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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)

August 2013

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ABBREVIATIONS

ADAF	age-dependent adjustment factor
AhR	aryl hydrocarbon receptor
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BPDE	benzo[a]pyrene-7,8-diol-9,10-epoxide
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
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
EROD	ethoxyresorufin-o-deethylase
ETS	environmental tobacco smoke
FSH	follicle stimulating hormone
GD	gestation day
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
IHD	ischemic heart disease
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MMAD	mass median aerodynamic diameter
MN	micronuclei
MPPD	Multi-Path Particle Deposition
NCEA	National Center for Environmental Assessment
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
OR	odds ratio
ORD	Office of Research and Development
PAH	polycyclic aromatic hydrocarbon
PBPK	physiologically based pharmacokinetic
PND	postnatal day
POD	point of departure
RBC	red blood cell
$RDDR_{ER}$	regional deposited dose ratio for extrarespiratory effects
RfC	inhalation reference concentration
RfD	oral reference dose
ROS	reactive oxygen species
RR	relative risk
s.c.	subcutaneous
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCSA	sperm chromatin structure assay
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SRBC	sheep red blood cells
SSB	single strand break
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
TWA	time-weighted average
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
WBC	white blood cell
WHO-NCTB	World Health Organization Neurobehavioral Core Test Battery

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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. Benzo[a]pyrene occurs in conjunction with other structurally related chemical compounds known as polycyclic aromatic hydrocarbons (PAHs).[1] Benzo[a]pyrene is universally present in these mixtures and is routinely analyzed and detected in environmental media contaminated with PAH mixtures, thus it is often used as an indicator chemical to measure exposure to PAH mixtures (Boström et al., 2002).

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 and a Maximum Contaminant Level Goal (MCLG) and enforceable Maximum Contaminant Level (MCL) have been established. In air, benzo[a]pyrene is regulated as a component in a class of chemicals referred to as Polycyclic Organic Matter, defined as a Hazardous Air Pollutant by the 1990 amendments to the Clean Air Act.

This assessment updates IRIS assessment of benzo[a]pyrene that was developed in 1987. The previous assessment included a cancer descriptor and oral slope factor. New information has become available, and this assessment reviews information on all health effects by all exposure routes. Organ/system-specific reference values are calculated based on developmental, reproductive and immune system toxicity data. These reference values may be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system. In addition, in consideration of the Agency's need to estimate the potential for skin cancer from dermal exposure (U.S. EPA, 2004), especially in children exposed to contaminated soil, this assessment includes the IRIS Program's first dermal slope factor.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to Toxicological Reviews. 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

[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.

information, and summaries of toxicity studies are provided as *Supplemental Information* to this assessment.

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

Chemical Properties 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 (ATSDR, 1995).

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, and silicon carbide, and in coal tar distillation. The major sources of non-occupational exposure are cigarettes and food. Additional information on benzo[a]pyrene exposure and chemical properties can be found in Appendix A.

Implementation of the 2011 National Research Council Recommendations

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

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

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

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

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS benzo[a]pyrene assessment is in Phase 2 and represents a significant advancement in implementing the NRC recommendations shown in Table F-1 in Appendix F. The Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table F-2 in Appendix F, including the development of a standardized approach to describe the strength of evidence for noncancer effects . On May 16, 2012, EPA announced (U.S. EPA, 2012c) that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

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 B. It is important to recognize that these assessments were prepared at different times, for different purposes, using different guidelines and methods, and that newer studies have been included in the IRIS assessment.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

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

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

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

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

2. Process for developing and peer-reviewing IRIS assessments

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

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

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

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

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

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

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

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

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

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

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

3. Identifying and selecting pertinent studies

3.1. Identifying studies

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

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

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

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

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

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

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

3.2. Selecting pertinent epidemiologic studies

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

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

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

3.3. Selecting pertinent experimental studies

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

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

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

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

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

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

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

4. Evaluating the quality of individual studies

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

4.1. Evaluating the quality of epidemiologic studies

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

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

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

4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each experimental animal study, in-vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991). Research involving human subjects is considered only if conducted according to ethical principles.

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

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

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991). 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, 1998, §3.1.1.4, 1991, §3.1.2.4.5.4).

4.3. Reporting study results

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

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

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

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

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

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

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

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

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

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

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

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

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

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

followed by a reduction of an adverse effect.

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

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

5.2. Evaluating evidence in humans

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

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

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

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

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

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

5.3. Evaluating evidence in animals

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

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

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

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

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

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

In evaluating evidence of genetic toxicity:

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

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

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

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

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

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

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

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

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

aspects of causation in addressing this question.

Is the hypothesized mode of action relevant to humans? The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

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

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (U.S. EPA, 2005a, §2.4.3.3). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 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 (U.S. EPA, 2005a, §2.3.5).

5.5. Characterizing the overall weight of the evidence

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Selecting studies for derivation of toxicity values

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

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

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.
- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model

can be used to extrapolate across exposure routes.

- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.
- Studies with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with 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, the EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

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

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

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

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

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

7.2. Modeling dose to sites of biologic effects

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

Because toxicokinetic modeling can require many parameters and more data than are typically available, the EPA has developed standard approaches that can be

applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration(U.S. EPA, 2005a, §3.1.1, 1991, §3.2).

- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.

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

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

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

7.3. Modeling response in the range of observation

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

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

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

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).

- For nonlinear approaches, both statistical and biologic considerations are taken into account.

- For dichotomous data, a response level of 10% extra risk is generally used for minimally adverse effects, 5% or lower for more severe effects.
- For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for minimally adverse effects, one-half standard deviation for more severe effects.

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

7.4. Extrapolating to lower doses and response levels

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

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

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

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

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

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

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

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

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

7.5. Considering susceptible populations and lifestages

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

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

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 toxicity values for application to susceptible individuals.

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

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

7.6. Reference values and uncertainty factors

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

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

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

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

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

10 is applied (U.S. EPA, 2011, 2002, §4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4).

Adverse-effect level to no-observed-adverse-effect level. If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, §4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4).

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

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

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

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

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

7.7. Confidence and uncertainty in the reference values

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

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

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

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

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

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

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EXECUTIVE SUMMARY

Occurrence and Health Effects

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

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

Studies in multiple animal species demonstrate that benzo[a]pyrene is carcinogenic at multiple tumor sites (alimentary tract, liver, kidney, respiratory tract, pharynx, and skin) by all routes of exposure. In addition, there is strong evidence of carcinogenicity in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as aluminum production, chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, and paving and roofing with coal tar pitch. An increasing number of occupational studies demonstrate a positive exposure-response relationship with cumulative benzo[a]pyrene exposure and lung cancer.

Effects Other Than Cancer Observed Following Oral Exposure

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

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

Oral Reference Dose (RfD) for Effects Other Than Cancer

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

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

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

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

Effect	Basis	RfD (mg/kg-d)	Confidence
Developmental	Neurobehavioral changes Gavage neurodevelopmental study in rats (PND 5-11) Chen et al. (2012)	3×10^{-4}	MEDIUM
Reproductive	Decreased ovary weight Gavage subchronic (60 d) reproductive toxicity study in rats Xu et al. (2010)	4×10^{-4}	MEDIUM
Immunological	Decreased thymus weight and serum IgM Gavage subchronic (35 d) study in rats 9 De Jong et al. (1999)	2×10^{-3}	LOW
Proposed Overall RfD	**Developmental toxicity**	3×10^{-4}	**MEDIUM**

Confidence in the Overall Oral RfD

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

Effects Other Than Cancer Observed Following Inhalation Exposure

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

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

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

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

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

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

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

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

Confidence in the Overall Inhalation RfC

The overall confidence in the RfC is low-to-medium. Confidence in the principal study (Archibong et al., 2002) is medium. The conduct and reporting of this developmental inhalation study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study and the lack of information on varied toxicity endpoints following subchronic and chronic inhalation exposure. However, confidence in the RfC is bolstered by consistent systemic effects observed by the oral route (including reproductive and

developmental effects) and similar effects observed in human populations exposed to PAH mixtures.

Evidence for Human Carcinogenicity

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

Quantitative Estimate of Carcinogenic Risk From Oral Exposure

Lifetime oral exposure to benzo[a]pyrene has been associated with forestomach, liver, oral cavity, jejunum or duodenum, and auditory canal tumors in male and female Wistar rats, forestomach tumors in male and female Sprague-Dawley rats, and forestomach, esophagus, tongue, and larynx tumors in female $B6C3F_1$ mice (male mice were not tested). Less-than-lifetime oral exposure to benzo[a]pyrene has also been associated with forestomach tumors in more than 10 additional bioassays with several strains of mice. The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. These studies included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group(~50/sex/group), treated animals for up to 2 years, and included detailed reporting methods and results (including individual animal data).

Time-weighted, average daily doses were converted to human equivalent doses on the basis of (body weight)$^{3/4}$ scaling (U.S. EPA, 1992). EPA then used the multistage-Weibull model for the derivation of the oral slope factor. This model was used because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination (slope factor = $0.1/BMDL_{10}$) reported by Kroese et al. (2001) and Beland and Culp (1998). The oral slope factor of **1 per mg/kg-day** based on the tumor response in the alimentary tract (forestomach, esophagus, tongue, and larynx) of

female $B6C3F_1$ mice (Beland and Culp, 1998) was selected as the factor with the highest value (most sensitive) among a range of slope factors derived.

Quantitative Estimate of Carcinogenic Risk From Inhalation Exposure

Inhalation exposure to benzo[a]pyrene has been associated with squamous cell neoplasia in the larynx, pharynx, trachea, 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 exhibit 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 (TWA) continuous exposure concentrations and the individual animal incidence data for tumors in the larynx, pharynx, trachea, esophagus, and forestomach. The inhalation unit risk of **5×10^{-4} per µg/m³** was calculated by linear extrapolation (slope factor = $0.1/BMCL_{10}$) from a $BMCL_{10}$ of 0.20 mg/m³ for the occurrence of upper respiratory and upper digestive tract tumors in male hamsters chronically exposed by inhalation to benzo[a]pyrene (Thyssen et al., 1981).

Quantitative Estimate of Carcinogenic Risk From Dermal Exposure

Skin cancer in humans has been documented to result from occupational exposure to complex mixtures of PAHs including benzo[a]pyrene, such as coal tar, coal tar pitches, unrefined mineral oils, shale oils, and soot. In animal models, numerous dermal bioassays have demonstrated an increased incidence of skin tumors with increasing dermal exposure of benzo[a]pyrene in all species tested (mice, rabbits, rats, and guinea pigs), although most benzo[a]pyrene bioassays have been conducted in mice. Due to the evidence supporting a hazard from exposure to benzo[a]pyrene by the dermal route (see Section 1.1.5) and the availability of quantitative information, a cancer slope factor for the dermal route was developed. The analysis in this assessment focuses on 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.

Both mouse skin tumor incidence data sets were modeled using the multistage-cancer model. 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 0.0005 per μg/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 some chemical-specific data exist for benzo[a]pyrene that demonstrate increased early life susceptibility to cancer, these data were not considered sufficient to develop separate risk estimates for childhood exposure. In the absence of adequate chemical-specific data to evaluate differences in age-specific susceptibility, the *Supplemental Guidance* (U.S. EPA, 2005b) recommends that age-dependent adjustment factors (ADAFs) be applied in estimating cancer risk. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to benzo[a]pyrene.

Regarding effects other than cancer, there are epidemiological studies that report associations between developmental effects (decreased postnatal growth, decreased head circumference, and neurodevelopmental delays), reproductive effects and internal biomarkers of exposure to benzo[a]pyrene. Studies in animals also indicate alterations in neurological development and heightened susceptibility to reproductive effects following gestational or early postnatal exposure to benzo[a]pyrene.

Key Issues Addressed in Assessment

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

LITERATURE SEARCH STRATEGY|STUDY SELECTION

The literature search strategy used to identify primary, peer-reviewed literature pertaining to benzo[a]pyrene was conducted using the databases listed in Table LS-1 (see Appendix C for the complete list of keywords used). References from previous assessments by EPA and other national and international health organizations were also examined. A comprehensive literature search was last conducted in February 2012.

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

Database	Keywords
Pubmed Toxcenter Toxline	Chemical name (CASRN): benzo[a]pyrene (50-32-8)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 Standard toxicology search keywords Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors
TSCATS ChemID Chemfinder CCRIS HSDB GENETOX RTECS	Searched by CASRNs and chemical names (including synonyms)

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

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

abstracts, and/or papers was then conducted. Notable exclusions from the Toxicological Review are large numbers of animal in vivo or in vitro studies designed to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of benzo[a]pyrene and toxicity studies of benzo[a]pyrene in nonmammalian species (e.g., aquatic species, plants).

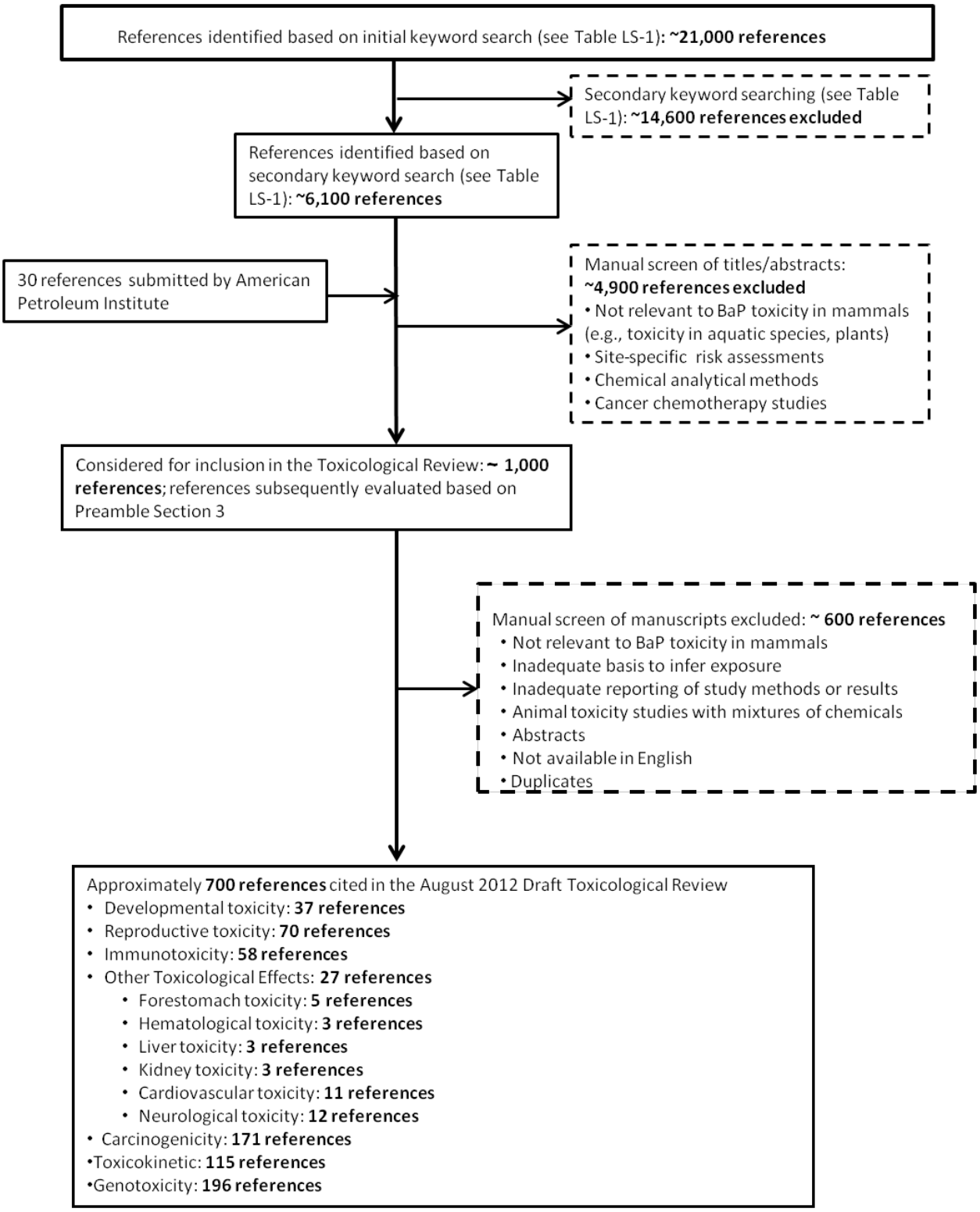

Figure LS-1. Study selection strategy.

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

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

The health effects literature for benzo[a]pyrene is extensive. All animal studies of benzo[a]pyrene involving repeated oral, inhalation, or dermal exposure that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to benzo[a]pyrene. 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] (http://hero.epa.gov/benzoapyrene).

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

1. HAZARD IDENTIFICATION

1.1. 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 routinely analyzed and detected in environmental media contaminated with PAH mixtures, thus it is often used as an indicator chemical to measure exposure to PAH mixtures (Boström et al., 2002).

1.1.1. Developmental Toxicity

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

Altered Birth Outcomes

Human and animal studies provide evidence that benzo[a]pyrene exposure may lead to altered outcomes reflecting growth and development in utero or in early childhood. Two? cohort studies in pregnant women in China and the United States examined cord blood levels of benzo[a]pyrene-7,8-diol-9,10 epoxide (BPDE)-DNA adducts in relation to measures of child growth following exposure to PAH mixtures (Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004) (Table 1-1). In the Chinese cohort, high benzo[a]pyrene-adduct levels were associated with reduced weight at 18, 24, and 30 months of age, but not at birth (Tang et al., 2006). In the U.S. cohort, an independent effect on birth weight was not observed with either benzo[a]pyrene-adducts or environmental tobacco smoke (ETS) exposure; however, a doubling of cord blood adducts in combination with ETS exposure in utero was seen, corresponding to an 8% reduction in birth weight (Perera et al., 2005b). ETS, also called secondhand smoke, is the smoke given off by a burning tobacco product and the smoke exhaled by a smoker that contains over 7,000 chemicals

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

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

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

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

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

Fertility in Offspring

Several studies suggest that gestational exposure to maternal tobacco smoke decreases the future fertility of female offspring (Ye et al., 2010; Jensen et al., 1998; Weinberg et al., 1989) (Table 1-1). In animal models, marked effects on the development of male and female reproductive organs and the fertility of animals exposed gestationally has also been demonstrated (Kristensen et al., 1995; Mackenzie and Angevine, 1981) (Table 1-2). In two studies examining reproductive effects in mice, decreased fertility and fecundity in F1 animals was observed following exposure to doses ≥10 mg/kg-day during gestation (Kristensen et al., 1995; Mackenzie and Angevine, 1981). When F1 females were mated with untreated males, a dose-related decrease in fertility of >30% was observed, in addition to a 20% decrease in litter size starting at the lowest dose tested of 10 mg/kg-day (Mackenzie and Angevine, 1981). A dose-related decrease in fertility was also observed in male mice treated gestationally with benzo[a]pyrene. At the lowest dose tested (10 mg/kg-day), a 35% decrease in fertility was observed when gestationally exposed animals were mated with untreated females (Mackenzie and Angevine, 1981). Similar effects on fertility were observed in another developmental study in mice (Kristensen et al., 1995). F1 females (bred continuously for 6 months) in this study had 63% fewer litters, and litters were 30% smaller as compared to control animals. The fertility of male offspring was not assessed in this study.

Reproductive Organ Effects in Offspring

The above mentioned studies also demonstrated dose-related effects on male and female reproductive organs in animals exposed gestationally to benzo[a]pyrene (Table 1-2). Testicular weight was decreased and atrophic seminiferous tubules and vacuolization were increased at ≥10 mg/kg-day in male mice exposed to benzo[a]pyrene gestationally from GD 7 to 16; severe atrophic seminiferous tubules were observed at 40 mg/kg-day (Mackenzie and Angevine, 1981).

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

Cardiovascular Effects in Offspring

Increased systolic and diastolic blood pressure was observed in adult animals following gestational treatment with benzo[a]pyrene (Jules et al., 2012) (Table 1-2). Approximate elevations in systolic and diastolic blood pressure of 20–30% and 50–80% were noted in the 0.6 and

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

Immune Effects in Offspring

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

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

Study Design and Reference	Results		
Tang et al. (2006) Tongliang, China	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed weight and height		
Birth cohort		Weight Beta (*p*-value)	Length (Height) Beta (*p*-value)
150 non-smoking women who delivered babies between March 2002 and June 2002	Birth	-0.007 (0.73)	-0.001 (0.89)
	18 mo	-0.048 (0.03)	-0.005 (0.48)
	24 mo	-0.041 (0.027)	-0.007 (0.28)
	30 mo	-0.040 (0.049)	-0.006 (0.44)
Exposure: mean hours per day exposed to ETS 0.42 (SD 1.19); lived within 2.5 km of power plant that operated from December 2001 to May 2002; benzo[a]pyrene-DNA adducts from maternal and cord blood samples; cord blood mean 0.33 (SD 0.14) (median 0.36) adducts/10^{-8} nucleotides; maternal blood mean 0.29 (SD 0.13) adducts/10^{-8} nucleotides	Adjusted for ETS, sex of child, maternal height, maternal weight, and gestational age (for measures at birth)		
Perera et al. (2005b); Perera et al. (2004) New York, United States	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed weight and length		

Study Design and Reference	Results		
		Weight Beta (*p*-value)	Length Beta (*p*-value)
	Interaction term	-0.088 (0.05)	-0.014 (0.39)
	Benzo[a]-pyrene-DNA adducts	-0.020 (0.49)	-0.005 (0.64)
	ETS in home	-0.003 (0.90)	-0.007 (0.32)
	Adjusted for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age		
Wu et al. (2010) Tianjin, China Case control study: 81 cases (96% participation rate)—fetal death confirmed by ultrasound before 14 wks gestation; 81 controls (91% participation rate)—elective abortions; matched by age, gestational age, and gravidity; excluded smokers and occupational PAH exposure Exposure: benzo[a]pyrene in aborted tissue and maternal blood samples (51 cases and controls, 2 of 4 hospitals)	*Benzo[a]pyrene adduct levels (/10^8 nucleotides), mean (±SD)*		
		Cases	Controls	(*p*-value)
	Maternal blood	6.0 (± 4.7)	2.7 (± 2.2)	(<0.001)
	Aborted tissue	4.8 (± 6.0)	6.0 (± 7.4)	(0.29)
	Low correlation between blood and tissue levels (r = -0.02 in cases, r = -0.21 in controls)			
	Association between benzo[a]pyrene adducts and miscarriage[a]			
			OR	(95% CI)
	Per unit increase in adducts		1.37	(1.12, 1.67)
	Dichotomized at median		4.56	(1.46, 14.3)
	[a]Conditional logistic regression, adjusted for maternal education, household income, and gestational age; age also considered as potential confounder			

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

Study Design and Reference	Results
Birth outcomes and postnatal growth	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/ dose 0, 10, 40, or 160 mg/kg-d by gavage GDs 7–16	↓ number of F0 females with viable litters: 46/60, 21/30, 44/60, and 13/30* ↓ F1 body weight at PND 20 % change from control: 0, 4, -7*, and -13* ↓ F1 body weight at PND 42 % change from control: 0, -6*, -6*, and -10* (no difference in pup weight at PND 4)
Kristensen et al. (1995) NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GDs 7–16	Exposed F0 females showed no gross signs of toxicity and no effects on fertility (data not reported)

Study Design and Reference	Results
Jules et al. (2012) Long-Evans rats, 6–17 F0 females/dose 0, 0.15, 0.3, 0.6, or 1.2 mg/kg-d by gavage GDs 14–17	No overt signs of toxicity in dams or offspring, differences in pup body weight, or number of pups/litter
McCallister et al. (2008) Long-Evans Hooded rats, 5–6/group 0 or 0.3 mg/kg-d by gavage GDs 14–17	No difference in number of pups/litter No overt maternal or pup toxicity No difference in liver:body weight Increased brain:body weight ratio at PNDs 15 and 30 (data not shown)
Brown et al. (2007) Long-Evans Hooded rats, 6/group 0, 0.025, or 0.15 mg/kg-d by gavage GDs 14–17	No difference in number of pups/litter or overt maternal or pup toxicity
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Statistically significant decrease in pup body weight (approximate 10–15% decrease) at 2 mg/kg-d measured on PND 36 and 71 (no significant alteration of pup weight during treatment period) No differences among treatment groups in developmental milestones: incisor eruption, eye opening, development of fur, testis decent, or vaginal opening
Archibong et al. (2002) F344 rats, 10 females/group 0, 25, 75, or 100 µg/m^3 nose-only inhalation for 4 hrs/d GDs 11–20	↓ fetal survival ([pups/litter]/[implantation sites/litter] × 100) % fetal survival: 97, 78*, 38*, and 34*%

Study Design and Reference	Results
Reproductive effects in offspring	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/ dose 0, 10, 40, or 160 mg/kg-d by gavage GDs 7–16	↓ number of F1 females with viable litters: 35/35, 23/35*, 0/55*, and 0/20* ↓F1 female fertility index (females pregnant/females exposed to males × 100): 100 , 66*, 0*, and 0* ↓ F1 male fertility index (females pregnant/females exposed to males × 100): 80, 52*, 5*, and 0* ↓ F2 litter size from F1 dams (20%) at 10 mg/kg-d (no litters were produced at high doses) ↓ size or absence of F1 ovaries (weights not collected) hypoplastic ovaries with few or no follicles and corpora lutea (numerical data not reported) ↓ testicular weight in F1 offspring % change from control: 0, -42, -82, and ND (statistical significance not reported) ↑ atrophic seminiferous tubules and vacuolization at ≥10 mg/kg-d; severe atrophic seminiferous tubules at 40 mg/kg-d (numerical data not reported)
Kristensen et al. (1995) NMRI mice, 9 F0 females/dose 0 or 10 mg/kg-d by gavage GDs 7–16	↓ number of F2 litters (-63%) ↓ F2 litter size (-30%) ↓ ovary weight (-31%) in F1 females Few or no small, medium, or large follicles and corpora lutea
Cardiovascular effects in offspring	
Jules et al. (2012) Long-Evans rats, 6–17 F0 females/dose 0, 0.15, 0.3, 0.6, or 1.2 mg/kg-d by gavage GDs 14–17	↑ systolic blood pressure (measured at PND 53) 15%* increase at 0.6 mg/kg-d 52%* increase at 1.2 mg/kg-d (other dose groups not reported) ↑ diastolic blood pressure (measured at PND 53) 33%* increase at 0.6 mg/kg-d 83% *increase at 1.2 mg/kg-d (other dose groups not reported) Altered heart rate 10% * increase at 0.6 mg/kg-day 8%* decrease at 1.2 mg/kg-day

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

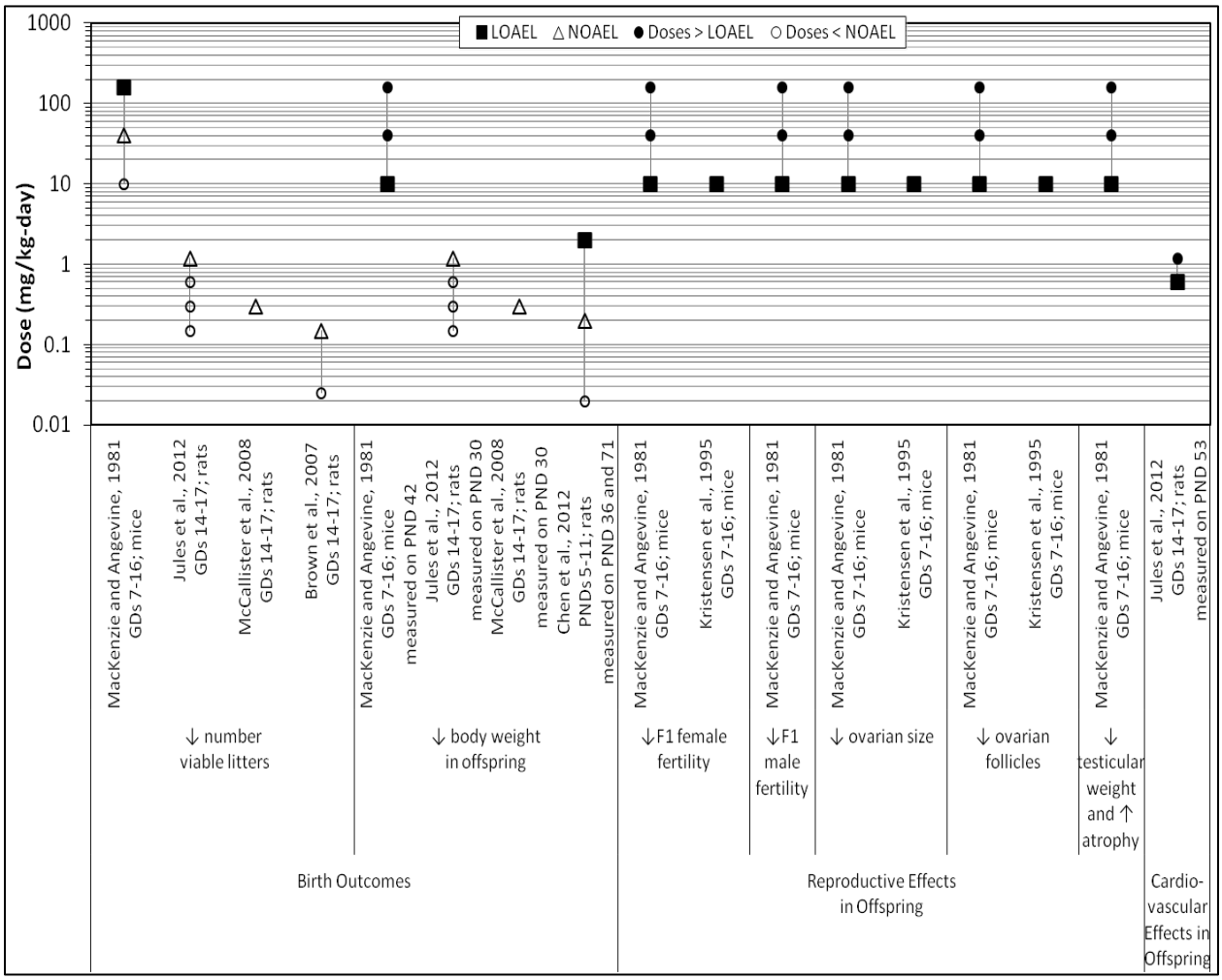

Figure 1-1. Exposure-response array for developmental effects following oral exposure to benzo[a]pyrene.

