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Provisional Peer-Reviewed Toxicity Values for

Benzo[e]pyrene (BeP)
(CASRN 192-97-2)

U.S. EPA Office of Research and Development
Center for Public Health and Environmental Assessment

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Center for Public Health and Environmental Assessment
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Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) website at https://ecomments.epa.gov/pprtv.

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
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
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-*S*-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure-activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR BENZO[E]PYRENE (CASRN 192-97-2)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund program. PPRTVs are derived after a review of the relevant scientific literature using established U.S. Environmental Protection Agency (U.S. EPA) guidance on human health toxicity value derivations.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

Currently available PPRTV assessments can be accessed on the U.S. EPA's PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing adverse human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

QUALITY ASSURANCE

This work was conducted under the U.S. EPA Quality Assurance (QA) program to ensure data are of known and acceptable quality to support their intended use. Surveillance of the work by the assessment managers and programmatic scientific leads ensured adherence to QA processes and criteria, as well as quick and effective resolution of any problems. The QA manager, assessment managers, and programmatic scientific leads have determined under the QA program that this work meets all U.S. EPA quality requirements. This PPRTV was written with guidance from the CPHEA Program Quality Assurance Project Plan (PQAPP), the QAPP titled *Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents (L-CPAD-0032718-QP),* and the PPRTV development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP).* As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

All PPRTV assessments receive internal peer review by at least two CPHEA scientists and an independent external peer review by at least three scientific experts. The reviews focus on whether all studies have been correctly selected, interpreted, and adequately described for the purposes of deriving a provisional reference value. The reviews also cover quantitative and qualitative aspects of the provisional value development and address whether uncertainties associated with the assessment have been adequately characterized.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and

limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. EPA programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA ORD CPHEA website at https://ecomments.epa.gov/pprtv.

1. INTRODUCTION

Benzo[e]pyrene (BeP), CASRN 192-97-2, belongs to the polycyclic aromatic hydrocarbon (PAH) class of chemicals. PAHs are typically complex mixtures of several PAH compounds that occur naturally in fossil fuels, crude oil, and bituminous coal (NLM, 2019a). BeP is also formed as a product of incomplete combustion of organic matter; it has been found in smoke from tobacco and marijuana cigarettes and in emissions from burning coal, wood, oil, and garbage. BeP has also been detected in both fresh and used motor oils, gasolines, and smoked and cooked food (NLM, 2019a). BeP is purified for use in research laboratories but has no other known uses and is not listed on the U.S. EPA's Toxic Substances Control Act (TSCA) public inventory (U.S. EPA, 2018b). It is registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program as a member of the PAH group of chemicals and has a Harmonised Classification Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation) listing, which requires manufacturers, importers, or downstream users to classify, label, and package BeP before placing it on the market (ECHA, 2019a, b).

The empirical formula for BeP is $C_{20}H_{12}$ and its structure is shown in Figure 1. A table of physicochemical properties for BeP is provided in Table 1. BeP is a crystalline solid with negligible water solubility and low vapor pressure. A Henry's law constant of 1.07×10^{-6} atm-m^3/mol calculated from the extrapolated vapor pressure of 5.7×10^{-9} mm Hg and measured water solubility of 1.89×10^{-8} mol/L indicates low volatilization of BeP from moist soil and water. No volatilization from dry soil is expected based on the vapor pressure. In the atmosphere, BeP will exist primarily in the particulate phase and to a lesser extent in the vapor phase. The particulate phase BeP in the atmosphere will be removed by wet and dry deposition. BeP absorbs ultraviolet (UV) wavelengths >290 nm; however, removal by direct photodegradation is not expected to be an important pathway based on a measured half-life of 21.1 days in the presence of simulated sunlight (NIST, 2019; Katz, 1979). The rate of photodegradation is expected to increase in the presence of oxidative species, such as oxygen and ozone. The chemical structure of BeP lacks functional groups susceptible to hydrolysis under environmental conditions; therefore, hydrolysis is not expected to be an important degradation route in aqueous environments. BeP is expected to adsorb suspended solids and sediment in water and will be immobile in soil based on a predicted K_{oc} value of 4.66×10^5 in soil. Sorption of PAH compounds, such as BeP, by soil organic matter will limit the potential for biodegradation (WHO, 1998). Limited biodegradation data for BeP indicate slow removal in water and soil under aerobic and anaerobic conditions, with a reported half-life of 875 days in petroleum-contaminated sediment and no removal in an anaerobic sludge digestion study [Kirk and Lesterm (1990) and Callahan et al. (1979) as cited in NLM (2019a)].

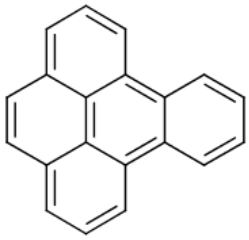


Figure 1. Benzo[e]pyrene (CASRN 192-97-2) Structure

Table 1. Physicochemical Properties of BeP (CASRN 192-97-2)[a]

Property (unit)	Value
Physical state	Solid
Boiling point (°C)	469 (predicted average)
Melting point (°C)	178
Density (g/cm^3)	1.28 (predicted average)
Vapor pressure (mm Hg)	5.7×10^{-9}
pH (unitless)	NA
Acid dissociation constant (pKa) (unitless)	NA
Solubility in water (mol/L)	1.89×10^{-8}
Octanol-water partition constant (log K_{ow})	6.44
Henry's law constant (atm-m^3/mol)	1.07×10^{-6} (predicted average)
Soil adsorption coefficient (K_{oc}) (L/kg)	4.66×10^{5} (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec)	3.45×10^{-11} (predicted average)
Atmospheric half-life (d)	21.10 (measured in simulated sunlight)[b]
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	252.316
Flash point (°C)	230 (predicted average)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard: Benzo[e]pyrene, CASRN 192-97-2; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3023764#properties; accessed February 8, 2021. Data presented are experimental averages unless otherwise noted.
[b]Katz (1979).

BeP = benzo[e]pyrene; NA = not applicable; NV = not available.

A summary of available toxicity values for BeP from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for BeP (CASRN 192-97-2)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference[c]
Noncancer			
IRIS	NV	NA	U.S. EPA (2020a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2018a)
ATSDR (MRL)	NV	No MRLs were derived for BeP	ATSDR (1995)
IPCS	NV	NA	IPCS (2020)
CalEPA	NV	NA	CalEPA (2019)
OSHA	NV	NA	OSHA (2020); OSHA (2020)
NIOSH	NV	NA	NIOSH (2018)
ACGIH	NV	NA	ACGIH (2020)
Cancer			
IRIS	NV	NA	U.S. EPA (2020a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2018a)
NTP	NV	NA	NTP (2016)
IARC (WOE)	Group 3: not classifiable as to its carcinogenicity to humans	Based on inadequate evidence in experimental animals for carcinogenicity	IARC (2010)
CalEPA	NV	NA	CalEPA (2019)
ACGIH	NV	NA	ACGIH (2020)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.
[b]Parameters: MRL = minimal risk level; WOE = weight of evidence.
[c]Reference date is the publication date for the database and not the date the source was accessed.

BeP = benzo[e]pyrene; NA = not applicable; NV = not available.

Literature searches were conducted in August 2019 and updated in August 2021 for studies relevant to the derivation of provisional toxicity values for BeP, CASRN 192-97-2. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE[1] (including TSCATS1), and Web of Science. The following resources were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists

[1]Note that this version of TOXLINE is no longer updated (https://www.nlm.nih.gov/databases/download/toxlinesubset.html); therefore, it was not included in the literature search update from August 2021.

(ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), U.S. EPA TSCATS2/TSCATS8e, U.S. EPA High Production Volume (HPV), Chemicals via IPCS INCHEM, Japan Existing Chemical Data Base (JECDB), Organisation for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

2. REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

As summarized in Tables 3A and 3B, no short-term, subchronic, or chronic studies as well as reproductive and developmental toxicity studies of BeP in humans or animals exposed by oral or inhalation routes adequate for deriving provisional toxicity values have been identified. The phrase "statistical significance" and term "significant," used throughout the document, indicate a *p*-value of < 0.05 unless otherwise specified.

Table 3A. Summary of Potentially Relevant Noncancer Data for BeP (CASRN 192-97-2)							
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**NOAEL**	**LOAEL**	**Reference (comments)**	**Notes**
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							

BeP = benzo[e]pyrene; LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level.

Table 3B. Summary of Potentially Relevant Cancer Data for BeP (CASRN 192-97-2)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference (comments)**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

BeP = benzo[e]pyrene; ND = no data.

2.1. HUMAN STUDIES

2.1.1. Oral Exposures

No studies have been identified.

2.1.2. Inhalation Exposures

No studies have been identified.

2.2. ANIMAL STUDIES

2.2.1. Oral Exposures

No studies have been identified.

2.2.2. Inhalation Exposures

No studies have been identified.

2.3. OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Available toxicity data for BeP include human occupational studies of exposure to complex mixtures that are of limited use for hazard identification and do not provide adequate exposure data for dose-response assessment. Two case-control studies found no associations between asthma or lung cancer incidence and serum BeP concentrations following BeP exposure. There is one subchronic study in apolipoprotein E knockout (apoE-KO) mice and two studies in avian models evaluating atherosclerosis. An acute oral study evaluated biochemical markers of hepatic damage. There are also dermal and injection studies evaluating immunotoxicity and carcinogenicity. Other available data include in vitro assays evaluating immunotoxic potential and retinal cell damage, genotoxicity assays, and toxicokinetic studies. Because the overall database for BeP was determined to be inadequate for direct derivation of oral or inhalation reference values, supporting studies described below were not considered for point of departure (POD) identification.

2.3.1. Genotoxicity

The genotoxicity of BeP has been evaluated in numerous in vitro studies and in a limited number of in vivo studies. Available studies are summarized below (see Table 4A for more details). The data indicate that BeP has mutagenic activity following metabolic activation and is not mutagenic in the absence of activation. In general, BeP did not cause chromosomal damage in vitro; however, findings from in vivo studies are mixed and suggest that, under certain conditions, BeP can cause chromosomal damage. BeP (or a metabolite) forms DNA adducts; however, most available in vitro and in vivo studies do not indicate that BeP alters DNA damage/synthesis/repair.

Mutagenicity

Of the available mutagenicity studies in *Salmonella typhimurium*, 15/20 reported that BeP was positive following metabolic activation in one or more strains (see Table 4A). In general, BeP yielded borderline or relatively low mutation rates compared with other mutagens (e.g., other PAHs) (Zeiger et al., 1992; De Flora et al., 1984; Dunkel et al., 1984; Haas et al., 1981; Kaden et al., 1979; Simmon, 1979; Wood et al., 1979; Andrews et al., 1978; McCann et al., 1975). One experiment that reported negative results for BeP with metabolic activation was part of a larger study comparing the results of experiments from four separate laboratories using the same protocol. The other three experiments all found positive results (Dunkel et al., 1984).

