporting evidence of carcinogenicity of inhaled benzo[a]pyrene; however, the use of intratracheal dosing alters the deposition, clearance, and retention of substances, and therefore studies utilizing this exposure technique are not as useful for the quantitative extrapolation of cancer risk from the inhalation of benzo[a]pyrene in the environment (Driscoll et al., 2000).

2.4.2. Dose Response Analysis—Adjustments and Extrapolations Methods

Biologically based dose-response models for benzo[a]pyene 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) 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. There were sufficient data to apply a time-to-tumor dose-response model, described in detail in Appendix C of the Supplemental Information.

The tumor incidence data used for dose-response modeling comprised the benign and malignant tumors in the pharynx and larynx. The pharynx and larynx are associated with the upper digestive tract and the upper respiratory tract, respectively. However, 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 squamous cell carcinomas in these tissues (U.S. EPA, 1990). Following EPA's *Cancer Guidelines* (Section 2.2.2.1.2; U.S. EPA, 2005a), incidence data for animals with malignant or benign tumors originating from the same cell type were selected for dose-response modeling based on the assumption that the benign tumors could develop into malignancies.

The availability of the raw chamber air monitoring data and individual times on study allowed the calculation of TWA continuous exposures for each hamster (U.S. EPA, 1990). Group averages of these concentrations were 0, 0.25, 1.01, and 4.29 mg/m^3, respectively, for the 0, 2, 10, and 50 mg/m^3 study concentrations.

A toxicokinetic model to assist in cross-species scaling of benzo[a]pyrene inhalation exposure was not available. In addition, default dosimetry adjustments utilized in the benzo[a]pyrene RfC calculation could not be applied because aerosol particle distribution data were not available for the hamster inhalation bioassay by Thyssen et al. (1981). The carrier particle used in Thyssen et al. (1981) was sodium chloride, a soluble hygroscopic particle, and the approaches presented in the RfC methodology guidelines (U.S. EPA 1994b) were developed for insoluble and nonhygroscopic particles. 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. This is equivalent to assuming that any metabolism of benzo[a]pyene is directly proportional to breathing rate and that the deposition rate is equal between species.

A time-to-tumor dose-response model was fit to the TWA exposure concentrations and the individual animal tumor and survival data for tumors in the larynx, pharynx, trachea, esophagus, and forestomach, using the computer software program MSW (U.S. EPA, 2010) as described in Appendix C of the Supplemental Information. Unlike in the available oral bioassays, Thyssen et al. did not determine cause of death for any of the animals. Since the investigators for the oral bioassays considered the same tumors to be fatal at least some of the time, bounding estimates for the Thyssen et al. (1981) data were developed by treating the tumors alternately as either all incidental or all fatal. Modeling results are provided in Appendix C of the Supplemental Information.

2.4.3. Derivation of the Inhalation Unit Risk

Because benzo[a]pyrene carcinogenicity involves a mutagenic mode of action, linear low dose extrapolation from the $BMCL_{10}$ was used (U.S. EPA, 2005a) to derive the inhalation unit risk. BMCs and BMCLs associated with an extra risk of 10% calculated using the multistage-Weibull model, based on the occurrence of upper respiratory and upper digestive tract tumors in male hamsters exposed to aerosols of benzo[a]pyrene for up to 132 weeks, were estimated under two bounding assumptions. The results are summarized in Table 2-9. At one extreme, taking the tumors to have been the cause of death of the experimental animals, the BMC_{10} and $BMCL_{10}$ were

0.648 and 0.461 mg/m^3, respectively. Then taking all of the tumors to have been incidental to the cause of death for each animal, the BMC_{10} and $BMCL_{10}$ were 0.285 and 0.198 mg/m^3, respectively, about twofold lower than the first case. Because the tumors were unlikely to have all been fatal, the lower $BMDL_{10}$ was selected for estimating the inhalation unit risk. Using linear extrapolation from the $BMCL_{10}$ of 0.198 mg/m^3, an inhalation unit risk of **0.5 per mg/m^3**, or **5×10^{-4} per µg/m^3** (rounding to one significant digit), was calculated.