Neurodevelopmental Effects

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

The mammalian brain undergoes 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, 1979, 1973) and the first 1–2 weeks of life in the rat and mouse neonate (Chen et al., 2011). 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 lactational exposure to benzo[a]pyrene (Bouayed et al., 2009a; McCallister et al., 2008; Wormley et al., 2004). These effects are described below.

Cognitive function

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

Animal studies have also provided evidence of altered learning and memory behaviors following lactational or direct postnatal exposure to benzo[a]pyrene (Chen et al., 2012; Bouayed et al., 2009a) (Table 1-4). In mice, spatial working memory was measured using the Y-maze spontaneous alternation test (Bouayed et al., 2009a). This test records alternations between arm entries in a Y-shaped maze as a measure of memory, as rodents typically prefer to investigate a new arm of the maze. To a lesser extent, this test can also reflect changes in sensory processing, novelty preference, and anxiety-related responses in rodents. An improvement in working memory was evident in mice, as exhibited by significant increases in spontaneous alternations in the Y-maze test in mice on PND 40 following lactational exposure to 2 mg/kg-day benzo[a]pyrene (but not 20 mg/kg-day) from PND 0 to 14 (Bouayed et al., 2009a). The total number of arm entries in the Y-maze was unaffected by lactational exposure, suggesting that changes in motor function were not driving this response. 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(time to find the hidden platform), as well as decreased time in the target quadrant and decreased number of platform crossings during a probe trial with the platform removed were observed in PND 39–40 rats following postnatal exposure to 2 mg/kg-day benzo[a]pyrene (Chen et al., 2012). These effects were more pronounced in animals tested at PNDs 74–75. No difference in swim speed was observed between treatment groups, suggesting the observed changes are not attributable to general motor impairment. These observations may indicate primary effects of benzo[a]pyrene on learning and/ or memory processes; however, the presented data were insufficient to attribute these findings to learning and memory processes alone. Specifically, visual examinations of the improvements in escape latency (slopes) over the four learning trial days were not noticeably affected by treatment dose, suggesting

that all groups learned at a similar rate. As 4 trials/ day were averaged for each animal at each trial day, it is unclear whether the dose-related increases in escape latency already observable at trial day 1 reflect effects on learning or other effects (e.g., altered anxiety or vision responses). Additionally, as it is not clear that the groups learned to a comparable extent in hidden platform tests, the results of the probe trial cannot be conclusively attributed to memory retention and likely involve other contributing factors.

Neuromuscular function, coordination, and sensorimotor development

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

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

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 (e.g. PNDs 3–12) and successful acquisition of these phenotypes is indicated when righting occurs. In rats, both phenotypes are nearly always established before PND 12. Chen et al. (2012) performed these tests as quantitative measures of sensorimotor function at PND 12 and beyond, with control animals already able to right within 0.8–1.8 seconds and orient 180° within 5–9 seconds. Although informative in terms of possible

delays in sensory motor development, the sensitivity of these measures at these later postnatal ages is difficult to interpret. 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 sometimes exhibit differences in the maturation of these developmental landmarks following challenge) were pooled for these measures.

Anxiety and activity

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 birth cohort study conducted in New York City (Perera et al., 2012b). The associations were stronger using the measures in cord blood compared with maternal samples, with indications of a fourfold increased risk (p = 0.051) of attention problems associated with cord blood adduct levels above the median compared with 2-fold increased risk associated with maternal blood adduct levels (Table 1-3). Exposure was treated as a dichotomy (i.e., detectable compared with non-detectable levels) in these analyses.

Decreased anxiety-like behavior was reported in both rat and mice following postnatal oral exposure to benzo[a]pyrene (Chen et al., 2012; Bouayed et al., 2009a) (Table 1-4). Anxiety-like behaviors were measured in both species using an elevated plus maze, where an increase in the time spent in the closed arms of the maze is considered evidence of anxious behavior. In mice, significant increases in the entries and time spent in open arms of the maze, as well as significantly decreased entries into closed arms of the maze (in the 2 mg/kg-day group), were observed on PND 32 following lactational exposure to ≥2 mg/kg-day benzo[a]pyrene (Bouayed et al., 2009a). The mice also exhibited decreased latency of the first entry into an open arm following lactational exposure to 20 mg/kg-day benzo[a]pyrene. There was no exposure-related effect on the total number of times the mice entered arms of the maze, indicating the lack of an effect on general locomotor activity. Decreased anxiety-like behavior was also reported in rats following oral benzo[a]pyrene exposure from PND 5 to 11, although sex-specific differences were observed (Chen et al., 2012). In females, postnatal exposure to ≥0.2 mg/kg-day benzo[a]pyrene was associated with a significant increase in the number of open arm entries and 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 needed to detect these responses were higher than females (i.e., increases at ≥2 mg/kg-day for open arm entries and ≥0.2 mg/kg-day for time spent in open arms). A significant decrease in latency to enter an open arm of the maze was observed in both male and female rat pups exposed to 2 mg/kg-day benzo[a]pyrene.

In contrast to results from the elevated plus maze, no altered reactions were observed in cliff aversion test paradigms (testing stress responses and integration of sensorimotor processes)

following postnatal oral exposure to benzo[a]pyrene (Chen et al., 2012; Bouayed et al., 2009a). Increased spontaneous locomotor activity in the open field on PND 69 has been reported in rats postnatally exposed to ≥0.2 mg/kg-day (Chen et al., 2012). Elevated activity in an open field is attributable primarily to either increased motor activity or decreased anxiety-like behavior, although the relative contributions of these two components could not be separated.

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), but not in mice exposed lactationally to doses up 20 mg/kg-day and tested on PND 15 (Bouayed et al., 2009a).

Electrophysiological changes

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

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

Reference and Study Design	Results		
Tang et al. (2008); Tang et al. (2006) Tongliang, China Birth cohort 150 non-smoking women, delivered March 2002–June 2002; lived within 2.5 km of power plant that operated from December 2001 to May 2002 Outcomes: head circumference at birth; Gesell Developmental Schedule, administered by physicians at 2 years of age (4 domains: motor, adaptive, language, and social); standardized mean score = 100 ± SD 15 (score < 85 = developmental delay) Exposure: mean hours per day exposed to ETS 0.42 (SD 1.19); lived within 2.5 km of power plant that operated from December 2001 to May 2002; benzo[a]pyrene-DNA adducts from maternal and cord blood samples; cord blood mean 0.33 (SD 0.14) (median 0.36) adducts/10^{-8} nucleotides; maternal blood mean 0.29 (SD 0.13) adducts/10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts and log-transformed head circumference		
		Beta (*p*-value)	
	Birth	-0.011 (0.057)	
	18 mo	-0.012 (0.085)	
	24 mo	-0.006 (0.19)	
	30 mo	-0.005 (0.31)	
	High versus low, dichotomized at median, adjusted for ETS, sex of child, maternal height, maternal weight, Cesarean section delivery, maternal head circumference, and gestational age (for measures at birth)		
Tang et al. (2008); Tang et al. (2006) (See above for population and exposure details); n = 110 for Developmental Quotient analysis. No differences between the 110 participants in this analysis and the nonparticipants with respect to maternal age, gestational age, birth weight, birth length, or birth head circumference. Higher maternal education (direction not reported, p = 0.056) Outcomes: Gesell Developmental Schedule, administered by physicians at 2 years of age (4 domains: motor, adaptive, language, and social); standardized mean score = 100 ± SD 15 (score < 85 = developmental delay)	Association between benzo[a]pyrene 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 benzo[a]pyrene adducts [b]Logistic regression of risk of developmental delay (defined as normalized score < 85) per 1 unit (0.1 adducts/10^{-8} nucleotides) increase in adducts Both analyses adjusted for sex, gestational age, maternal education, ETS, and cord lead levels CI = confidence level; OR = odds ratio		
Perera et al. (2012a); Tang et al. (2008); Tang et al. (2006) (See above for population and exposure details); 132 (83%) followed through age 5; 100 of these had complete data for	Environmental tobacco smoke (ETS) measure not correlated with benzo[a]pyrene adduct measures (i.e., absolute value of Spearman r < 0.10) Relation between cord blood benzo[a]pyrene-DNA adducts, ETS exposure, and IQ measures		

Reference and Study Design	Results		
analysis. No differences between the 100 participants in this analysis and the nonparticipants with respect to adduct levels, environmental tobacco smoke exposure, IQ measures, maternal age, gestational age, or infant gender. Higher maternal education (60% and 35% with ≥ high school, respectively, in participants and non-participants, $p < 0.05$) Outcomes: Wechsler Preschool and Primary Intelligence Quotient scale (Shanghai version)	Beta (95% CI)		
		Main Effect	With ETS Interaction term
	Full scale	-2.42 (-7.96, 3.13)	-10.10 (-18.90, -1.29)
	Verbal	-1.79 (-7.61, 4.03)	-10.35 (-19.61, -1.10)
	Performance	-2.57 (-8.92, 3.79)	-7.78 (-18.03,2.48)
	Beta per 1 unit increase in log-transformed cord adducts, adjusted for ETS exposure, gestational age, maternal education, cord lead, maternal age, and gender		
Perera et al. (2012b); Perera et al. (2005b); Perera et al. (2004) United States, New York Birth cohort 265 pregnant women : African-American and Dominican non-smoking women that delivered babies between April 1998 – October 2002 (253 and 207 for behavior and head circumference analysis, respectively); approximately 40% with smoker in the home Outcomes: head circumference at birth Exposure: benzo[a]pyrene-DNA adducts from maternal and cord blood samples; mean 0.22 (SD 0.14) adducts/10^{-8} nucleotides; median of detectable values 0.36 adducts/10^{-8} nucleotides	Relation between cord blood benzo[a]pyrene-DNA adducts, environmental tobacco smoke exposure (ETS), and log-transformed head circumference		
			Beta (*p*-value)
	Interaction term		-0.032 (0.01)
	benzo[a]pyrene-DNA adducts		-0.007 (0.39)
	ETS in home		-0.005 (0.43)
	High versus low, dichotomized at 0.36 adducts/10^{-8} nucleotides, adjusted for ethnicity, sex of newborns, maternal body mass index, dietary PAHs, and gestational age		
Perera et al. (2012b); n = 215 with outcome data and no missing covariate data). No differences between the participants in this analysis and the nonparticipants with respect to adduct levels, environmental tobacco smoke exposure, maternal age, gestational age, and socioeconomic variables; participants more likely to be female and African-American Outcomes: Child Behavior Checklist (118 items), completed by mothers for children ages 6–7 yrs. Two domains: anxious/ depression, attention problems (normalized T-score ≤65 = borderline or clinical syndrome); also used for scales of anxiety problems and attention deficit hyperactivity problems based on DSM classification	Logistic regression of risk of borderline or clinical status in relation to detectable levels of benzo[a]pyrene adducts		
		Maternal	Cord blood
	Prevalence	OR (95% CI)	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 ETS, ethnicity, age, heating season, prenatal demoralization, and HOME inventory		

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

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

Reference and Study Design	Results[a]
Cognitive function	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Hidden Platform test in Morris water maze (day 4 of testing): PND 39: significant increase in escape latency at 2 mg/kg-d only PND 74: significant increase in escape latency at ≥0.2 mg/kg-d Increases in latency were observed in trials 1-3 (PND 36-38 or PND 71-73): ~30^{+}% greater than controls at 2 mg/kg-day (note: all experimental groups exhibited similar decreases in escape latency-slopes were visually equivalent, across the four trial days) Probe test in the Morris water maze (day 5): Time spent in the target quadrant: PND 40: significant decrease at 2 mg/kg-d only PND 75: significant decrease at ≥0.2 mg/kg-d Number of platform crossings: PND 40: significant decrease at 2 mg/kg-d only PND 75: significant decrease at ≥0.2 mg/kg-d (in females) and 2 mg/kg-d (in males)
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d maternal gavage PNDs 0–14 (lactational exposure)	Significant increase in the percent of spontaneous alternations in the Y-maze alternation test at 2 mg/kg-d but not at 20 mg/kg-d No effect on the total number of arm entries in the Y-maze alternation test
Neuromuscular function, coordination, and sensorimotor development	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	Latency in the surface righting reflex test PND 12: significant increase at 0.2 mg/kg-d only PND 14: significant increase at 0.02 and 2 mg/kg-d only PND 16: significant difference at 2 mg/kg-d only PND 18: no significant difference Latency in the negative geotaxis test PND 12: significant increase at all doses PND 14: significant increase at 2 mg/kg-d only PNDs 16 and 18: no significant difference No effect on duration of forelimb grip in forelimb grip strength test

Reference and Study Design	Results[a]
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d maternal gavage PNDs 0–14 (lactational exposure)	Significant increase in righting time in the surface righting reflex test at both doses on PNDs 3 and 5 (but not PNDs 7 and 9) Significant increase in latency in the negative geotaxis time for 20 mg/kg-d dose group at PNDs 5, 7, and 9 (no significant difference at PND 11) Significant increase in duration of forelimb grip in forelimb grip strength test at both dose groups on PND 9 (statistically significant at PND 11 only at high dose) Significant increase in pole grasping latency in male pups in the water escape pole climbing test at 20 mg/kg-d No effect on climbing time in the water escape pole climbing test Significant increase in pole escape latency in the water escape pole climbing test in male rats at 20 mg/kg-d
Anxiety and/or motor activity	
Chen et al. (2012) Sprague-Dawley rats, 20 pups (10 male and 10 female)/group 0, 0.02, 0.2, or 2 mg/kg-d by gavage PNDs 5–11	*Elevated plus maze* Significant increase in the number of entries into open arms at PND 70 at ≥0.2 mg/kg-d (in females) and 2 mg/kg-d (in males) (no difference at PND 35) Significant decrease in the number of entries into closed arms at PND 70 at ≥0.2 mg/kg-d (in females) and 2 mg/kg-d (in males) (no difference at PND 35) Significant increase in the time spent in open arms at PND 35 at 2 mg/kg-d in females and at PND 70 at doses ≥0.02 mg/kg-d in females and ≥0.2 mg/kg-d in males Significant decrease in latency time to first enter an open arm on PND 70 at ≥0.2 mg/kg-d (no difference at PND 35) Open field test Significant increase in the number of squares: PND 34- significant increase at 2 mg/kg-d; PND 69; significant increase at ≥0.02 mg/kg-d (no difference at PNDs 18 and 20) Significant increase in rearing activity at 0.2 mg/kg-d on PND 69 (no difference at PNDs 18, 20, and 34) Cliff aversion test No effect on the latency to retract from the edge
Bouayed et al. (2009a) Female Swiss albino mice, 5/group 0, 2, or 20 mg/kg-d maternal gavage PNDs 0–14 (lactational exposure)	*Elevated plus maze* Significantly increased time in open arms at ≥2 mg/kg-d Significantly increased percentage of entries into open arms at ≥2 mg/kg-d Significantly decreased entries into closed at 2 mg/kg-d, but not at 20

Reference and Study Design	Results[a]
	mg/kg-d Significantly decreased latency time to enter an open arm at 20 mg/kg-d No effect on the total number of arm entries Open field test No significant change in activity on PND 15
Electrophysiological changes	
McCallister et al. (2008) Long-Evans Hooded rats, 5–6/group 0 or 0.3 mg/kg-d by gavage GDs 14–17	Statistically significant decreases in stimulus-evoked cortical neuronal activity on PNDs 90–120 Reduction in the number of spikes in both the short and long latency periods on PNDs 90–120 (numerical data not presented)
Wormley et al. (2004) F344 rats, 10 females/group 0 or 100 µg/m^3 nose-only inhalation for 4 hrs/d GDs 11–21	Electrophysiological changes in the hippocampus: Consistently lower long term potentiation following gestational exposure (statistical analysis not reported) % change relative to control: -26%

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

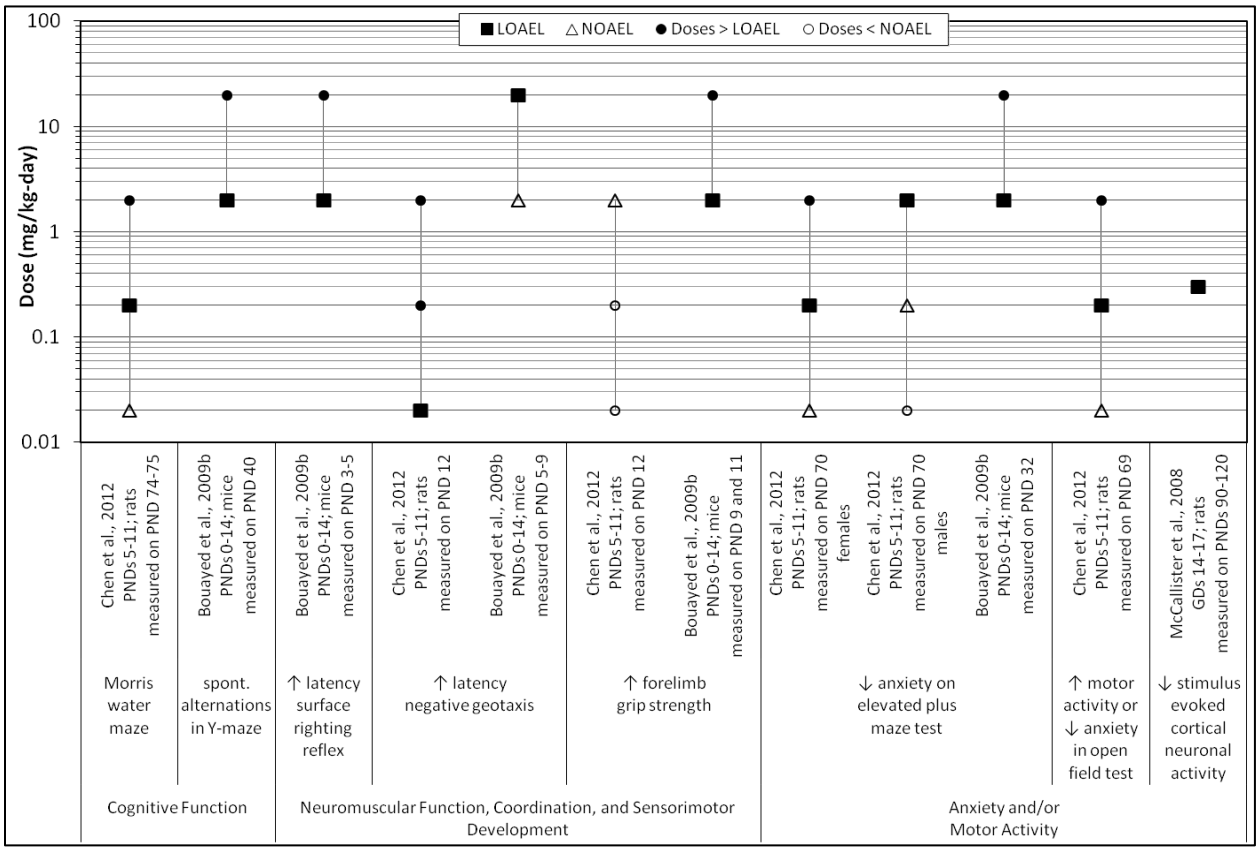

Figure 1-2. Exposure-response array for neurodevelopmental effects following oral exposure.

Mode of Action Analysis- Developmental Toxicity

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

It is plausible that developmental effects of benzo[a]pyrene may be mediated by altered cell signaling through the Ah receptor (AhR). Benzo[a]pyrene is a ligand for the AhR and activation of this receptor regulates downstream gene expression including the induction of CYP enzymes important in the conversion of benzo[a]pyrene into reactive metabolites. Studies in AhR knock-out mice indicate that AhR signaling during embryogenesis is essential for normal liver, kidney, vascular, hematopoietic, and immune development (Schmidt et al., 1996; Fernandez-Salguero et al., 1995). In experiments in AhR responsive and less-responsive mice, the mice with the less responsive AhR were protected from renal injury as adults following gavage treatment with 0.1 or

0.5 mg/kg-day benzo[a]pyrene from GD 10–13. Renal injury was indicated by an increase in urinary albumin and a decrease in glomerular number (Nanez et al., 2011).

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

No clear mode(s) of action for the observed neurodevelopmental and neurobehavioral changes following benzo[a]pyrene exposure have been demonstrated. General hypothesized mechanisms with limited evidentiary support are related to altered CNS neurotransmission. These mechanisms involve altered neurotransmitter gene expression, and neurotransmitter levels, in regions associated with spatial learning, anxiety, and aggression, such as the hippocampus, striatum, and hypothalamus (Li et al., 2012; Qiu et al., 2011; Tang et al., 2011; Xia et al., 2011; Bouayed et al., 2009a; Grova et al., 2008; Brown et al., 2007; Grova et al., 2007; Stephanou et al., 1998). Specifically, benzo[a]pyrene exposure caused changes associated with potential modifications to the dopaminergic and serotonergic systems (Bouayed et al., 2009a; Stephanou et al., 1998), as well as NMDA receptor signaling (Qiu et al., 2011). Increased oxidative stress in these same regions has also been proposed as a mechanism (Saunders et al., 2006).

Summary of Developmental Effects

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

The available human and animal data also support the conclusion that benzo[a]pyrene is a developmental neurotoxicant. Human studies of environmental PAH exposure in two cohorts have observed neurotoxic effects, including suggestions of reduced head circumference (Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004), impaired cognitive ability (Perera et al., 2009; Tang et al., 2008), impaired neuromuscular function (Tang et al., 2008), and increased attention problems and anxious/depressed behavior following prenatal exposure (Perera et al., 2012b). These effects were seen in birth cohort studies in different populations (New York City and China),

in studies using specific benzo[a]pyrene measures (i.e., adduct levels measured in cord blood samples) (Perera et al., 2012b; Tang et al., 2008; Tang et al., 2006; Perera et al., 2005b; Perera et al., 2004). This type of measure covers a relevant time window of exposure with respect to gestational development. The analytical method was the same 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; 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 neonatal toxicity (Chen et al., 2012; Bouayed et al., 2009a).

In conclusion, the available human and animal data suggest that developmental toxicity and developmental neurotoxicity are hazards of benzo[a]pyrene exposure.

Susceptible Populations and Lifestages

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

Studies in humans and experimental animals indicate that exposure to PAHs in general, and benzo[a]pyrene in particular, may impact neurological development. Observational studies in humans have suggested associations between gestational exposure to PAHs and later measures of neurodevelopment (Perera et al., 2009; Tang et al., 2008). In the Perera et al. (2009) study, the exposure measures are based on a composite of 8 PAHs measured in air. In Tang et al., (2008), increased levels of benzo[a]pyrene-DNA adducts in cord blood were associated with decreased developmental quotients in offspring (Tang et al., 2008).

Evidence in animals of the effects of benzo[a]pyrene on neurological development includes: (1) 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 GDs 11–21 (Wormley

et al., 2004; Wu et al., 2003a); (2) decreased evoked response in the field cortex and decreased cerebrocortical levels of messenger RNA for the N-methyl-D-aspartate receptor subunit in offspring of Long-Evans rats exposed to 300 μg/kg on GDs 14–17 (McCallister et al., 2008); and (3) decreased righting reflex and altered anxiety-like behavior in offspring of lactating rats exposed to oral doses of 2 or 20 mg/kg-day on PNDs 1–14 (Bouayed et al., 2009a).

1.1.2. Reproductive Toxicity

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

Male Reproductive Effects

Fertility

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

Sperm parameters

Effects on semen quality have been demonstrated in populations exposed to mixtures of PAHs including coke oven workers and smokers (Soares and Melo, 2008; Hsu et al., 2006). Coke oven workers had higher frequency of oligospermia (19 versus 0% in controls) and twice the number of morphologically abnormal sperm (Hsu et al., 2006). Elevated levels of BPDE-DNA adducts have been measured in the sperm of populations exposed to PAHs occupationally (Gaspari et al., 2003) and through cigarette smoke (Phillips, 2002; Zenzes et al., 1999). A higher concentration of BPDE-DNA adducts was observed in sperm not selected for intrauterine insemination or in vitro fertilization based on motility and morphology in patients of fertility clinics

(Perrin et al., 2011b; Perrin et al., 2011a). An association between benzo[a]pyrene exposure levels and increased sperm DNA fragmentation using the sperm chromatin structure assay was observed by Rubes et al. (2010). However, it is currently unclear whether the sperm chromatin structure assay, which measures sperm fragmentation following denaturation, is predictive of fertility (Sakkas and Alvarez, 2010; ASRM, 2008).