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* TA100	0, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, 1,000 µg/plate	NS	+	Plate incorporation method. Criterion for positive response was dose-response (significant linear regression). The study authors reported that BeP was positive with S9 activation. The number of revertants was increased >twofold at 1,000 µg with S9 activation (data not reported for lower doses). Results without metabolic activation were not specified.	Andrews et al. (1978)
Mutation	*S. typhimurium* TA98, TA100, and TA104	0, 0.1, 0.5, 1.0, 2.5, 5.0, 10 µg/plate	NDr	+ (TA100) – (TA98, TA104)	Plate incorporation method. Criterion for positive response was not reported.[b] Metabolic activation was tested at three S9 concentrations (0.08, 0.22, and 0.95 mg/plate). Based on graphically presented data, the number of revertants was increased >twofold at 10 µg/plate at the highest S9 concentration in TA100 only.	Ball et al. (1991)
Mutation	*S. typhimurium* TA98	0, 25, 50, 100, 200 nmol/plate	–	NDr	Plate incorporation method. Criterion for positive response was not reported.[b] No evidence of mutagenicity without metabolic activation. A positive control was not used (primary purpose of study was to evaluate influence of PAHs on mutagenicity of 1-nitropyrene).	Cherng et al. (1996)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, TA1538	Various dilutions using geometric ratio of 2, starting from solubility or toxicity limit	+ (TA98, TA100) ± (TA97, TA1537) – (TA1535, TA1538)		Plate incorporation method. Criterion for positive response was >threefold increase in revertants (two- to threefold increase considered weak positive, 1.5- to twofold increase considered "reproducible," <1.5-fold considered negative). Based on this criterion, TA98 and TA100 were positive, TA97 and TA1537 were "reproducible," and TA1535 and TA1538 were negative; S9 activation increased mutagenicity. It is not clear from data presentation if reported fold-changes are with or without S9 activation. Mutagenetic potential in TA100 = 1.6 revertants/nmol with S9 activation.	De Flora et al. (1984)
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0, 333.3 µg/plate	–	+ (TA98, TA100, TA1538) ± (TA1535) – (TA1537)	Plate test. IRI laboratory. All strains tested with metabolic activation (using rat [R], mouse [M], and hamster [H] liver S9; either uninduced [U] or Arochlor induced [I]) and without activation. Criterion for positive response was ≥twofold increase at two or more doses; ≥twofold increase in revertants at only one dose was considered equivocal. BeP was positive with metabolic activation in TA98 (RI), TA100 (RI), and TA1538 (HU) and equivocal with metabolic activation in TA1535 (MU). No evidence of mutagenicity without metabolic activation.	Dunkel et al. (1984)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0, 333.3 µg/plate	–	–	Plate test. LBI laboratory. All strains tested with metabolic activation (RU, RI, MU, MI, HU, HI) and without activation. Criterion for positive response was ≥twofold increase at two or more doses; ≥twofold increase in revertants at only one dose was considered equivocal. No evidence of mutagenicity with or without metabolic activation. Results for positive controls (sodium azide, 9-aminoacridine, 2-nitrofluorene, 2-aminoanthracene) were as expected.	Dunkel et al. (1984)
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0, 333.3 µg/plate	–	+ (TA98) ± (TA1537, TA1538) – (TA100, TA1535)	Plate test. NYM laboratory. All strains tested with metabolic activation (RU, RI, MU, MI, HU, HI) and without activation. Criterion for positive response was ≥twofold increase at two or more doses; ≥twofold increase in revertants at only one dose was considered equivocal. BeP was positive with metabolic activation in TA98 (RI) and equivocal with metabolic activation in TA1537 (MI) and TA1538 (HU). No evidence of mutagenicity without metabolic activation.	Dunkel et al. (1984)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0, 333.3 µg/plate	–	+ (TA98, TA100, TA1537) ± (TA1538) – (TA1535)	Plate test. SRI laboratory. All strains tested with metabolic activation (RU, RI, MU, MI, HU, HI) and without activation. Criterion for positive response was ≥twofold increase at two or more doses; ≥twofold increase in revertants at only one dose was considered equivocal. BeP was positive with metabolic activation in TA98 (RI, MU), TA100 (RI) and TA1537 (MI) and equivocal with metabolic activation in TA1538 (MI). No evidence of mutagenicity without metabolic activation.	Dunkel et al. (1984)
Mutation	*S. typhimurium* TA98, TA100	0.2−2.5 µg/plate (TA98) 0.2−1.5 µg/plate (TA100)	NDr	+ (TA100) – (TA98)	Plate incorporation method. Criterion for positive response was not reported, but the study authors reported a "significant" mutagenic response in TA100 with S9 activation. No evidence of mutagenicity in TA98 with S9 activation.	Haas et al. (1981)
Mutation	*S. typhimurium* TM677	NS	NDr	+	8-Azaguanine resistance forward mutation assay. Criterion for positive response was ≥3 SDs from the background mean. A positive response was observed at ≥90 µM with S9 activation.	Kaden et al. (1979)
Mutation	*S. typhimurium* TA98, TA100	0, 10, 20 µg/plate	NDr	+ (TA100) – (TA98)	Plate incorporation method. Criterion for positive response was not reported.[b] Revertants increased >twofold in TA100 with S9 activation. No evidence of mutagenicity in TA98 with S9 activation.	Lavoie et al. (1979)
Mutation	*S. typhimurium* TA100	10−333 µg/plate	NDr	–	Plate incorporation method. Criterion for positive response was reversion frequency >2 times the spontaneous rate. BeP was negative with metabolic activation using liver homogenates from mice pretreated with corn oil, BeP, or TCDD.	Ma et al. (1991)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA100	NS	NDr	+	Plate incorporation method. Criterion for positive response was >0.1 revertants/mol, weak response was <0.1 but >0.01 revertants/mol, and negative response was <0.01 revertants/mol. BeP mutation rate with S9 activation was 0.60 revertants/nmol (143 revertants/60 µg tested).	McCann et al. (1975)
Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	0.1−1,000 µg/plate	– (TA100)	± (TA100)	Plate incorporation method. Criterion for positive response was >twofold increase in revertants. A twofold increase in revertants was observed in TA100 with S9 activation. Mutagenic response was reported as highest in TA100; number of revertants/plate not reported for other strains.	Salamone et al. (1979)
Mutation	*S. typhimurium* TA97, TA98, TA100	0, 5, 10, 50, 250 µg/plate	–	+	Plate incorporation method. Criterion for positive response was not reported.[b] Revertants increased >twofold in all strains with S9 activation. No evidence of mutagenicity without S9 activation.	Sakai et al. (1985)
Mutation	*S. typhimurium* TA1535, TA1536, TA1537, TA1538, TA98, TA100	≤50 µg/plate	–	+ (TA1538, TA98, TA100) – (TA1535, TA1536, TA1537)	Plate incorporation method. Criterion for positive result was a reproducible, dose-related increase in the number of revertants. A positive result was observed for TA1538, TA98, and TA100 with S9 activation. No evidence of mutagenicity without S9 activation.	Simmon (1979)
Mutation	*S. typhimurium* TA1535, TA1536, TA1537, TA1538	0, 50 µg/plate	NDr	–	Plate incorporation method. Criterion for positive response was a significant increase in the number of revertants. BeP was negative with metabolic activation using liver homogenates from rats pretreated with phenobarbital. A positive control was not used, but other tested PAHs induced significant increases in the number of revertants.	Teranishi et al. (1975)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA98, TA100	10 nmol/plate	NDr	±	Plate incorporation method. Criterion for positive response was not reported, but BeP was reported as weakly mutagenic in TA100 with S9 activation (rat liver microsomes), although not when incubated with purified rat hepatic CYP450. BeP was also weakly mutagenic in TA98 when activated by purified rat hepatic CYP450, but not S9.	Wood et al. (1979)
Mutation	*S. typhimurium* TA98 and its NR-deficient strains TA98NR and TA98/I,8-DNP6	0, 10 µg/plate	–	NDr	Plate incorporation assay. Criterion for positive response was ≥twofold increase in revertants. Mutagenic potential tested with and without irradiation (artificial sunlight or cool-white light). No evidence of mutagenicity with or without irradiation. Positive control (BaP) produced expected increase in revertants without irradiation (not tested with irradiation).	White et al. (1985a)
Mutation	*S. typhimurium* TA98, TA100	0, 3.3, 10, 33, 100, 333, 1,000, 2,000 µg/plate	–	+	Preincubation assay. Criterion for a positive response was a reproducible, dose-related response in replicate trials. BeP was positive in TA98 and TA100 with rat S9 activation. No evidence of mutagenicity without metabolic activation or with hamster S9 activation.	Zeiger et al. (1992)
Mutation	*Escherichia coli* WP2 *uvr*A	0, 0.3, 1.0, 3.3, 10.0, 33.3, 100.0, 333.3 µg/plate	–	–	Plate test. *E. coli* was tested with metabolic activation (RU, RI, MU, MI, HU, HI) and without activation at four separate laboratories (IRI, LBI, NYM, SRI). All laboratories: No evidence of mutagenicity with or without metabolic activation.	Dunkel et al. (1984)
DNA repair	*E. coli* WP2, WP67, C871	≤50 µg (eight twofold dilutions)	–	–	Liquid micromethod. The minimal inhibitory concentration was >50 µg with or without S9 activation.	De Flora et al. (1984)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
DNA repair	*E. coli* WP2, WP67, C871	≤50 µg/10^3 bacteria	–	±	2-h preincubation assay (treat-and-plate method). Survival did not differ between repair-deficient and wild-type strains without metabolic activation. Findings were equivocal with S9 activation.	De Flora et al. (1984)
DNA damage	*E. coli* PQ37	0, 0.156, 0.625, 2.5, 10.0 µg/assay	–	±	SOS chromotest. 1.9-fold increase in DNA damage with S9 activation (>twofold considered positive). Findings were negative without S9 activation.	Mersch-Sundermann et al. (1993); Mersch-Sundermann et al. (1992)
Genotoxicity studies in mammalian cells—in vitro						
Mutation	Human B-lymphoblastoid cells (h1A1v2); RPMI 1640 medium with 9% horse serum	0, 10, 100, 1,000, 10,000 ng/mL	+	NDr	Forward mutation assay (thymidine kinase locus). A twofold increase in mutations was observed at 10,000 ng/mL; this dose was associated with 33% cytotoxicity. The minimum mutagenic concentration was calculated to be 8,000 ng/mL (dose that exceeds the 99% upper confidence limit of historical negative control). No exogenous metabolic activation was used, but h1A1v2 cells constitutively express cytochrome P4501A1.	Durant et al. (1996)
Mutation	Adult rat liver epithelial (ARL18) cells; culture media NS	0, 100 µM	–	NDr	*Hgprt* forward mutation assay. No evidence of mutagenicity without exogenous metabolic activation (ARL18 cells have endogenous metabolic capabilities). Cell survival was not reported.	Ved Brat et al. (1983)
Mutation	Chinese hamster lung fibroblast (V79 cells); Eagle's medium and 5−10% FBS	0, 1 µg/mL	NDr	–	Cell-mediated mutagenesis assay. No evidence of mutagenicity with metabolic activation (V79 cells were co-cultured with metabolically competent rodent cells to produce reactive metabolites during exposure). Cell survival was not reported.	Huberman (1977)
SCE	Adult rat liver epithelial (ARL18) cells; culture media NS	0, 10 µM	–	NDr	No evidence of SCE induction without exogenous activation (ARL18 cells have endogenous metabolic capabilities).	Ved Brat et al. (1983)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
SCE	SHE cells; DMEM with 0, 10, or 40% FBS	0, 0.1 µg/mL	±	NDr	SHE cells were exposed to 10 µg BeP for 24 hours; postexposure, the organic phase was extracted to the culture medium and added to V79 cells (BeP was not metabolized by SHE cells during organic phase). A 1.4-fold increase in SCEs was observed when cells were exposed to organic phase extraction with 0% serum in the media; SCE induction was prevented with 10−40% FBS in the media. The study authors propose the observed decrease in cellular uptake was due to binding of BeP to serum proteins.	Coulomb et al. (1981)
SCE	Chinese hamster V79 cells; serum-free M199 medium	0, 1 µg/mL	–	–	No induction of SCEs with or without metabolic activation (V79 cells were cocultured with metabolically competent rat mammary epithelial cells to produce reactive metabolites during exposure).	Mane et al. (1990)
MN	Wistar rat skin fibroblasts (UGT-normal or UGT-deficient); DMEM with 13% FBS	0, 10 µM	– (UGT-deficient)	– (UGT-normal)	No induction of MN in UGT-normal or UGT-deficient fibroblasts.	Vienneau et al. (1995)
DNA damage	Human skin fibroblasts; MEM with 20% FBS	0, 13 µM	–	NDr	No significant induction of DNA breaks.	Milo et al. (1978)
DNA damage	Chinese hamster V79 cells; DMEM with 5% FBS	0, 10, 20, 50, 100 µM	+	NDr	Comet test (alkaline version). DNA strand breaks increased >twofold without metabolic activation at 100 µM.	Platt et al. (2008)
DNA synthesis	Human skin fibroblasts; MEM with 20% FBS	0, 13 µM	–	NDr	^{3}HTdR incorporation assay. No significant inhibition of scheduled DNA synthesis.	Milo et al. (1978)
DNA synthesis	Rat mammary epithelial cells; serum-free M199 medium	5 µg/mL	–	NDr	^{3}HTdR incorporation assay. No significant inhibition of scheduled DNA synthesis.	Mane et al. (1990)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
DNA synthesis	F344 rat hepatocytes; MEM with 25% HPRS	0, 1, 10, 100 µM	–	NDr	^{3}HTdR incorporation assay. No significant inhibition of scheduled DNA synthesis.	Novicki et al. (1985)
DNA synthesis	Sprague Dawley rat hepatocytes; L-15 medium with 10% FBS	0, 1, 100, 1,000 nM	–	NA	^{3}HTdR incorporation assay. No significant inhibition of scheduled DNA synthesis.	Zhao and Ramos (1995)
UDS	Adult rat hepatocytes; serum-free William's medium E	≤100 nmol/mL	–	NDr	Hepatocyte primary culture-DNA repair test. No evidence of increased UDS in the absence of exogenous metabolic activation (hepatocytes are metabolically competent).	Probst et al. (1981)
UDS	Adult mouse lung fibroblasts; DMEM with 10% FBS	0, 1, 10, 100 µM	–	–	No evidence of increased UDS with or without S9 activation.	Schmitt et al. (1984)
UDS	SHE cells; serum- and arginine-free medium	0, 2.5, 5, 10, 20 µg/mL	–	NDr	No evidence of increased UDS.	Casto et al. (1976)
UDS	Syrian hamster tracheal organ cultures; serum-free CMRL medium 1066	0, 0.1, 1.0 µg/mL	–	NDr	No evidence of increased UDS in the absence of exogenous metabolic activation (tracheal organ cultures are metabolically competent).	Schiff et al. (1983)
Cell transformation	SHE cells; DMEM with 10% FBS	0, 10 µg/mL	±	NDr	BeP treatment produced 0.6% transformation and was considered "slightly effective." Cloning efficiency (6.8%) was comparable to control (7.0%).	DiPaolo et al. (1969)
Genotoxicity studies—in vivo						
CA (bone marrow)	Female Long-Evans rats exposed to BeP via i.v. infusion and sacrificed 24 h postinjection	0, 40 mg/kg	+	NA	>Threefold increase in chromosomal breaks at 24 h postinjection.	Rees et al. (1970)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
CA (bone marrow)	Male and female Chinese hamsters were exposed to BeP via i.p. injection; hamsters were administered two injections, 24 h apart; hamsters were sacrificed 24 h after 2nd injection	0, 450 mg/kg	–	NA	No significant induction of CAs.	Roszinsky-Köcher et al. (1979)
SCE (bone marrow)	Male and female Chinese hamsters were exposed to BeP via i.p. injection; hamsters were administered two injections, 24 h apart; hamsters were sacrificed 24 h after 2nd injection	0, 450 mg/kg	+	NA	Significant 1.4-fold increase in SCEs per metaphase.	Roszinsky-Köcher et al. (1979)
MN (bone marrow)	Female mice (C57BL/6, BALB/c, DBA/2, BDF1, and CDF1 hybrids) were exposed once to BeP via i.p. injection and sacrificed 24, 48, or 72 h postinjection	0, 100 mg/kg	–	NA	No significant induction of MN in any mouse strain at any time point.	Sato et al. (1987)
DNA damage (liver)	Female Sprague Dawley rats exposed twice to BeP via gavage in corn oil; the 1st dose was administered 21 h prior to sacrifice and the 2nd dose was administered 4 h prior to sacrifice	0, 18 mg/kg	–	NA	Alkaline elution assay. No significant hepatic DNA damage.	Kitchin et al. (1993); Kitchin et al. (1992)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
DNA synthesis (thymus, spleen, bone marrow)	Male Wistar rats exposed to BeP via i.p. injection in olive oil and sacrificed at 24 and 48 h postinjection. ^{3}HTdR i.m. injections given 30 min prior to sacrifice	0.19 µmol/g	+	NA	^{3}HTdR incorporation analysis. DNA synthesis was significantly decreased by 64.5−67.8% in thymus and 31.0−50.3% in spleen at 24- and 48-h postexposure. DNA synthesis was significantly decreased by 21.2% in bone marrow at 24-h postexposure only. The study authors note that decreased ^{3}HTdR incorporation may reflect cytotoxicity instead of decreased DNA synthesis.	Prodi et al. (1975)
DNA synthesis (liver)	Male Wistar rats exposed to BeP via i.p. injection in olive oil 2 h after a partial hepatectomy; rats sacrificed 22 h postexposure; ^{3}HTdR i.m. injections given 30 min prior to sacrifice	0.19 µmol/g	+	NA	^{3}HTdR incorporation analysis. DNA synthesis was significantly decreased by 39.4% in regenerating liver following BeP exposure. The study authors note that decreased ^{3}HTdR incorporation may reflect cytotoxicity instead of decreased DNA synthesis.	Prodi et al. (1975)
DNA adducts	Female BALB/c mice exposed to BeP via four topical applications at 0, 6, 30, and 54 h; sacrificed 24 h after last treatment	1.2 µmol	+	NA	^{32}P-postlabeling analysis. Five adducts were detected in mouse skin at a level of 1 adduct per $>10^{7}$ normal nucleotides.	Reddy et al. (1984)