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 and digestive tracts; all treated as cause of death Thyssen et al., 1981	Male Hamsters	Multistage Weibull	10%	0.648	0.461	0.22
Upper respiratory and digestive tracts; all treated as incidental to death Thyssen et al., 1981	Male Hamsters	Multistage Weibull	10%	0.285	0.198	0.51

[a] Human equivalent unit risk = 0.10/BMCL10; see Appendix C for details of modeling results.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

Only one animal cancer bioassay by the inhalation route is available that describes the dose-response relationship for respiratory tract tumors with chronic inhalation exposure to benzo[a]pyrene (Thyssen et al., 1981). Although corroborative information on dose-response relationships in other animal species is lacking, the findings for upper respiratory tract tumors are consistent with findings in other hamster studies with intratracheal administration of benzo[a]pyrene, and with some of the portal of entry effects in oral exposure studies. This study is adequate for dose-response analysis and derivation of an inhalation unit risk estimate, but some associated uncertainty includes the inability to apply U.S. EPA (1994b) dosimetry approaches to extrapolate inhaled doses from animals to humans, due to the use of a soluble hygroscopic carrier particle (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 (Varghese and Gangamma, 2009; Asgharian, 2004; Ferron, 1994; Xu and Yu, 1985). 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.

The exposure measures entailed some uncertainty, in that the exposure concentrations for about half of the study period were documented to vary above and below the average achieved by

about twofold in all exposed groups. One possibility that cannot be ruled out, due to lack of data, is that peak exposure above some concentration may be associated with the observed effects. Use of Haber's Law (equal cumulative exposures being expected to lead to similar outcomes) to estimate continuous equivalent exposures may be not justified. There is not enough information available to estimate an impact on the estimated unit risk due to this uncertainty.

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

Consideration	Impact on unit risk	Decision	Justification
Data set and target organ	No IUR 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 dose-response relationships for cancer from inhaled benzo[a]pyrene. Intratracheal implantation study supports the association of benzo[a]pyrene exposure with respiratory tract tumors. Oral exposure resulted in similar tumors.
Dose metric	Alternatives could ↓ or ↑ unit risk	Administered dose as time-weighted average	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor	The carcinogenicity observed was portal-of-entry, but data to implement RfC methodology (EPA, 1994) were lacking. Cross-species scaling was not applied.	There are no data to support alternatives. Equal risk per µg/m3 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 point of departure (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	↓ IUR 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 on administered exposure at 10% extra risk of respiratory tract tumors.

Sensitive subpopulations	↑ IUR 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 Inhalation Unit Risk

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

2.5. Dermal Slope Factor for Cancer

Evidence in humans and animal studies demonstrates an increased incidence of skin tumors with increasing dermal exposure to polycyclic aromatic hydrocarbons (PAHs) mixtures or to benzo[a]pyrene alone. Thus, this assessment for benzo[a]pyrene derives a dermal slope factor, a quantitative risk estimate that is a plausible upper bound on the estimate of risk per µg/day of dermal exposure. This is the first derivation of a dermal slope factor for the 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; WHO, 1998; Boffetta et al., 1997; ATSDR, 1995); however, 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 mice, rats, rabbits, and guinea pigs. However, no lifetime chronic bioassays of dermal benzo[a]pyrene exposure were located in these species. Therefore, this analysis focuses on chronic carcinogenicity bioassays in several strains of mice demonstrating increasing incidence of benign and malignant skin tumors, as well as earlier occurrence of tumors with increasing exposure, following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime. These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984, 1983; Habs et al., 1984, 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959) (see Tables B-15 to B-23 in the Supplemental Information).

Additional carcinogenicity studies in mice were considered, but not used, in the dose-response analysis. These 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 Hoffman, 1959; Wynder et al., 1957); (2) initiation-promotion studies utilizing acute dosing of benzo[a]pyrene followed by repeated exposure to a potent tumor promoter (sometimes benzo[a]pyrene at a lower dose than the initiation step), because they are not as relevant for calculating risks from constant benzo[a]pyrene exposure alone; (3) bioassays with one benzo[a]pyrene dose level or with only dose levels inducing 90–100% incidence of mice with tumors, because they provide relatively little information about the shape of the dose-response relationship (e.g., Wilson and Holland, 1988); (4) studies with shorter exposure and

observation periods (i.e., <1 year) (Higginbotham et al., 1993; Albert et al., 1991; Nesnow et al., 1983; Emmett et al., 1981; Levin et al., 1977) which are less relevant for characterizing lifetime risk; and (5) studies involving vehicles expected to interact with or enhance benzo[a]pyrene carcinogenicity (e.g., Bingham and Falk, 1969), which precludes assessment of carcinogenic risks of benzo[a]pyrene alone.