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

Decreases in epididymal sperm counts (25–50% compared to controls) have been reported in Sprague-Dawley rats and C57BL6 mice treated with 1–5 mg/kg-day benzo[a]pyrene by oral exposure for 42 or 90 days (Chen et al., 2011; Mohamed et al., 2010). 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 because the authors dosed the animals with 0.001, 0.01, and 0.1 mg/kg-day benzo[a]pyrene, but only reported on sperm parameters at the mid-dose, and no other available studies demonstrated findings in the range of the mid- and high-dose. In rats, an oral short-term study and a subchronic inhalation study lend support for the endpoint of decreased sperm count (Arafa et al., 2009; Archibong et al., 2008; Ramesh et al., 2008). Significantly decreased sperm count and daily sperm production (~40% decrease from control in each parameter) were observed following 10 days of gavage exposure to 50 mg/kg-day benzo[a]pyrene in rats (Arafa et al., 2009). In addition, a 69% decrease from controls in sperm count was observed in rats following inhalation exposure to 75 μg/m^3 benzo[a]pyrene for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

Both oral and inhalation exposure of rodents to benzo[a]pyrene have been shown to lead to decreased epididymal sperm motility and altered morphology. Decreased motility of 20–30% compared to controls was observed in C57BL6 mice (≥1 mg/kg-day) and Sprague-Dawley rats (0.01 mg/kg-day) (Chung et al., 2011; Mohamed et al., 2010). The effective doses spanned two orders of magnitude; however, as noted above, reporting is limited in the study that observed effects at 0.01 mg/kg-day benzo[a]pyrene (Chung et al., 2011). A short-term oral study in rats also reported a significantly decreased number of motile sperm (~40% decrease) following 10 days of gavage exposure to 50 mg/kg-day benzo[a]pyrene (Arafa et al., 2009). In addition, decreased sperm motility was observed following inhalation exposure to 75 μg/m^3 benzo[a]pyrene in rats for 60 days (Archibong et al., 2008; Ramesh et al., 2008) and to ≥75 μg/m^3 for 10 days (Inyang et al., 2003). Abnormal sperm morphology was observed in Sprague-Dawley rats treated with 5 mg/kg-day benzo[a]pyrene by gavage for 84 days (Chen et al., 2011) and in rats exposed to 75 μg/m^3 benzo[a]pyrene by inhalation for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

Testicular changes

Several studies have demonstrated dose-related effects on male reproductive organs in adult animals exposed subchronically to benzo[a]pyrene (Table 1-5). Decreases in testicular weight of approximately 35% have been observed in a 10-day gavage study in adult Swiss albino 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 50 mg/kg-day (Kroese et al., 2001) F344 rats exposed for 90 days to dietary doses up to 100 mg/kg-day (Knuckles et al., 2001); or Sprague-Dawley rats exposed for 90 days to gavage doses up to 0.1 mg/kg-day (Chung et al., 2011). Strain differences may have contributed to differences in response, however, F344 rats exposed to benzo[a]pyrene via inhalation showed effects on testicular weight (Archibong et al., 2008; Ramesh et al., 2008). In addition, decreased testicular weight has also been observed in offspring following in utero exposure to benzo[a]pyrene as discussed in Section 1.1.1.

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

Epididymal changes

In addition to testicular effects, histological effects in the epididymis have been observed following 90-day gavage exposure to benzo[a]pyrene (Chung et al., 2011) (Table 1-5). 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., 2011).

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 approximately 40% in intratesticular testosterone and 70% in serum testosterone 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 (Chung et al., 2011) and in F344 rats following inhalation exposure to 75 μg/m^3 benzo[a]pyrene for 60 days (Archibong et al., 2008; Ramesh et al., 2008).

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

Reference and Study Design	Results
Sperm quality	
Mohamed et al. (2010) C57BL/6 mice, 10 males/dose (treated before mating with unexposed females) 0, 1, or 10 mg/kg-d, daily gavage (F0 males only) 42 d	↓ epididymal sperm count in F0 mice Approximate % change from control: 0, -50*, and -70* (data reported graphically) ↓ epididymal sperm motility in F0 mice Approximate % change from control: 0, -20*, and -50* (data reported graphically) ↓ epididymal sperm count in untreated F1 and F2 generations (data reported graphically) No effects were observed in the F3 generation
Chen et al. (2011) Sprague-Dawley rats, 10 males/dose 0 or 5 mg/kg-d by gavage 84 d	↓ epididymal sperm count (% change from control) 0 and -29* ↑ % abnormal epididymal sperm 5 and 8*
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↓ epididymal sperm motility (% change relative to control; reported only for 0.01 mg/kg-d) 0 and -30* No statistically significant decrease in epididymal sperm count

Reference and Study Design	Results
Archibong et al. (2008); Ramesh et al. (2008) F344 rats, 10 males/group 0 or 75 µg/m^3, 4 hrs/d by inhalation 60 d	↓ epididymal sperm motility (% change from control) 0 and -73* ↓ epididymal sperm count (% change from control) 0 and -69* ↑ % abnormal epididymal sperm 33 and 87* ↓ spermatids/g testis (approximate % change from control; numerical data not reported) 0 and -45*
Testicular changes (weight, histology)	
Mohamed et al. (2010) C57BL/6 mice, 10 males/dose (treated before mating with unexposed females) 0, 1, or 10 mg/kg-d, daily gavage (F0 males only) 42 d	↓ seminiferous tubules with elongated spermatids (approximate % change from control; numerical data not reported) 0, -20*, and -35* No statistically significant change in area of seminiferous epithelium of testis (approximate % change from control; numerical data not reported) 0, 5, and 20
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↑ number of apoptotic germ cells per tubule (TUNEL or caspase 3 positive) No change in testis weight or histology
Chen et al. (2011) Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-d by gavage 84 d	↑ testicular lesions characterized as irregular arrangement of germ cells and absence of spermatocytes (numerical data not reported) No change in testis weight
Archibong et al. (2008); Ramesh et al. (2008) F344 rats, 10 adult males/group 0 or 75 µg/m^3, 4 hrs/d by inhalation 60 d	↓ decreased testis weight (% change from control) 0 and 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-d by gavage, 5d/wk 35 d	No change in testis weight
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d in diet 90 d	No change in testis weight

Reference and Study Design	Results
Epididymal changes (weight, histology)	
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, or 0.1 mg/kg-d by gavage 90 d	↓ diameter of caput epididymal tubule (n = 5; numerical data not reported) ↓ diameter of cauda epididymal tubule (n = 5; numerical data not reported)
Chen et al. (2011) Sprague-Dawley rats, 10/dose 0 or 5 mg/kg-d by gavage 84 d	No change in epididymis weight
Hormone changes	
Chung et al. (2011) Sprague-Dawley rats, 20–25 males/dose 0, 0.001, 0.01, 0.1 mg/kg-d by gavage 90 d	↓ Intratesticular testosterone (approximate % change from control; data reported graphically) 0, -12, -25, and -40* ↓ Serum testosterone (approximate % change from control ; numerical data not reported) 0, 0, -35, and -70* ↑ serum LH (approximate % change from control; numerical data not reported) 0, 33, 67*, and 87* ↓ 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-d by gavage 90 d	↓ Intratesticular testosterone (approximate % change from control; numerical data not reported) 0, -15, and -15*
Archibong et al. (2008); Ramesh et al. (2008) F344 rats, 10 adult males/group 0 or 75 µg/m^3, 4 hrs/d by inhalation 60 d	↓ intratesticular testosterone (approximate % change from control; numerical data not reported) 0 and -80* ↓ serum testosterone (approximate % change from control) 0 and -60* ↑ serum LH (approximate % change from control) 0 and 50*

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

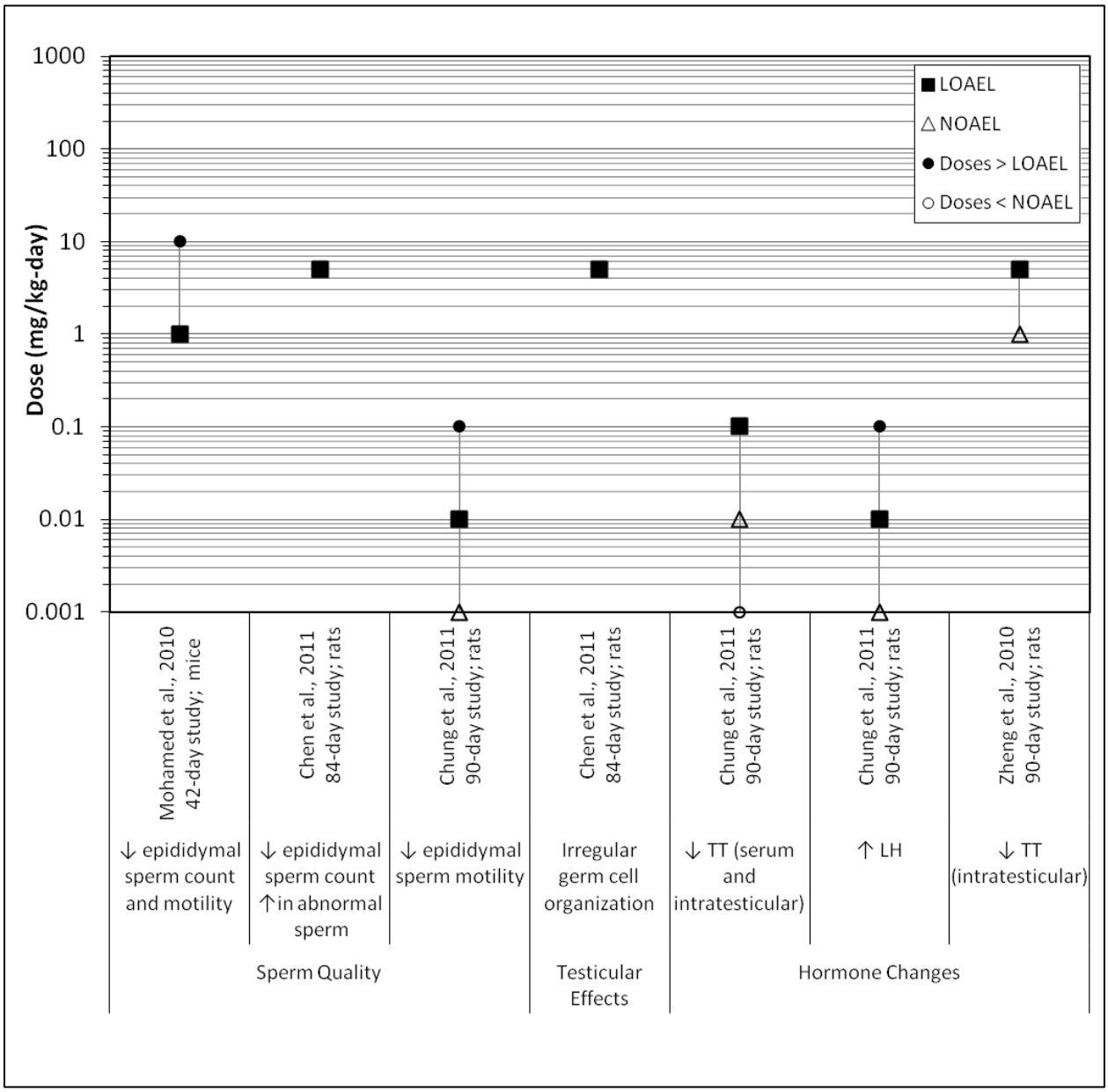

Figure 1-3. Exposure-response array for male reproductive effects following oral exposure in adult animals.

Mode of action analysis—male reproductive effects

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

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

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

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

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

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

Female Reproductive Effects

Fertility

In women, exposure to cigarette smoke has been shown to affect fertility, including effects related to pregnancy, ovulatory disorders, and spontaneous abortion (as reviewed in Waylen et al., 2009; Cooper and Moley, 2008; Soares and Melo, 2008). In addition, several studies suggest that in utero exposure to maternal tobacco smoke also decreases the future fertility of female offspring (Ye

et al., 2010; Jensen et al., 1998; Weinberg et al., 1989). Benzo[a]pyrene levels in follicular fluid and benzo[a]pyrene-DNA adducts in granulosa-lutein cells and oocytes and in human cervical cells have been associated with smoking status and with amount smoked (Neal et al., 2008; Mancini et al., 1999; Melikian et al., 1999; Zenzes et al., 1998; Shamsuddin and Gan, 1988).

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

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

Ovarian effects

Human epidemiological studies that directly relate ovotoxicity and benzo[a]pyrene exposure are not available; however, smoking, especially during the time of the peri-menopausal transition, has been shown to accelerate ovarian senescence (Midgette and Baron, 1990). Benzo[a]pyrene-induced ovarian toxicity has been demonstrated in animal studies. In adult female rats treated by gavage, statistically significant, dose-related decreases in ovary weight 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 ovary weight were not observed in two subchronic studies in rats. Specifically, no changes in ovary weight were seen in Wistar rats exposed for 35 days to gavage doses up to 50 mg/kg-day (Kroese et al., 2001) or in F344 rats exposed for 90 days to dietary doses up to 100 mg/kg-day (Knuckles et al., 2001).

In adult female rats treated by gavage, dose-related decreases in the number of primordial follicles have been observed in female rats treated for 60 days at doses ≥2.5 mg/kg-day, with a

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

Hormone levels

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

Cervical effects

One subchronic animal study is available that investigated effects in the cervix following oral exposure to benzo[a]pyrene (Table 1-7). 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; Gao et al., 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. (2011a) considered the hyperplasia responses to be preneoplastic lesions. Cervical neoplasia was not reported in the available chronic bioassays, but this tissue was not subjected to histopathology examination in either bioassay (Kroese et al., 2001; Beland and Culp, 1998). Thus, the relationship of the cervical lesions to potential development of neoplasia is uncertain.

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

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

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

Reference and Study Design	Results[a]
Fertility	
Mackenzie and Angevine (1981) CD-1 mice, 30 or 60 F0 females/dose 0, 10, 40, or 160 mg/kg-d by gavage	↓ number of F0 females with viable litters 46/60, 21/30, 44/60, and 13/30*

Reference and Study Design	Results[a]
GDs 7–16	
Kristensen et al. (1995) NMRI mice, 9 females/dose 0 or 10 mg/kg-d by gavage GDs 7–16	No changes in fertility of F0 females
Ovarian effects (weight, histology, follicle numbers)	
Xu et al. (2010) Sprague-Dawley rats, 6 females/ dose 0, 5 or 10 mg/kg by gavage every other day (2.5 and 5 mg/kg-d, adjusted) 60 d	↓ ovary weight (% change from control) 0, -11*, and -15* ↓ number of primordial follicles (20%* decrease at high dose) ↑ increased apoptosis of ovarian granulosa cells (approximate % apoptosis) 2, 24*, and 14*
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d in diet 90 d	No changes in ovary weight
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk 35 d	No changes in ovary weight
Hormone levels	
Xu et al. (2010) Sprague-Dawley rats, 6 females/ dose 0, 5, or 10 mg/kg by gavage every other day (2.5 and 5 mg/kg-d, adjusted) 60 d	↓ serum estradiol (approximate % change from control) 0, -16, and -25* Altered estrous cyclicity
Archibong et al. (2002) F344 rats, 10 females/group 0, 25, 75, or 100 μg/m^3 by inhalation 4 hrs/d GDs 11–20 (serum hormones tested at GD 15 and 17 in 0, 25, and 75 μg/m^3 dose groups)	↓ F0 estradiol, approximately 50% decrease at 75 μg/m^3 at GD 17 ↓ F0 prolactin, approximately 70% decrease at 75 μg/m^3 at GD 17 ↑ F0 plasma progesterone approximately 17% decrease at 75 μg/m^3 at GD 17
Cervical effects	
Gao et al. (2011a) ICR mice, 26 females/dose 0, 2.5, 5, or 10 mg/kg by gavage 2 d/wk 98 d	↑ cervical epithelial hyperplasia: 0/26, 4/26, 6/25*, and 7/24* ↑ cervical atypical hyperplasia: 0/26, 0/26, 2/25, and 4/24* ↑ inflammatory cells in cervical epithelium: 3/26, 10/26, 12/25*, and 18/24*

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

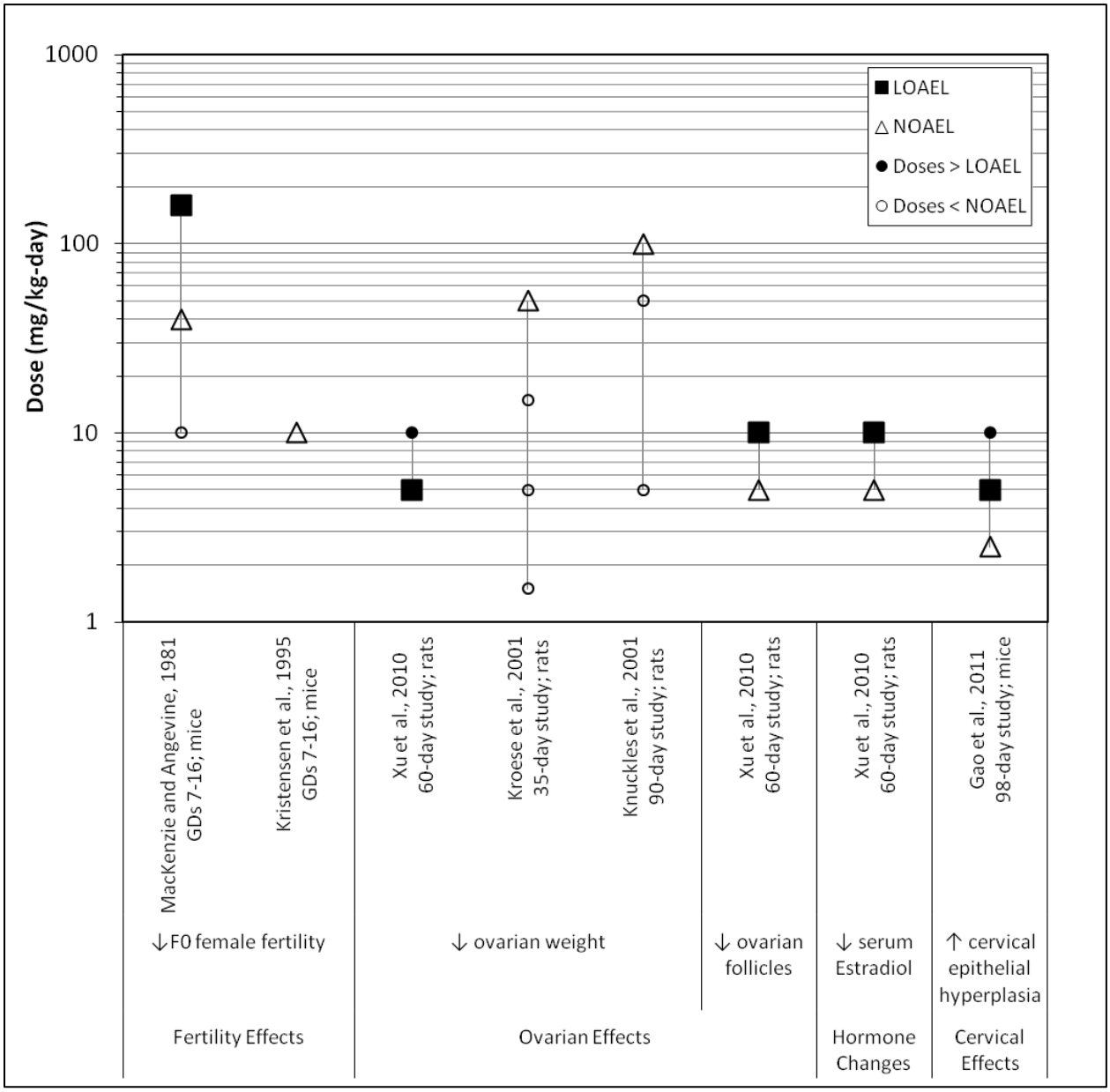

Figure 1-4. Exposure-response array for female reproductive effects following oral exposure in adult animals.

Mode-of-action analysis—female reproductive effects

Although the mechanisms underlying female reproductive effects following benzo[a]pyrene exposure are not fully established, associations with stimulation of apoptosis, impairment of steroidogenesis, and cytotoxicity have been made. Ovarian lesions in benzo[a]pyrene-exposed rats

have been associated with increased apoptosis in ovarian granulosa cells and alteration in hormone-mediated regulation of folliculogenesis (Xu et al., 2010), and results from in vitro experiments provide support for an association between benzo[a]pyrene exposure and impaired folliculogenesis, steroidogenesis, and oocyte maturation (Sadeu and Foster, 2011; Neal et al., 2007). A growing body of research suggests that benzo[a]pyrene triggers the induction of apoptosis in oocytes through AhR-driven expression of pro-apoptotic genes, including Bax (Kee et al., 2010; Neal et al., 2010; Pru et al., 2009; Matikainen et al., 2002; Matikainen et al., 2001; Robles et al., 2000). Other proposed mechanisms include the impairment of folliculogenesis from reactive metabolites (Takizawa et al., 1984; Mattison and Thorgeirsson, 1979, 1977) or by a decreased sensitivity to FSH-stimulated follicle growth (Neal et al., 2007). Based on findings that an ERα antagonist counteracted effects of subcutaneously administered benzo[a]pyrene on uterine weight (decreased in neonatal rats and increased in immature rats), interactions with ERα have been proposed, possibly via occupation of ERα binding sites or via AhR-ER-crosstalk (Kummer et al., 2008; Kummer et al., 2007). However, several in vitro studies have demonstrated low affinity binding of benzo[a]pyrene to the estrogen receptor and alteration of estrogen-dependent gene expression (Liu et al., 2006; van Lipzig et al., 2005; Vondrácek et al., 2002; Fertuck et al., 2001; Charles et al., 2000), so the role of the ER in benzo[a]pyrene-induced reproductive toxicity is unclear.

Summary of Reproductive Effects

Male reproductive effects

Exposure to benzo[a]pyrene in laboratory animals induces male reproductive effects including decreased levels of testosterone and increased levels of LH, decreased sperm count and motility, histological changes in the testis, and decreased reproductive success. These findings in animals are supported by decrements in sperm quality and decreased fertility in human populations exposed to PAH mixtures (Soares and Melo, 2008; Hsu et al., 2006). In laboratory animals, male reproductive toxicity has been observed after oral and inhalation exposure to rats or mice. Effects seen after oral exposures include impaired fertility, effects on sperm parameters, decreased reproductive organ weight, testicular lesions, and hormone alterations (Chen et al., 2011; Chung et al., 2011; Mohamed et al., 2010; Zheng et al., 2010; Mackenzie and Angevine, 1981). In addition to oral exposure, male reproductive effects of benzo[a]pyrene have also been observed following inhalation exposure in rats (Archibong et al., 2008; Ramesh et al., 2008; Inyang et al., 2003). The male reproductive effects associated with benzo[a]pyrene exposure are considered to be biologically plausible and adverse. 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]pyrene impacts fertility through the disruption of folliculogenensis. This finding is supported, albeit indirectly, by observations of premature ovarian senescence in women exposed to cigarette smoke (Midgette and Baron, 1990). Evidence for female reproductive toxicity of benzo[a]pyrene comes from studies of human populations exposed to PAH mixtures as well as laboratory animal and in vitro studies. In addition, two human studies observed associations specifically between benzo[a]pyrene measures and two fertility-related endpoints: decreased ability to conceive (Neal et al., 2008; Neal et al., 2007) and increased risk of early fetal death (i.e., before 14 weeks 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., 2008; El-Nemr et al., 1998) and that prenatal exposure to cigarette smoking is associated with reduced fertility of women later in life (Weinberg et al., 1989). A case-control study in a Chinese population has also indicated that women with elevated levels of benzo[a]pyrene-DNA adducts in maternal blood were 4 times more likely to have experienced a miscarriage (Wu et al., 2010).

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

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

Reductions in female fertility associated with decreased ovary weight and follicle number following gestational exposure (as discussed in Section 1.1.1) are supported by observations of: (1) destruction of primordial follicles (Borman et al., 2000; Mattison et al., 1980) and decreased corpora lutea (Miller et al., 1992; Swartz and Mattison, 1985) in adult female mice following i.p.

exposure; (2) decreased ovary weight in adult female rats following oral exposure (Xu et al., 2010); and (3) stimulation of oocyte apoptosis (Matikainen et al., 2002; Matikainen et al., 2001) or by a decreased sensitivity to FSH-stimulated follicle growth (Neal et al., 2007).

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

1.1.3. Immunotoxicity

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

Thymus Effects

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

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

Spleen Effects

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

are supported by observations of reduced spleen cellularity and decreased spleen weights following i.p. injection or in utero benzo[a]pyrene exposure to doses ranging from 50 to 150 mg/kg (Holladay and Smith, 1995; Urso et al., 1988).

In addition to physical effects on the spleen, several studies have demonstrated functional suppression of the spleen following benzo[a]pyrene exposure. Dose-related decreases in sheep red blood cell (SRBC) specific serum IgM levels after SRBC challenge were reported in rats (10 or 40 mg/kg-day) and mice (5, 20,or 40 mg/kg-day) following s.c. injection of benzo[a]pyrene for 14 days (Temple et al., 1993). Similarly, reduced spleen cell responses, including decreased numbers of plaque forming cells and reduced splenic phagocytosis to SRBC and lipopolysaccharide challenge, were observed in $B6C3F_1$ mice exposed to doses ≥40 mg/kg-day benzo[a]pyrene by i.p. or s.c. injection for 4–14 days (Lyte and Bick, 1985; Dean et al., 1983; Munson and White, 1983) or by intratracheal instillation for 7 days (Schnizlein et al., 1987).

Immunoglobulin Alterations

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

Decreases in serum IgM (13–33% compared to controls) and IgA levels (22–61% compared to controls) were observed in male Wistar rats exposed to 3–90 mg/kg-day benzo[a]pyrene by gavage for 35 days (De Jong et al., 1999); however, these reductions were not dose-dependent. Similarly, reductions in IgA (9–38% compared to controls) were also observed in male and female B6C3F$_1$ mice exposed to doses of 5–40 mg/kg benzo[a]pyrene by s.c. injection for 14 days (Munson and White, 1983). Reductions in serum IgG levels of 18–24%, although not statistically significant, were observed in female B6C3F$_1$ mice exposed to doses ≥50 mg/kg benzo[a]pyrene by i.p. injection for 14 days (Dean et al., 1983).

Immune Suppression and Sensitization

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

Results of functional immune assays in laboratory animals following short-term i.p. and s.c. exposures add to the evidence for benzo[a]pyrene immunotoxicity. Resistance to Streptococcus pneumonia or Herpes simplex type 2 was dose dependently reduced in B6C3F$_1$ mice following s.c. injection of ≥5 mg/kg-day benzo[a]pyrene for 14 days (Munson et al., 1985). Reduced cell proliferation, IFN-γ release, and IL-4 release were observed in male and female C56BL/6 mice following short-term exposure to a gavage dose of 13 mg/kg benzo[a]pyrene as measured in a modified local lymph node assay (van den Berg et al., 2005). A statistically significant decrease in natural killer cell activity was observed in male Wistar rats (Effector:Target cell ratio was 40.9 ± 28.4% that of controls) exposed to 90 mg/kg-day by gavage for 35 days (De Jong et al., 1999); however, splenic natural killer cell activity was not affected in B6C3F$_1$ mice after s.c. injection of 40mg/kg-day benzo[a]pyrene for 14 days (Munson et al., 1985). The magnitude of the dose and duration of the exposure may account for the discrepancy between these two studies. Single i.p. injections of 50 mg/kg benzo[a]pyrene decreased pro- and/or pre-B-lymphocytes and neutrophils in the bone marrow of C57BL/6J mice without affecting the numbers of immature and mature B-lymphocytes or GR-1+ myeloid cells (Galván et al., 2006).

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

Developmental Immunotoxicity

As noted above, several i.p. injection studies suggest that cell-mediated and humoral immunity may be altered by exposure to high doses of benzo[a]pyrene during gestation. Suppression of the mixed lymphocyte response, the graft-versus-host response, and suppression of the plaque-forming cell response to SRBCs was observed in mice exposed in utero to 150 mg/kg during mid (GDs 11–13), late (GDs 16–18), or both (GDs 11–17) stages of gestation; these effects persisted until 18 months of age (Urso and Gengozian, 1984, 1982, 1980). Fetal thymic atrophy, as assessed by reductions in cellularity (74–95%, compared to controls), was observed in mice exposed to 50–150 mg/kg benzo[a]pyrene from GD 13 to 17, when examined on GD 18 (Holladay and Smith, 1994). Analysis of cell surface markers (e.g., CD4, CD8) from the same study indicate that benzo[a]pyrene may inhibit and/or delay thymocyte maturation, possibly contributing to the observed thymic atrophy (Holladay and Smith, 1994). Consistent with these findings, several other studies have noted decreased thymocyte numbers and disrupted T cell maturation after in utero exposure to benzo[a]pyrene (Rodriguez et al., 1999; Holladay and Smith, 1995; Lummus and Henningsen, 1995; Urso et al., 1992; Urso and Johnson, 1987).