Table 4A. Summary of BeP (CASRN 192-97-2) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies in invertebrates-in vivo						
DNA repair	*Drosophila melanogaster*; *Rec*$^-$ males and *Rec*$^+$ females were exposed as larvae and scored as adults	Up to 100 mg/mL	–	NA	A reduction in *Rec*$^-$ larvae would indicate DNA damage from the test compound. There was no significant reduction in the *Rec*$^-$:*Rec*$^+$ ratio.	Fujikawa et al. (1993)
Genotoxicity studies in subcellular systems						
DNA adducts	Human DNA	100 µM	NDr	+	^{32}P-postlabeling assay with nuclease P1 treatment or butanol extraction for enrichment of adducts. BeP adducts were detected with both procedures, but ~50% more adducts were detected with butanol extraction.	Segerbäck and Vodicka (1993)

[a]+ = positive; ± = weakly positive/equivocal; – = negative.
[b]For bacterial mutagenicity studies that did not report criteria for positive results, an induction of revertants >twofold was considered positive for the purposes of this review.

^{3}HTdR = [^{3}H]Thymidine; BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; CA = chromosomal aberration; CMRL = Connaught Medical Research Laboratories Medium 1066; CYP450 = cytochrome P450; DMEM = Dulbecco's modified minimal essential medium; DNA = deoxyribonucleic acid; FBS = fetal bovine serum; HI = Arochlor-induced hamster liver S9 protein; HPRS = hepatectomized rat serum; HU = uninduced hamster liver S9 protein; i.m. = intramuscular; i.p. = intraperitoneal; IRI = Inveresk Research International; i.v. = intravenous; LBI = Litton Bionetics, Inc.; MEM = minimal essential medium; MI = Arochlor-induced mouse liver S9 protein; MN = micronuclei; MU = uninduced mouse liver S9 protein; NA = not applicable; NDr = not determined; NR = nitroreductase; NS = not specified; NYM = New York Medical College; PAH = polycylic aromatic hydrocarbon; RI = Arochlor-induced rat liver S9 protein; RU = uninduced rat liver S9 protein; SCE = sister chromatid exchange; SD = standard deviation; SHE = Syrian hamster embryo; SRI = SRI International; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UDS = unscheduled DNA synthesis; UGT = uridine 5′-diphospho-glucuronosyltransferase.

Of the remaining studies, BeP was not mutagenic when metabolic activation was from liver homogenates of rats pretreated with phenobarbital (Teranishi et al., 1975) or corn oil, BeP, or tetrachlorodibenzo-p-dioxin (TCDD) (Ma et al., 1991) as alternatives to the standard Arochlor-induced S9 liver fraction. In the absence of metabolic activation, BeP was consistently reported as nonmutagenic in *S. typhimurium* (Cherng et al., 1996; Zeiger et al., 1992; Sakai et al., 1985; White et al., 1985a; Dunkel et al., 1984; Salamone et al., 1979; Simmon, 1979). BeP was not mutagenic in *Escherichia coli* with or without metabolic activation (Dunkel et al., 1984).

Mutagenicity findings in mammalian cells were negative at noncytotoxic concentrations. In metabolically competent human B-lymphoblastoid cells, forward mutations were induced at the thymidine kinase (*tk*) locus at a BeP concentration associated with >30% cytotoxicity (10,000 ng/mL); mutations were not observed at noncytotoxic concentrations ≤1,000 ng/mL (Durant et al., 1996). In other assays, BeP did not induce forward mutations in metabolically competent adult rat liver epithelial (ARL18) cells at the hypoxanthine-guanine phosphoribosyltransferase (*Hgprt*) locus (Ved Brat et al., 1983) or cell-mediated mutagenicity in Chinese hamster lung fibroblasts (V79 cells) cocultured with metabolically competent rodent cells (Huberman, 1977).

Clastogenicity

Evidence of clastogenicity from in vitro studies in mammalian cells is predominately negative. Coulomb et al. (1981) reported a weak induction of sister chromatid exchanges (SCEs) by BeP in Chinese hamster V79 cells in serum-free culture media, but not in media containing 10−40% fetal bovine serum (FBS). The study authors proposed that the presence or absence of serum in the media may influence test results, because dose-related decreases in cellular uptake of BeP by Syrian hamster embryo (SHE) cells were observed with increasing levels of FBS in culture media (possibly due to BeP binding to serum lipoproteins) (Coulomb et al., 1981). However, Mane et al. (1990) reported that SCEs were not induced by BeP in Chinese hamster V79 cells with or without metabolic activation in serum-free medium. SCEs were also not induced by BeP in metabolically competent ARL18 cells (culture medium composition not reported) (Ved Brat et al., 1983). Micronuclei (MN) were not induced by BeP in normal rat skin fibroblasts or uridine 5′-diphospho-glucuronosyltransferase (UGT)-deficient rat skin fibroblasts cultured with 13% FBS (Vienneau et al., 1995).

Evidence of clastogenicity from in vivo studies in rodents is mixed. BeP induced chromosomal aberrations (CAs) in rat bone marrow following a single intravenous (i.v.) injection of 40 mg/kg (Rees et al., 1970). And in hamsters, BeP induced SCEs (but not CAs) in bone marrow cells following a single i.p. injection of 450 mg/kg (Roszinsky-Köcher et al., 1979). In mice, BeP did not increase micronucleated erythrocytes in bone marrow following i.p. injection of 100 mg/kg (Sato et al., 1987).

DNA Damage and Repair

Findings were generally negative in in vitro assays of DNA damage and repair. In *E. coli*, BeP did not induce DNA damage or repair activity without metabolic activation; with activation, findings were negative or equivocal/borderline (Mersch-Sundermann et al., 1993; Mersch-Sundermann et al., 1992; De Flora et al., 1984). In mammalian cells, DNA strand breaks were reported in Chinese hamster V79 cells following exposure to BeP in media containing 5% FBS (Platt et al., 2008); however, DNA strand breaks were not induced by BeP in human skin fibroblasts in media containing 20% FBS (Milo et al., 1978). As discussed above, the higher levels of serum in the human cell assay could have affected test results due to decreased cellular

uptake of BeP with increasing serum levels in culture media (Coulomb et al., 1981). Additionally, the presence of serum (a mitogen) may skew the utilization of alternative DNA damage-and-repair pathways that are only active in actively cycling cell populations. It is unclear how the use of different molecular DNA damage-and-repair pathways may influence the dynamics of these repair processes.

In other mammalian cell studies, BeP did not inhibit scheduled DNA synthesis in human or rat cells with serum present in the culture media or rat cells in the absence of serum (Zhao and Ramos, 1995; Mane et al., 1990; Novicki et al., 1985; Milo et al., 1978). Similarly, BeP did not induce unscheduled DNA synthesis in rat or hamster cells in serum-free media or in mouse lung fibroblasts in the presence of serum (Schmitt et al., 1984; Schiff et al., 1983; Probst et al., 1981; Casto et al., 1976).

Findings were also generally negative in in vivo assays of DNA damage and repair. *Drosophila melanogaster* did not show increased rates of DNA repair after exposure to BeP (Fujikawa et al., 1993). No evidence of hepatic DNA damage was observed in rats following two gavage doses of 18 mg/kg (Kitchin et al., 1993; Kitchin et al., 1992). Another rat study reported inhibited ^{3}H-thymidine uptake in thymus, spleen, bone marrow, and regenerating liver 24−48 hours after i.p. exposure to BeP, suggesting decreased rates of DNA synthesis (Prodi et al., 1975). However, the study authors noted that findings may reflect general cytotoxicity of BeP, rather than decreased DNA synthesis.

DNA adducts were identified in mouse skin following repeated topical application of BeP to shaved skin (four exposures over 54 hours) (Reddy et al., 1984). Isolated human DNA has also been shown to form BeP DNA adducts in vitro (Segerbäck and Vodicka, 1993).

Cell Transformation

BeP was a weak inducer of cell transformation in SHE cells in the absence of metabolic activation in medium containing 10% FBS (DiPaolo et al., 1969).

2.3.2. Supporting Human Studies

Several occupational studies examined populations with exposures to multiple compounds in the absence of BeP-specific exposure analyses. These studies are of limited use for BeP hazard identification and do not provide data for dose-response analysis. Reported effects include subjective health complaints (skin irritation, headaches, shortness of breath, nausea, cough, phlegm) and altered markers of immune function in coke oven workers exposed to various PAHs in coal tar sludge and coal dust (Winker et al., 1997; NIOSH, 1987, 1986). Other effects include skin phototoxicity and skin and respiratory tract irritation in petroleum refining workers exposed to various PAHs in petroleum pitch (Vandervort and Lucas, 1974), impaired lung function (e.g., increased residual volume, reduced total lung capacity) in rubber factory workers exposed to suspended particulate matter containing various PAHs (Gupta et al., 1998; Gupta et al., 1994), and increased markers of oxidative deoxyribonucleic acid (DNA) damage in roofers exposed to various PAHs in hot asphalt (Serdar et al., 2016). Another study reported elevated biomarkers of chromosomal and DNA damage in nonsmoking female city hall clerks employed and living in the most polluted urban region of Silesia, Poland (high PAH exposure levels), compared with nonsmoking female controls living in a less polluted urban region of Białystok, Poland (low PAH exposure levels) (Motykiewicz et al., 1998).

In population-based, case-control studies, no significant differences were observed in serum BeP levels between control and asthmatic children in Saudi Arabia (Al-Daghri et al., 2014) or lung tissue BeP levels between patients with tuberculosis (control) and patients with lung carcinoma in 1961−1962 or 1991−1996 in Japan (Tokiwa et al., 1998).

2.3.3. Supporting Animal Studies

Acute Toxicity

Kitchin et al. (1993) and Kitchin et al. (1992) evaluated hepatic biochemical endpoints in female rats following acute oral exposure to BeP. The rats were exposed twice to BeP at 0 or 18 mg/kg via gavage in corn oil; the first dose was administered 21 hours prior to sacrifice and the second one 4 hours prior to sacrifice. All rats survived. No exposure-related changes in serum alanine aminotransferase (ALT) activity, hepatic ornithine decarboxylase activity, or hepatic cytochrome P450 (CYP450) content were observed.

Cardiovascular Toxicity

There is a body of evidence describing cardiotoxicity induced by environmentally relevant PAHs in non-human model systems (Brette et al., 2017). Specifically, there is concern that BeP may cause cardiovascular effects because the U.S. EPA (2017c) concluded that available animal studies for benzo[a]pyrene (BaP), a PAH with similar structure and physicochemical properties, provided suggestive evidence of cardiovascular toxicity (i.e., atherosclerosis). Curfs et al. (2005) conducted a study directly comparing BeP- and BaP-induced atherosclerotic plaque formation in mice genetically predisposed to develop plaques. Both compounds showed a similar capability to promote atherosclerosis. Following oral exposure to BaP or BeP (5 mg/kg-day) via once weekly gavage for 24 weeks, the location and total number of initial and advanced plaques (combined) per aortic arch were similar in treated and control mice, but the total area of advanced plaques/arch was significantly ($p<0.05$) increased by 1.4- to 1.5-fold in BaP- and BeP-treated mice compared with controls. In the thoracic aorta, there were no exposure-related changes in number or size of plaques; however, both BaP- and BeP-treated groups exhibited significantly increased plaque total inflammatory cells (CD45-positive cells) and plaque T lymphocytes (CD3-positive cells). The only clear difference between BaP- and BeP-treated mice was significant DNA-adduct formation in the lung of BaP-treated mice compared with BeP-treated and control mice. This suggests potential differences in distribution and/or potency in lung tissue; however, the relevance of this data to human PAH-mediated cardiotoxicity is unclear due to the use of transgenic knockout mice. Immunostaining for TGFβ1 in aortic arch plaques—a phenomenon observed in stable atherosclerotic lesions—was also increased in BaP- and BeP-treated groups compared with controls (Toma and McCaffrey, 2012). In contrast, in vitro treatment of the murine monocyte/macrophage RA W364.7 cell line with BaP or BeP revealed no apparent PAH treatment-related effect on release of tumor necrosis factor-alpha (TNF-α) or expression of transforming growth factor beta 1 (TGFβ1).

Avian models have also been used to study the promotion of atherosclerosis by PAH compounds. Penn and Snyder (1988) demonstrated that both BaP (40 mg/kg-day) and BeP (20 mg/kg-day) promoted atherosclerotic plaque development parameters in cockerels following weekly intramuscular (i.m.) injection for 16 weeks. A study in pigeons, however, reported an increase in aortic plaque size and number, for BaP only, following weekly i.m. injections of 100 mg/kg BaP or BeP for 3 or 6 months (Revis et al., 1984).