Study designs and the extent of data reported varied across the studies identified above. Because no particular studies stood out as superior for developing a dermal slope factor, these data sets were considered as a group in order to assess the overall evidence. Each data set was examined for limitations that would not support dose-response evaluation. Only the data from Poel (1960) were not considered further for modeling. The three data sets reported by Poel (1960) demonstrated high mortality and 100% tumor incidence at doses higher than those in any of the other studies under consideration, used the smallest group sizes of the available studies, and did not provide sufficient information to estimate the duration of exposure for the dose groups with less than 100% tumor incidence (see Table B-16 in the Supplemental Information).

The remaining studies varied in complete reporting of: incidence of benign and malignant tumors or only malignancies; times of first tumor occurrence, which informs whether earlier mortality impacts the number at risk of tumor development; and duration of exposure by dose group. With the exception of Poel (1959), summarized next, the rest of the studies considered for dose-response modeling contained groups which survived the majority of a two-year exposure period. These studies support dose-response modeling, with some limitations that indicate the slope factors could be underestimates (e.g., possible over-estimation of animalas at risk or exposure associated with tumor response). The study by Poel (1959) included nine dose groups and also demonstrated high mortality and tumor incidence at higher exposure levels. All mice in dose groups with >3.8 μg/application died by week 44 of the study. Therefore, these five dose groups were omitted prior to dose-response modeling because of the relatively large uncertainty associated with characterizing lifetime cancer risk from short exposures (<1 year). Four dose groups in addition to control remained, all with exposures lasting at least 83 weeks.

2.5.2. Dose Response Analysis – Adjustments and Extrapolations Methods

As with the oral and inhalation benzo[a]pyrene carcinogenicity data, benzo[a]pyrene's dermal exposure carcinogenicity data were generally characterized by earlier occurrence of tumors and increased mortality with increasing exposure level. However, individual animal data were not available for any of the identified studies. Therefore, time-to-tumor modeling was not possible. Each of the dermal data sets was modeled using the multistage model.

For all studies, administered doses were converted to average daily doses using the equation:

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

Next, lifetime equivalent doses were estimated for study groups that were reported to end before 104 weeks by multiplying the relevant average daily doses by $(L_e/104)^3$, where L_e is the length of exposure, based on observations that tumor incidence tends to increase with age (Doll, 1971). Note that exposure periods <52 weeks would lead to a relatively large adjustment [i.e., $(52/104)^3 = 0.125$, or an eightfold lower dose than administered], reflecting considerable uncertainty in lifetime equivalent dose estimates generated from relatively short studies. This adjustment was relevant for Poel (1959), Roe et al. (1970), Habs et al. (1980), and Sivak et al. (1997).

Concerning the incidence data, some of these studies reported incidences of skin tumor-bearing animals for tumors thought to be malignant (Roe et al., 1970; Poel, 1959) or without clear designation of the relative percentages of animals with carcinomas and papillomas (Habs et al., 1980). In the other studies, incidences of animals with skin papillomas and skin carcinomas were clearly reported, showing that skin tumors from life-time exposure to benzo[a]pyrene were predominately malignant (Sivak et al., 1997; Habs et al.,1984; Grimmer et al., 1984, 1983; Schmähl et al., 1977; Schmidt et al., 1973). Following EPA's *Cancer Guidelines* (Section 2.2.2.1.2; U.S. EPA, 2005a), incidence data for animals with malignant or benign skin tumors were selected for dose-response modeling based on the evidence that skin papillomas can develop into malignant skin tumors. The data sets as modeled are presented in Tables C-24 through C-27 in the Supplemental Information.

The multistage-cancer model was then fit to each data set. If there was no adequate fit using the multistage-cancer model, then other dichotomous models were considered. 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) for candidate dermal slope factors.

2.5.3. Derivation of the Dermal Slope Factor

Adequate model fits were found using the multistage model for all but one of the mouse skin tumor incidence data sets (see Appendix C of the Supplemental Information). The data from Grimmer et al. (1984) could not be adequately fit by the multistage model initially, and the other dichotomous models available in BMDS were considered. Due to the supralinear shape of the dose-response data, only the log-logistic and dichotomous Hill models provided adequate fits. Also due to the supralinear dose-response shape, the POD for slope factor derivation was identified near the lowest response of ~70%, because of the lack of data to inform the dose-response relationship at lower doses.