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

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

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

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

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

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

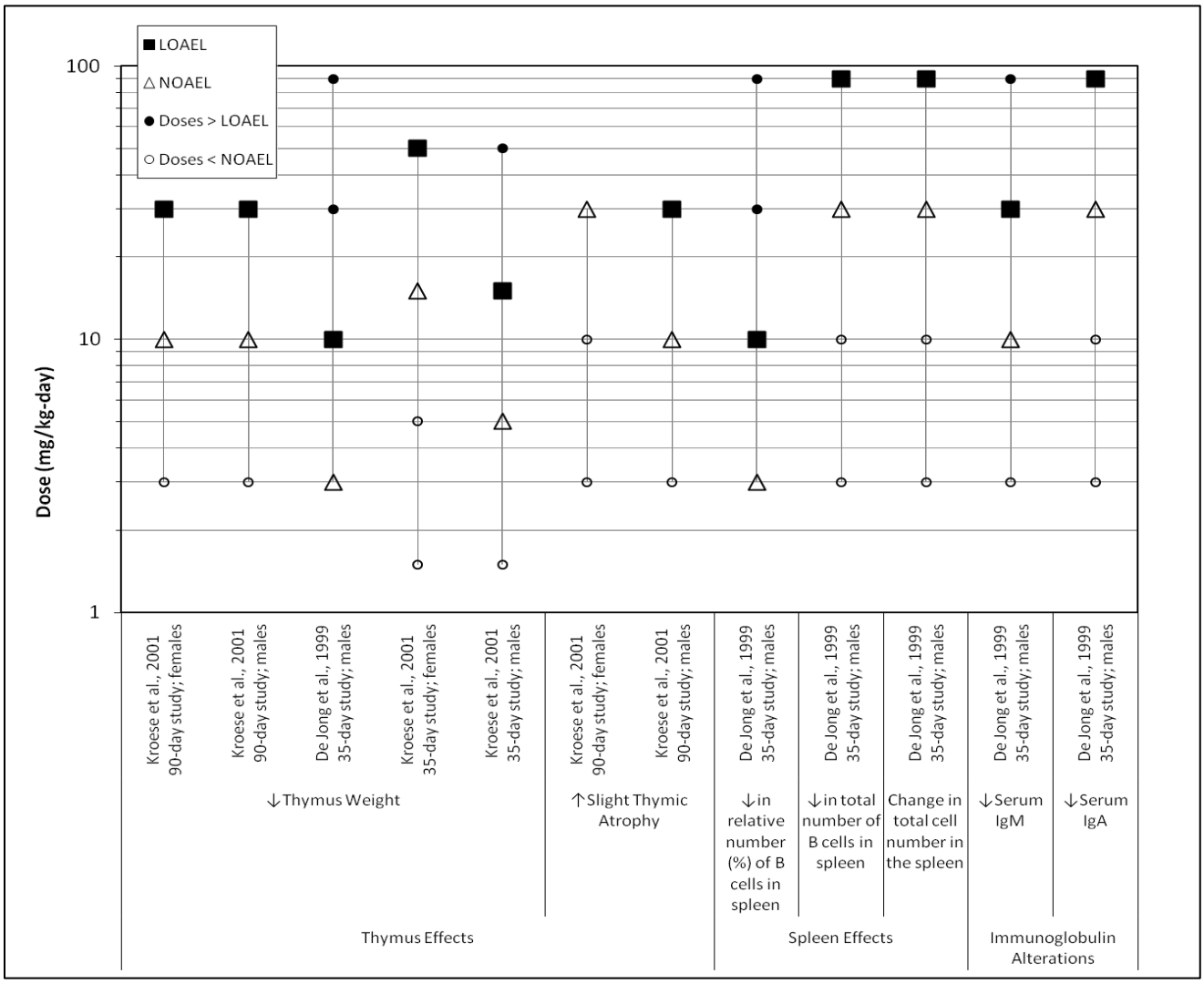

Figure 1-5. Exposure-response array for immune effects following oral exposure.

Mode-of-Action Analysis—Immune Effects

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

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

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

Summary of Immune Effects

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

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

Susceptible Populations and Lifestages

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

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

1.1.4. Other Toxicity

There is some evidence that benzo[a]pyrene can produce effects in the forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters. However, there is less evidence for these effects compared to organ systems described earlier in Section 1.1.1 to 1.1.3.

Forestomach Toxicity

Lesions have been observed in the forestomach following subchronic and chronic oral exposure to benzo[a]pyrene (Table 1-10). Increases in the incidence of forestomach hyperplasia have been observed in Wistar rats following shorter-term, subchronic, and chronic gavage exposure (Kroese et al., 2001; De Jong et al., 1999) and in B6C3F$_1$ mice following chronic dietary exposure (Beland and Culp, 1998; Culp et al., 1998).

Following chronic gavage exposure, increased incidences of forestomach hyperplasia were observed in male and female rats at 3 and 10 mg/kg-day; at the highest dose, a lower incidence of hyperplasia was reported (Kroese et al., 2001). However, only the highest-level lesion (hyperplasia, papilloma, or carcinoma) observed in each organ was scored, such that hyperplasia observed in the forestomach, in which tumors were also observed, was not scored. The majority of animals in the

high-dose group exhibited forestomach tumors; therefore, the hyperplasia was not scored and the incidence of forestomach hyperplasia in the study is more uncertain at the highest dose. Shorter-term studies (Kroese et al., 2001; De Jong et al., 1999) showed dose-related increases in forestomach hyperplasia at doses ≥10 mg/kg-day in Wistar rats. In addition, following chronic dietary exposure, a dose-dependent increase in the incidence of forestomach hyperplasia and hyperkeratosis was observed in female mice at ≥0.7 mg/kg-day (Beland and Culp, 1998; Culp et al., 1998). Forestomach tumors were also observed at ≥0.7 mg/kg-day by Beland and Culp (1998) and Culp et al. (1998).

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

Hematological Toxicity

Altered hematological parameters, including decreases in red blood cell (RBC) count, hemoglobin, and hematocrit have been observed in laboratory animals following benzo[a]pyrene exposure (Table 1-9. Statistically significant decreases in RBC count, hemoglobin, and hematocrit were observed in male Wistar rats at doses ≥10 mg/kg-day for 35 days (De Jong et al., 1999). A minimal, but statistically significant increase in mean cell volume and a decrease in mean cell hemoglobin were observed at the highest dose (90 mg/kg-day), which may indicate dose-related toxicity for the RBCs and/or RBC precursors in the bone marrow (De Jong et al., 1999). Similarly, male and female F344 rats also showed maximal decreases in RBC counts, hematocrit, and hemoglobin levels between 10-12% in a 90-day dietary study (Knuckles et al., 2001). Findings were significant for RBC counts and hematocrit in males at ≥ 50 mg/kg-day, while decreased RBC counts and hematocrit in females and hemoglobin levels in both sexes were only significant in the

100 mg/kg-day group (Knuckles et al., 2001). Small, but not statistically significant, decreases in RBC counts and hemoglobin were observed in both 35- and 90-day studies in Wistar rats (Kroese et al., 2001). It should be noted that when observed, the magnitudes of the decreases in RBCs, hemoglobin, and hematocrit were generally small; about 18% at 90 mg/kg-day and <10% at lower doses (De Jong et al., 1999) and about 10% in F344 rats (Knuckles et al., 2001). A decrease in white blood cells (WBCs), attributed to reduced numbers of lymphocytes and eosinophils, was also observed at 90 mg/kg-day following gavage exposure for 35 days (De Jong et al., 1999). The mode of action by which benzo[a]pyrene exposure may lead to altered hematological parameters is undetermined.

Liver Toxicity

Liver effects other than cancer associated with benzo[a]pyrene exposure primarily include changes in liver weight and abnormal histopathology (Table 1-10). 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 1,000 mg/kg) in an accompanying acute study (Knuckles et al., 2001). A statistically significant increase in liver weight was also observed in male Wistar rats given 90 mg/kg-day benzo[a]pyrene by gavage for 35 days (De Jong et al., 1999). Consistent with the findings by De Jong et al. (1999), a statistically significant increased liver weight (about 18%) was also observed in both male and female Wistar rats at the highest dose (50 mg/kg-day) given by gavage in a 35-day study (Kroese et al., 2001).

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

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

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

35-day study (Kroese et al., 2001). However, at the highest dose tested, with the greatest fold induction in EROD activity, there was no evidence of associated adverse histopathologic findings. The finding of increased liver weight across multiple studies of varying exposure durations, as well as histopathological changes in the liver provide evidence of the liver as a target of benzo[a]pyrene-induced toxicity. The mode of action by which benzo[a]pyrene induces these effects is unknown.

Kidney Toxicity

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

Cardiovascular Toxicity

Atherosclerotic vascular disease and increased risk of cardiovascular mortality have been associated with cigarette smoking (Ramos and Moorthy, 2005; Miller and Ramos, 2001; Thirman et al., 1994) and, to a more limited degree, occupational exposure to PAH mixtures (Friesen et al., 2010; Friesen et al., 2009; Burstyn et al., 2005; Chau et al., 1993). Elevated mortality due to cardiovascular disease was observed in a PAH-exposed occupational population (coke oven plant workers), but elevated cardiovascular mortality was also observed in the non-exposed or slightly exposed populations (Chau et al., 1993). Elevated risks of ischemic heart disease (IHD) were associated with past cumulative benzo[a]pyrene exposure among aluminum smelter workers (with a 5-year lag), although the trend was not statistically significant; there was no observed association with more recent benzo[a]pyrene exposure (Friesen et al., 2010). Elevated risk of mortality from IHD was also associated with cumulative benzo[a]pyrene exposure in a cohort of male asphalt workers (although not statistically significant); the trend in average benzo[a]pyrene exposure and

association with IHD was statistically significant, with an approximately 60% increase in risk between the lowest and highest exposure groups (Burstyn et al., 2005). The two studies that associate benzo[a]pyrene exposure with cardiovascular effects (Friesen et al., 2010; Burstyn et al., 2005) rely on statistical models to create exposure groups rather than direct measurement of the cohort under examination. Additionally, while these studies used benzo[a]pyrene exposure groupings for analysis, they cannot address co-exposures that may have occurred in the occupational setting (asphalt or aluminum smelters) or exposures that occurred outside the workplace.

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

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

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

Neurological Toxicity

Impaired learning and memory, as well as neurochemical alterations, have been observed in humans following occupational exposure to PAH mixtures (Niu et al., 2010). 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 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.

Alterations in neuromuscular, autonomic, sensorimotor, aggression, and electrophysiological endpoints have been reported in rats and mice following acute or short-term exposure to benzo[a]pyrene (Bouayed et al., 2009b; Grova et al., 2008; Grova et al., 2007; Saunders et al., 2006; Liu et al., 2002; Saunders et al., 2002; Saunders et al., 2001). Impaired Morris water maze performance was observed following subchronic oral gavage in adult rats (Chen et al., 2011; Chengzhi et al., 2011) and following short-term i.p. exposure in adult mice (Grova et al., 2007); however, the former study was conducted with only a single dose group, while the latter did not evaluate possible changes in locomotion and reported unexplained decreases in escape latency on trial day 1 following benzo[a]pyrene exposure. Decreased anxiety-like behavior in hole board and elevated plus maze tests has been observed following short-term i.p. exposure (Grova et al., 2008). In addition, a 28-day gavage study in male mice observed an increase in aggressive behavior (as measured by the resident intruder test) and an increase in consummatory sexual behavior in mice treated with 0.02 mg/kg-day (Bouayed et al., 2009b). These data suggest that benzo[a]pyrene exposure could be neurotoxic in adults; however, only limited data are available to inform the neurotoxic potential of repeated subchronic or chronic exposure to benzo[a]pyrene via the oral route (Table 1-9).

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

Reference and Study Design	Results[a]
Forestomach toxicity	
Kroese et al. (2001) Wistar (Riv:TOX) rats: male and female (52/sex/dose group) 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 104 wks (chronic)	Forestomach hyperplasia (basal cell hyperplasia) Incidences[b]: M: 2/50; 8/52; 8/52; and 0/52 F: 1/52; 8/51; 13/51; and 2/52
Wistar (Riv:TOX) rats: male and female (10/sex/dose group) 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d (subchronic)	Forestomach hyperplasia (slight basal cell hyperplasia) Incidences: M: 2/10; 0/10; 6/10; and 7/10 F: 0/10; 2/10; 3/10; and 7/10
Wistar (specific pathogen-free Riv:TOX) rats (10/sex/dose group) 0, 1.5, 5, 15, or 50 mg/kg bw by gavage 5 d/wk 5 wks (shorter-term)	Forestomach hyperplasia (basal cell hyperplasia) Incidences: M: 1/10; 1/10; 4/10; 3/10; and 7/10 F: 0/10; 1/10; 1/10; 3/10; and 7/10*

Reference and Study Design	Results[a]
Beland and Culp (1998); Culp et al. (1998) B6C3F$_1$ mice: female (48/dose group) 0, 5, 25, or 100 ppm in the diet (average daily doses[b]: 0, 0.7, 3.3, and 16.5 mg/kg-d) 2 years	Forestomach hyperplasia Incidences: 13/48; 23/47; 33/46*; and 38/47* Forestomach hyperkeratosis Incidences: 13/48, 22/47, 33/46*, 38/47*
De Jong et al. (1999) Wistar rats: male (8/ dose group) 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 5 wks	Forestomach hyperplasia (basal cell hyperplasia) statistically significantly increased incidences at 30 and 90 mg/kg-d were reported, but incidence data were not provided
Hematological toxicity	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk for35 d	RBC count and hemoglobin changes not statistically significant in males or females at any dose (numerical data not reported) RBC count: changes not statistically significant (numerical data not reported) Hemoglobin: changes not statistically significant (numerical data not reported)
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↓ RBC count Females (% change from control): statistically significant at 100 mg/kg-d (numerical data not reported) Males (% change from control): statistically significant at 50 and 100 mg/kg-d (numerical data not reported) ↓ hematocrit Females (% change from control): statistically significant at 100 mg/kg-d (numerical data not reported) Males (% change from control): statistically significant at 50 and 100 mg/kg-d (numerical data not reported) ↓ hemoglobin Females: statistically significant at 100 mg/kg-d (numerical data not reported) Males: statistically significant at 100 mg/kg-d (numerical data not reported)

Reference and Study Design	Results[a]
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↓ RBC count % change from control: 0, -1,- 5*, -10*, and -18* ↓ hemoglobin % change from control: 0, -1, -7*, -10*, and -18* ↓ hematocrit % change from control: 0, 0, -6*, -8*, and -14* ↓ WBC count % change from control: 0, -8, -9, -9, and -43* ↑ mean cell volume % change from control: 0, 0, -3, 0, and 3* ↓ mean corpuscular hemoglobin concentration % change from control: 0, -1, -1, -1, and -3*
Liver toxicity	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 3, 10, or 30 mg/kg-d by gavage 5 d/wk 90 d Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk for 35 d	↑ liver weight Females (% change from control): 0, -2, 4, and 17* Males (% change from control): 0, 7, 15*, and 29* Liver histopathology: no effects reported ↑ liver weight Females (% change from control): 0, 3, 2, 9, and 18* Males (% change from control): 0, 2, 1, 3, and 18* Liver histopathology: no effects reported
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↑ liver:body weight ratio Females: no change (numerical data not reported) Males (% change from control): 23% change reported at 100 mg/kg-d (numerical data not reported)
De Jong et al. (1999) Wistar rats, 8 males/dose 0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	↑ liver weight % change from control: 0, -9, 7, 5, and 15* ↑ liver oval cell hyperplasia (numerical data not reported) reported as significant at 90 mg/kg-d;
Kidney effects	
Knuckles et al. (2001) F344 rats, 20/sex/dose 0, 5, 50, or 100 mg/kg-d by diet 90 d	↑ abnormal tubular casts Females: not statistically significant (numerical data not reported) Males: apparent dose-dependent increase (numerical data not reported)
De Jong et al. (1999) Wistar rats, 8 males/dose	↓ kidney weight % change from control: 0, -11*, -4, -10*, and -18*

Reference and Study Design	Results[a]
0, 3, 10, 30, or 90 mg/kg-d by gavage 5 d/wk 35 d	
Kroese et al. (2001) Wistar rats, 10/sex/dose 0, 1.5, 5, 15, or 50 mg/kg-d by gavage 5 d/wk 35 d	kidney weight: no change (data not reported)
Neurological toxicity	
Chengzhi et al. (2011) Sprague-Dawley rats, male, 32/dose 0 or 2 mg/kg-d by gavage 90 d	↑ time required for treated rats to locate platform in water maze (data reported graphically)
Bouayed et al. (2009b) Swiss albino mice, male, 9/group 0, 0.02, or 0.2 mg/kg by gavage 28 d	Significant decrease in latency to attack and increase in the number of attacks in the resident-intruder test at 0.02 mg/kg-d (but not at high dose) Significant increase in mount number in the copulatory behavior test at 0.02 and 0.2 mg/kg-d

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

1.1.5. Carcinogenicity

Evidence in Humans

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. All identified studies have co-exposures to other PAHs. The identified studies were separated into tiers according to the extent and quality of the exposure analysis and other study design features:

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

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

For lung cancer, each of the Tier 1 studies observed increasing risks of lung cancer with increasing cumulative exposure to benzo[a]pyrene (measured in µg/m^3-years), and each of these

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

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

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% confidence interval [CI] 0.26, 2.48) [22 cases) in (Spinelli et al., 2006) and 0.58 (95% CI 0.12, 1.7) in Gibbs et al. (2007)[3 cases]. 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 years (Hammond et al., 1976). In addition to this study, three 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, and power linesmen) using incidence data from population registries (Pukkala, 1995; Karlehagen et al., 1992; Törnqvist et al., 1986).

The standardized incidence ratio (SIR) estimates were 1.5 (95% CI: 0.7, 2.6) based on five exposed cases, 2.37 (95% CI: 1.08, 4.50) based on nine cases, and 4.64 (95% CI: 1.51, 10.8) based

on five cases, respectively, in Törnqvist et al. (1986), Karlehagen et al. (1992), and Pukkala (1995). These studies provide support for the association between dermal PAH exposure, including benzo[a]pyrene exposure, and skin cancer.

In addition to cohorts of workers occupationally exposed to PAH mixtures, populations exposed to benzo[a]pyrene through topical coal tar formulations for the treatment of psoriasis, eczema, and dermatitis have also been studied. In the majority of studies with greater than 20 years of follow-up, coal tar treatment was not significantly associated with skin cancer (Roelofzen et al., 2010; Pittelkow et al., 1981; Maughan et al., 1980). However, in populations of patients with co-exposure to psoralen and ultraviolet-A light therapy (later determined to be carcinogenic), high exposure to coal tar treatments was associated with an increased risk of non-melanoma skin cancer (Stern et al., 1998; Stern et al., 1980).

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

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

PAH-Related Mixture or Occupation	Sites with *Sufficient Evidence* in Humans	Sites with *Limited Evidence* in Humans	Reference
Aluminum production	Lung Urinary bladder		Baan et al. (2009)
Carbon electrode manufacture		Lung	IARC (2010)
Coal gasification	Lung		Baan et al. (2009)
Coal tar distillation	Skin		Baan et al. (2009)
Coal tar pitch (paving and roofing)	Lung	Urinary bladder	Baan et al. (2009)
Coke production	Lung		Baan et al. (2009)
Creosotes		Skin	IARC (2010)
Diesel exhaust	Lung	Urinary bladder	Benbrahim-Tallaa et al. (2012)
Indoor emissions from household combustion of biomass fuel (primarily wood)		Lung	Secretan et al. (2009)
Indoor emissions from household combustion of coal	Lung		Secretan et al. (2009)

PAH-Related Mixture or Occupation	Sites with *Sufficient Evidence* in Humans	Sites with *Limited Evidence* in Humans	Reference
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 1-11. Summary of epidemiologic studies of benzo[a]pyrene (direct measures) in relation to lung cancer risk: Tier 1 studies

Reference and Study Design	Results			
Armstrong and Gibbs (2009) (Quebec, Canada) Cohort, aluminum smelter workers, seven plants 16,431 (15,703 men; 728 women) Duration: minimum 1 yr, began work 1966–1990 Follow-up: through 1999 (mean ~30 yrs) Smoking information collected from medical records Exposure: Job exposure matrix ~5,000 personal benzo[a]pyrene measures from the 1970s to 1999 Related references: Lavoué et al. (2007) (exposure data); Gibbs and Sevigny (2007a); Gibbs et al. (2007); Gibbs and Sevigny (2007b); Armstrong et al. (1994)	SMR 1.32 (1.22, 1.42) [677 cases] Lung cancer risk by cumulative benzo[a]pyrene exposure			
	Median benzo[a]-pyrene 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)
	RR = relative risk 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.			
Spinelli et al. (2006) (British Columbia, Canada) Cohort, aluminum smelter workers 6,423 (all men) Duration: minimum ≥3 yrs; began work 1954–1997 Follow-up: through 1999 (14% loss to follow-up; mean ~24 yrs) Smoking information from self-administered questionnaire Exposure: Job exposure matrix using 1,275 personal	SMR 1.07 (0.89, 1.28) [120 cases] SIR 1.10 (0.93, 1.30) [147 cases] Lung cancer risk by cumulative benzo[a]pyrene exposure			
	Benzo[a]-pyrene µg/m^3-years	n cases	RR (95% CI)[a]	
	0–0.5	25	1.0 (referent)	
	0.5–20	42	1.23 (0.74, 2.03)	

benzo[a]pyrene measures from 1977 to 2000 (69% for compliance monitoring) Related references: Friesen et al. (2006) (exposure data); Spinelli et al. (1991)	20–40	23	1.35	(0.76, 2.40)
	40–80	25	1.36	(0.78, 2.39)
	≥80	32	1.79	(1.04, 3.01)
	[a] Adjusting for smoking category; trend $p < 0.001$			
Xu et al. (1996) (China) Nested case-control in iron-steel worker cohort 610 incident cases (96% participation); 959 controls (94% participation) (all men) Duration: data not reported Smoking information collected from interviews; next-of-kin interviews with 30% of lung cancer cases and 5% of controls Exposure: Job exposure matrix 82,867 historical monitoring records, 1956–1992	Lung cancer risk by cumulative benzo[a]pyrene exposure			
	Benzo[a]-pyrene (μg/m^3-years)	n cases	RR (95% CI)[a]	
	<0.84	72	1.1 (0.8, 1.7)	
	0.85–1.96	117	1.6 (1.2, 2.3)	
	1.97–3.2	96	1.6 (1.1, 2.3)	
	≥3.2[b]	105	1.8 (1.2, 2.5)	
	[a]Adjusting for birth year and smoking category; trend $p < 0.004$. Referent group is "nonexposed" (employed in administrative or low-exposure occupations) [b]Study table IV unclear; could be ≥3.0 for this category			

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

Reference and Study Design	Results			
Limited follow-up period (≤20 yrs)				
Friesen et al. (2009) (Australia) Cohort, aluminum smelter workers 4,316 (all men) Duration: minimum 90 d; began work after 1962 Follow-up: through 2002, mean 16 yrs (maximum 20 yrs) Smoking information from company records if employed before 1995 and study interviews if employed after 1994 Exposure: Job/task exposure matrix using TWA benzo[a]pyrene measures (n=655), 1977–2004 (79% from 1990 to 2004)	RR 1.2 (0.7, 2.3) [19 cases in exposed; 20 in unexposed]			
	Lung cancer risk by cumulative benzo[a]pyrene exposure			
	Benzo[a]-pyrene μg/m^3-yrs	n cases	RR	(95% CI)[a]
	0	20	1.0 (referent)	
	>0–0.41	6	0.7 (0.3, 1.8)	
	0.41–10.9	6	1.4 (0.6, 3.5)	
	>10.9	7	1.7 (0.7, 4.2)	
	[a]Poisson regression, adjusting for smoking; trend $p = 0.22$.			
Proxy measure				
Olsson et al. (2010) (Denmark, Norway, Finland, Israel) Nested case-control, asphalt workers 433 lung cancer cases (65% participation); 1,253 controls (58% participation), matched by year of birth, country (all men) Duration: minimum ≥2 seasons, median 8 seasons;	Lung cancer risk by cumulative coal tar exposure[a]			
	Coal tar unit-yrs[a]	n cases	RR	(95% CI)
	0.39– 4.29	43	1.31	(0.87, 2.0)
	4.30–9.42	32	0.98	(0.62, 1.6)
	9.43–16.88	30	0.97	(0.61, 1.6)

Reference and Study Design	Results
began work 1913–1999 Follow-up: from 1980 to 2002–2005 (varied by country) Smoking information from interviews Exposure: compilation of coal tar exposure measures, production characteristics, and repeat measures in asphalt industry in each country used to develop exposure matrix Related references: Boffetta et al. (2003); Burstyn et al. (2000)	16.89–196.48 54 1.60 (1.09, 2.4) (trend p-value) (0.07) [a]Adjusting for set, age, country, tobacco pack-years.
Costantino et al. (1995) (United States, Pennsylvania) Cohort, coke oven workers 5,321 and 10,497 unexposed controls (non-oven steel workers; matched by age, race, date of first employment) (all men) Duration: data not reported; worked in 1953 Follow-up through 1982 (length data not reported) Exposure: average daily exposure coal tar pitch volatiles: 3.15 mg/m^3 top-side full-time jobs, 0.88 mg/m^3 side jobs; used to calculate weighted cumulative exposure index Related reference: Dong et al. (1988) (exposure data)	SMR 1.95 (1.59, 2.33) [255 cases] Lung cancer risk by cumulative exposure Coal tar pitch volatiles (mg/m^3-mo) / n cases / RR (95% CI)[a] 0 / 203 / 1.0 (referent) 1–49 / 34 / 1.2 (0.85, 1.8) 50–199 / 43 / 1.6 (1.1, 2.3) 200–349 / 59 / 2.0 (1.5, 2.8) 350–499 / 39 / 2.0 (1.6, 3.2) 500–649 / 27 / 2.7 (2.0, 4.6) ≥650 / 56 / 3.1 (2.4, 4.6) [a]Adjusting for age, race, coke plant, period of follow-up; trend $p < 0.001$.
Limited exposure information	
Liu et al. (1997)(China) Cohort, various carbon plants and aluminum smelter workers 6,635 (all men) Duration: minimum 15 yrs; began work before 1971 Follow-up: through 1985 (mean ~14 yrs) Smoking information from questionnaire Exposure: Area samples from one carbon plant, 1986–1987	SMR 2.2 (1.1, 2.8) [50 cases] Lung cancer risk by exposure category Exposure category / Mean benzo[a]-pyrene µg/m^3 / n cases / SMR (95% CI)[a] None / -- / 13 / 1.49 (0.83, 2.5) Low / -- / 6 / 1.19 (0.48, 2.5) Moderate / 0.30 / 5 / 1.52 (0.55, 3.4) High / 1.19 / 26 / 4.30 (2.9, 6.2) [a]Calculated by EPA from data in paper
Berger and Manz (1992) (Germany) Cohort, coke oven workers 789 (all men)	SMR 2.88 (2.28, 3.59) [78 cases]