Immunotoxicity

The immunotoxic potential of BeP has been evaluated in numerous in vivo and in vitro studies (see Table 4B for more details). Most of the in vivo studies reported lack of immunosuppressive effects of BeP on parameters of humoral or cell-mediated immunity in mice or rats given BeP via subcutaneous (s.c.) or intraperitoneal (i.p.) injection. Effects measured included antibody responses to antigens (Munson and White, 1992, 1986; White et al., 1985b; White and Holsapple, 1984; Dean et al., 1983; Munson and White, 1983); delayed hypersensitivity (White et al., 2012; Munson and White, 1992, 1986, 1983); lymphoproliferative response to mitogens (Munson and White, 1992; Wojdani and Alfred, 1984; Dean et al., 1983; Munson and White, 1983); macrophage function (Munson and White, 1992, 1983); host resistance to bacteria, viruses, or tumor cells (Munson et al., 1985); and cell-mediated cytotoxicity (Wojdani and Alfred, 1984; Wojdani et al., 1984). Exposure to BeP reduced the T cell-dependent antibody response to sheep red blood cells (sRBCs) in spleen cells cultured from exposed mice in one study (Blanton et al., 1986). Each of these studies compared the response of BeP to the immunosuppressive response of the canonical PAH BaP administered at the same dose levels (highest dose of 40 mg/kg-day). It is unclear whether BeP would have elicited an immunosuppressive response if administered at higher doses. Dermal exposure to BeP was shown to elicit a contact hypersensitivity response in mouse skin (Anderson et al., 1995).

In vitro studies provided some evidence of BeP immunotoxicity, including inhibition of mitogenesis in human lymphocytes (Davila et al., 1996) and decreased B-cell lymphopoiesis in murine bone marrow (Hardin et al., 1992). Inconsistent findings, however, were reported for inhibition of T-cell and B-cell antibody responses in mouse spleen cells (Blanton et al., 1986; White and Holsapple, 1984), and no effect was reported after BeP exposure on the differentiation of human monocytes into macrophages (van Grevenynghe et al., 2003). Mechanistic studies have investigated the possible involvement of altered protein tyrosine kinase activity, calcium uptake and/or retention, glutathione (GSH) depletion, or aryl hydrocarbon hydrolase (AHH) activity; however, no clear relationship between these parameters and BeP immunotoxicity was reported (Davila et al., 1999; Romero et al., 1997; Krieger et al., 1995; Krieger et al., 1994; Gurtoo et al., 1979).

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
In vivo animal studies				
Contact hypersensitivity	C3H/HeN mice (3−5/group, sex not reported); 1,000 µg BeP was applied to the shaved abdominal skin (covered by permeable membrane); 5 d later, 200 µg BeP was applied to the dorsal aspect of the ear; ear thickness was measured immediately following challenge and daily thereafter for up to 5 d; negative controls received challenge application only; AHH activity was measured in microsomes isolated from abdominal skin of two mice 24 h following application of 1,000 µg BeP.	Maximum ear thickness was increased by 3.2×10^{-2} mm over negative controls; AHH activity was 5% higher than controls.	BeP produced a contact hypersensitivity response in mouse skin but was a poor inducer of AHH activity.	Anderson et al. (1995)
T cell-dependent and independent antibody responses	Female B6C3F1 mice (number per group not reported) received daily s.c. injections containing 0 (polyvinylpyrrolidone vehicle) or 40 mg/kg-d for 7 or 14 d; spleen cell responses to sRBCs or LPS were measured in culture (measured as PFCs).	Response to sRBCs was reduced by 48% at 7 d and 51% at 14 d; slight inhibition of antibody response to LPS (data not shown).	Exposure to BeP reduced the T cell-dependent antibody response to sRBCs in spleen cells cultured from exposed mice; inhibition of the B cell-mediated antibody response was minimal.	Blanton et al. (1986)
T cell-dependent and independent antibody responses; lymphoproliferative response to mitogens	Female B6C3F1 mice (6−10/group per assay) received daily s.c. injections containing 0 (corn oil vehicle), 5, 20, or 40 mg/kg-d for 14 d; parameters evaluated included body and organ weights, histopathology, hematology, antibody PFC response to sRBCs and LPS administered intravenously, and lymphoproliferative responses to mitogens (PHA, LPS).	No effects on body or organ weights or hematological parameters; no effects on antibody PFC responses or lymphoproliferative responses to mitogens.	BeP did not affect the T cell-dependent antibody response to sRBCs, the B-cell response to LPS, or the lymphoproliferative response to mitogens in mice.	Dean et al. (1983)

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Multiple assays of humoral and cell-mediated immunity and macrophage function	Male and female B6C3F1 mice (72−140/sex/group) received daily s.c. injections containing 0 (corn oil vehicle), 5, 20, or 40 mg/kg-d for 14 d; endpoints evaluated included body and organ weights, hematology, humoral immunity evaluations (spleen IgM response to sRBCs, serum immunoglobulins and complement, and spleen B-cell response to LPS), cell-mediated immunity evaluations (delayed hypersensitivity response to KLH and sRBCs, popliteal lymph node response to sRBCs, spleen T-cell response to Con A and acute inflammatory response to carrageenan), and macrophage function (vascular clearance and uptake of radiolabeled sRBCs).	Absolute and relative liver weights increased by 19 and 7%, respectively, in males at 40 mg/kg-d; absolute spleen weight increased by 26 and 18% in males at 20 and 40 mg/kg-d, respectively; absolute spleen weight decreased by 36% in females at 40 mg/kg-d (no changes in relative spleen weight); and relative lung weight decreased by 11−12% in males at all doses (no change in absolute lung weight). Leukocyte count decreased by 68% in males at 40 mg/kg-d. No other significant treatment-related effects were observed.	BeP did not impair humoral immunity, cell-mediated immunity, or macrophage function in mice.	Munson and White (1992); Munson and White (1983); Munson and White (1983) (unpublished)
Host resistance assays	Female B6C3F1 mice (8/group) received daily s.c. injections containing 0 (corn oil vehicle) or 40 mg/kg-d for 14 d; *Listeria monocytogenes*, *Streptococcus pneumoniae*, herpes simplex type 2, influenza A2, or B16F10 melanoma cells injected on Day 15 (four concentrations of each pathogen); host resistance measured as percent mortality or incidence and multiplicity of tumors.	No change in mortality incidence in response to *L. monocytogenes*, *S. pneumoniae*, herpes simplex type 2, or influenza A2; no change in tumor incidence and multiplicity following injection of B16F10 melanoma cells.	BeP did not alter host resistance to bacteria, viruses, or tumor cells in mice.	Munson et al. (1985) (unpublished)

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results	Conclusions	References
T cell-dependent antibody response and delayed hypersensitivity response	Male F344 rats (8/group) received daily s.c. injections containing 0 (corn oil vehicle) or 20 mg/kg-d for 14 d; parameters evaluated included body and organ weights, hematology, spleen IgM response to sRBCs, and delayed hypersensitivity response to KLH.	No change in body weight; 18% increase in relative liver weight (no other organ-weight changes); 26% decrease in leukocytes, 67% decrease in monocytes; no change in the spleen IgM response to sRBCs; and no change in the delayed hypersensitivity response to KLH and sRBCs.	BeP did not alter T cell-dependent antibody response to sRBCs or the delayed hypersensitivity response to KLH or sRBCs.	Munson and White (1986) (unpublished)
T cell-dependent antibody response	Female B6C3F1 mice (8/group) received daily s.c. injections containing 0 (corn oil vehicle), 5, 20, or 40 mg/kg-d for 14 d; spleen IgM response was evaluated 4 d after injection with sRBCs (PFCs).	No change in the spleen IgM response to sRBCs.	BeP did not impair humoral immunity in mice.	White and Holsapple (1984)
T cell-dependent antibody response	Female B6C3F1 mice (8/group) received daily s.c. injections containing 0 (corn oil vehicle) or 40 mg/kg-d (160 µmol/kg-d) for 14 d; spleen IgM response was evaluated 4 d after injection with sRBCs (spleen weight, cellularity, and number of PFCs).	No change in the spleen IgM response to sRBCs.	BeP did not impair humoral immunity in mice.	White et al. (1985b)
Candida albicans DTH test	Female B6C3F1 mice (7−8/group) received daily s.c. injections containing 0 (corn oil vehicle), 5, 20, or 40 mg/kg-d for 28 d; the *C. albicans* DTH test was performed; *C. albicans* was injected into the right flank of mice on Day 21; mice were challenged with injection of *C. albicans* antigen (chitosan) into the right footpad; footpad thickness was measured pre- and postchallenge.	BeP did not increase footpad swelling above vehicle controls.	BeP did not impair cell-mediated immunity in mice.	White et al. (2012)

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Multiple assays of cell-mediated immunity	C3H, C57BL/6, and DBA mice (6/group; sex not reported) were given an i.p. injection of PHA; 96 h later, BeP was administered by i.p. injection at doses of 0 (corn oil), 2.5, 10, or 50 mg/kg; mice were sacrificed 24 h later; cell-mediated immunity parameters included blastogenesis of splenic lymphocytes, cell-mediated cytotoxicity (percent ^{51}Cr release from target cells), and percent of monocyte-macrophages in spleen cell suspensions.	BeP did not induce lymphoblastogenesis or increase cell-mediated cytotoxicity or the percent monocyte-macrophages in spleen cell suspensions.	BeP did not impair cell-mediated immunity in mice.	Wojdani and Alfred (1984)
Cytotoxic lymphocyte response	C3H and C57BL/6 mice (6/group, sex not reported) were preimmunized with P815 tumor cells (i.p.) and treated 10 d later with a single i.p. injection of BeP at doses of 0 (corn oil), 0.5, 5, or 50 mg/kg; after 24 h, splenic lymphocytes and peritoneal exudate lymphocytes were isolated and used to measure binding and killing of P815 target cells.	BeP reduced percent binding and percent killing by splenic and peritoneal lymphocytes in both mouse strains at 5 and 50 mg/kg; however, effects were small in magnitude.	BeP produced minimal effects on cell-mediated immunity in mice.	Wojdani et al. (1984)
In vitro studies				
T cell-dependent and polyclonal B-cell antibody responses	Spleen cells from B6C3F1 mice cultured with and without sRBCs were exposed to 0 (polyvinylpyrrolidone vehicle), 0.2, 2, 20, or 200 µg/mL BeP; T cell-dependent antibody response to sRBCs; polyclonal antibody responses to LPS and PPD (B cell); measured on Day 5.	Dose-dependent reduction in response to sRBCs (79 and 100% inhibition at 20 and 200 µg/mL, respectively); reduction in response to LPS (83% decrease) and PPD (46% decrease) at 20 µg/mL.	BeP inhibited both T cell-dependent and polyclonal antibody responses in spleen cells in vitro.	Blanton et al. (1986)
Lymphocyte mitogenesis	Human peripheral blood mononuclear cells were incubated with PHA in the presence of BeP at concentrations of 0 (DMSO vehicle), 0.1, 1, or 10 µM for 72 h; lymphocyte proliferation (i.e., mitogenesis) was measured for each donor (4−8 donors/group).	Lymphocyte mitogenesis was significantly reduced in 1/4 (94% of control), 3/8 (94% of control), and 7/8 (76% control) donors at 0.1, 1, or 10 µM, respectively.	BeP inhibited mitogenesis in human lymphocytes.	Davila et al. (1996)

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results	Conclusions	References
PTK activation in human T cells	Human HPB-ALL T cells (a leukemia cell line) were exposed to 0 (DMSO vehicle) or 10 µM BeP; total and Fyn- and Zap-70-specific PTK activity were measured in cell lysates after 10 min exposure; total PTK activity was measured in a time course experiment at 5 min, 30 min, and 18 h.	Total PTK activity was increased at 18 h only; total and Fyn- and Zap-70-specific PTK activity were similar to control after 10 min of exposure.	BeP significantly increased total PTK activity in human HPB-ALL T cells after 18 h exposure; the role of increased total PTK activity in BeP immunotoxicity is unclear from the data presented.	Davila et al. (1999)
AHH activity in fresh mitogen activated human lymphocytes	Lymphocytes from a single human donor were suspended in a medium containing pokeweed mitogen and PHA; cells were exposed to 0, 10^{-7}, 10^{-6}, or 10^{-5} M BeP for 24 h and AHH activity was measured immediately thereafter.	10^{-5} M BeP inhibited AHH activity in human lymphocytes in vitro (~40% of control, data shown graphically); no effect was observed at lower concentrations (10^{-7} or 10^{-6} M).	The role of AHH inhibition in BeP immunotoxicity is unclear from the data presented.	Gurtoo et al. (1979)
B-cell lymphopoiesis	C57BL/6 murine bone marrow cells were cultured under conditions that favor the growth of pre-B cells; cultures were treated with 0 (acetone vehicle), 10^{-7}, 10^{-6}, 10^{-5}, or 10^{-4} M BeP, and pre-B cells were recovered and counted after 2 and 7 d.	10^{-4} M BeP decreased the number of pre-B cells after 7 d (~65% of control lymphocytes, data shown graphically); no effect was observed at lower concentrations (10^{-7}, 10^{-6}, or 10^{-5} M).	BeP inhibited B-cell lymphopoiesis.	Hardin et al. (1992)
Calcium elevation in human T cells	Human HPB-ALL T cells (a leukemia cell line) were exposed to 0 (DMSO vehicle) or 10 µM BeP for 3 min or 4 h; calcium mobilization was determined by a fluo-3 fluorescence flow cytometry assay.	BeP did not induce calcium mobilization in human T cells after 3 min or 4 h.	Calcium elevation was not identified as a mechanism of immunotoxicity in human T cells in this study.	Krieger et al. (1994)
Calcium uptake and activity of calcium ATPases in vesicles prepared from human T cells	ATP-dependent $^{45}Ca^{2+}$ uptake was measured in microsomes prepared from human HPB-ALL T cells after 5 min of incubation with 0 (DMSO vehicle), 0.1, 1, or 10 µM BeP; microsomal Ca^{2+}-ATPase activity was measured 30 min following incubation with 10 µM BeP.	Microsomal calcium uptake was inhibited by 19, 25, and 20% of control at 0.1, 1, or 10 µM BeP; no effect was observed on Ca^{2+}-ATPase activity.	BeP inhibited calcium uptake in T-cell microsomes; however, the effect was not treatment related. No effect was observed on Ca^{2+}-ATPase activity in human T-cell microsomes.	Krieger et al. (1995)

Table 4B. Immunotoxicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results	Conclusions	References
GSH levels in human peripheral blood mononuclear cells (lymphocytes)	Human peripheral blood mononuclear cells obtained from five donors were incubated with 0 (DMSO), 1, or 10 µM for 6, 48, or 72 h.	BeP did not significantly affect GSH in human lymphocytes.	GSH depletion was not identified as a mechanism of immunotoxicity.	Romero et al. (1997)
Differentiation of human monocytes into macrophages	Monocyte cultures prepared from human peripheral blood were exposed to 10 µM BeP for 6 d; formation of adherent macrophages and expression of phenotypic markers (CD71) were monitored.	BeP did not decrease the number of adherent cells or increase the expression of CD71.	BeP did not alter differentiation of human monocytes into macrophages.	van Grevenynghe et al. (2003)
Antibody production by spleen cells in vitro	Spleen cell cultures were prepared from untreated female B6C3F1 mice; cells were incubated with 0 (corn oil vehicle), 0.5, 5, or 50 µg/culture BeP for 4 d; antibody response to sRBCs was measured as AFCs/10^6 spleen cells (with or without metabolic activation with Arochlor-induced S9).	BeP did not alter the antibody response to sRBCs (with or without metabolic activation).	No effect on humoral immunity in vitro.	White and Holsapple (1984)

AFC = antibody-forming colony(ies); AHH = aryl hydrocarbon hydroxylase; ATP = adenosine triphosphate; BeP = benzo[e]pyrene; Con A = Concanavalin A; DMSO = dimethylsulfoxide; DTH = delayed-type hypersensitivity; GSH = reduced glutathione; IgM = immunoglobin M; i.p. = intraperitoneal; KLH = keyhole limpet hemocyanin; LPS = lipopolysaccharide; PFC = plaque-forming cell; PHA = phytohemagglutinin; PPD = purified protein derivative; PTK = protein tyrosine kinase; s.c. = subcutaneous; sRBC = sheep red blood cell.