Dermal slope factors, calculated in units of risk per (μg/day) using linear extrapolation from the BMDL values, ranged from 0.25 to 1.8 per μg/day, a roughly sevenfold range (see Table 2-11). A number of differences among studies may contribute to this range, including solvent choice, sex and strain of mice studied, dose ranges, varying group sizes, and level of detail reported. Mouse strains

were not repeated across sexes among these studies, so it cannot be established whether Swiss or NRMI mice are more or less sensitive than other strains. In addition, different solvents were used in the various studies with varying strain and sex combinations tested. For example, toluene was used in one male study only, and all of the female studies used acetone. Thus, any possible impact of the solvents used is not clear. As noted earlier, incomplete mortality information in several of the female mouse studies (Grimmer et al., 1984, 1983; Habs et al., 1984, 1980; Schmähl et al., 1977; Schmidt et al., 1973) suggests that the dermal slope factors derived from those studies may underestimate cancer risk.

The estimates derived from the two studies in male mice (Poel, 1959; Sivak et al. 1997) were at the higher end of the range of slope factors derived. The available information is too limited to conclude that males are more sensitive than females, in view of the similar result from the Habs et al. (1984) data and the limitations of the remaining studies that suggested risk might be underestimated. Nevertheless, the studies in male mice were among the stronger studies. They included at least three exposure levels, the lowest doses tested, and better reporting of intercurrent mortality. Both studies yielded very similar BMDs, with Poel (1959) providing a higher $BMDL_{10}$, associated with its larger group sizes (~50/dose group). Thus these two results were combined by estimating the midpoint between the $BMDL_{10}$s, 0.068 µg/day.

Table 2-11. Summary of Dermal Slope Factor Derivations

Reference	Sex/ strain of mouse	Selected Model[a]	BMR	BMD (µg/d)	BMDL (µg/d)	Dermal slope factor[b] $(µg/d)^{-1}$
Sivak et al., 1997	Male C3H/HeJ	Multistage 2°	10%	0.11	0.058	1.7
Poel, 1959	Male C57L	Multistage 3°	10%	0.12	0.078	1.3
Habs et al., 1984	Female NMRI	Multistage 3°	10%	0.078	0.056	1.8
Grimmer et al., 1984	Female CFLP	Log-logistic	70%	1.07	0.48	1.5
Schmahl et al., 1977	Female NMRI	Multistage 2°	10%	0.23	0.15	0.67
Schmidt et al., 1973	Female Swiss	Multistage 3°	10%	0.28	0.22	0.45
Grimmer et al., 1983	Female CFLP	Multistage 1°	10%	0.24	0.21	0.48
Habs et al., 1980	Female NMRI	Multistage 3°	10%	0.29	0.22	0.45
Schmidt et al., 1973	Female NMRI	Multistage 2°	10%	0.33	0.29	0.34
Roe et al., 1970	Female Swiss	Multistage 2°	10%	0.69	0.39	0.25

[a] See Appendix C for details of modeling results.
[b] Unadjusted for interspecies differences

The $BMDL_{10}$ of 0.068 µg/day, based on the tumor responses in C57L male mice (Poel, 1959) and in C3H/HeJ male mice (Sivak et al., 1997), was chosen for developing a human dermal slope factor.

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, 1994b). No established methodology exists to adjust for interspecies differences in dermal toxicity at the point of contact; however, 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. A comparison of these alternatives is provided in Appendix D of the Supplemental Information.

The POD_M derived from the mouse studies of Poel (1959) and Sivak et al. (1997) is adjusted to a HED as follows:

$$POD_{HED}\ (\mu g/day) = POD_M\ (\mu g/day) \times (BW_H / BW_M)^{3/4}$$
$$= 0.068\ \mu g/day \times (70\ kg / 0.035\ kg)^{3/4}$$
$$= 20.3\ \mu g/day$$

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

$$\text{Dermal slope factor} = 0.1/POD_{HED} = 0.1/(20.3\ \mu g/day) = \mathbf{0.005\ (\mu g/day)^{-1}}$$

Note that the dermal slope factor should only be used with lifetime human exposures <20 µg/day, the human equivalent of the POD_M, because above this level, the dose-response relationship may not 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., 1998), lifetime skin cancer bioassays that have included pathological examination of other organs have not found elevated incidences of tumors at distal sites (Higginbotham et al., 1993; Habs et al., 1980; Schmahl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959). In addition, benzo[a]pyrene tends to bind to targets within the skin rather than enter the plasma receptor fluid (a surrogate measure of systemic absorption) in in vitro human skin experiments. These data are consistent with benzo[a]pyrene's metabolism to reactive metabolites within the viable layers of the skin (Wester et al., 1990). Some studies indicate that the fraction of benzo[a]pyrene left within the viable layers of the skin is a large portion of the applied dose (Moody et al., 2007, 1995). Taken together, these data support the conclusion that the risk of skin cancer following dermal exposure likely outweighs cancer risks at distal organs.