Reference and Study Design	Results
Duration: minimum 10 yrs (mean 27 yrs); began work 1900–1989 Follow-up through 1989 (length data not reported) Smoking information from plant records and interviews Exposure: mean benzo[a]pyrene: 28 (range 0.9–89) µg/m^3	
Hansen (1991); (Hansen, 1989) (Denmark) Cohort, asphalt workers 679 workers (applicators) (all men) Duration: data not reported; employed 1959 to 1980 Follow-up to 1986 (mean ~11 yrs) Smoking information from 1982 surveys of industry and general population Exposure: asphalt fume condensate, 35 personal samples during flooring: median 19.7 (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 yr, median 15 yrs; employed 1965–1972 Follow-up: 1966–1986 (mortality); 1966–1983 (incidence; mean ~15 yrs) Smoking information from interviews with older workers Exposure: area sampling - top of ovens. Benzo[a]pyrene, 1,964 mean 4.3 (range 0.007–33); 1,965 mean 0.52, (0.021–1.29) µg/m^3	SMR 0.82 (0.22, 2.1) [4 cases] (referent group = employed men) SIR 1.35 (0.36, 3.5) [4 cases]
Moulin et al. (1989) (France) Cohort and nested case-control, two carbon electrode plants 1,302 in Plant A (all men), employed in 1975; follow-up 1975–1985 (incidence); smoking information from plant records 1,115 in Plant B (all men); employed in 1957; follow-up 1957–1984 (mortality) Duration of employment and follow-up: data not reported Exposure: benzo[a]pyrene, 19 area samples and 16 personal samples in Plant A (personal sample mean 2.7; 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 yr duration: Plant A: OR 3.42 (0.35, 33.7) [7 cases, 21 controls] Plant B: OR 0.49 (0.12. 2.0) [13 cases, 33 controls]

Reference and Study Design	Results
Hammond et al. (1976) (United States) Cohort, asphalt – roofers 5,939 (all men) Duration: minimum 9 yrs, began before 1960 Follow-up: through 1971 Exposure: 52 personal samples (masks with filters) during specific jobs and tasks; mean benzo[a]pyrene 16.7 µg per 7-hr d	SMR 1.6 (1.3, 1.9) [99 cases] (≥20 yrs since joining union) (CIs calculated by EPA from data in paper)

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

Reference and Study Design	Results
Tier 1 studies	
Burstyn et al. (2007) (Denmark, Norway, Finland, Israel) Cohort, asphalt workers 7,298 all men) Duration: minimum ≥2 seasons, median 8 seasons; began work 1913–1999 Follow-up: began around 1960, ended around 2000 (years varied by country); median 21 yrs Smoking information not collected Exposure: compilation of benzo[a]pyrene measures, production characteristics, and repeat measures in asphalt industry in each country used to develop exposure matrix Related references: (Boffetta et al., 2003); Burstyn et al. (2000)	48 incident bladder cancer cases (39 cases in analyses with 15-yr lag) Bladder cancer risk by cumulative benzo[a]pyrene exposure[a] Benzo[a]-pyrene µg/m^3-yrs[a] / n cases / RR (95% CI) (no lag)[b] / RR (95% CI) (15 yr lag)[c] 0–0.253 / 12 / 1.0 (referent) / 1.0 (referent) 0.253–0.895 / 12 / 0.69 (0.29, 1.6) / 1.1 (0.44, 2.9) 0.895–1.665 / 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-yr lag (RR 1.5, 2.7, 1.9 in second through fourth quartile; trend p = 0.15)
Gibbs and Sevigny (2007a); Gibbs et al. (2007); Gibbs and Sevigny (2007b) (Quebec, Canada) Cohort, aluminum smelter workers, 7 plants 16,431 (15,703 men; 728 women) Duration: minimum 1 yr, began work 1966–1990 Follow-up: through 1999 (mean ~30 yrs) Smoking information collected from medical records Exposure: Job exposure matrix using ~5,000 personal benzo[a]pyrene measures from the 1970s to 1999 Related references: Lavoué et al. (2007) (exposure	Hired before 1950: SMR 2.24 (1.77, 2.79) [78 cases] Bladder cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-yrs[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

Reference and Study Design	Results
data); Armstrong et al. (1994); Gibbs (1985); Gibbs and Horowitz (1979)	240 30 6.4 (4.3, 9.2) 480 12 23.9 (12.2, 41.7) [a]Category midpoint [b]CIs not reported; highest category is ≥80 µg/m^3-yrs (n observed = 57). Mortality risk reduced in cohort hired in 1950–1959, SMR = 1.23. Similar patterns seen in analysis of bladder cancer incidence.
Spinelli et al. (2006) (British Columbia, Canada) See Table 1-11 for study details; this study is considered a "Tier 2") study for bladder cancer because of the smaller number of bladder cancer cases (n = 12) compared with lung cancer cases (n = 120)	SMR 1.39 (0.72, 2.43) [12 cases] SIR 1.80; CI 1.45–2.21 [90 cases, including in situ] Bladder cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-years; n cases; RR (95% CI)[a] 0–0.5: 17; 1.0 (referent) 0.5–20: 20; 0.83 (0.43, 1.59) 20–40: 13; 1.16 (0.56, 2.39) 40–80: 18; 1.50 (0.77, 2.94) ≥80: 22; 1.92 (1.02, 3.65) [a]Adjusting for smoking category; trend $p < 0.001$
Tier 2 studies	
Friesen et al. (2009) (Australia) See Table 1-12 for study details	RR 0.6 (0.2, 2.0) [five cases in exposed; eight in unexposed] Bladder cancer risk by cumulative benzo[a]pyrene exposure Benzo[a]-pyrene µg/m^3-yrs; n cases; RR (95% CI)[a] 0: 8; 1.0 (referent) >0–0.41: 1; 0.2 (0.03, 1.9) 0.41–10.9: 2; 0.7 (0.2, 3.7) >10.9: 2; 1.2 (0.2, 5.6) [a]Poisson regression, adjusting for smoking category; trend $p = 0.22$
Costantino et al. (1995) (United States, Pennsylvania) See Table 1-12 for study details	SMR 1.14 (0.61, 2.12) (16 cases)
Hammond et al. (1976) (United States)	SMR 1.7 (0.94, 2.8) (13 cases) (≥20 yrs since joining union) (CIs calculated by EPA from data in paper)

Reference and Study Design	Results
See Table 1-12 for study details	
Moulin et al. (1989) (France) See Table 1-12 for study details	Plant A: 0 observed cases; expected <1.0 Plant B: SMR 1.94 (0.40, 5.0) (3 cases)
Gustavsson et al. (1990) (Sweden) See Table 1-12 for study details	SMR 2.85 (0.30, 10.3) (2 cases) (referent group = employed men)

Evidence in Animals

Oral exposure

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

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

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

Chronic oral exposure to benzo[a]pyrene resulted in a dose-dependent increased incidence of liver carcinomas in both sexes of Wistar rats, with the first liver tumors detected in week 35 in high-dose male rats; liver tumors were described as complex, with a considerable proportion

(59/150 tumors) metastasizing to the lungs (Kroese et al., 2001). Treatment-related hepatocellular tumors were not observed in mice (Beland and Culp, 1998; Culp et al., 1998).

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

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

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

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

2

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

*Indicates statistical significance as identified in study.
[a]Based on the assumption that daily benzo[a]pyrene intake at 5 ppm was one-fifth of the 25-ppm intake (about 21 µg/day) and using TWA body weights of 0.032 kg for the control, 5- and 25-ppm groups and 0.026 kg for the 100-ppm group.
[b]Incidences are for number of rats with tumors compared with number of tissues examined histologically. Auditory canal tissue was examined histologically when abnormalities were observed on macroscopic examination.
[c]Two malignant forestomach tumors were observed (one each in the mid- and high-dose groups).

Inhalation exposure

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

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

Chronic inhalation exposure to benzo[a]pyrene resulted in the development of tumors in the respiratory tract and pharynx in Syrian golden hamsters (Table 1-15). Concentration-dependent increased incidences of tumors in the upper respiratory tract, including the larynx and trachea, were reported by Thyssen et al. (1981) at measured exposure concentrations of ≥9.5 mg/m^3. 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-concentration, but the incidences were not dose-dependently increased. A concentration-related increase in tumors in the upper digestive tract (pharynx and esophagus) was also reported. In addition, a single forestomach tumor was observed in each of the mid- and high-concentration groups, in animals with a tumor in either the larynx or pharynx; 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. The authors stated that the rates of tumors of other organs generally corresponded to the rates in controls.

Under contract to the U.S. EPA, Clement and Associates U.S. EPA (1990b) 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 concentration-dependent increased incidence of tumors in the larynx and pharynx (U.S. EPA, 1990a, b). 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 U.S. EPA (1990b).

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

Reference and Study Design	Results[b]
Thyssen et al. (1981) Syrian golden hamsters: male (20–30 animals/group) 0, 2.2, 9.5, or 46.5 mg/m^3 on NaCl particles by nose only inhalation for 3–4.5 hrs/5–7 d/wk (TWA exposure concentrations[a]: 0, 0.25, 1.01, and 4.29 mg/m^3) Treated until moribund or dead (up to 130 wks) MMAD: not reported	Larynx incidences: 0/27; 0/27; 8/26; and 13/25 earliest observation of tumor[c]: 107 and 68 wks Pharynx incidences: 0/27; 0/27; 6/26; and 14/25 earliest observation of tumor: 97 and 68 wks Trachea incidences: 0/27; 0/27; 1/26; and 3/25 earliest observation of tumor: 115 and 63 wks Nasal cavity incidences: 0/27; 0/27; 3/26; and 1/25 earliest observation of tumor: 116 and 79 wks Esophagus incidences: 0/27; 0/27; 0/27; and 2/25 earliest observation of tumor: 71 wks Forestomach incidences: 0/27; 0/27; 1/26; and 1/25 earliest observation of tumor: 119 and 72 wks
	Revised tumor incidence data[d] Larynx incidences: 0/27; 0/27; 11/26; and 12/34 Pharynx incidences: 0/27; 0/27; 9/26; and 18/34 Larynx and pharynx (combined)[e] incidences: 0/27; 0/27; 16/26; and 18/34

[a]Duration adjusted inhalation concentrations calculated from exposure chamber monitoring data and exposure treatment times obtained by Clement Associates and reported in U.S. EPA (1990b). Daily exposure times: 4.5 hours/day, 5 days/week on weeks 1–12; 3 hours/day, 5 days/week on weeks 13–29; 3.7 hours/day, 5 days/week on week 30; 3 hours/day, 5 days/week on weeks 31–41; and 3 hours/day, 7 days/week for reminder of the experiment.
[b]Statistical significance not reported by study authors.
[c]Earliest observation of tumor provided for 9.5 and 46.5 mg/m^3 concentration groups.
[d]Revised tumor incidence data based on original study pathology reports obtained by Clement Associates and reported in U.S. EPA (1990b).
[e]Nasal, forestomach, esophageal, and tracheal tumors occurred in hamsters that also had tumors in the larynx or pharynx, except for two animals in the mid-concentration 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]pyrene to skin (in the absence of exogenous promoters) has been demonstrated to induce skin tumors in mice, rats, rabbits, and guinea pigs. These studies have been reviewed by multiple national and international health agencies (IARC, 2010; IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973). Mice have been the most extensively studied species in dermal carcinogenesis studies of benzo[a]pyrene because of evidence that they may be more sensitive than other animal species; however, comprehensive comparisons of species differences in sensitivity to lifetime dermal exposure are not available. Systemic tumors in benzo[a]pyrene-treated mice were not increased compared to controls in lifetime dermal bioassays in which macroscopic examination of internal organs was included (Higginbotham et al., 1993; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959).

The analysis in this document focuses on chronic carcinogenicity bioassays in several strains of mice following repeated dermal exposure to benzo[a]pyrene for the animals' lifetime (Table 1-16). These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959).

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

One study applied benzo[a]pyrene (topically once a week for 6 months) to immunocompromised mice with human skin xenografts (n=10) and did not observe tumors, whereas all three control mice (mice with no xenografts) developed skin tumors (Urano et al., 1995). The authors concluded this result indicates that human skin is much less susceptible to benzo[a]pyrene than mouse skin. However, it is unclear that this human skin xenograft model preserves the physiological and morphological properties of human skin in vivo (Kappes et al., 2004).

1 **Table 1-16. Tumor observations in dermal animal bioassays**

Reference and Study Design	Results[a]
Poel (1959) C57L mice: male (13–56/dose) 0, 0.15, 0.38, 0.75, 3.8, 19, 94, 188, 376, or 752 µg Dermal; 3 times/wk for up to 103 wks or until the appearance of a tumor by gross examination	Skin tumors (gross skin tumors and epidermoid carcinoma); dose-dependent decreased time of tumor appearance Incidences: Gross skin tumors: 0/33; 5/55; 11/55; 7/56; 41/49; 38/38; 35/35; 12/14; 14/14; and 13/13 Epidermoid carcinoma: 0/33; 0/55; 2/55; 4/56; 32/49; 37/38; 35/35; 10/14; 12/14; and 13/13
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/wk until mice died or a skin tumor was observed	Skin tumors and dose-dependent decreased time of first tumor appearance Incidences: SWR: 0/20; 0/25; 2/22; 15/18; 12/17; 16/16; 16/17; and 14/14 C3HeB: 0/17; 0/19; 3/17; 4/17; 11/18;/ 17/17; 18/18; and 17/17 A/He mice: 0/17; 0/18; 0/19; 0/17; 0/17; 21/23; 11/16; and 17/17
Roe et al. (1970) Swiss mice: female (50/dose) 0, vehicle, 0.1, 0.3, 1, 3, or 9 µg Dermal; 3 times/wk for up to 93 wks	Skin tumors; malignant skin tumors were observed in 4/41 and 31/40 mice in the two high dose groups, respectively incidences: 0/43; 0/47; 1/42; 0/42; 1/43; 8/41; and 34/46
Schmidt et al. (1973) NMRI mice: female (100/group) Swiss mice: female (100/group) 0, 0.05, 0.2, 0.8, or 2 µg Dermal; 2 times/wk until spontaneous death occurred or until an advanced carcinoma was observed	Skin tumors (carcinomas) incidences: NMRI: 2/100 at 2 µg (papillomas); 2/100 at 0.8 µg and 30/100 at 2 µg (carcinomas) Swiss: 3/80 at 2 µg (papillomas); 5/80 at 0.8 µg and 45/80 at 2 µg (carcinomas)
Schmähl et al. (1977) NMRI mice: female (100/group) 0, 1, 1.7, or 3 µg Dermal; 2 times/wk until natural death or until they developed a carcinoma at the site of application	Skin tumors (papillomas and carcinomas) incidences: 0/81; 1/77; 0/88; and 2/81 (papillomas) 0/81; 10/77; 25/88; and 43/81 (carcinomas)
Habs et al. (1980) NMRI mice: female (40/group) 0, 1.7, 2.8, or 4.6 µg Dermal; 2 times/wk until natural death or gross observation of infiltrative tumor growth	Skin tumors and dose-dependent increase in age-standardized tumor incidence incidences: 0/35; 8/34; 24/35; and 22/36 age-standardized tumor incidence: 0, 24.8, 89.3, and 91.7%

Reference and Study Design	Results[a]
Grimmer et al. (1984); Grimmer et al. (1983) CFLP mice: female (65–80/group) 0, 3.9, 7.7, or 15.4 µg (1983 study) 0, 3.4, 6.7, or 13.5 µg (1984 study) Dermal; 2 times/wk for 104 wks	Skin tumors (papillomas and carcinomas) with a decrease in tumor latency incidences: 1983: 0/80; 7/65; 5/64; and 2/64 (papillomas) 0/80; 15/65; 34/64; and 54/64 (carcinomas) 1984: 0/65; 6/64; 8/65; and 4/65 (papillomas) 0/65; 37/64; 45/65; and 53/65 (carcinomas)
Habs et al. (1984) NMRI mice: female (20/group) 0, 2, or 4 µg Dermal; 2 times/wk for life	Skin tumors (papillomas and carcinomas) with a decrease in mean survival time incidences: 0/20; 2/20; and 0/20 (papillomas) 0/20; 7/20; and 17/20 (carcinomas)
Sivak et al. (1997) C3H/HeJ mice: male (30/group) 0, 0.05, 0.5, or 5 µg Dermal; 2 times/wk for 104 wks	Skin tumors (papillomas and carcinomas) incidences: 0/30; 0/30; 5/30 (2 papillomas, 3 carcinomas); and 27/30 (1 papilloma, 28 carcinomas)

[a]Statistical significance not reported by study authors.

Mode of Action Analysis—Carcinogenicity

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

Benzo[a]pyrene is a complete carcinogen, in that it can act as both an initiator and a promoter of carcinogenesis. Initiation via direct DNA damage (key event 2) can occur via all three metabolites of benzo[a]pyrene. DNA damage that is not adequately repaired leads to mutation (key event 3), and these mutations can undergo clonal expansion (key event 4) enabled by multiple mechanisms also induced by benzo[a]pyrene, including AhR binding leading to an upregulation of genes related to biotransformation, growth, and differentiation, and regenerative cell proliferation resulting from cytotoxicity and a sustained inflammatory response. However, there is not sufficient

evidence that these mechanisms, which contribute to the promotion and progression phases of cancer development, act independently of DNA damage and mutation to produce benzo[a]pyrene-induced tumors (please see *Other possible modes of action*, below). The available human, animal, and in vitro evidence supports a mutagenic mode of action as the primary mode by which benzo[a]pyrene induces carcinogenesis.

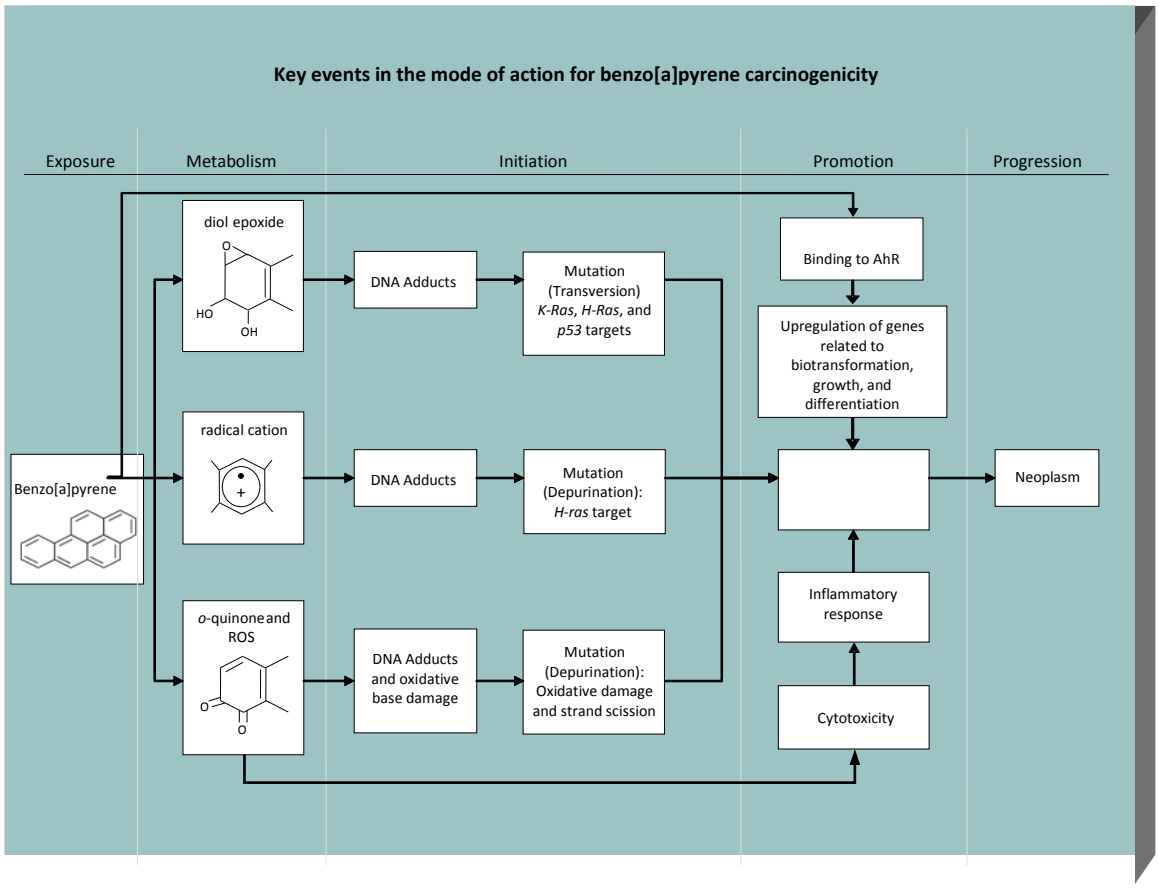

Figure 1-6. Proposed metabolic activation pathways and key events in the carcinogenic mode of action for benzo[a]pyrene.

Data in Support of the Mode of Action

Summary of metabolic activation pathways

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

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

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

o-Quinone/ROS pathway. The o-quinone metabolites of PAHs are formed by enzymatic dehydrogenation of dihydrodiols (Bolton et al., 2000; Penning et al., 1999; Harvey, 1996; ATSDR, 1995) (see Appendix D of the Supplemental Information). Dihydrodiol dehydrogenase enzymes are members of the α-keto reductase gene superfamily. o-Quinone metabolites are potent cytotoxins, are weakly mutagenic, and are capable of producing a broad spectrum of DNA damage. These metabolites can interact directly with DNA as well as result in the production of ROS (i.e., hydroxyl and superoxide radicals) that may produce further cytotoxicity and DNA damage. The o-quinone/ROS pathway also can produce depurinated DNA adducts from benzo[a]pyrene metabolites. In this pathway, and in the presence of $NAD(P)^+$, aldo-keto reductase oxidizes benzo[a]pyrene-7,8-diol to a ketol, which subsequently forms benzo[a]pyrene-7,8-dione. This and other PAH o-quinones react with DNA to form unstable, depurinating DNA adducts. In the presence of cellular reducing equivalents, o-quinones can also activate redox cycles, which produce ROS (Penning et al., 1996). DNA damage in in vitro systems following exposure to benzo[a]pyrene-7,8-dione or other o-quinone PAH derivatives occurs through the 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; Flowers et al., 1996).

Summary of genotoxicity and mutagenicity

The ability of metabolites of benzo[a]pyrene to cause mutations and other forms of DNA damage in both in vivo and in vitro studies is well documented (see genotoxicity tables in Appendix D in Supplemental Information). With metabolic activation (e.g., the inclusion of S9), benzo[a]pyrene is consistently mutagenic in the prokaryotic Salmonella/Ames and Escherichia coli assays. In mammalian in vitro studies, benzo[a]pyrene is consistently mutagenic and clastogenic,

and induces cell transformation both with and without metabolic activation. Cytogenetic damage in the form of chromosomal aberrations (CAs), micronuclei (MN), sister chromatid exchanges (SCEs), and aneuploidy are commonplace following benzo[a]pyrene exposure as are DNA adduct formation, single strand breaks (SSB), and induction of DNA repair and unscheduled DNA synthesis. 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; (2005a)] describe a procedure for evaluating mode-of-action data for cancer. A framework for analysis of mode of action information is provided and followed below.

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

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

Additional supporting evidence of a mutagenic mode of action for benzo[a]pyrene carcinogenicity is the extensive database of in vitro and in vivo studies demonstrating the genotoxicity and mutagenicity of benzo[a]pyrene following metabolic activation (Table 1-17). In vitro studies overwhelmingly support the formation of DNA adducts, mutagenesis in bacteria, yeast, and mammalian cells, several measures of cytogenetic damage (CA, SCE, MN), and DNA damage. In vivo systems in animal models are predominantly positive for somatic mutations following benzo[a]pyrene exposure.

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

Support for the *o*-quinone/ROS pathway contributing to tumor initiation via mutagenic events includes in vitro demonstration that several types of DNA damage can occur from *o*-quinones and ROS (Park et al., 2006; Balu et al., 2004; Mccoull et al., 1999; Flowers et al., 1997; Flowers et al., 1996). In addition, benzo[a]pyrene-7,8-dione can induce mutations in the *p53* tumor suppressor gene using an in vitro yeast reporter gene assay (Park et al., 2008; Shen et al., 2006; Yu et al., 2002), and dominant *p53* mutations induced by benzo[a]pyrene-7,8-dione in this system corresponded to *p53* mutational hotspots observed in human lung cancer tissue (Park et al., 2008).

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

Bennett et al. (1999) demonstrated a dose-response relationship between smoking history/intensity and the types of *p53* mutations associated with benzo[a]pyrene (G→T

transversions) in human lung cancer patients (Table 1-17). In lung tumors of non-smokers, 10% of *p53* mutations were G→T transversions, versus 40% in lung tumors from smokers with >60 pack-years of exposure.

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

Another study examined the dose-response relationship and the time course of benzo[a]pyrene-induced skin damage (Table 1-17), DNA adduct formation, and tumor formation in female mice. Mice were treated dermally with 0, 16, 32, or 64 μg of benzo[a]pyrene once per week for 29 weeks (Albert et al., 1991). Indices of skin damage and levels of BPDE-DNA adducts in skin reached plateau levels in exposed groups by 2–4 weeks of exposure. With increasing dose level, levels of BPDE-DNA adducts (fmol/μg DNA) initially increased in a linear manner and began to plateau at doses ≥32 μg/week. Tumors began appearing after 12–14 weeks of exposure for the mid- and high-dose groups and at 18 weeks for the low-dose group. At study termination (35 weeks after start of exposure), the mean number of tumors per mouse was approximately one per mouse in the low- and mid-dose groups and eight per mouse in the high-dose group. The time-course data indicate that benzo[a]pyrene-induced increases in BPDE-DNA adducts preceded the appearance of skin tumors, consistent with the formation of DNA adducts as a precursor event in benzo[a]pyrene-induced skin tumors.

(Culp et al., 1996) compared dose-response relationships for BPDE-DNA adducts and tumors in female $B6C3F_1$ mice exposed to benzo[a]pyrene in the diet at 0, 18.5, 90, or 350 μg/day for 28 days (to examine adducts) or 2 years (to examine tumors) (Table 1-17). The benzo[a]pyrene dose-tumor response data showed a sharp increase in forestomach tumor incidence between the 18.5 μg/day group (6% incidence) and the 90 μg/day group (78% incidence). The BPDE-DNA adduct levels in forestomach showed a relatively linear dose-response throughout the benzo[a]pyrene dose range tested. The appearance of increased levels of BPDE-DNA adducts in the target tissue at 28 days is temporally consistent with the contribution of these adducts to the initiation of forestomach tumors. Furthermore, about 60% of the examined tumors had mutations in the *K-ras* oncogene at codons 12 and 13, which were G→T or G→C transversions indicative of BPDE reactions with DNA (Culp et al., 1996).