Effects on Retinal Cells

Several in vitro studies have been performed in retinal cell preparations to evaluate potential PAH-mediated mechanisms for increased risk of age-related macular degeneration in cigarette smokers, because cigarette combustion is a relevant source of exposure to complex PAH mixtures. BaP, the most well-studied PAH, is demonstrated to be metabolized into its diol epoxide by bovine retinal pigment epithelial (RPE) cells, resulting in concomitant DNA-adduct formation and subsequent inhibition of cell growth and replication (Patton et al., 2002). Because the structurally related compound BeP has been characterized at higher concentrations in cigarette smoke than BaP (Patil et al., 2009), several studies were conducted to specifically evaluate the effects of BeP exposure on retinal cell preparations. BeP exposure produced a concentration-dependent reduction in the viability of human retinal pigment epithelial (ARPE-19) cells after 24 hours of treatment (percent viability was 96, 59, 36, and 20% at concentrations of 100, 200, 400, and 1,000 µM, respectively) (Sharma et al., 2008). Apoptosis was induced through the activation of multiple caspase pathways, and inhibition of caspase activation by preincubation with genistein, resveratrol, and memantine was shown to protect the viability of the ARPE-19 cells (Mansoor et al., 2010; Sharma et al., 2008). These data suggest that the decreased viability of human retinal pigment epithelial cells exposed to BeP was due to increased apoptosis mediated through increased caspase signaling.

Pretreatment of ARPE-19 cells with 17β-estradiol was also shown to protect against cytotoxicity of BeP and to reduce markers of apoptosis after BeP exposure. Estrago-Franco et al. (2016) reported that reactive oxygen/reactive nitrogen species (ROS/RNS) and inflammatory cytokines (i.e., IL-6, GM-CSF) were increased in ARPE-19 cells exposed to 200 µM BeP for 24 hours. BeP treatment for 24 hours also reduced cell viability in human microvascular endothelial cells (HMVECs) at concentrations ≥200 µM and in retinal neurosensory (R28) cells at concentrations ≥400 µM (Patil et al., 2009). The mechanism of cytotoxicity differed by cell type and concentration (i.e., no caspase activation in HMVECs; activation of caspases at 100 and 200 µM only in R28 cells) (Patil et al., 2009). Given the multiple modes of genotoxic activity exhibited by PAHs and the complex interplay of DNA damage, metabolic CYP activity, and apoptotic cell death, it is unclear how the cell-specific activity of these molecular pathways will influence cell fate determination described across the different model systems.

In an ex vivo study using porcine retinal arterioles, intraluminal administration of 100 µM BeP for 180 minutes reduced endothelium-dependent, nitric oxide-induced vasodilation via a mechanism involving superoxide production (Kamiya et al., 2017).

Cancer

The dermal, lung implantation, and i.p. injection studies that evaluated the carcinogenic potential of BeP in rodents are summarized in Table 4C.

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results (tumor incidence)	References
Dermal complete carcinogenicity study	Female ICR/Ha Swiss mice (100 controls, 50 treated); 0 or 15 µg (0.06 µmol) per application (acetone vehicle); 3 times/wk for 53 wk	Skin papillomas 0 µmol = 0/100 (0%) 0.06 µmol BeP = 0/50 (0%)	Van Duuren and Goldschmidt (1976)
Dermal complete carcinogenicity study	Female Swiss Millerton (*n* = 20); 0.1% BeP (acetone vehicle); 3 times/wk for up to 13 mo; no negative control was used; dose (µmol) of BeP could not be calculated because application volume was not reported	A total of 2/20 (10%) mice developed skin papillomas and 3/20 (15%) mice developed skin carcinomas over the course of the study. The first papilloma was observed at 9 mo, when there were only eight survivors, and the first carcinoma at 13 mo, when there were only five survivors. While a negative control was not used, no tumors were induced in 20 mice similarly exposed to 0.1% fluoranthene (9 survivors at 14 mo). In contrast, 19/20 mice similarly treated with 0.01% BaP developed both skin papillomas and carcinomas (first tumors observed at 4 mo; all mice died by 11 mo). The authors characterized their results as showing "very weak" carcinogenic activity for BeP.	Wynder and Hoffmann (1959)
Dermal initiation-promotion study	Female Swiss ICR/HA mice (20/group); single application of 1,000 µg BeP (4 µmol); starting 2 wk later, 25 µg croton resin, 3 times/wk, up to 64 wk; two control groups were used (croton resin only) with no BeP exposure; 25 µg croton resin, 3 times/wk, for 60 or 66 wk; both control groups are presented because neither were exposed for the exact duration as the BeP group	Skin papillomas 0 µmol (60 wk group) = 1/20 (5%) 0 µmol (66 wk group) = 5/20 (25%) 4 µmol BeP (64 wk) = 2/20 (10%) Skin carcinomas 0 µmol (60 wk group) = 0/20 (0%) 0 µmol (66 wk group) = 1/20 (5%) 4 µmol BeP (64 wk) = 0/20 (0%)	Van Duuren et al. (1968)

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results (tumor incidence)	References
Dermal initiation-promotion study	Female CD-1 mice (30/group); single application of 0 (acetone/DMSO vehicle), single application of 1, 2.5, or 6 µmol BeP; 2.5 or 6 µmol BeP 4,5-dihydrodiol; 1, 2.5, or 6 µmol BeP 9,10-dihydrodiol; 6 µmol BeP H_4-9,10-diol; or 2.5 µmol 9,10-H_2 BeP; starting 1 wk later, 16 nmol TPA 2 times/wk for 35 wk	Skin papillomas 0 (vehicle control) = 7% 1 µmol BeP = 15% 2.5 µmol BeP = 11% 6 µmol BeP = 14% 2.5 µmol BeP 4,5-dihydrodiol = 14% 6 µmol BeP 4,5-dihydrodiol = 12% 1 µmol BeP 9,10-dihydrodiol = 14% 2.5 µmol BeP 9,10-dihydrodiol = 0% 6 µmol BeP 9,10-dihydrodiol = 11% 6 µmol BeP H_4-9,10-diol = 18% 2.5 µmol 9,10-H_2 BeP = 67%[†] *Note: Incidence data reported only as percentages. The study authors indicated that at least 27 mice/group survived to 25 wk. Actual incidences could not be estimated due to unknown animal number/group.*	Buening et al. (1980)
Dermal initiation-promotion study	Female CD-1 mice (20/group BeP, 30/group untreated controls); single application of 10 µmol BeP; starting 1 wk later, 5 µmol (exposed) or 10 µmol (untreated controls) TPA 2 times/wk for 35 wk	Skin papillomas 0 µmol = 0/30 (0%) 10 µmol BeP = 17/20 (85%)*	Scribner (1973)

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)			
Test	**Materials and Methods**	**Results (tumor incidence)**	**References**
Dermal initiation-promotion study	Female CD-1 mice (30/group); single application of 0 (TPA only), 100, or 252 µg BeP (0, 0.4, or 1 µmol); starting 1 wk later, 10 µg TPA 2 times/wk for 30 and 40 wk	Skin papillomas at 30 wk: 0 µmol = 4/30 (14%) 0.4 µmol BeP = 0/30 (0%) 1 µmol BeP = 6/30 (19%) Skin carcinomas at 40 wk: 0 µmol = 0/30 (0%) 0.4 µmol µg BeP = 0/30 (0%) 1 µmol µg BeP = 0/30 (0%) *Note: Incidence data reported only as percentages; actual incidences estimated from number of animals placed on study.*	Slaga et al. (1979)
Dermal initiation-promotion study	Female SENCAR mice (30/group); single application of 0 (acetone), 0.4, or 0.8 µmol BeP; starting 2 wk later, 3.4 nmol TPA 2 times/wk for 20 wk	Skin papillomas 0 µmol = 6/30 (19%) 0.4 µmol BeP = 4/30 (14%) 0.8 µmol BeP = 5/30 (18%) *Note: Incidence data reported only as percentages; actual incidences estimated from number of animals placed on study.*	Sawyer et al. (1987)
Dermal initiation-promotion study	Female SENCAR mice (30/group); single application of 0 (TPA only) or 2 µmol BeP, BeP 9,10-dihydrodiol, or BeP 9,10-diol-11,12-epoxide; starting 1 wk later, 2 µg TPA 2 times/wk for 15 wk	Skin papillomas 0 µg = 3/30 (10%) 2 µmol BeP = 5/29 (17%) 2 µmol BeP 9,10-dihydrodiol = 9/28 (32%) 2 µmol BeP 9,10-diol-11,12-epoxide = 2/29 (7%)	Slaga et al. (1980)
Lung implantation study	Female Osborne-Mendel rats (35/group); implantation of 0, 200, 1,000, or 5,000 µg BeP (0, 0.8, 4, or 20 µmol) in a beeswax/trioctanoin pellet; follow-up until moribund or dead	Lung carcinomas and sarcomas 0 µmol = 0/35 (0%) 0.8 µmol BeP = 0/35 (0%) 4 µmol BeP = 1/30 (3%) 20 µmol BeP = 1/35 (3%)	Deutsch-Wenzel et al. (1983)

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results (tumor incidence)	References
i.p. injection study	Experiment 1: Newborn male and female Swiss-Webster BLU:HA (ICR) mice (80/group); total dose of 0 or 2.8 µmol BeP, BeP 4,5-dihydrodiol, or BeP 9,10-dihydrodiol; injections given on PNDs 1, 8, and 15; mice were sacrificed during PNWs 62−66 Experiment 2: Newborn male and female Swiss-Webster BLU:HA (ICR) mice (120/group); total dose of 0 or 5.6 µmol BeP; injections given on PNDs 1, 8, and 15; mice were sacrificed during PNWs 62−66	Experiment 1: Pulmonary tumors at PNW 62−66 sacrifice Females: 0 µmol = 12/21 (57%) 2.8 µmol BeP = 12/30 (40%) 2.8 µmol BeP 4,5-dihydrodiol = 11/23 (48%) 2.8 µmol BeP 9,10-dihydrodiol = 9/32 (28%) Males: 0 µmol = 16/38 (42%) 2.8 µmol BeP = 12/30 (41%) 2.8 µmol BeP 4,5-dihydrodiol = 10/18 (56%) 2.8 µmol BeP 9,10-dihydrodiol = 12/28 (43%) Hepatic tumors at PNW 62−66 sacrifice Females: 0 µmol = 0/21 (0%) 2.8 µmol BeP = 0/30 (0%) 2.8 µmol BeP 4,5-dihydrodiol = 0/23 (0%) 2.8 µmol BeP 9,10-dihydrodiol = 0/32 (0%) Males: 0 µmol = 4/38 (11%) 2.8 µmol BeP = 6/30 (21%) 2.8 µmol BeP 4,5-dihydrodiol = 3/18 (17%) 2.8 µmol BeP 9,10-dihydrodiol = 17/28 (61%)*	Buening et al. (1980)

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)			
Test	**Materials and Methods**	**Results (tumor incidence)**	**References**
Continued:	Continued:	Continued: Experiment 2: <u>Pulmonary tumors at PNW 62−66 sacrifice</u> Females: 0 µmol = 12/30 (40%) 5.6 µmol BeP = 6/23 (26%) Males: 0 µmol = 9/29 (31%) 5.6 µmol BeP = 12/25 (48%) <u>Hepatic tumors at PNW 62−66 sacrifice</u> Females: 0 µmol = 0/30 (0%) 5.6 µmol BeP = 0/23 (0%) Males: 0 µmol = 0/29 (0%) 5.6 µmol BeP = 3/25 (12%)	Continued:

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results (tumor incidence)	References
i.p. injection study	Newborn male and female Swiss-Webster BLU:HA (ICR) mice (80 or 100/group); total dose of 0 µmol, 0.07 µmol BeP H_4-9,10-epoxide or 0.7 µmol BeP, BeP 4,5-oxide, 9,10-H_2 BeP, BeP diol epoxide (diastereomer 1 and 2); injections given on PNDs 1, 8, and 15; mice were sacrificed during PNWs 39−43	Pulmonary tumors PNW 39−43 sacrifice Females: 0 µmol = 3/24 (13%) 0.07 µmol BeP H_4-9,10-epoxide = 2/19 (11%) 0.7 µmol BeP = 5/25 (20%) 0.7 µmol BeP 4,5-oxide = 7/30 (23%) 0.7 µmol 9,10-H_2 BeP = 8/32 (25%) 0.7 µmol BeP diol epoxide 1 = 8/31 (26%) 0.7 µmol BeP diol epoxide 2 = 5/21 (24%) Males: 0 µmol = 8/37 (22%) 0.07 µmol BeP H_4-9,10-epoxide = 2/36 (6%) 0.7 µmol BeP = 5/31 (16%) 0.7 µmol BeP 4,5-oxide = 2/37 (5%) 0.7 µmol 9,10-H_2 BeP = 14/42 (33%) 0.7 µmol BeP diol epoxide 1 = 10/21 (48%) 0.7 µmol BeP diol epoxide 2 = 7/28 (25%) Hepatic tumors PNW 39−43 sacrifice Females: 0 µmol = 0/24 (0%) 0.07 µmol BeP H_4-9,10-epoxide = 0/19 (0%) 0.7 µmol BeP = 0/25 (0%) 0.7 µmol BeP 4,5-oxide = 0/30 (0%) 0.7 µmol 9,10-H_2 BeP = 0/32 (0%) 0.7 µmol BeP diol epoxide 1 = 0/31 (0%) 0.7 µmol BeP diol epoxide 2 = 0/21 (0%)	Chang et al. (1981)

Table 4C. Other Route Carcinogenicity Studies of BeP (CASRN 192-97-2)

Test	Materials and Methods	Results (tumor incidence)	References
Continued:	Continued:	Continued: Males: 0 µmol = 0/37 (0%) 0.07 µmol BeP H_4-9,10-epoxide = 1/36 (3%) 0.7 µmol BeP = 2/31 (6%) 0.7 µmol BeP 4,5-oxide = 0/37 (0%) 0.7 µmol 9,10-H_2 BeP = 2/42 (5%) 0.7 µmol BeP diol epoxide 1 = 1/21 (5%) 0.7 µmol BeP diol epoxide 2 = 6/28 (21%)*	Continued:

†Reported to be "significant" by study authors, although statistics not shown and data reporting inadequate to support independent analysis.
*Statistically significant at $p < 0.05$ based on two-tailed Fisher's exact probability test conducted for this review.

9,10-H_2 BeP = 9,10-dihydrobenzo[e]pyrene; BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; BeP 4,5-dihydrodiol = *trans*-4,5-dihydroxy-4,5-dihydrobenzo[e]pyrene; BeP 4,5-oxide = benzo[e]pyrene 4,5-oxide; BeP 9,10-dihydrodiol = *trans*-9,10-dihydroxy-9,10-dihydrobenzo[e]pyrene; BeP 9,10-diol-11,12-epoxide = *trans*-9,10-dihydroxy-anti-11,12-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene; BeP diol epoxide 1 = (±)-9β,10α-dihydroxy-11β,12β-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene; BeP diol epoxide 2 = (±)-9β,10α-dihydroxy-11α,12α-epoxy-9,10,11,12-tetrahydro-benzo[e]pyrene; BeP H_4-9,10-diol = *trans*-9,10-dihydroxy-9,10,11,12-tetrahydrobenzo[e]pyrene; BeP H_4-9,10-epoxide = 9,10-epoxy-9,10,11,12-tetrahydrobenzo[e]pyrene; DMSO = dimethyl sulfoxide; i.p. = intraperitoneal; PND = postnatal day; PNW = postnatal week; TPA = 12-*O*-tetradecanoylphorbol-13 acetate.

Available skin painting studies do not indicate that BeP is a complete dermal carcinogen. Skin tumors were not observed (0/50) following dermal application of 0.06 µmol BeP in acetone to mice 3 times/week for 53 weeks (Van Duuren and Goldschmidt, 1976). According to the authors of an earlier study (Wynder and Hoffmann, 1959), BeP showed "very weak" carcinogenic activity. In this study, mice treated dermally with an unknown dose of BeP (0.1% in acetone) 3 times/week for up to 13 months developed low incidences of skin papillomas (2/20) and skin carcinomas (3/20 mice). The study was inadequate, however, due to small initial group size (n = 20), high early mortality unrelated to tumor development (only eight survivors at 9 months, when the first tumor was observed), and lack of a negative control group.

The majority of other BeP cancer assays were also negative. BeP was not a skin tumor initiator at single exposure doses up to 6 µmol with 12-*O*-tetradecanoylphorbol-13 acetate (TPA) as a promoter or up to 4 µmol with croton resin as a promotor (Sawyer et al., 1987; Buening et al., 1980; Slaga et al., 1979; Van Duuren et al., 1968). One study, however, reported significantly increased skin papillomas in mice given BeP as an initiator at 10 µmol with TPA as a promoter (17/20) compared with mice given TPA alone (0/30) (Scribner, 1973). Lung implantation of BeP did not significantly increase the formation of pulmonary tumors in rats (Deutsch-Wenzel et al., 1983). Similarly, i.p. injections of BeP in newborn Swiss Webster mice (on Postnatal Days [PNDs] 1, 8, and 15) at total doses up to 5.6 µmol did not result in an increase in pulmonary or hepatic tumors in adulthood (Chang et al., 1981; Buening et al., 1980).

Similar studies evaluating carcinogenic potential of BeP metabolites were also generally negative. In dermal initiation-promotion studies, only 9,10-dihydrobenzo[e]pyrene was positive, resulting in an increased number of papillomas (67%) compared with vehicle control (7%) following promotion with TPA when tested at 2.5 µmol (Buening et al., 1980). The 9,10-dihydrodiol metabolite resulted in a borderline (p = 0.05) increase in the number of papillomas (9/28) compared with vehicle control (3/30) when tested at 2 µmol using TPA as a promoter (Slaga et al., 1980), but was negative when similarly tested at doses up to 6 µmol in a second study (Buening et al., 1980). Other tested metabolites (n = 3, see Table 4C) were not initiators at doses up to 6 µmol using TPA as a promoter (Buening et al., 1980; Slaga et al., 1980). In injection studies in newborn mice, exposure to the 9,10-dihydrodiol or a diol epoxide on PNDs 1, 8, and 15 (total doses of 2.8 or 0.7 µmol, respectively) resulted in a significant increase in hepatic tumors in adult males, but not hepatic tumors in females or pulmonary tumors in either sex (Chang et al., 1981; Buening et al., 1980). Other tested BeP metabolites (n = 5; see Table 4C) did not induce pulmonary or hepatic tumors following neonatal i.p. injection (Chang et al., 1981; Buening et al., 1980).

Taken together, these data suggest that BeP has low carcinogenic potential. BeP is not a complete dermal carcinogen and did not induce tumors in lung implantation or i.p. studies; however, 1/6 studies reported that BeP was a tumor initiator when administered dermally at high doses. Evidence regarding the carcinogenic potential of BeP metabolites is limited; 1/5 tested metabolites (9,10-dihydrobenzo[e]pyrene) was positive for initiation of skin tumors and 2/7 tested metabolites (a dihydrodiol and a diol epoxide) were positive for induction of tumors following neonatal injection. The relevance of these findings to the oral and inhalation routes of exposure is unclear but do suggest that metabolic processing is required for BeP-mediated carcinogenic activity.

2.3.4. Metabolism/Toxicokinetic Studies

No data on absorption or distribution are available for BeP. Because absorption and distribution of a chemical in the body are determined largely by physical and chemical properties related to chemical size and general structure (e.g., lipophilicity, vapor pressure, etc.), it is reasonable to assume that BeP will be absorbed and distributed similarly to other PAHs of similar size and structure with similar physical and chemical properties (e.g., BaP).

Based on data for BaP and other similar PAHs (U.S. EPA, 2017b), BeP is expected to be absorbed through inhalation, oral, and dermal routes of exposure, and generally through diffusion across cell membranes. For inhalation exposure, BeP is expected to adsorb onto particulates in the air and deposit in the respiratory tract with the associated carrier particles. As for other PAHs (IARC, 2010), particle size, region of deposition, and rate of dissolution or desorption from particles are all expected to affect the rate and extent of BeP absorption from the respiratory tract. In general, absorption through airways will likely be biphasic, with rapid absorption occurring through thin epithelia in the alveoli and slower absorption occurring in thicker regions of the airways (ATSDR, 1995). A significant proportion of inhaled BeP is likely to be transported to the gut via the mucociliary escalator (IARC, 2010). For oral exposure, studies with BaP indicate gastrointestinal absorption ranging from 10 to 60% (U.S. EPA, 2017b; ATSDR, 1995). Oral absorption of PAHs in animals is enhanced by the presence of lipophilic compounds, such as oils and fats, and by the presence of bile in the gastrointestinal tract (ATSDR, 1995). Dermal absorption studies with BaP reported rapid and near complete absorption in rats, mice, monkeys, and guinea pigs using vehicles, such as crude oil or acetone, that enhanced absorption (U.S. EPA, 2017b). Dermal absorption was decreased with higher viscosity oil vehicles and in the presence of soils with high organic carbon content (U.S. EPA, 2017b).

Like BaP (U.S. EPA, 2017c; IARC, 2010; ATSDR, 1995), BeP is expected to show widespread, systemic tissue distribution, with initial rapid uptake into highly diffused tissues, such as lung, kidney, liver, and blood, followed by accumulation and retention in fat, with subsequent slow release. Exposure of pregnant rats to BaP both orally and via inhalation indicates that limited placental transfer can occur (U.S. EPA, 2017b; ATSDR, 1995).

PAHs, in general, are metabolized in multiple tissues in the body into more soluble metabolites, including dihydrodiols, phenols, quinones, and epoxides, that form conjugates with glucuronide, glutathione (GSH), or sulfate (U.S. EPA, 2017b; IARC, 2010; ATSDR, 1995). The metabolism of BeP has been investigated in several in vitro studies using hamster or mouse embryo cells, rat liver homogenates, or purified microsomal fractions (Jacob et al., 1985; Jacob et al., 1983; MacLeod et al., 1982; Selkirk et al., 1982; MacLeod et al., 1979; Selkirk and MacLeod, 1979; Wood et al., 1979; Sims, 1970a, b; Duncan et al., 1969). BeP is oxidized via the CYP450 system (Jacob et al., 1985; Jacob et al., 1983; Wood et al., 1979). The primary oxidative metabolites include the k-region 4,5-dihydrodiol and phenolic metabolites, such as 3-hydroxybenzo[e]pyrene (3-OH-BeP) and other phenols with uncertain identities (1-OH, 4-OH, 9-OH, and 10-OH-BeP); BeP quinones have also been identified (Jacob et al., 1985; Jacob et al., 1983; MacLeod et al., 1979; Selkirk and MacLeod, 1979; Sims, 1970a, b). 4,5-Dihydrodiol and the phenols conjugate with uridine diphosphate glucuronic acid to their respective glucuronide conjugates (MacLeod et al., 1982; MacLeod et al., 1979). In contrast to BaP, there is little evidence of direct bay-region activation of BeP, although the characteristic bay region is present within BeP's structure. The 9,10-dihydrodiol metabolite was detected in trace amounts with rat liver microsomes, generally only after pretreatment with potent monooxygenase inducers,

including BaP (Jacob et al., 1985; Jacob et al., 1983; Wood et al., 1979). When the 9,10-dihydrodiol did form, subsequent oxidation was observed to occur at the 4,5-position to form 4,5,9,10-tetrahydroxy-4,5,9,10-tetrahydrobenzyo[e]pyrene, and not the carcinogenic 7,8-diol-9,10-epoxide (Jacob et al., 1985; Jacob et al., 1983). A study with human liver microsomes reported a higher percentage of the 9,10-dihydrodiol metabolite, approximately 12% of the total metabolites, compared with only 1% using liver microsomes from rats pretreated with PAHs (Jacob et al., 1985). Confounding metabolic potential through pretreatment with PAHs is likely to result in decreased rates of individual PAH metabolite production due to the likelihood of other available PAHs acting as a molecular sink for CYP activity. Given the unique and varied metabolic capacity of individual model systems and the discrepancy in production of mutagenic metabolites across those model systems, the significance and contribution of bay region-directed metabolism of BeP remains unclear. Because Jacob et al. (1985) described an increase in production of the proximal carcinogen (9,10-dihydrodiol) in human microsomes compared with that from rodent microsomes, future efforts should be directed at quantifying production of the ultimate diol-epoxide carcinogen within human models systems, as this metabolic step is likely to underlie the moderate carcinogenic potential observed for BeP.

In both mouse and hamster embryo cells, metabolism of BeP was essentially complete by 48 hours (MacLeod et al., 1982; Duncan et al., 1969). In liver microsomes from non-induced rats, the total metabolic rate of BeP was low (2 nmol/mg microsomal protein); metabolic rates of BeP increased significantly in liver microsomes when rats were pretreated with CYP450 inducers (Jacob et al., 1983; Sims, 1970b). Metabolic profiles were similar between rat liver and lung microsomes, and between hepatic microsomes from both rats and mice (Jacob et al., 1985). Metabolism studies with hamster embryo cells indicate the majority (71.7%) of BeP metabolites distribute to extracellular space, while smaller amounts (1.1 and 0.18%) are retained in the cytoplasm and nucleus, respectively (MacLeod et al., 1979).

Based on studies of BaP in multiple species (U.S. EPA, 2017b; IARC, 2010; ATSDR, 1995), excretion, mainly of conjugated metabolites, occurs primarily via biliary excretion to feces, and to a lesser extent, by urine. BaP has also been detected in small amounts in milk (U.S. EPA, 2017b). Excretion half-lives for BaP ranged from 22 to 30 hours in rats following inhalation and dermal exposures, respectively (U.S. EPA, 2017b; ATSDR, 1995).