2.5.5. Uncertainties in the Derivation of the Dermal Slope Factor

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 seven-fold difference (0.056–0.39 μg/day) in magnitude. There is some indication that the recommended dermal slope factor may underestimate cancer risk, due to inadequate data to take the observed decreasing tumor latency with increasing exposure level into account. Reliance on studies with the lowest exposure levels where early mortality due to benzo[a]pyrene exposure was low and exposures continued for approximately 104 weeks tends to minimize this source of uncertainty.

Human dermal exposure to benzo[a]pyrene in the environment likely occurs predominantly through soil contact. The available mouse dermal bioassays of benzo[a]pyrene relied on delivery of benzo[a]pyrene to the skin in a solvent solution (typically acetone or toluene). The use of 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 considered treated animals 2-3 times a 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 which saturate or slow detoxifying metabolic steps.

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 adduct levels have been detected in WBCs following dermal exposure to benzo[a]pyrene, indicating that dermally applied benzo[a]pyrene enters systemic circulation (Godschalk et al., 1998). Although none of the lifetime dermal bioassays in mice, which included macroscopic examination of internal organs, reported an elevation of systemic tumors in benzo[a]pyrene-treated mice compared to controls (Higginbotham et al., 1993; Habs et al., 1980; Schmahl 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 benzo[a]pyrene cancer dermal slope factor (DSF)

Consideration	Impact on unit risk	Decision	Justification
Data set	↓ DSF if alternative data set were selected	Poel (1959); Sivak et al. (1997)	Poel (1959) included lowest doses among available studies (where intercurrent mortality was less likely to impact the number at risk) and used adequate group sizes.
Target organ	No DSF 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;
Dose metric	Alternatives could ↓ or ↑ slope factor	Administered dose, as time-weighted average in µg/day.	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor	Total daily dose scaled by BW3/4	Alternatives discussed in Appendix C. 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, metabolic processes were assumed to scale allometrically without evidence to the contrary.

Consideration	Impact on unit risk	Decision	Justification
Dose-response modeling	Alternatives could ↓ or ↑ slope factor	Multistage model	No biologically based models for benzo[a]pyrene were available. The multistage model is consistent with biological processes and is the preferred model for IRIS cancer assessments (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 MOA)	Available mode of action data support linearity (mutagenicity is a primary mode of action of benzo[a]pyrene);
Sensitive subpopulations	↑ DSF 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 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 0.5 per mg/m^3, and dermal slope factor of 0.005 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. 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)	Exposure concentration (mg/kg-d)	Duration adjustment	Cancer risk for specific exposure duration scenarios
0–<2 yrs	10	1	0.001	2 yrs/70 yrs	0.0003
2–<16 yrs	3	1	0.001	14 yrs/70 yrs	0.0006
≥16 yrs	1	1	0.001	54 yrs/70 yrs	0.0007
				Total risk	0.002

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 partial cancer risk based on the early age-specific group. The cancer risk for each age group is the product of the four factors in columns 2–5. Therefore, the cancer risk following daily benzo[a]pyrene oral exposure in the age group 0–<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 partial risks for the three age groups would be 3×10^{-4}, 6×10^{-4}, and 2×10^{-4}, which would result in a total risk estimate of 1×10^{-3}.

In calculating the cancer risk for a 30-year constant exposure to benzo[a]pyrene at an exposure level of 0.001 mg/kg-day for ages 20–50 years, the duration adjustments would be 0/70, 0/70, and 30/70. The partial risks for the three groups are 0, 0, and 4×10^{-4}, which would result in a total risk estimate of 4×10^{-4}.

Consistent with the approaches for the oral route of exposure (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. 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)	Exposure concentration (µg/m^3)	Duration adjustment	Cancer risk for specific exposure duration scenarios
0–<2 yrs	10	5×10^{-4}	1	2 yrs/70 yrs	0.0001
2–<16 yrs	3	5×10^{-4}	1	14 yrs/70 yrs	0.0003
≥16 yrs	1	5×10^{-4}	1	54 yrs/70 yrs	0.0004
				Total risk	0.0008

Table 2-15. 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)	Exposure concentration (µg/d)	Duration adjustment	Cancer risk for specific exposure duration scenarios
0–<2 yrs	10	0.005	0.001	2 yrs/70 yrs	1×10^{-6}
2–<16 yrs	3	0.005	0.001	14 yrs/70 yrs	3×10^{-6}
≥16 yrs	1	0.005	0.001	54 yrs/70 yrs	4×10^{-6}
				Total risk	8×10^{-6}

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