Biological plausibility and coherence. The evidence for a mutagenic mode of action for benzo[a]pyrene is consistent with the current understanding that mutations in *p53* and *ras*

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

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

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

2. Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage.
Evidence that benzo[a]pyrene metabolites induce key events: • Reactive benzo[a]pyrene metabolites have demonstrated genotoxicity in most in vivo and in vitro systems in which they have been tested, including the bacterial mutation assay, transgenic mouse assay, dominant lethal mutations in mice, BPDE-DNA adduct detection in humans and animals, and DNA damage, CAs, MN formation, and SCE in animals (Appendix D in Supplemental Information) • Multiple in vivo benzo[a]pyrene animal exposure studies have demonstrated DNA adduct formation in target tissues that precede tumor formation and increase in frequency with dose (Culp et al., 1996; Talaska et al., 1996; Albert et al., 1991) • Benzo[a]pyrene diol epoxide metabolites interact preferentially with the exocyclic amino groups of deoxyguanine and deoxyadenine in DNA (Geacintov et al., 1997; Jerina et al., 1991; Koreeda et al., 1978; Jeffrey et al., 1976) • Benzo[a]pyrene o-quinone metabolites are capable of activating redox cycles and producing ROS that cause oxidative base damage (Park et al., 2006; Balu et al., 2004; Mccoull et al., 1999; Flowers et al., 1997; Flowers et al., 1996) *Human evidence that key events are necessary for carcinogenesis:* • Detection of benzo[a]pyrene diol epoxide-specific DNA adducts is associated with increased cancer risk in humans that are occupationally exposed (see *Evidence in Humans* section) • These benzo[a]pyrene diol epoxides formed BPDE-DNA adducts preferentially at guanine residues that have been detected in tissues of humans with cancer who were exposed to PAHs (Vineis and Perera, 2007; Rojas et al., 2004; Godschalk et al., 2002; Li et al., 2001; Pavanello et al., 1999; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992)
3. Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation.
Evidence that benzo[a]pyrene metabolites induce key events: • Several in vivo exposure studies have observed benzo[a]pyrene diol epoxide-specific mutational spectra (e.g., G→T transversion mutations) in *K-ras*, *H-ras*, and *p53* in forestomach or lung tumors (Culp et al., 2000; Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993) • Multiple animal exposure studies have identified benzo[a]pyrene-specific mutations in *H-ras*, *K-ras*, and *p53* in target tissues preceding tumor formation (Liu et al., 2005; Culp et al., 1996);(Wei et al., 1999) *Human evidence that key events are necessary for carcinogenesis:* • DNA adducts formed by the benzo[a]pyrene diol epoxide reacting with guanine bases lead predominantly to G→T transversion mutations; these specific mutational spectra have been identified in PAH-associated tumors in humans at mutational hotspots, including oncogenes (*K-ras*) and tumor suppressor genes (*p53*) (Liu et al., 2005; Keohavong et al., 2003; Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; DeMarini et al., 2001; Hainaut and Pfeifer, 2001; Bennett et al., 1999; Denissenko et al., 1996; Puisieux et al., 1991; Marshall et al., 1984; Koreeda et al., 1978; Jeffrey et al., 1976)

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

Other possible modes of action

The carcinogenic process for benzo[a]pyrene is likely to be related to some combination of molecular events resulting from the formation of several reactive metabolites that interact with DNA to form adducts and produce DNA damage resulting in mutations in cancer-related genes, such as tumor suppressor genes or oncogenes. These events may reflect the initiation potency of benzo[a]pyrene. However, benzo[a]pyrene possesses promotional capabilities that may be related to AhR affinity, immune suppression, cytotoxicity, and the formation of ROS, 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. The promotional effects of PAHs appear to be related to AhR affinity and the upregulation of genes related to growth and differentiation (Boström et al., 2002). The genes regulated by this receptor belong to two major functional groups (i.e., induction of metabolism or regulation cell differentiation and proliferation). PAHs bind to the cytosolic AhR in complex with heat shock protein 90. The ligand-bound receptor is then transported to the nucleus in complex with the AhR nuclear translocator protein. The AhR complex interacts with AhR elements of DNA to increase the transcription of proteins associated with induction of metabolism and regulation of cell differentiation and proliferation. Following benzo[a]pyrene exposure, disparities have been observed in the tumor pattern and toxicity of Ah-responsive and Ah-nonresponsive mice, as Ah-responsive mice were more susceptible to tumorigenicity in target tissues such as liver, lung, and skin (Ma and Lu, 2007; Talaska et al., 2006; Shimizu et al., 2000).

Benzo[a]pyrene has both inflammatory and immunosuppressive effects that may function to promote tumorigenesis. Inflammatory responses to cytotoxicity may contribute to the tumor promotion process; for example, benzo[a]pyrene quinones (1,6-, 3,6-, and 6,12-benzo[a]pyrene-quinone) generated ROS and increased cell proliferation by enhancing the epidermal growth factor receptor pathway in cultured breast epithelial cells (Burdick et al., 2003). In addition, several studies have demonstrated that exposure to benzo[a]pyrene increases the production of inflammatory cytokines, which may contribute to cancer progression (N'Diaye et al., 2006; Tamaki et al., 2004; Garçon et al., 2001b; Garçon et al., 2001a). Conversely, the immunosuppressive effects

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

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

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

Conclusions about the hypothesized mode of action

There is sufficient evidence to conclude that the major mode of action for benzo[a]pyrene carcinogenicity involves mutagenicity mediated by DNA reactive metabolites. The evidence for a mutagenic mode of action for benzo[a]pyrene is consistent with the current understanding that mutations in *p53* and *ras* oncogenes are associated with increased risk of tumor initiation. The benzo[a]pyrene database provides strong and consistent evidence for BPDE-induced mutations associated with tumor initiation in cancer tissue from humans exposed to complex mixtures containing benzo[a]pyrene, in animals exposed to benzo[a]pyrene, and in in vitro systems. Supporting evidence suggests that contributions to tumor initiation through potential mutagenic events can be made by the radical cation and *o*-quinone/ROS metabolic activation pathways. Other processes may contribute to the carcinogenicity of benzo[a]pyrene via the promotion and

progression phases of cancer development (e.g., inflammation, cytotoxicity, sustained regenerative cell proliferation).

Support for the Hypothesized Mode of Action in Test Animals

Benzo[a]pyrene induces gene mutations in a variety of in vivo and in vitro systems and produces tumors in all animal species tested and by all routes of exposure (see Appendix D in Supplemental Information). Strong, consistent evidence in animal models supports the postulated key events: the metabolism of benzo[a]pyrene to DNA-reactive intermediates, the formation of DNA adducts, the subsequent occurrence of mutations in oncogenes and tumor suppressor genes, and the clonal expansion of mutated cells.

Relevance of the Hypothesized Mode of Action to Humans

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

Populations or Lifestages Particularly Susceptible to the Hypothesized Mode of Action

A mutagenic mode of action for benzo[a]pyrene-induced carcinogenicity is considered relevant to all populations and lifestages. The current understanding of biology of cancer indicates that mutagenic chemicals, such as benzo[a]pyrene, are expected to exhibit a greater effect in early life exposure versus later life exposure (U.S. EPA, 2005b; Vesselinovitch et al., 1979). The EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action. Given that a determination benzo[a]pyrene acts through a mutagenic mode of carcinogenic action has been made, ADAFs should be applied along with exposure information to estimate cancer risks for early-life exposure.

Toxicokinetic information suggest early lifestages may have lower levels of some CYP enzymes than adults (Ginsberg et al., 2004; Cresteil, 1998), suggesting that lower levels of mutagenic metabolites may be formed in early lifestages. Though expression of bioactivating enzymes is believed to be lower in the developing fetus and children, metabolism of

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

In addition, newborn or infant mice develop liver and lung tumors more readily than young adult mice following acute i.p. exposures to benzo[a]pyrene (Vesselinovitch et al., 1975). These results indicate that exposure to benzo[a]pyrene during early life stages presents additional risk for cancer, compared with exposure during adulthood, despite lower metabolic activity in early lifestages. Population variability in metabolism and detoxification of benzo[a]pyrene, in addition to DNA repair capability, may affect cancer risk. Polymorphic variations in the human population in CYP1A1, CYP1B1, and other CYP enzymes have been implicated as determinants of increased individual cancer risk in some studies (Ickstadt et al., 2008; Aklillu et al., 2005; Alexandrov et al., 2002; Perera and Weinstein, 2000). Some evidence suggests that humans lacking a functional GSTM1 gene have higher BPDE-DNA adduct levels and are thus at greater risk for cancer (Binkova et al., 2007; Vineis et al., 2007; Pavanello et al., 2005; Pavanello et al., 2004; Alexandrov et al., 2002; Perera and Weinstein, 2000). In addition, acquired deficiencies or inherited gene polymorphisms that affect the efficiency or fidelity of DNA repair may also influence individual susceptibility to cancer from environmental mutagens (Wang et al., 2010; Ickstadt et al., 2008; Binkova et al., 2007; Matullo et al., 2003; Shen et al., 2003; Cheng et al., 2000; Perera and Weinstein, 2000; Wei et al., 2000; Amos et al., 1999). In general, however, available support for the role of single polymorphisms in significantly modulating human PAH cancer risk from benzo[a]pyrene or other PAHs is relatively weak or inconsistent. Combinations of polymorphisms, on the other hand, may be critical determinants of a cumulative DNA-damaging dose, and thus indicate greater susceptibility to cancer from benzo[a]pyrene exposure (Vineis et al., 2007).

Analysis of Toxicogenomics Data

An analysis of pathway-based transcriptomic data was conducted to help inform the cancer mode of action for benzo[a]pyrene (see the Supplemental Information for details of this analysis). These data support a mutagenic and cellular proliferation mode of action that follows three candidate pathways: aryl hydrocarbon signaling; DNA damage regulation of the G1/S phase transition; and/or Nrf2 regulation of oxidative stress. Specifically, the analysis showed that benzo[a]pyrene may activate the AhR, leading to the formation of oxidative metabolites and radicals which may lead to oxidative damage and DNA damage. Subsequently, DNA damage can

occur and activate p53 and p53 target genes, including p21 and MDM2. In addition, the data indicate that p53 signaling may be decreased under these conditions, as ubiquitin and MDM2 are both upregulated, and work together to degrade p53. Furthermore, the transcriptional upregulation of cyclin D may result in enough cyclin D protein to overcome the p21 inhibitory competition for CDK4, allowing for G1/S phase transition to occur. The data also supports the hypothesis that an upregulation of PCNA in combination with the upregulation of ubiquitin indicates that cells are moving towards the G1/S phase transition. Although the alterations to the Nrf2 pathway suggest cells are preparing for a pro-apoptotic environment, there is no transcriptional evidence that the apoptotic pathways are being activated.

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

1.2. SUMMARY AND EVALUATION

1.2.1. Weight of Evidence for Effects Other than Cancer

The weight of the evidence from human and animal studies indicates that the strongest evidence for potential hazard following benzo[a]pyrene exposure is 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 on benzo[a]pyrene report associations between particular health endpoints and concentrations of benzo[a]pyrene-DNA adducts, with fewer noncancer studies correlating health effects with external measures of exposure. 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 1.1.1 to 1.1..4.

In animals, evidence of developmental and reproductive toxicity and immunotoxicity has been observed across species and dosing regimens. The available evidence from mice and rats treated by gavage during gestation or in the early postnatal period demonstrate developmental effects including decreased body weight, decreased fetal survival, decreased fertility, atrophy of reproductive organs, and altered neurobehavioral outcomes (Chen et al., 2012; Jules et al., 2012; Bouayed et al., 2009a; Kristensen et al., 1995; Mackenzie and Angevine, 1981). 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 considered further for dose-response analysis and the derivation of reference values.

1.2.2. Weight of Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), benzo[a]pyrene is "carcinogenic to humans." EPA's *Cancer Guidelines* (U.S. EPA, 2005b) 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 1-18.

a) Strong human evidence of cancer or its precursors

There is a large body of evidence for human carcinogenicity for complex PAH mixtures containing benzo[a]pyrene, including soot, coal tars, coal-tar pitch, mineral oils, shale oils, and smoke from domestic coal burning (IARC, 2010; Baan et al., 2009). There is also evidence of

carcinogenicity, primarily of the lung and skin, in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, aluminum production, and paving and roofing with coal tar pitch (IARC, 2010; Baan et al., 2009; Straif et al., 2005). Increased cancer risks have been reported among other occupations involving exposure to PAH mixtures such as carbon black and diesel exhaust (Benbrahim-Tallaa et al., 2012; Bosetti et al., 2007). There is extensive evidence of the carcinogenicity of tobacco smoke, of which benzo[a]pyrene is a notable constituent. The methodologically strongest epidemiology studies (in terms of exposure assessment, sample size, and follow-up period) provide consistent evidence of a strong association between benzo[a]pyrene exposure and lung cancer. Three large epidemiology studies in different geographic areas, representing two different industries, observed increasing risks of lung cancer with increasing cumulative exposure to benzo[a]pyrene (measured in μg/m^3-years), with approximately a 2-fold increased risk at the higher exposures; each of these studies addressed potential confounding by smoking (Armstrong and Gibbs, 2009; Spinelli et al., 2006; Xu et al., 1996) (Table 1-11). Although the relative contributions of benzo[a]pyrene and of other PAHs cannot be established, the exposure-response patterns seen with the benzo[a]pyrene measures make it unlikely that these results represent confounding by other exposures. Similarly, for bladder cancer, two of the three cohort studies with detailed exposure data observed an increasing risk with exposures above >80 μg/m^3-years(Gibbs and Sevigny, 2007a; Gibbs et al., 2007; Gibbs and Sevigny, 2007b; Spinelli et al., 2006) (Table 1-13). The exposure range was much lower in the third study (Burstyn et al., 2007; Gibbs and Sevigny, 2007a; Gibbs et al., 2007; Gibbs and Sevigny, 2007b), such that the highest exposure group only reached the level of exposure seen in the lowest exposure categories in the other studies. Data pertaining to non-melanoma skin cancer is limited to studies with more indirect exposure measures, e.g., based on occupations with likely dermal exposure to creosote (i.e., timber workers, brick makers, and power linesmen); the relative risk estimates seen in the four available studies that provide risk estimates for this type of cancer ranged from 1.5 to 4.6, with three of these four estimates greater than 2.5 and statistically significant (Pukkala, 1995; Karlehagen et al., 1992; Törnqvist et al., 1986; Hammond et al., 1976). These four studies provide support for the association between dermal PAH exposure, including benzo[a]pyrene exposure, and skin cancer.

Although it is likely that multiple carcinogens present in PAH mixtures contribute to the carcinogenic responses, strong evidence is available from several studies of humans exposed to PAH mixtures supporting a contributing role for benzo[a]pyrene diol epoxide in inducing key mutagenic precursor cancer events in target tissues. Elevated BPDE-DNA adducts have been reported in smokers compared to non-smokers, and the increased adduct levels in smokers are typically increased twofold compared with non-smokers (Phillips, 2002). Elevated BPDE-DNA adduct levels have been observed in WBCs of groups of coke oven workers and chimney sweeps, occupations with known elevated risks of cancer (Rojas et al., 2000; Bartsch et al., 1999; Pavanello et al., 1999; Bartsch et al., 1998; Rojas et al., 1998), and in lung tissue from tobacco smokers with

lung cancer (Rojas et al., 2004; Godschalk et al., 2002; Bartsch et al., 1999; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992).

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

Similarly, investigations of mutagenesis following specific exposures to benzo[a]pyrene (as opposed to PAH mixtures) have consistently observed that the benzo[a]pyrene diol epoxide is very reactive with guanine bases in DNA, and that G→T transversions are the predominant type of mutations caused by benzo[a]pyrene diol epoxide in several biological test (Pfeifer and Hainaut, 2003; Hainaut and Pfeifer, 2001). Following treatment of human HeLa cells with benzo[a]pyrene diol epoxide, Denissenko et al. (1996) reported that the distribution of BPDE-DNA adducts within *p53* corresponded to mutational hotspots observed in *p53* in human lung cancers. Benzo[a]pyrene exposure induced mutations in embryonic fibroblasts from human *p53* "knock-in" mice that were similar to those found in smoking related human cancers, with a predominance of G→T transversions that displayed strand bias and were also located in the same mutational hotspots found in *p53* in human lung tumors (Liu et al., 2005). These results, combined with a mechanistic understanding that mutations in *p53* (which encodes a key transcription factor in DNA repair and regulation of cell cycle and apoptosis) may be involved in the initiation phase of many types of cancer, are consistent with a common mechanism for mutagenesis following exposures to PAH mixtures and provide evidence of a contributing role of benzo[a]pyrene diol epoxide in the carcinogenic response of humans to coal smoke and tobacco smoke.

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

b) Extensive animal evidence

In laboratory animals (rats, mice, and hamsters), exposures to benzo[a]pyrene via the oral, inhalation, and dermal routes have been associated with carcinogenic responses both systemically and at the site of administration. Three 2-year oral bioassays are available that associate lifetime benzo[a]pyrene exposure with carcinogenicity at multiple sites. These bioassays observed forestomach, liver, oral cavity, jejunum, kidney, auditory canal (Zymbal gland), and skin or mammary gland tumors in male and female Wistar rats (Kroese et al., 2001); forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981); and forestomach, esophagus, tongue, and larynx tumors in female B6C3F$_1$ mice (Beland and Culp, 1998; Culp et al., 1998). Repeated or short-term oral exposure to benzo[a]pyrene was associated with forestomach tumors in additional bioassays with several strains of mice (Weyand et al., 1995; Benjamin et al., 1988; Robinson et al., 1987; El-Bayoumy, 1985; Triolo et al., 1977; Wattenberg, 1974; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967; Berenblum and Haran, 1955). EPA has considered the uncertainty associated with the relevance of forestomach tumors for estimating human risk from benzo[a]pyrene exposure. While humans do not have a forestomach, squamous epithelial tissue similar to that seen in the rodent forestomach exists in the oral cavity and upper two-thirds of the esophagus in humans (IARC, 2003; Wester and Kroes, 1988). Human studies, specifically associating exposure to benzo[a]pyrene with the alimentary tract tumors are not currently available. However, benzo[a]pyrene-DNA adducts have been detected in oral and esophageal tissue obtained from smokers (reviewed by Phillips, 2002) and several epidemiological studies have identified increased exposure to PAHs as an independent risk factor for esophageal cancer (Abedi-Ardekani et al., 2010; Szymańska et al., 2010; Gustavsson et al., 1998; Liu et al., 1997). Thus, EPA concluded that forestomach tumors in rodents are relevant for assessing the carcinogenic risk to humans.

Chronic inhalation exposure to benzo[a]pyrene was associated primarily with tumors in the larynx and pharynx of male Syrian golden hamsters exposed to benzo[a]pyrene:NaCl aerosols (Thyssen et al., 1981). Additionally, less-than-lifetime oral exposure cancer bioassays in mice provide supporting evidence that exposure to benzo[a]pyrene is associated with an increased incidence of lung tumors in mice (Weyand et al., 1995; Robinson et al., 1987; Wattenberg, 1974). In additional studies with hamsters, intratracheal instillation of benzo[a]pyrene was associated with upper and lower respiratory tract tumors (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). 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; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959). Skin tumors in rats, rabbits, and guinea pigs have also been associated with repeated application of benzo[a]pyrene to skin in the absence of exogenous promoters (IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973). When followed by repeated exposure to a potent tumor promoter, acute dermal exposure to benzo[a]pyrene induced skin tumors in numerous studies of mice, indicating that benzo[a]pyrene is a strong tumor-initiating agent in the mouse skin model (Weyand et al., 1992; Cavalieri et al., 1991; Rice et al., 1985; El-Bayoumy et al., 1982; Lavoie et al., 1982; Raveh et al., 1982; Cavalieri et al., 1981; Slaga et al., 1980; Wood et al., 1980; Slaga et al., 1978; Hoffmann et al., 1972).

Carcinogenic responses in animals exposed to benzo[a]pyrene by other routes of administration include: (1) liver or lung tumors in newborn mice given acute postnatal i.p. injections (Lavoie et al., 1994; Busby et al., 1989; Weyand and Lavoie, 1988; Lavoie et al., 1987; Wislocki et al., 1986; Busby et al., 1984; Buening et al., 1978; Kapitulnik et al., 1978); (2) increased lung tumor multiplicity in A/J adult mice given single i.p. injections (Mass et al., 1993); (3) injection site tumors in mice following s.c. injection (Nikonova, 1977; Pfeiffer, 1977; Homburger et al., 1972; Roe and Waters, 1967; Grant and Roe, 1963; Steiner, 1955; Rask-Nielsen, 1950; Pfeiffer and Allen, 1948; Bryan and Shimkin, 1943; Barry et al., 1935); (4) injection site sarcomas in mice following intramuscular injection(Sugiyama, 1973); (5) mammary tumors in rats with intramammilary administration (Cavalieri et al., 1991; Cavalieri et al., 1988c; Cavalieri et al., 1988b; Cavalieri et al., 1988a); (6) cervical tumors in mice with intravaginal application (Näslund et al., 1987); and (7) tracheal tumors in rats with intratracheal implantation (Topping et al., 1981; Nettesheim et al., 1977).

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

c) 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 i.p. injection (Nesnow et al., 1998a; Nesnow et al., 1998b; Nesnow et al., 1996, 1995; Mass et al., 1993). Mutations in these same genes have also been reported in lung tumors of human cancer patients, bearing distinctive mutation spectra (G→T transversions) that correlate with exposures to coal smoke (Keohavong et al., 2003; DeMarini et al., 2001) or tobacco smoke (Pfeifer and Hainaut, 2003; Pfeifer et al., 2002; Hainaut and Pfeifer, 2001; Bennett et al., 1999).

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

Evidence	Reference
a) Strong human evidence of cancer or its precursors	
• Increased risk of lung, bladder, and skin cancer in humans exposed to complex PAH mixtures containing benzo[a]pyrene	IARC (2010, 2004); Secretan et al. (2009);Baan et al. (2009); Benbrahim-Tallaa et al. (2012)
• Benzo[a]pyrene-specific biomarkers detected in humans exposed to PAH mixtures associated with increased risk of cancer	
– BPDE-DNA adducts in WBCs of coke oven workers and chimney sweeps	Rojas et al. (2000); Bartsch et al. (1999); Pavanello et al. (1999); Bartsch et al. (1998); Rojas et al. (1998)
– BPDE-DNA adducts in smokers	Phillips (2002)
• Benzo[a]pyrene-specific DNA adducts have been detected in target tissues in humans exposed to PAH mixtures	
– BPDE-DNA adducts in non-tumor lung tissues of cigarette smokers with lung cancer and in skin eczema patients treated with coal tar	Rojas et al. (2004); Godschalk et al. (2002); Bartsch et al. (1999); Godschalk et al. (1998b); Rojas et al. (1998); Andreassen et al. (1996); Alexandrov et al. (1992)
– BPDE-DNA adduct formation in *p53* in human cells in vitro corresponds to mutational hotspots at guanine residues in human lung tumors	Denissenko et al. (1996); Puisieux et al. (1991)
• Benzo[a]pyrene-specific mutational spectra identified in PAH-associated tumors in humans	
– GC→TA transversions and GC→AT transitions at hprt locus in T-lymphocytes of humans with lung cancer	Hackman et al. (2000)
– G→T transversions in exposed human- *p53* knock-in mouse fibroblasts at the same mutational hotspot in *p53* from smoking-related lung tumors in humans	Liu et al. (2005)
– G→T transversions at the same mutational hotspot in *p53* and *K-ras* in human lung tumors associated with smoky coal exposures	Keohavong et al. (2003); DeMarini et al. (2001)
– Increased percentage of G→T transversions in *p53* in smokers versus non-smokers	Pfeifer and Hainaut (2003); Pfeifer et al. (2002); Hainaut and Pfeifer (2001); Bennett et al. (1999)
b) Extensive animal evidence	

Evidence	Reference
Oral exposures	
• Forestomach tumors in male and female rats and in female mice following chronic exposure	Kroese et al. (2001); Beland and Culp (1998); Culp et al. (1998); Brune et al. (1981)
• Forestomach tumors in mice following less-than-lifetime exposures	Weyand et al. (1995); Benjamin et al. (1988); Robinson et al. (1987); El-Bayoumy (1985); Triolo et al. (1977); Wattenberg (1974); Roe et al. (1970); Biancifiori et al. (1967); Chouroulinkov et al. (1967); Fedorenko and Yansheva (1967); Neal and Rigdon (1967); Berenblum and Haran (1955)
• Alimentary tract and liver tumors in male and female rats following chronic exposure	Kroese et al. (2001)
• Kidney tumors in male 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	
• Upper respiratory tract 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); Grimmer et al. (1984); Habs et al. (1984); Grimmer et al. (1983); Habs et al. (1980); Schmähl et al. (1977); Schmidt et al. (1973); Roe et al. (1970); Poel (1960, 1959)
• Skin tumors in rats, rabbits, and guinea pigs following subchronic exposures	(IPCS, 1998; ATSDR, 1995; IARC, 1983, 1973)
Other routes of exposure	
• Respiratory tract tumors in hamsters following intratracheal instillation	(Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972)
• Liver or lung tumors in newborn mice given acute postnatal i.p. injections	(Lavoie et al., 1994; Busby et al., 1989; Weyand and Lavoie, 1988; Lavoie et al., 1987; Wislocki et al., 1986; Busby et al., 1984; Buening et al., 1978; Kapitulnik et al., 1978)
• Lung tumor multiplicity in A/J adult mice given single i.p. injections	Mass et al. (1993)
c) Identification of key precursor events have been identified in animals	
• Bioactivation of benzo[a]pyrene to DNA-reactive metabolites has been shown to occur in multiple species and tissues by all routes of exposure • Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage • Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation	See 'Experimental Support for Hypothesized Mode of Action' section

Evidence	Reference
d) Strong evidence that the key precursor events are anticipated to occur in humans	
• Mutations in *p53* or *ras* oncogenes have been observed in forestomach or lung tumors from mice exposed to benzo[a]pyrene	Culp et al. (2000); Nesnow et al. (1998a); Nesnow et al. (1998b); Nesnow et al. (1996, 1995); Mass et al. (1993)
– G→T transversions in *ras* oncogenes or the *p53* gene have been observed in lung tumors of human cancer patients exposed to coal smoke	Keohavong et al. (2003); DeMarini et al. (2001)
– Higher frequency of G→T transversions in lung tumors from smokers versus non-smokers	Pfeifer and Hainaut (2003); Pfeifer et al. (2002); Hainaut and Pfeifer (2001); Bennett et al. (1999)

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2. DOSE-RESPONSE ANALYSIS

2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The oral reference dose (RfD) (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95 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 data suggest that benzo[a]pyrene exposure may pose health hazards other than cancer including reproductive and developmental effects such as infertility, miscarriage, and reduced birth weight (Wu et al., 2010; Neal et al., 2008; Tang et al., 2008; Perera et al., 2005b; Perera et al., 2005a) and cardiovascular effects (Friesen et al., 2010; Burstyn et al., 2005). However, 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, and exposure is likely to have occurred by multiple routes. In addition, uncertainty exists due to concurrent exposure to other PAHs and other components of the mixture (such as metals).

Animal studies were evaluated to determine which provided the most relevant routes and durations of exposure; multiple exposure levels to provide information about the shape of the dose-response curve; and power to detect effects at low exposure levels (U.S. EPA, 2002). The oral database for benzo[a]pyrene includes a variety of studies and datasets that are suitable for use in deriving reference values. Specifically, chronic effects associated with benzo[a]pyrene exposure in animals include observations of organ weight and histological changes and hematological

parameters observed in several oral cancer bioassays (Kroese et al., 2001; Beland and Culp, 1998). Multiple subchronic studies are available which characterize a variety of effects other than cancer. In addition, several developmental studies are available which help inform hazards of exposure during sensitive developmental windows.

Developmental Toxicity

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

Chen et al. (2012) observed increased latency in negative geotaxis, increased motor activity in the open field test, decreased anxiety-like behaviors in the elevated plus maze test, and impaired performance in the Morris water maze test as measured by an increase in latency time to find a hidden platform. Chen et al. (2012) also observed increased latencies for treated pups to right themselves in the surface righting test and the negative geotaxis test. The data from the open field test, negative geotaxis test, and surface righting test were considered less informative as male and female data were pooled (male and female rats sometimes show differences in the maturation of these developmental landmarks following challenge). In addition, altered behaviors and locomotion in open field tests could be attributed to anxiety responses due to open spaces and bright light, as well as changes to motor system function. Chen et al. (2012) reported increased quadrants crossed, which could indicate either increased motor activity or decreased anxiety (less fear of the open spaces/bright lights). EPA considered the elevated plus maze and Morris water maze tests (which did report responses for each sex) to be the most informative and appropriate measures of neurobehavioral function performed by Chen et al. (2012).