3. DERIVATION OF PROVISIONAL VALUES

3.1. DERIVATION OF PROVISIONAL REFERENCE DOSES

No studies have been located regarding toxicity of BeP to humans by oral exposure. Animal studies of oral exposure to BeP are limited to an acute study with limited hepatic endpoints and a subchronic cardiovascular study in apoE-KO mice of inadequate design and scope to support derivation of a subchronic or chronic provisional reference dose (p-RfD). As a result of the limitations of the available oral toxicity data for BeP, subchronic and chronic p-RfDs were not derived directly. Instead, screening subchronic and chronic p-RfDs are derived in Appendix A using an alternative analogue approach. Based on the overall analogue approach presented in Appendix A, BaP was selected as the most appropriate analogue for BeP for deriving a screening subchronic and chronic p-RfD (see Table 5).

3.2. DERIVATION OF PROVISIONAL REFERENCE CONCENTRATIONS

No adequate studies have been located regarding toxicity of BeP to humans via inhalation exposure. Identified studies included occupational studies in workers exposed to complex mixtures that lacked BeP-specific exposure data and case-control studies of asthma and lung cancer that did not find associations with biomarkers of BeP exposure. No animal studies of inhalation exposure to BeP were identified. As a result of the limitations of the available inhalation toxicity data for BeP, subchronic and chronic provisional reference concentrations (p-RfCs) were not derived directly. Instead, screening subchronic and chronic p-RfCs are derived in Appendix A using an alternative analogue approach. Based on the overall analogue approach presented in Appendix A, BaP was selected as the most appropriate analogue for BeP for deriving a screening subchronic and chronic p-RfC (see Table 5).

3.3. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

A summary of the noncancer provisional reference values is shown in Table 5.

Table 5. Summary of Noncancer Reference Values for BeP (CASRN 192-97-2)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED/HEC)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat/M, F	Neurodevelopmental changes following early postnatal exposure	9×10^{-5}	$BMDL_{1SD}$	0.092[a] (based on analogue POD)	1,000	Chen et al. (2012) as cited in U.S. EPA (2017c)
Screening chronic p-RfD (mg/kg-d)	Rat/M, F	Neurodevelopmental changes following early postnatal exposure	9×10^{-5}	$BMDL_{1SD}$	0.092[a] (based on analogue POD)	1,000	Chen et al. (2012) as cited in U.S. EPA (2017c)
Screening subchronic p-RfC (mg/m^3)	Rat/F	Decreased embryo/fetal survival	2×10^{-6}	LOAEL	0.0046 (based on analogue POD)	3,000	Archibong et al. (2002) as cited in U.S. EPA (2017c)
Screening chronic p-RfC (mg/m^3)	Rat/F	Decreased embryo/fetal survival	2×10^{-6}	LOAEL	0.0046 (based on analogue POD)	3,000	Archibong et al. (2002) as cited in U.S. EPA (2017c)

[a]The POD was not converted into an HED using $BW^{3/4}$ because it is unknown whether allometric scaling is appropriate for exposure in early postnatal animals (see Appendix A for more details).

BeP = benzo[e]pyrene; BMDL = benchmark dose lower confidence limit; BW = body weight; F = female(s); HEC = human equivalent concentration; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; M = male(s); POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; SD = standard deviation; UF_C = composite uncertainty factor.

3.4. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Although the scientific literature provides information on the mutagenicity and genotoxicity of BeP, no oral or inhalation studies have been conducted to assess its carcinogenicity. Available dermal, lung implantation, and i.p. carcinogenicity studies in animals suggest that BeP has some carcinogenic potential, although findings are inconsistent across studies, and the relevance of these findings to oral or inhalation exposure is unclear. Under the U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is *"Inadequate Information to Assess Carcinogenic Potential"* of BeP by oral or inhalation exposure (see Table 6). Within the current U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is no standard methodology to support the identification of a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates for data-poor chemicals using an analogue approach. In the absence of an established framework, a screening evaluation of potential carcinogenicity is provided using the methodology described in Appendix B. This evaluation determined that there was a qualitative level of *concern for potential carcinogenicity* for BeP (see Appendix C).

Table 6. Cancer WOE Descriptor for BeP (CASRN 192-97-2)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	No human data are available.
"Likely to Be Carcinogenic to Humans"	NS	NA	No adequate chronic-duration animal cancer bioassays are available.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	No adequate chronic-duration animal cancer bioassays are available.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**No adequate animal cancer bioassays are available. Dermal, lung implantation, and i.p. studies in animals provide limited evidence of carcinogenic potential.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No evidence of noncarcinogenicity is available. No adequate chronic-duration animal cancer bioassays are available.

BeP = benzo[e]pyrene; i.p. = intraperitoneal; NA = not applicable; NS = not selected; WOE = weight of evidence.

3.5. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of suitable data precludes development of cancer risk estimates for BeP (see Table 7).

Table 7. Summary of Cancer Risk Estimates for BeP (CASRN 192-97-2)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Risk Estimates	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

BeP = benzo[e]pyrene; NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. SCREENING NONCANCER PROVISIONAL VALUES

Due to the lack of evidence described in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional toxicity values for benzo[e]pyrene (BeP) because the limited database on the toxicity of BeP is insufficient to support direct derivation. However, some information is available for this chemical, which although insufficient to support deriving a provisional toxicity value under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the provisional reference values to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there could be more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the CPHEA.

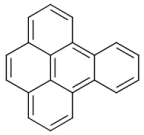
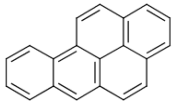
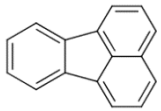
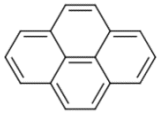
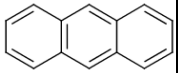
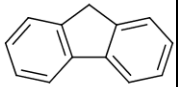
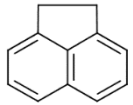
APPLICATION OF AN ALTERNATIVE ANALOGUE APPROACH

The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012). Three types of potential analogues (structural, metabolic, and toxicity-like) are identified to facilitate the final analogue chemical selection. The analogue approach may or may not be route specific or applicable to multiple routes of exposure. All information was considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable analogue both toxicologically and chemically.

Structural Analogues

An initial analogue search focused on the identification of structurally similar chemicals with toxicity values from the Integrated Risk Information System (IRIS), PPRTV, the Agency for Toxic Substances and Disease Registry (ATSDR), or the California Environmental Protection Agency (CalEPA) databases to take advantage of the well-characterized chemical-class information. Under Wang et al. (2012), structural similarity for analogues is typically evaluated using U.S. EPA's DSSTox database (DSSTox, 2018) and the National Library of Medicine's (NLM) ChemIDplus database (ChemIDplus, 2021). However, DSSTox is no longer available to the public, and there is no date available for the implementation of its replacement dashboard. In lieu of DSSTox scores, the Organisation for Economic Co-operation and Development (OECD) Toolbox was used to calculate structural similarity using the Dice method and default fingerprint settings. Six structural analogues to BeP that have oral noncancer toxicity values were identified (benzo[a]pyrene [BaP], fluoranthene, pyrene, anthracene, fluorene, and acenaphthene). Table A-1 summarizes the analogues' physicochemical properties and similarity scores. The analogues are presented in order of decreasing molecular weight.

Table A-1. Physicochemical Properties of BeP (CASRN 192-97-2) and Candidate Structural Analogues[a]

Property	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Structure							
CASRN	192-97-2	50-32-8	206-44-0	129-00-0	120-12-7	86-73-7	83-32-9
Molecular weight	252.316	252.316	202.256	202.256	178.234	166.223	154.212
OECD Toolbox similarity score (%)[b]	100	85	88.9	88.9	58.8	60.6	50
ChemIDplus similarity score (%)[c]	100	100	54	94	67	<50	<50
Melting point (°C)	178	177	108	150	215	115	93.9
Boiling point (°C)	469 (predicted average)	495	380	399	340	295	279
Vapor pressure (mm Hg)	5.7×10^{-9}	5.49×10^{-9}	9.22×10^{-6}	4.5×10^{-6}	6.53×10^{-6}	6.0×10^{-4}	2.15×10^{-3}
Henry's law constant (atm-m^3/mole)	1.07×10^{-6}	4.57×10^{-7}	8.86×10^{-6}	1.19×10^{-5}	5.56×10^{-5}	9.62×10^{-5}	1.84×10^{-4}
Water solubility (mol/L)	1.89×10^{-8}	8.40×10^{-9}	1.24×10^{-6}	6.65×10^{-7}	3.38×10^{-7}	1.15×10^{-5}	4.64×10^{-5}
Octanol-water partition coefficient (log K_{ow})	6.44	6.13	5.16	4.88	4.45	4.18	3.92
Acid dissociation constant (pKa)	NA	NA	NA	NA	NA	NA	NA

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard:
benzo[e]pyrene, CASRN 192-97-2; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3023764#properties; accessed February 8, 2021;
benzo[a]pyrene, CASRN 50-32-8; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID2020139#properties; accessed February 8, 2021;
fluoranthene, CASRN 206-44-0; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3024104#properties; accessed February 8, 2021;
pyrene, CASRN 129-00-0; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3024289#properties; accessed February 8, 2021;
anthracene, CASRN 120-12-7; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID0023878#properties; accessed February 8, 2021;
fluorene, CASRN 86-73-7; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID8024105#properties; accessed February 8, 2021;
acenaphthene, CASRN 83-32-9; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3021774#properties; accessed February 8, 2021;
all presented values are experimental averages unless otherwise noted.
[b]OECD (2019).
[c]ChemIDplus advanced similarity scores (ChemIDplus, 2021).

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; NA = not applicable; OECD = Organization for Economic Co-operation and Development.

All of the candidate structural analogues contain two or more benzene rings and range in molecular weight from 154.21 to 252.32 g/mol. The analogues are solids that have negligible to low solubility in water. Measured octanol-water partition coefficient (log K_{ow}) values for the analogues ranged from 3.92 for acenaphthene to 6.13 for BaP. Log K_{ow} values >4 correspond to hydrophobic chemicals that are not very soluble in water, and compounds with the highest log K_{ow} values are more likely to partition to fat compartments in the body following absorption. Similar to the target compound BeP, the four analogues with higher molecular weights have low vapor pressures (see Table A-1), so they are expected to have low volatility from dry surfaces and will exist as particulates in the atmosphere. Chemicals with low vapor pressures of $<1 \times 10^{-6}$ mm Hg, such as BaP, have low potential for inhalation exposure as gases or vapors. The two analogues with moderate vapor pressures, acenaphthene and fluorene, are expected to have some volatility from dry surfaces and may exist in the atmosphere as a gas/particulate mixture. However, all of the analogues have the potential for moderate volatilization from water to air based on their reported Henry's law constant values. Differences in absorption and distribution between the potential analogues are not expected to be significant for the four candidate structural analogues with higher molecular weights and lower vapor pressures; however, acenaphthene and fluorene are more volatile and more soluble in water than the other analogues by a minimum of one order of magnitude. BaP is the preferred structural analogue for BeP due to its physical and chemical properties that closely resemble BeP.

Metabolic Analogues

Table A-2 summarizes the available toxicokinetic data for BeP and the structurally similar compounds identified as potential analogues. Absorption occurs via all routes, with the rate and extent of absorption dependent on the exposure medium (i.e., enhanced in the presence of oils and fats). Oral absorption appears to be more rapid and extensive for analogues with lower molecular weight (e.g., anthracene). No absorption data were identified for fluorene and acenaphthene. Polycyclic aromatic hydrocarbon (PAH) analogues are widely distributed in the body, with preferential accumulation in fat as suggested by the log K_{ow} values (see above). PAH analogues undergo oxidative metabolism to diols, dihydroxy, and hydroxy metabolites, which are common across all analogues. The presence of a bay region in both BeP and BaP indicates the potential for metabolism to dihydrodiols and diol epoxides, which are electrophilic and covalently bind to proteins and deoxyribonucleic acid (DNA) (U.S. EPA, 2017b, c); see "Genotoxicity" summary in the "Other Data" section in the main body of the PPRTV document. However, experimental data indicate that the potential for BeP to generate these reactive metabolites is much lower than for BaP (see Table A-2 and "Metabolism" discussion in "Other Data" section for more details). PAH analogues with lower molecular weight appear to be excreted more rapidly in the urine (e.g., anthracene) while larger molecular weight compounds (i.e., BaP) are excreted primarily in feces via the biliary system. BaP is the preferred metabolic analogue for BeP due to the potential—albeit limited for BeP—to form reactive dihydrodiol and diol epoxide metabolites. Further, the rates of absorption and excretion are expected to be similar for BeP and BaP because their chemical and physical properties are comparable because of their similar structure and identical molecular weight.