Significant, dose-related effects were reported in an established test of spatial learning and memory (Morris water maze). Specifically, increased escape latency in each of four hidden platform trial days and decreased time spent in the target quadrant during a probe trial were observed following benzo[a]pyrene exposure. Due to the altered baseline performance of treated

animals on day 1 of the hidden platform trials these findings cannot be specifically attributed to impaired learning. In fact, the slopes of the lines across trial days are nearly identical for the treatment groups suggesting the lack of an effect on learning. The impaired Morris water maze performance of treated animals could be due to effects on several other components of neurological function besides learning, including anxiety, vision, and locomotion. Similarly, as escape latencies were not comparable across groups after learning acquisition (i.e., the end of the hidden platform trials), differences in probe trial performance are likely to be influenced by factors other than impaired memory retention. As a result, although they identify significant effects of benzo[a]pyrene exposure, the Morris water maze data were considered less informative than the results from the elevated plus maze test. Chen et al. (2012) reported an increase in the number of open arm entries in the elevated plus maze test, an indicator of decreased anxiety-like behavior. These results indicate effects on a single, discreet neurological function which are unlikely to be complicated by changes in other processes such as motor activity (total arm entries calculated by summing open and closed arm entries were unchanged with treatment). This finding is considered adverse, is supported by similar observations in developing (Bouayed et al., 2009a) and adult (Grova et al., 2008) mice, and may be indirectly related to observations of increased aggression in mice (Bouayed et al., 2009b) as well as attention and anxiety problems in PAH-exposed children (Perera et al., 2012b).

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

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

Reproductive Toxicity

Male reproductive toxicity was demonstrated in numerous subchronic studies (Chen et al., 2011; Chung et al., 2011; Mohamed et al., 2010; Zheng et al., 2010). Chung et al. (2011) was not

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

Female reproductive toxicity was demonstrated in two subchronic studies (Gao et al., 2011a; Xu et al., 2010). Specifically, Xu et al., 2010 demonstrated altered ovary weights and follicle numbers, and Gao et al. (2011a) demonstrated cervical epithelial cell hyperplasia following oral exposure to benzo[a]pyrene. These studies were identified as the most informative studies on female reproductive toxicity for dose-response analysis. Gao et al. (2011a) 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., 2011a; Gao et al., 2010). Cervical effects of increasing severity (including epithelial hyperplasia, atypical hyperplasia, apoptosis, and necrosis) were also observed at higher doses (Gao et al., 2011a; Gao et al., 2010). There are no data on cervical effects in other species or in other mouse strains. However, Gao et al. (2011a) 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 (Phillips, 2002; Melikian et al., 1999). However, data to support that cervical hyperplasia following oral benzo[a]pyrene exposure progresses to cervical tumors were not available (no cervical tumors were noted in the two available chronic oral cancer bioassays). Thus, in the absence of these data, cervical hyperplasia is presented as a noncancer effect.

Xu et al. (2010) identified biologically and statistically significant decreases in ovary weight, estrogen, and primordial follicles, and altered estrus cycling in treated animals. These reductions in female reproductive parameters are supported by a large database of animal studies indicating that benzo[a]pyrene is ovotoxic with effects including decreased ovary weight, decreased primordial follicles, and reduced fertility (Borman et al., 2000; Kristensen et al., 1995; Miller et al., 1992; Swartz and Mattison, 1985; Mackenzie and Angevine, 1981; Mattison et al., 1980). Additionally,

epidemiology studies indicate that exposure to complex mixtures of PAHs, such as through cigarette smoke, is associated with measures of decreased fertility in humans (Neal et al., 2008; El-Nemr et al., 1998). Specific associations have also been made between infertility and increased levels of benzo[a]pyrene in follicular fluid in women undergoing in vitro fertilization (Neal et al., 2008).

Immunotoxicity

As described in Section 1.1.3, the immune system was identified as a 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 only available studies to support development of an RfD were conducted by Kroese et al. (2001) and De Jong et al. (1999). These are subchronic studies with multiple exposure levels and adequate power to detect effects. In comparing these studies, the Kroese et al. (2001) study is preferred for dose-response analysis due to its longer duration (90 days).

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

2.1.2. Methods of Analysis

No biologically based dose-response models are available for benzo[a]pyrene. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), the benchmark dose (BMD) and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data in the absence of information regarding what level of change is considered

biologically significant, and also to facilitate a consistent basis of comparison across endpoints, studies, and assessments. The estimated BMDLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best fit model for each endpoint can be found in Appendix E of the Supplemental Information.

Among the endpoints identified as representative of the hazards of benzo[a]pyrene exposure, the data for neurobehavioral changes in the elevated plus maze and Morris water maze tests (Chen et al., 2012), decreased ovary weight (Xu et al., 2010), increased cervical hyperplasia (Gao et al. (2011a), and decreased thymus weight (Kroese et al., 2001) 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 E of the Supplemental Information for details of statistical analyses).

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

Human equivalent doses (HEDs) for oral exposures were derived from the PODs estimated from the laboratory animal data as described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011). In this guidance, EPA advocates a hierarchy of approaches for deriving HEDs from data in laboratory animals, with the preferred approach being physiologically-based toxicokinetic modeling. Other approaches can include using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. As discussed in Appendix D of the Supplemental Information, several animal physiologically based pharmacokinetic (PBPK) models for benzo[a]pyrene have been developed and published, but a validated human PBPK model for benzo[a]pyrene for extrapolating doses from animals to humans is not available. In lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., $BW^{3/4}$) approach is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD. $BW^{3/4}$ scaling was not employed for deriving HEDs from studies in which doses were administered directly to early postnatal animals because of the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans due to presumed toxicokinetic and/or toxicodynamic differences between lifestages (U.S. EPA, 2011; Hattis et al., 2004).

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

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Developmental							
Neurobehavioral changes Chen et al. (2012)	Female Sprague-Dawley rats	Exponential (M4)[a]	1SD	0.18	0.09	0.09	0.09
Cardiovascular effects Jules et al. (2012)	Long-Evans rats	LOAEL (0.6 mg/kg-d) (15% ↑ in systolic BP; 33% ↑ in diastolic BP)				0.6	0.15
Reproductive							
Decreased ovary weight Xu et al. (2010)	Female Sprague-Dawley rats	Linear[a]	1SD	2.3	1.5	1.5	0.37
Decreased intratesticular testosterone Zheng et al. (2010)	Male Sprague-Dawley rats	NOAEL (1 mg/kg-d) (15% ↓ in testosterone)				1	0.24
Decreased sperm count and motility Mohamed et al. (2010)	Male C57BL/6 mice	LOAEL (1 mg/kg-d) (50% ↓ in sperm count; 20% ↓ in sperm motility)				1	0.15

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

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

2.1.3. Derivation of Candidate Values

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

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

to quantitatively estimate variability in human susceptibility; therefore, the full value for the intraspecies UF was retained.

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

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

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

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

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

Endpoint and Reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Developmental									
Neurobehavioral changes in rats Chen et al. (2012)	0.09	$BMDL_{1SD}$	10	10	1	1	3	300	3×10^{-4}
Cardiovascular effects in rats Jules et al. (2012)	0.15	LOAEL	3	10	10	1	3	1,000	2×10^{-4}
Reproductive									
Decreased ovary weight in rats Xu et al. (2010)	0.37	$BMDL_{1SD}$	3	10	1	10	3	1,000	4×10^{-4}
Decreased intratesticular testosterone in rats Zheng et al. (2010)	0.24	NOAEL	3	10	1	10	3	1,000	2×10^{-4}
Decreased sperm count and motility in mice Mohamed et al. (2010)	0.15	LOAEL	3	10	10	10	3	10,000	Not calculated due to UF > 3000[a]
Cervical epithelial hyperplasia in mice Gao et al. (2011a)	0.06	$BMDL_{10}$	3	10	1	10	3	1,000	6×10^{-5}

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

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

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

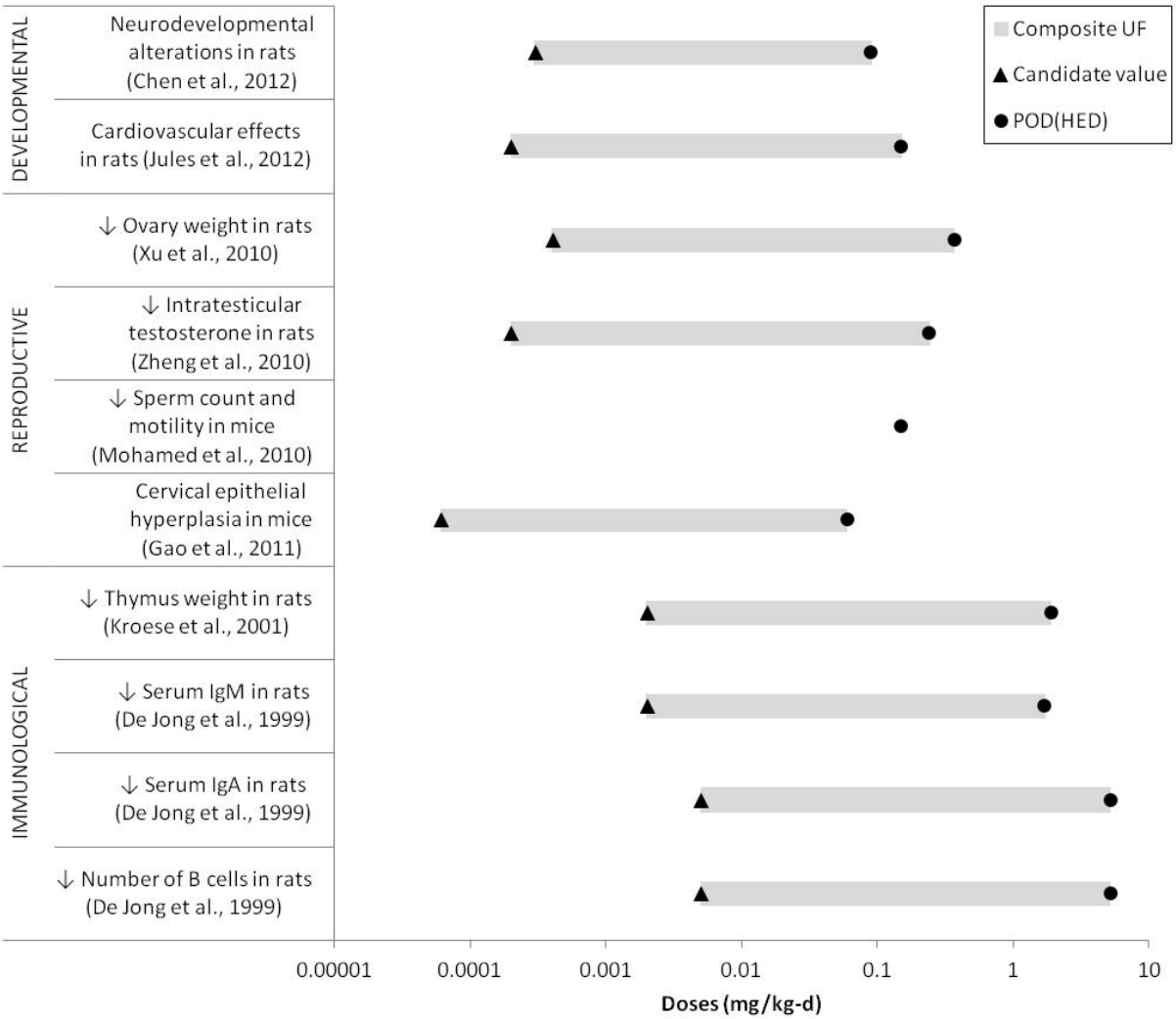

Figure 2-1. Candidate values with corresponding PODs and composite UFs.

2.1.4. Derivation of Organ/System-specific Reference Doses

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

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

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

Developmental Toxicity

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

Reproductive Toxicity

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

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

Immunotoxicity

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

2.1.5. Selection of the Proposed Overall Reference Dose

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

The overall reference dose is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002). Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD.

Furthermore, certain exposure scenarios may require particular attention to the risk-assessment population of interest in order to determine whether a reference value based on toxicity following developmental exposure is warranted. For example, the use of an RfD based on developmental effects may not be appropriate for a risk assessment in which the population of interest is post-reproductive age adults.

2.1.6. Confidence Statement

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

Confidence in the principal study (Chen et al., 2012) is medium-to-high. The study design included randomized experimental testing, blinded observations, culling of pups to account for nutritional availability, treatment-randomization, and controls for litter and nursing bias. Some informative experimental details were, however, omitted including the sensitivity of some assays at the indicated developmental ages 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 IRIS Assessment: Reference Dose

An RfD was not derived in the previous IRIS assessment.

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

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

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

In Section 1.2.1, developmental and reproductive toxicities were 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 an RfC. Although preferred for derivation of reference values, human studies were not considered because of the contribution to the observed hazard of multiple PAHs across multiple routes of exposure and uncertainty due to concurrent exposure to other PAHs and other components of the mixtures (such as metals).

Animal studies were evaluated to determine which provided the most relevant routes and durations of exposure, multiple exposure levels to provide information about the shape of the dose response curve, and relative ability to detect effects at low exposure levels. The only chronic animal inhalation study available for benzo[a]pyrene, Thyssen et al. (1981), was designed as a cancer bioassay and did not report other effects; however, the inhalation database for benzo[a]pyrene includes several shorter duration studies that are sufficient for use in deriving reference values (U.S. EPA, 2002). Specifically, several reproductive toxicity studies are available for the inhalation route, including one subchronic study Archibong et al. (2008). Furthermore, several developmental studies are available which help identify hazards of exposure during sensitive developmental windows (Wormley et al., 2004; Archibong et al., 2002). In addition, a four week inhalation study in rats is available which investigated but did not detect lung injury (Wolff et al., 1989). The inhalation database for benzo[a]pyrene is less extensive than the database of studies by the oral route, however, the types of non-cancer effects observed are consistent between routes and are supported by studies in human populations (see Sections 1.1.1, 1.1.2, and 1.1.3).

Developmental Toxicity

Developmental toxicity, as represented by decreased fetal survival and developmental neurotoxicity, was observed by Archibong et al. (2002) and (Wu et al., 2003a). (Wu et al., 2003a) was not considered for dose-response analysis due to lack of study details related to number of dams and litters per group and lack of reporting of numerical data. 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 decreased fetal survival at the lowest dose tested by the inhalation route on GDs 11-20 (i.e., LOAEL of 25 μg/m^3). This study indicates that the developing fetus is a sensitive target following inhalation exposure to benzo[a]pyrene. The observed decrease in fetal survival is supported by the oral database for benzo[a]pyrene (e.g., decreased survival of litters in mice following in utero exposure to benzo[a]pyrene on GDs 7–16) (Mackenzie and Angevine, 1981). In addition, a single exposure inhalation study by Wormley et al. (2004) demonstrated developmental neurotoxicity, represented by electrophysiological changes in the hippocampus, as a result of gestational exposure. This dose group appears to be the high exposure group from Archibong et al. (2002) study (as indicated by identical outcomes for fetal survival), and at this exposure level, a 66% reduction in fetal survival was observed. Due to the apparent overt toxicity at this exposure concentration, Wormley et al. (2004) was not considered 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. However, this single-dose subchronic study is consistent with male reproductive effects observed across multiple studies by the oral route and with human studies in PAH exposed populations (see Section 1.1.2). The endpoints of decreased testes weight and sperm count and motility reported in Archibong et al. (2008) were selected for dose-response analysis as both represent sensitive endpoints of male reproductive toxicity and are indicators of potentially decreased fertility. These effects are also consistent with human studies in PAH exposed populations as effects on male fertility and semen quality have been demonstrated in epidemiological studies of smokers (reviewed by Soares and Melo, 2008).

2.2.2. Methods of Analysis

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

By definition, the RfC is intended to apply to continuous lifetime exposures for humans (U.S. EPA, 1994). EPA recommends that adjusted continuous exposures be used for inhalation developmental toxicity studies as well as for studies of longer durations (U.S. EPA, 2002). The LOAELs identified from Archibong et al. (2002) and Archibong et al. (2008) were adjusted to account for the discontinuous daily exposure as follows:

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

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

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

where:

BW = body weight (kg)

V_E = ventilation rate (L/minute)

F_{TOT} = total fractional deposition

The total fractional deposition includes particle deposition in the nasal-pharyngeal, tracheobronchial, and pulmonary regions. F_{TOT} for both animals and humans was calculated using the Multi-Path Particle Dosimetry model, a computational model used for estimating human and rat airway particle deposition and clearance (Multi-Path Particle Dosimetry [MPPD]; Version 2.0 © 2006, publicly available through the Hamner Institute). F_{TOT} was based on the average particle size of 1.7 ± 0.085 μm (mass median aerodynamic diameter [MMAD] ± geometric SD) as reported in Wu et al. (2003a) for the exposure range 25–100 μm^3. For the model runs, the Yeh-Schum 5-lobe model was used for the human and the asymmetric multiple path model was used for the rat (see Appendix E for MPPD model output). Both models were run under nasal breathing scenarios with the inhalability adjustment selected. A geometric SD of 1 was used as the default by the model because the reported geometric SD of 0.085 was ≤1.05.

The human parameters used in the model for calculating F_{TOT} and in the subsequent calculation of the POD_{HEC} were as follows: human body weight, 70 kg; V_E, 13.8 L/minute; breathing frequency, 16 per minute; tidal volume, 860 mL; functional residual capacity, 3,300 mL; and upper respiratory tract volume, 50 mL. Although the most sensitive population in (Archibong et al., 2002) is the developing fetus, the adult rat dams were directly exposed. Thus, adult rat parameters were used in the calculation of the HEC. The parameters used for the rat were body weight, 0.25 kg (a generic weight for male and female rats); V_E, 0.18 L/minute; breathing frequency, 102 per minute; tidal volume, 1.8 mL; functional residual capacity, 4 mL; and upper respiratory tract volume, 4.42 mL. All other parameters were set to default values (see Appendix E).

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

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

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

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

Table 2-4. Summary of derivation of PODs

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

[a]PODs were adjusted for continuous daily exposure: POD_{ADJ}= POD × hours exposed per day/24 hours.

[b]POD_{HEC} calculated by adjusting the POD_{ADJ} by the RDDR calculated using particle size reported in Hood et al. (2000) using MPPD software as detailed in Section 2.2.2 and Appendix E in the Supplemental Information.

2.2.3. Derivation of Candidate Values

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

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

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

Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994).

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

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

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

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

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

additional data were available. Therefore, a database UF of 10 for the benzo[a]pyrene inhalation database was applied to account for the lack of a multigenerational study and the lack of a developmental neurotoxicity study.

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

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

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

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

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

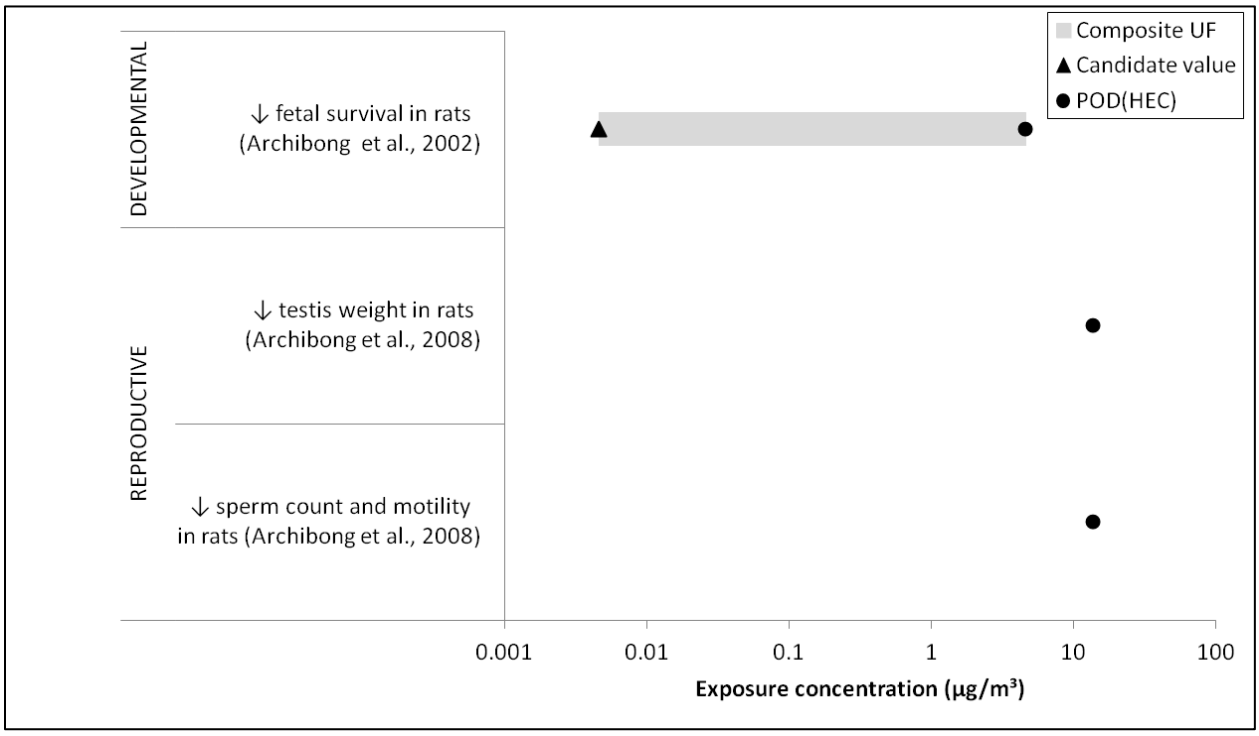

Figure 2-2. Candidate values with corresponding PODs and composite UFs.

2.2.4. Derivation of Organ/System-specific Reference Concentrations

Table 2-6 distills the candidate values from Table 2-5 into a single value for each organ or system. These organ or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. The candidate values for reproductive toxicity 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 UF in four or more areas of extrapolation should be avoided.

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

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

2.2.5. Selection of the Proposed Reference Concentration

For benzo[a]pyrene, the derivation of multiple organ/system-specific reference doses were considered for effects observed following inhalation exposure and identified as potential hazards of benzo[a]pyrene including developmental and reproductive toxicity. However, an organ/system-specific RfC to represent reproductive toxicity could not be derived due to a composite UF of >3,000 from the application of uncertainty factors to cover several areas of extrapolation.

An overall RfC of **2×10^{-6} mg/m^3** was selected based on the hazard of developmental toxicity. The study by Archibong et al. (2002) was selected as the study used for the derivation of the proposed overall RfC, as it observed biologically significant effects at the lowest dose tested by the inhalation route. This study indicates that the developing fetus is a sensitive target following inhalation exposure to benzo[a]pyrene and the observed decreased fetal survival/litter is the most sensitive noncancer effect observed following inhalation exposure to benzo[a]pyrene. Additional support for this endpoint of decreased fetal survival is provided by a developmental/reproductive study conducted via the oral route (Mackenzie and Angevine, 1981).

This overall RfC is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002). Decisions concerning averaging exposures over time for comparison with the RfC should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfC.

Furthermore, certain exposure scenarios may require particular attention to the risk-assessment population of interest in order to determine whether a reference value based on toxicity following developmental exposure is warranted. For example, the use of an RfC based on developmental effects may not be appropriate for a risk assessment in which the population of interest is post-reproductive age adults.

2.2.6. Confidence Statement

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

The overall confidence in the RfC is low-to-medium. Confidence in the principal study (Archibong et al., 2002) is medium. The conduct and reporting of this developmental study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study, the lack of studies on developmental neurotoxicity and 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 IRIS Assessment: Reference Concentration

An RfC was not derived in the previous IRIS assessment.

2.2.8. Uncertainties in the Derivation of the RfD and RfC

The following discussion identifies uncertainties associated with the RfD and RfC for benzo[a]pyrene. To derive the RfD, the UF approach (U.S. EPA, 2000, 1994) was applied to a POD based on neurobehavioral changes in rats treated developmentally. To derive the RfC, this same approach was applied to a POD from a developmental study for the effect of decreased fetal survival. UFs were applied to the POD to account for extrapolating from an animal bioassay to human exposure, the likely existence of a diverse population of varying susceptibilities, and database deficiencies. These extrapolations are carried out with default approaches given the lack of data to inform individual steps.

The database for benzo[a]pyrene contains limited human data. The observation of effects associated with benzo[a]pyrene exposure in humans is complicated by several factors including the existence of benzo[a]pyrene in the environment as one component of complex mixtures of PAHs, exposure to benzo[a]pyrene by multiple routes of exposure within individual studies, and the difficulty in obtaining accurate exposure information. Data on the effects of benzo[a]pyrene alone are derived from a large database of studies in animal models. The database for oral benzo[a]pyrene exposure includes two 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). In addition, subchronic and short-term inhalation studies are available, which examine developmental and reproductive endpoints in rats. Developmental studies by the inhalation route identified biologically significant reductions in the number of pups/litter and percent fetal survival and possible neurodevelopmental effects (e.g., diminished electrophysiological responses to stimuli in

the hippocampus) following gestational exposures. Additionally, a 60-day oral study in male rats reported male reproductive effects (e.g., decreased testes weight and sperm production and motility), but provides limited information to characterize dose-response relationships with chronic exposure scenarios.

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

One area of uncertainty in the database pertains to the lack of information regarding fertility in animals exposed gestationally to benzo[a]pyrene, especially in light of developmental studies by the oral route indicating reduced fertility in the F1 generation and decreased reproductive organ weights. The database also lacks a multigenerational reproductive study via the inhalation route. Areas of uncertainty include the lack of chronic inhalation studies focusing on noncancer effects, limited data on dose-response relationships for impaired male or female fertility with gestational exposure or across several generations, and limited data on immune system endpoints with chronic exposure to benzo[a]pyrene.

The toxicokinetic and toxicodynamic differences for benzo[a]pyrene between the animal species in which the POD was derived and humans are unknown. PBPK models can be useful for the evaluation of interspecies toxicokinetics; however, the benzo[a]pyrene database lacks an adequate model that would inform potential differences. There is some evidence from the oral toxicity data that mice may be more susceptible than rats to some benzo[a]pyrene effects (such as ovotoxicity) (Borman et al., 2000), although the underlying mechanistic basis of this apparent difference is not understood. Most importantly, it is unknown which animal species may be more comparable to humans.

2.3. ORAL SLOPE FACTOR FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure.

2.3.1. Analysis of Carcinogenicity Data

The database for benzo[a]pyrene contains numerous cancer bioassays that identify tumors, primarily of the alimentary tract including the forestomach, following oral exposure in rodents. Three 2-year oral bioassays are available that associate lifetime benzo[a]pyrene exposure with

carcinogenicity at multiple sites: forestomach, liver, oral cavity, jejunum, kidney, auditory canal (Zymbal gland) tumors, and skin or mammary gland tumors in male and female Wistar rats (Kroese et al., 2001); forestomach tumors in male and female Sprague-Dawley rats (Brune et al., 1981); and forestomach, esophageal, tongue, and larynx tumors in female B6C3F$_1$ mice (Beland and Culp, 1998; Culp et al., 1998).

In addition to these 2-year cancer bioassays, there are studies available that provide supporting evidence of carcinogenicity but are less suitable for dose-response analysis due to one or more limitations in study design: (1) no vehicle control group, (2) only one benzo[a]pyrene dose group, or (3) a one-time exposure to benzo[a]pyrene (Benjamin et al., 1988; Robinson et al., 1987; El-Bayoumy, 1985; Wattenberg, 1974; Roe et al., 1970; Biancifiori et al., 1967; Chouroulinkov et al., 1967; Berenblum and Haran, 1955). Of the controlled, multiple dose-group, repeat-dosing studies that remain, most treated animals for <1 year, which is less optimal for extrapolating to a lifetime exposure (Weyand et al., 1995; Triolo et al., 1977; Fedorenko and Yansheva, 1967; Neal and Rigdon, 1967).

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

The Kroese et al. (2001) and Beland and Culp (1998)studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. The rat bioassay by Kroese et al. (2001) and the mouse bioassay by Beland and Culp (1998) were conducted in accordance with Good Laboratory Practice as established by the Organisation for Economic Co-operation and Development (OECD). These studies included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results (including individual animal data).

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

- Squamous cell carcinomas (SCCs) or papillomas of the forestomach or oral cavity in male and female rats;
- SCCs or papillomas of the forestomach, tongue, larynx, or esophagus in female mice;
- Auditory canal carcinomas in male and female rats;
- Kidney urothelial carcinomas in male rats;

- Jejunum/duodenum adenocarcinomas in female and male rats;
- Hepatocellular adenomas or carcinomas in male and female rats; and
- SCCs or basal cell tumors of the skin or mammary gland in male rats.

These tumors were generally observed earlier during the study with increasing exposure levels, and showed statistically significantly increasing trends in incidence with increasing exposure level (Cochran-Armitage trend test, $p \leq 0.001$). These data are summarized in Appendix D of the Supplemental Information. As recommended by the National Toxicology Program (NTP) (McConnell et al., 1986) and as outlined in EPA's *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 the only mid-dose male with an auditory canal tumor did not also have a forestomach or oral cavity squamous cell tumor, and no auditory canal tumors were observed in low-dose male or female rats, the data are insufficient to conclude that the auditory canal tumors occur independently of other tumors. The investigators did not suggest that these tumors were metastases from other sites (in which case, the auditory canal tumors would be repetitions of other tumors, or statistically dependent). Therefore dose-response analysis was not pursued for this site, either separately or in combination with another tumor type.

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

2.3.2. Dose Response Analysis – Adjustments and 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 HEDs on the basis of (body weight)$^{3/4}$ (U.S. EPA, 1992). This was accomplished by multiplying administered doses by (animal body weight (kg)/70 kg)$^{1/4}$ (U.S. EPA, 1992), where the animal body weights were TWAs from each group, and the U.S. EPA (1988) reference body weight for humans is 70 kg. It was not necessary to adjust the administered doses for lifetime equivalent exposure prior to modeling for the groups terminated early, because the multistage-Weibull model characterizes the tumor incidence as a function of time, from which it provides an extrapolation to lifetime exposure.

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

2.3.3. Derivation of the Oral Slope Factor

The PODs estimated for each tumor site are summarized in Table 2-7. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination and are listed in Table 2-7.

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

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

[a]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix E of the Supplemental Information for details of modeling results.

[b]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 twofold higher than the oral slope factor derived from the alimentary tract tumors in male rats.

Although the time-to-tumor modeling helps to account for competing risks associated with decreased survival times and other causes of death including other tumors, considering the tumor sites individually still does not convey the total amount of risk potentially arising from the sensitivity of multiple sites—that is, the risk of developing any combination of the increased tumor types. A method, for estimating overall risk, involving the assumption that the variability in the slope factors could be characterized by a normal distribution, is detailed in Appendix E of the Supplemental Information. The resulting composite slope factor for all tumor types for male rats was 0.5 per mg/kg-day, about 25% higher than the slope factor based on the most sensitive tumor site, oral cavity and forestomach, while for female rats, the composite slope factor was equivalent to that for the most sensitive site (Table 2-7; see Appendix E of Supplemental Information for composite slope factor estimates).

The overall risk estimates from rats and mice spanned about a threefold range. As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result was used to derive the oral slope factor. The recommended slope factor for assessing human cancer risk associated with 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 estimating human risk from benzo[a]pyrene exposure. The rodent forestomach serves to store foods and liquids for several hours before contents continue to the stomach for further digestion (Clayson et al., 1990; Grice et al., 1986). Thus, tissue of the forestomach in rodents may be exposed to benzo[a]pyrene for longer durations than analogous human tissues in the oral cavity and esophagus. This suggests that the rodent forestomach may be quantitatively more sensitive to the development of squamous epithelial tumors in the forestomach compared to oral or esophageal tumors in humans. .

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

the mouse alimentary tract data was about threefold higher than the overall oral slope factor based on male rat data (Table 2-8). These comparisons show that the selection of target organ, animal species, and interspecies extrapolation can impact the oral cancer risk estimate. However, all of the activation pathways implicated in benzo[a]pyrene carcinogenicity have been observed in human tissues, and associations have been made between the spectra of mutations in tumor tissues from benzo[a]pyrene-exposed animals and humans exposed to complex PAH mixtures containing benzo[a]pyrene (see Section 1.1.5).

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

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

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

2.3.5. Previous IRIS Assessment: Oral Slope Factor

The previous cancer assessment for benzo[a]pyrene was posted on the IRIS database in 1992. At that time, benzo[a]pyrene was classified as a probable human carcinogen (Group B2) based on inadequate data in humans and sufficient data in animals via several routes of exposure. An oral slope factor was derived from the geometric mean of four slope factor estimates based on studies of dietary benzo[a]pyrene administered in the diet for approximately 2 years in ten week old Sprague-Dawley rats (Brune et al., 1981) and administered for up 7 months in two week to 5 month old CFW-Swiss mice (Neal and Rigdon, 1967). A single slope factor estimate of 11.7 per mg/kg-day, using a linearized multistage procedure applied to the combined incidence of forestomach, esophageal, and laryngeal tumors, was derived from the Brune et al. (1981) study (see Section 1.1.5 for study details). Three modeling procedures were used to derive risk estimates from the Neal and Rigdon (1967) bioassay (see Section 1.1.5). In an analysis by Clement Associates, commissioned by EPA, U.S. EPA (1990b) fit a two-stage response model, based on exposure-dependent changes in both transition rates and growth rates of preneoplastic cells, to derive a value of 5.9 per mg/kg-day. U.S. EPA (1991b) derived a value of 9.0 per mg/kg-day by linear extrapolation from the 10% response point to the background response in a re-analysis of the 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, which reflected extrapolation to humans assuming surface area equivalence (BW $^{2/3}$ scaling) were within threefold of each other and were judged to be of equal merit. Consequently, the geometric mean of these four estimates, 7.3 per mg/kg-day, was recommended as the oral slope factor.

2.4. INHALATION UNIT RISK FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose

extrapolation procedure. If derived, the inhalation unit risk is a plausible upper bound on the estimate of risk per μg/m^3 air breathed.

2.4.1. Analysis of Carcinogenicity Data

The inhalation database demonstrating carcinogenicity of benzo[a]pyrene consists of a lifetime inhalation bioassay in male hamsters (Thyssen et al., 1981) and intratracheal instillation studies, also in hamsters (Feron and Kruysse, 1978; Ketkar et al., 1978; Feron et al., 1973; Henry et al., 1973; Saffiotti et al., 1972). The intratracheal instillation studies provide supporting evidence of carcinogenicity of inhaled benzo[a]pyrene; however, the use of this exposure method alters the deposition, clearance, and retention of substances, and therefore, studies utilizing this exposure technique are not as useful for the quantitative extrapolation of cancer risk from the inhalation of benzo[a]pyrene in the environment (Driscoll et al., 2000).

The bioassay by Thyssen et al. (1981) represents the only lifetime inhalation cancer bioassay available for describing exposure-response relationships for cancer from inhaled benzo[a]pyrene. As summarized in Section 1.1.5, increased incidences of benign and malignant tumors of the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach were seen with increasing exposure concentration. In addition, survival was decreased relative to control in the high-exposure group; mean survival times in the control, low-, and mid-concentration groups were 96.4, 95.2, and 96.4 weeks, respectively, and 59.5 weeks in the high-exposure group animals. Overall, tumors occurred earlier in the highest benzo[a]pyrene exposure group than in the mid-exposure group.

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

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

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

Second, weekly averages of chamber concentration measurements varied two- to fivefold from the overall average for each group, which exceeds the limit for exposure variability of <20%

for aerosols recommended by OECD (2009). For risk assessment purposes, EPA generally assumes that cancer risk is proportional to cumulative exposure, and therefore to lifetime average exposure as estimated here, when there is no information to the contrary. Under this assumption, the variability of the chamber concentrations has little impact on the estimated exposure-response relationship. The impact of alternative assumptions are considered in Section 2.4.4.

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

2.4.2. Dose Response Analysis—Adjustments and Extrapolation Methods

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

The tumor incidence data used for dose-response modeling comprised the benign and malignant tumors in the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach (tumors of the upper respiratory and digestive tracts; see Table D-16). The tumors in these sites were judged to be sufficiently similar to combine as joint incidences by the following reasoning. While the pharynx and larynx are associated with the upper digestive tract and the upper respiratory tract, respectively, these sites are close anatomically and in some cases where both tissues were affected, the site of origin could not be distinguished (U.S. EPA, 1990a). 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, 1990a). Consequently, 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, as outlined in EPA's Cancer Guidelines (Section 2.2.2.1.2; U.S. EPA, 2005a).

A toxicokinetic model to assist in cross-species scaling of benzo[a]pyrene inhalation exposure was not available. EPA's RfC default dosimetry adjustments (U.S. EPA, 1994) were utilized in the benzo[a]pyrene RfC calculation (see Section 2.2.2) but could not be applied to the aerosols generated for the inhalation bioassay by Thyssen et al. (1981) as the approaches presented in the RfC methodology guidelines (U.S. EPA, 1994) were developed for insoluble and nonhygroscopic particles, not the sodium chloride particle used in Thyssen et al. (1981). Consequently, without data to inform a basis for extrapolation to humans, it was assumed that equal risk for all species would be associated with equal concentrations in air, at least at anticipated environmental concentrations. This is equivalent to assuming that any metabolism of benzo[a]pyrene is directly proportional to breathing rate and that the deposition rate is equal between species.

The multistage-Weibull model was fit to the TWA exposure concentrations and the individual animal tumor and survival data for tumors in the larynx, pharynx, trachea, esophagus, or forestomach (tumors of the upper respiratory and digestive tracts), using the software program MSW (U.S. EPA, 2010). Modeling results are provided in Appendix E of the Supplemental Information.

Because benzo[a]pyrene carcinogenicity involves a mutagenic mode of action, linear low-exposure extrapolation from the $BMCL_{10}$ was used to derive the inhalation unit risk (U.S. EPA, 2005a).

2.4.3. Inhalation Unit Risk Derivation

The results from modeling the inhalation carcinogenicity data from Thyssen et al. (1981) are summarized in Table 2-9. Taking the tumors to have been the cause of death of the experimental animals with tumors, the BMC_{10} and $BMCL_{10}$ 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 with a tumor, the BMC_{10} and $BMCL_{10}$ values 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 $BMDL_{10}$ from the incidental deaths analysis, 0.198 mg/m^3, is recommended for the calculation of 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/m³)	POD= BMCL (mg/m³)	Unit Risk[a] (mg/m³)$^{-1}$
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/$BMCL_{10}$; see Appendix E for details of modeling results.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

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

The hamster inhalation bioassay by Thyssen et al. (1981) observed upper respiratory tract tumors, but not lung tumors. The lack of a lung tumor response in hamsters, given the strong association of inhaled PAH mixtures with lung cancer in humans across many studies (see Section 1.1.5) suggests that this study may not be ideal for extrapolating to humans. Hamsters have an apparent lower sensitivity to lung carcinogenesis than rats and mice and a tendency to give false negatives for particles classified as carcinogenic to humans by IARC (Mauderly, 1997). However, hamster laryngeal tumors have been used as an indication of the carcinogenic hazard of cigarette smoke for more than 50 years (IARC, 2002). For example, a large study investigating the inhalation of cigarette smoke in hamsters (n=4400) indicated that the larynx was the most responsive tumor site, which the authors indicated was due to a large difference in particle deposition between the larynx and the lung (Dontenwill et al., 1973). EPA's Guidelines for Carcinogen Assessment (U.S. EPA, 2005a) stress that site concordance between animals and humans need not always be assumed. Therefore, the robust tumor response in the upper respiratory tract of Syrian golden hamsters was considered to be supportive of the use of the Thyssen et al. (1981) study for the derivation of an inhalation unit risk.

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

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

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

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

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

2.4.5. Previous IRIS Assessment: Inhalation Unit Risk

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

2.5. DERMAL SLOPE FACTOR FOR CANCER

Human and animal studies of exposure to PAH mixtures or benzo[a]pyrene alone demonstrate an increased incidence of skin tumors with increasing dermal exposure. This assessment for benzo[a]pyrene derives a dermal slope factor, a quantitative risk estimate that is a plausible upper bound on the estimate of risk per μg/day of dermal exposure. This derivation provides the first 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; IPCS, 1998; Boffetta et al., 1997; ATSDR, 1995). Although studies of human exposure to benzo[a]pyrene alone are not available, repeated application of benzo[a]pyrene to skin (in the absence of exogenous promoters) has been demonstrated to induce skin tumors in guinea pigs, rabbits, rats, and mice. Given the availability of chronic bioassays of dermal benzo[a]pyrene exposure in mice, this analysis focuses on chronic carcinogenicity bioassays in several strains of mice demonstrating predominantly malignant skin tumors, as well as earlier occurrence of tumors with increasing exposure, following repeated dermal exposure to benzo[a]pyrene for the majority of typical two-year chronic study durations. These studies involved 2- or 3-times/week exposure protocols, at least two exposure levels plus controls, and histopathological examinations of the skin and other tissues (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1960, 1959) (see Tables D-15 to D-23 in the Supplemental Information for study details).

Other carcinogenicity studies in mice were considered as supportive of the studies listed above, but were not considered 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 Hoffmann, 1959; Wynder et al., 1957); (2) bioassays with one benzo[a]pyrene dose level or with only dose levels inducing 90–100% incidence of mice with tumors, which provide relatively little information about the shape of the dose-response relationship (e.g., Wilson and Holland, 1988); and (3) 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.

Regarding study design, these data sets varied in terms of number of exposure levels used (two to nine, compared with three in typical NTP bioassays) and in number of mice per group (from ~17 to 100 mice /dose group, compared with 50 used in most NTP bioassays). While the largest studies would be expected to have greater ability to detect low responses at low doses (Schmidt et al., 1973), studies with smaller group sizes also showed significant dose-response trends involving

lower doses (Sivak et al., 1997; Poel, 1959). One of the data sets, Poel (1960), did not generate data between background and maximal responses (see Table C-16), and thus was not considered further for dose-response modeling, given the availability of other data sets with graded responses which could better support low-dose extrapolation.

Additionally, an important consideration was that none of the available studies reported time on study or tumor status for individual animals. When exposure is associated with early mortality, this level of detail helps to understand the number at risk of development of tumors and the extent of exposure associated with tumor development, and helps to minimize under-estimation of cancer risk. Investigators did report that mortality was increased at higher exposures and that tumors occurred earlier with increasing exposure, but reporting was mostly at the level of dose groups rather than individual animals. These details facilitated some refinement of dose-response results through data adjustments (described in Section 2.5.2), allowing for evaluation of results on a more comparable basis across studies.

Finally, the available studies included different mouse strains, sexes, and vehicles. However, for any given mouse strain only one sex and only one vehicle was tested. Thus, the studies did not support evaluation of whether any particular vehicle solvent enhanced or diminished carcinogenicity, or whether one sex was more sensitive.

Given these considerations, the studies by Roe et al. (1970), Sivak et al. (1997) and Poel (1959) showed the strongest study designs for supporting dose-response analysis, in that they included at least three exposure levels and the lowest doses tested, and reported the actual duration of exposure for each dose group. All but one (Habs et al., 1980) of the remaining studies provided incomplete exposure duration information for approximating lifetime (104-week) equivalent exposures. The study with the most uncertainty for extrapolating to lower exposures may be the Habs et al. (1984) study, which used only two exposure levels, the lower of which was more than tenfold higher than the lowest doses used by Roe et al. (1970), Sivak et al. (1997) and Poel (1959). The reported duration of exposure in the higher dose group was approximately 80 weeks. The high tumor response at the lower dose (~50%) and the uncertainty in characterizing a 104-week equivalent exposure suggests that low-dose extrapolation would be relatively uncertain. Nevertheless, all of these studies were included in the dose-response analysis in order to help characterize similarities among the studies on a quantitative basis.

2.5.2. Dose Response Analysis – Adjustments and Extrapolation 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. Because individual animal data were not available for any of the identified studies, time-to-tumor modeling was not possible. Each of the dermal data sets was modeled using the multistage model, incorporating data adjustments where appropriate, as summarized below.

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

Average daily dose/day = (μg/application) × (number of applications/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 all dose groups in Poel (1959) and Roe et al. (1970), and the highest dose group in Habs et al. (1980), and in Sivak et al. (1997).

Another adjustment made to minimize confounding by mortality was to omit the dose groups with nearly 100% mortality occurring early in the study from dose-response modeling. Poel (1960, 1959) exposed multiple strains of male mice to 7–9 levels of benzo[a]pyrene, with all mice in the dose groups with >3.8 μg/application dying by week 44. For three of the strains—C57L (Poel, 1959), SWR and C3HeB (Poel, 1960)—the remaining four dose groups in addition to control survived most of the two-year exposure period and showed a graded dose response, adequate to support derivation of a slope factor for chronic exposure.

Concerning the incidence data, some of these studies reported incidences of skin tumor-bearing animals for tumors thought to be malignant only (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 lifetime exposure to benzo[a]pyrene were predominantly malignant (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Schmähl et al., 1977; Schmidt et al., 1973). Following EPA's *Guidelines for Carcinogen Risk Assessment* (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, including adjustments, are presented in Tables E-19 through E-22 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 used. 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.

Several data sets provided incomplete information about the length of exposure (Habs et al., 1984; Grimmer et al., 1983; Schmähl et al., 1977; Schmidt et al., 1973; Poel, 1960). Accordingly, dermal slope factors derived from those studies may underestimate cancer risk, due to

overestimation of exposure associated with the development of tumors for the higher exposure groups. In addition, several study designs generated only relatively high dose-response points (Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980), increasing the extent of low-dose extrapolation relative to the other studies. Under the assumption that duration of exposure was not impacted at lower exposures, linear extrapolation from the lowest dose-response point in a study, if the response was higher than 10%, was also used.

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 E 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 used. 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. Overall, model fits demonstrated low statistical variability at the PODs, with BMDLs generally less than twofold lower than corresponding BMDs.

For data sets designed with higher overall exposure ranges (Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980), consideration of PODs based on the lowest response in each study, rather than based on an extrapolated 10% extra risk, led to slope factors less than twofold lower than when based on the $BMDL_{10}$ (see non-shaded portion of Table 2-11). The alternate slope factors (based on BMRs greater than 10%) are less impacted by interpretation of the responses and estimated exposures of the higher exposure groups, but necessarily reflect only an assumption of linearity between the lowest exposure and background responses, which may not be supported.

Dermal slope factors, calculated using linear extrapolation from the $BMDL_{10}$s, ranged from 0.25 to 1.8 per μg/day, a roughly sevenfold range (see Table 2-11). Among the stronger studies (shaded entries in Table 2-11), values for male mice ranged from 0.9 to 1.7 per μg/day, and for female mice from 0.25 to 0.67 per μg/day. Results from the remaining studies (shaded entries in Table 2-11) suggest that some female mouse strains may be as sensitive as some male mouse strains, but the associated uncertainties—e.g., increased extent of low-dose extrapolation and incomplete exposure information—provide less support for relying on many of these values. These four female mice data sets were considered to be the most uncertain because of dose ranges covered and incomplete information regarding length of exposure (Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980), and are summarized in the shaded portion of Table 2-11. In particular, the data set reported by Habs et al. (1984) yielded the most uncertain result with only two dose-response points; the lowest response was 40%, and the slope estimate is determined by the characterization of both exposure estimates.

There was insufficient information to conclude that males were more sensitive because both sexes were not tested for any strain. However, among the three better designed and reported studies (Sivak et al., 1997; Roe et al., 1970; Poel, 1959), male mice were more sensitive than female mice. Consequently, without any information indicating which data set is more relevant for extrapolation to humans, the male mouse results from the higher quality studies (Sivak et al., 1997; Poel, 1959) were selected for the proposed dermal slope factor. The female mouse results (Roe et al., 1970) were not considered further. The average of the $BMDL_{10}$s for the two male data sets was 0.068 µg/day.

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

Reference	Mouse Strain	Selected Model[a]	BMR	BMD (µg/d)	POD= BMDL (µg/d)	Candidate Dermal Slope Factors[b] $(\mu g/d)^{-1}$	Comments
Male mice							
Sivak et al. (1997)	C3H/HeJ	Multistage 2°	10%	0.11	0.058	1.7	Grouped survival data reported
Poel (1959)[a,c]	C57L	Multistage 3°	10%	0.13	0.078	1.3	Grouped survival data reported
Poel (1960)[a,c]	SWR	Multistage 3°	10%	0.13	0.11	0.91	No characterization of survival/exposure duration
Poel (1960)[a,c]	C3HeB	Multistage 1°	10%	0.16	0.11	0.91	No characterization of survival/exposure duration
Female mice							
Roe et al. (1970)	Swiss	Multistage 2°	10%	0.69	0.39	0.25	Grouped survival data reported
Schmidt et al. (1973)	Swiss	Multistage 3°	10%	0.28	0.22	0.45	No characterization of exposure duration
Schmidt et al. (1973)	NMRI	Multistage 2°	10%	0.33	0.29	0.34	No characterization of exposure duration
Schmähl et al. (1977)	NMRI	Multistage 2°	10%	0.23	0.15	0.67	No characterization of exposure duration
Habs et al. (1980)	NMRI	Multistage 4°	10% 30%	0.36 0.49	0.24 0.44	0.42 0.69	Higher overall exposure range; unclear overall duration of exposure
(Habs et al., 1984)	NMRI	Multistage 1°	10% 50%	0.078 0.51	0.056 0.37	1.8 1.4	No characterization of exposure duration for high exposure; high response at lowest exposure limits usefulness of low-dose extrapolation
Grimmer et al. (1983)	CFLP	Multistage 1°	10% 40%	0.24 1.2	0.21 1.0	0.48 0.40	No characterization of exposure duration

Grimmer et al. (1984)[a,c]	CFLP	Log-logistic	70%	1.07	0.48	1.5	No characterization of exposure duration; high response at lowest exposure limits usefulness of low-dose extrapolation

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

2.5.4. Dermal Slope Factor Cross-Species Scaling

Different methodologies have been established for interspecies scaling of PODs used to derive oral slope factors and inhalation unit risks. Cross-species adjustment of oral doses is based on allometric scaling using the ¾ power of body weight. This adjustment accounts for more rapid distribution, metabolism, and clearance in small animals (U.S. EPA, 2005a). Cross-species extrapolation of inhalation exposures is based on standard dosimetry models that consider factors such as solubility, reactivity, and persistence (U.S. EPA, 1994). 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 E 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:

Dermal slope factor = BMR/POD_{HED} = 0.1/(20.3 μg/day) = **0.005 per μg/day**

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., 1998a), 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; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959). This may be because benzo[a]pyrene tends to bind to targets within the skin rather than enter the plasma receptor fluid (a surrogate measure of systemic absorption) in in vitro human skin experiments. These data are consistent with 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; Moody and Chu, 1995). Taken together, these data support the conclusion that the risk of skin cancer following dermal exposure likely outweighs cancer risks at distal organs.

2.5.5. Uncertainties in the Derivation of the Dermal Slope Factor

Uncertainty in the recommended dermal slope factor is partly reflected in the range of POD values derived from the modeled mouse skin tumor data sets: the lowest and highest $BMDL_{10}$ values listed in Table 2-11 show a sevenfold difference (0.058–0.39 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 with a more complex model, such as a time-to-tumor model. Reliance on studies with the lowest exposure levels having low early mortality due to benzo[a]pyrene exposure and exposures continuing 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 that saturate or diminish 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 adducts 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., 1998a). Although none of the lifetime dermal bioassays in mice, which included macroscopic examination of internal organs, reported an elevation of systemic tumors in benzo[a]pyrene-treated mice compared to controls (Higginbotham et al., 1993; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1959), most of these studies attempted to remove animals with grossly observed skin tumors from the study before the death of the animal, possibly minimizing the development of more distant tumors with longer latency. The risk of benzo[a]pyrene-induced point-of-contact tumors in the skin possibly competes with systemic risk of tumors. Currently, the potential contribution of dermally absorbed benzo[a]pyrene to systemic cancer risk is unclear.

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

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of data set ↓ dermal slope factor if alternative data set were selected	Sivak et al. (1997); Poel (1959)	Both studies included lowest doses among available studies (where intercurrent mortality was less likely to impact the number at risk) and used typical group sizes (up to 50/group).
Selection of target organ No dermal slope factor if skin tumor studies not used	Selection of skin tumors	Skin tumors were replicated in numerous studies of male or female mice. No studies were available indicating that other tumors occur following dermal exposure.

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of dose metric Alternatives could ↓ or ↑ slope factor	Administered dose, as TWA in µg/d	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified.
Interspecies extrapolation Alternatives could ↓ or ↑ slope factor	Total daily dose scaled by $BW^{3/4}$	Alternatives discussed in Appendix E. An established methodology does not exist to adjust for interspecies differences in dermal toxicity at the point of contact. Benzo[a]pyrene metabolism is known to occur in the dermal layer. Viewing the skin as an organ, metabolic processes were assumed to scale allometrically without evidence to the contrary.
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 mode of action)	Available mode of action data support linearity (mutagenicity is a primary mode of action of benzo[a]pyrene).
Sensitive subpopulations ↑ dermal slope factor to unknown extent	ADAFs are recommended for early life exposures	No chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity.

2.5.6. Previous IRIS Assessment: Dermal Slope Factor

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

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

Based on sufficient support in laboratory animals and relevance to humans, benzo[a]pyrene is determined to be carcinogenic by a mutagenic mode of action. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* ("*Supplemental Guidance*") (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to have increased risk for cancer. The oral slope factor of 1 per mg/kg-day, inhalation unit risk of 0.5 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. Sample application of ADAFs for the estimation of benzo[a]pyrene cancer risk following lifetime (70-year) oral exposure

Age Group	ADAF	Unit Risk (per mg/kg-d)	Sample Exposure Concentration (mg/kg-d)	Duration Adjustment	Cancer Risk for 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	0.0008
Total risk					0.002

The example exposure duration scenarios include full lifetime exposure (assuming a 70-year lifespan). Table 2-13 lists the four factors (ADAFs, cancer risk estimate, assumed exposure, and duration adjustment) that are needed to calculate the age-specific cancer risk based on the early age-specific group. The cancer risk for each age group is the product of the four factors in columns 2–5. Therefore, the cancer risk following daily benzo[a]pyrene oral exposure in the age group 0–<2 years is the product of the values in columns 2–5 or $10 \times 1 \times 0.001 \times 2/70 = 3 \times 10^{-4}$. The cancer risk for specific exposure duration scenarios that are listed in the last column are added together to get the total risk. Thus, a 70-year (lifetime) risk estimate for continuous exposure to 0.001 mg/kg-day benzo[a]pyrene is 2×10^{-3}, which is adjusted for early-life susceptibility and assumes a 70-year lifetime and constant exposure across age groups.

In calculating the cancer risk for a 30-year constant exposure to benzo[a]pyrene at an exposure level of 0.001 mg/kg-day for ages 0–30 years, the duration adjustments would be 2/70, 14/70, and 14/70, and the age-specific risks for the three age groups would be 3×10^{-4}, 6×10^{-4}, and 2×10^{-4}, which would result in a total risk estimate of 1×10^{-3}.

In calculating the cancer risk for a 30-year constant exposure to benzo[a]pyrene at an exposure level of 0.001 mg/kg-day for ages 20–50 years, the duration adjustments would be 0/70,

0/70, and 30/70. The age-specific risks for the three groups are 0, 0, and 4×10^{-4}, which would result in a total risk estimate of 4×10^{-4}.

Consistent with the approaches for the oral route of exposure (Table 2-13), the ADAFs should also be applied when assessing cancer risks for subpopulations with early life exposures to benzo[a]pyrene via the inhalation and dermal routes (presented in Tables 2-14 and 2-15).

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

Age Group	ADAF	Unit Risk (per µg/m^3)	Sample Exposure Concentration (µg/m^3)	Duration Adjustment	Cancer Risk for 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. Sample application of ADAFs for the estimation of benzo[a]pyrene cancer risk following lifetime (70-year) dermal exposure

Age Group	ADAF	Unit Risk (per µg/d)	Sample Exposure Concentration (µg/d)	Duration Adjustment	Cancer Risk for 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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