Table A-2. Comparison of ADME Data for BeP (CASRN 192-97-2) and Candidate Analogues

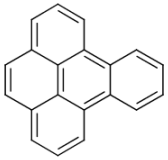
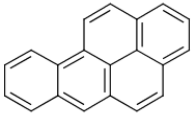
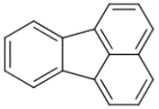
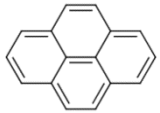
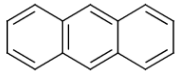
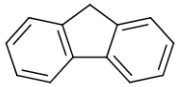
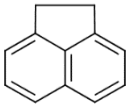
BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
						
CASRN 192-97-2	CASRN 50-32-8	CASRN 206-44-0	CASRN 129-00-0	CASRN 120-12-7	CASRN 86-73-7	CASRN 83-32-9
Absorption						
ND	**Laboratory animals (oral, inhalation, dermal):** • Absorbed by oral, inhalation, and dermal exposure • Rate and extent of absorption is variable, depending on exposure medium (e.g., oral and dermal absorption enhanced in presence of oils and fats; dermal absorption decreased in presence of soils with high organic carbon content) • Significant mucociliary clearance of inhaled particulate to gut • Absorption from gut depends on presence of bile in intestinal lumen	**Rats (oral):** • Peak blood level, higher than that for pyrene, achieved ~2 h after oral dosing • Absorption from GI tract enhanced by administration in lipophilic vehicle (dietary fat)	**Rodents (oral, i.t., dermal):** • Peak blood level achieved ~1 h after oral dosing • Extensive oral absorption (68−92% in one study) • Absorbed through tracheal epithelium more rapidly than BaP following i.t. exposure • Rapid and extensive dermal absorption in acetone (disappearance half-time of radiolabel from skin of 0.5−0.8 d; ~50% of applied radiolabel recovered in urine and feces within 6 d of application) • Dermal absorption of 94% in guinea pigs	**Rats (oral, dermal):** • More extensive oral absorption than BaP in one study (53−74% vs. 38−58%) • Absorption from gut much less dependent on the presence of bile than BaP • ~52% of applied radiolabel recovered in urine, feces, and tissues within 6 d of skin application	ND	ND

Table A-2. Comparison of ADME Data for BeP (CASRN 192-97-2) and Candidate Analogues

BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Distribution						
ND	**Laboratory animals (all routes):** • Widely distributed throughout the body • Initial rapid uptake into well-perfused tissues (e.g., lung, kidney, liver) • Subsequent accumulation, retention, and slow release from fat • High levels in gut (from any route) due to mucociliary clearance from respiratory tract and hepatobiliary excretion of metabolites • Limited placental transfer	**Rats (oral, i.v.):** • Widely distributed throughout the body • Initial rapid uptake into well-perfused tissues (e.g., lung, kidney, liver) • Levels in fat and testes were lower and peaked later • Coadministration with saturated fat extended duration of observed peaks relative to administration with no fat (controls) or unsaturated fat	**Rats (all routes):** • Widely distributed throughout the body • Initial rapid uptake into well-perfused tissues (e.g., lung, kidney, liver) • Subsequent accumulation, retention, and slow release from fat • High levels in gut (from any route) due to mucociliary clearance from respiratory tract and hepatobiliary excretion of metabolites • Limited placental transfer	**Rats (dermal):** • 6 d after application, radiolabel was located primarily in liver and kidney	**Rats (i.p.):** • Widely distributed throughout the body (radiolabel found in all tissues examined) • Highest amounts of radiolabel found in gut, gut contents, kidney, and liver 1−8 d after injection (fat not tested)	ND

Table A-2. Comparison of ADME Data for BeP (CASRN 192-97-2) and Candidate Analogues

BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Metabolism						
In vitro: • Metabolism is rapid (complete within 48 h) • Oxidized via CYP450 to the k-region 4,5-dihydrodiol, phenols and quinones • Oxidative metabolism can be induced by CYP450 inducers • Bay-region 9,10-dihydrodiol is formed only in small amounts and then oxidized at the 4,5-position, rather than forming the 9,10-expoxide in rodent model systems • Oxidative metabolites conjugated with glucuronic acid	**Laboratory animals (all routes), in vitro:** • Metabolism is rapid and occurs in many tissues throughout the body • Oxidized via CYP450; primary metabolites are 9,10-, 7,8-, 4,5-, and 2,3-dihydrodiols and epoxides, as well as various phenols, quinones, and derivatives • Oxidative metabolism can be induced by CYP450 inducers • Oxidative metabolites conjugated with GSH, glucuronic acid, and sulfate esters	**Rats (oral):** • Primary reactive metabolites identified were *trans*-2,3-dihydro-hydroxy-fluoranthene and *trans*-2,3-dihydroxy-1,10b-epoxy-1,2,3,10b-tetrahydro-fluoranthene; primary nonreactive metabolites identified were 3-hydroxy-fluoranthene and 8-hydroxy-fluoranthene • Levels of reactive metabolites in plasma and tissues were slightly higher when coadministered with saturated fat relative to administration with no fat (controls) or unsaturated fat	ND	**Rats (oral):** • Urinary metabolites include *trans*-1,2-dihydro-1,2-dihydroxy- and 1,2-dihydroxy-anthracene and *trans*-9,10-dihydro-9,10-dihydroxy-anthracene, excreted mainly as sulphate and glucuronide conjugates	**Laboratory animals (oral, i.p.):** • Primary urinary metabolites were 2- and 9-fluorenol (hydroxyfluorene) glucuronide and sulfate conjugates	**Rats, rabbits (oral):** • Urinary metabolites include *cis*- and *trans*-acenaphthene-1,2-diols and naphthalene-1,8-dicarboxylic acid, the latter indicating fission of the 5-carbon ring

Table A-2. Comparison of ADME Data for BeP (CASRN 192-97-2) and Candidate Analogues						
BeP	**BaP**	**Fluoranthene**	**Pyrene**	**Anthracene**	**Fluorene**	**Acenaphthene**
Excretion						
ND	**Laboratory animals (all routes):** • Excretion is rapid, with half-times of 22−30 h • Primary route is biliary excretion to feces; urine is secondary route • Excreted mainly as conjugated metabolites • Small amounts excreted in milk	**Rodents (oral, i.v.):** • Excretion is rapid • Coadministration with saturated fat increased excretion half-time relative to administration with unsaturated fat	**Rodents (oral, dermal):** • Excretion is rapid • Excreted in urine and feces in similar amounts	**Rat (dermal):** • Excreted in slightly higher amounts in urine than in feces	**Laboratory animals (i.p.):** • Excretion is rapid • Excreted primarily in urine, with lower amounts in feces • Excreted mainly as conjugated metabolites	**Laboratory animals (all routes):** • Excretion is rapid, with half-times of 22−30 h • Primary route is biliary excretion to feces; urine is secondary route • Excreted mainly as conjugated metabolites • Small amounts excreted in milk
NA	U.S. EPA (2017b); U.S. EPA (2017c); IARC (2010); ATSDR (1995)	Walker et al. (2007); Lipniak and Brandys (1993)	IARC (2010); ATSDR (1995); Lipniak and Brandys (1993)	IARC (2010); ATSDR (1995); Sims (1964)	Grantham (1963); Neish (1948)	Chang and Young (1943)

ADME = absorption, distribution, metabolism, and excretion; BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; CYP450 = cytochrome P450; GI = gastrointestinal; GSH = glutathione; i.p. = intraperitoneal; i.t. = intratracheal; i.v. = intravenous; NA = not applicable; ND = no data.

Toxicity-Like Analogues—Oral

No adequate studies were located regarding toxicity of BeP via oral exposure. Table A-3 summarizes available oral toxicity values for the potential structural analogues of BeP. The critical effects for potential analogues include neurodevelopmental effects, hematological effects, and kidney and liver toxicity. BaP is the most well-studied of the analogue compounds, with dose-response data available for each of the critical effects (see exposure-response arrays in Figures A-1, A-2, A-3, and A-4). The oral data for the other analogue compounds are limited to one or two subchronic gavage studies in mice. These studies provide dose-response data for hematological effects and kidney and liver toxicity only (see Figures A-1, A-2, A-3, and A-4). A review of the available dose-response data for the candidate analogues shows the lowest effect levels for BaP for each critical effect examined.

In developing the oral reference dose (RfD) for BaP, the U.S. EPA compared candidate values for developmental, reproductive, and immunological effects (U.S. EPA, 2017b, c). The overall RfD, based on neurodevelopmental effects in rats exposed during the early postnatal period, was supported by numerous human and animal studies and was considered protective of all types of health effects (U.S. EPA, 2017b, c). The mode of action (MOA) for neurodevelopmental effects of BaP is not fully understood; however, possible mechanisms may include covalent protein binding of oxidative metabolites (IARC, 2010; ATSDR, 2005), oxidative stress, and the formation of reactive oxygen species, aryl hydrocarbon (Ah) receptor-mediated effects on cell growth and differentiation, DNA damage of germ cells, stimulation of apoptosis, altered neurotransmitter levels, and changes in the balance of reproductive hormones (U.S. EPA, 2017c). In the absence of repeated-exposure oral toxicity data for BeP, there is no information with which to clearly identify or rule out candidate analogues based on toxicity comparisons.

Toxicity-Like Analogues—Inhalation

No adequate studies were located regarding toxicity of BeP via inhalation exposure. Table A-4 summarizes available inhalation toxicity values for the potential structural analogues of BeP. BaP is the only potential analogue with an inhalation toxicity value. Candidate reference concentration (RfC) values were derived for both reproductive and developmental effects of BaP (U.S. EPA, 2017b, c). The overall RfC, based on decreased embryo/fetal survival following prenatal inhalation exposure, was considered protective of all types of health effects (U.S. EPA, 2017b, c). The MOA for BaP effects on fertility may involve a decrease in prolactin and decidual luteotropin levels, leading to decreases in plasma progesterone and estradiol-17β levels [Archibong et al. (2002) as cited in U.S. EPA (2017c)]. Fetal mortality may result from a subsequent decrease in the levels of uterine progesterone receptors. In the absence of repeated-exposure inhalation toxicity data for BeP, there is no information with which to clearly identify or rule out BaP as an appropriate analogue based on toxicity comparisons.

Table A-3. Comparison of Available Oral Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

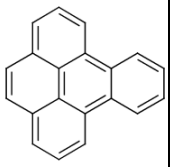
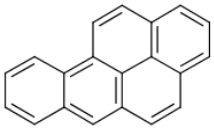
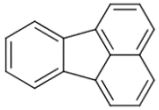
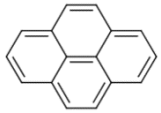
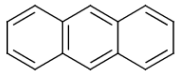
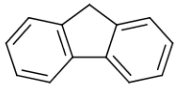
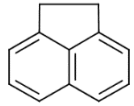
Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Structure							
CASRN	192-97-2	50-32-8	206-44-0	129-00-0	120-12-7	86-73-7	83-32-9
Subchronic oral toxicity values							
POD (mg/kg-d)	ND	ND *Note: The POD for the chronic RfD is also applicable to subchronic exposure because it is based on a developmental study (see further details below)*	124	75	1,000	125	161
POD type	ND	ND	$BMDL_{10}$	NOAEL	NOAEL	LOAEL	$BMDL_{10}$
Subchronic UF_C	ND	ND	1,000 (UF_A, UF_D, UF_H)	300 (UF_A, UF_D, UF_H)	1,000 (UF_A, UF_D, UF_H)	300 (UF_A, UF_H, UF_L)	1,000 (UF_A, UF_D, UF_H)
Subchronic p-RfD/MRL (mg/kg-d)	ND	ND	1×10^{-1}	3×10^{-1}	1×10^{0}	4×10^{-1}	2×10^{-1}

Table A-3. Comparison of Available Oral Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Critical effects	ND	ND	Nephropathy at ≥250 mg/kg-d	Kidney effects (renal tubular pathology, decreased kidney weights) at ≥125 mg/kg-d	No adverse effects on mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross or microscopic pathology	Increased liver weight	Increased relative liver weight in female mice
Species	ND	ND	Mouse	Mouse	Mouse	Mouse	Mouse
Duration	ND	ND	13 wk	13 wk	13 wk	13 wk	13 wk
Route (method)	ND	ND	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)
Source	NA	NA	U.S. EPA (2012) (PPRTV)	U.S. EPA (2007) (PPRTV)	U.S. EPA (2009) (PPRTV)	ATSDR (1995) (Intermediate MRL)	U.S. EPA (2011c) (PPRTV)
Chronic oral toxicity values							
POD (mg/kg-d)	ND	0.092	125	75	1,000	125	175
POD type	ND	$BMDL_{1SD}$	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL
Chronic UF_C	ND	300 (UF_A, UF_D, UF_H)	3,000 (UF_A, UF_D, UF_H, UF_S)	3,000 (UF_A, UF_D, UF_H, UF_S)	3,000 (UF_A, UF_D, UF_H, UF_S)	3,000 (UF_A, UF_D, UF_H, UF_S)	3,000 (UF_A, UF_D, UF_H)
Chronic RfD/p-RfD (mg/kg-d)	ND	3×10^{-4}	4×10^{-2}	3×10^{-2}	3×10^{-1}	4×10^{-2}	6×10^{-2}

Table A-3. Comparison of Available Oral Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Critical effects	ND	Neurodevelopmental effects (increased open field crossed squares at PND 69; elevated plus maze open arm entries at PND 70; increased Morris water maze hidden platform trial escape latency at PNDs 71−74	Nephropathy, increased absolute and relative liver weights, hematological and clinical chemistry alterations (decreased albumin:globulin ratio, increased ALT, decreased packed cell volume and eosinophils) at ≥250 mg/kg-d	Kidney effects (renal tubular pathology, decreased kidney weights) at ≥125 mg/kg-d	No adverse effects on mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross or microscopic pathology at ≤1,000 mg/kg-d	Decreased RBC, packed cell volume, and hemoglobin	Hepatotoxicity accompanied by increased liver weight (which was considered adaptive)
Species	ND	Rat	Mouse	Mouse	Mouse	Mouse	Mouse
Duration	ND	PNDs 5−11	13 wk	13 wk	13 wk	13 wk	13 wk
Route (method)	ND	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)
Source	NA	U.S. EPA (2017b); U.S. EPA (2017c)	U.S. EPA (1990d) (IRIS)	U.S. EPA (1990f) (IRIS)	U.S. EPA (1990b) (IRIS)	U.S. EPA (1990e) (IRIS)	U.S. EPA (1990a) (IRIS)
Acute oral lethality data							
Oral LD_{50} (mg/kg)	ND	ND	800 (mouse) 2,700 (rat)	ND	>17,000 (mouse)	2,000 (rat)	ND
Toxicity at rat LD_{50}	ND	ND	Behavioral effects (excitement, muscle contraction or spasticity); eye irritation	ND	Fatty degeneration of the liver	ND	ND

Table A-3. Comparison of Available Oral Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Source	NLM (2019e)	NLM (2019d)	U.S. EPA (1990d) (IRIS); NLM (2019f)	U.S. EPA (1990f) (IRIS); NLM (2019h)	NLM (2019c)	NLM (2019g)	NLM (2019b)

ALT = alanine aminotransferase; BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; BMDL = benchmark dose lower confidence limit; IRIS = Integrated Risk Information System; LD_{50} = median lethal dose; LOAEL = lowest-observed-adverse-effect level; MRL = minimal risk level; NA = not applicable; ND = no data; NOAEL = no-observed-adverse-effect level; PND = postnatal day; POD = point of departure; (p-)RfD = (provisional) reference dose; PPRTV = provisional peer-reviewed toxicity value; RBC = red blood cell; SD = standard deviation; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

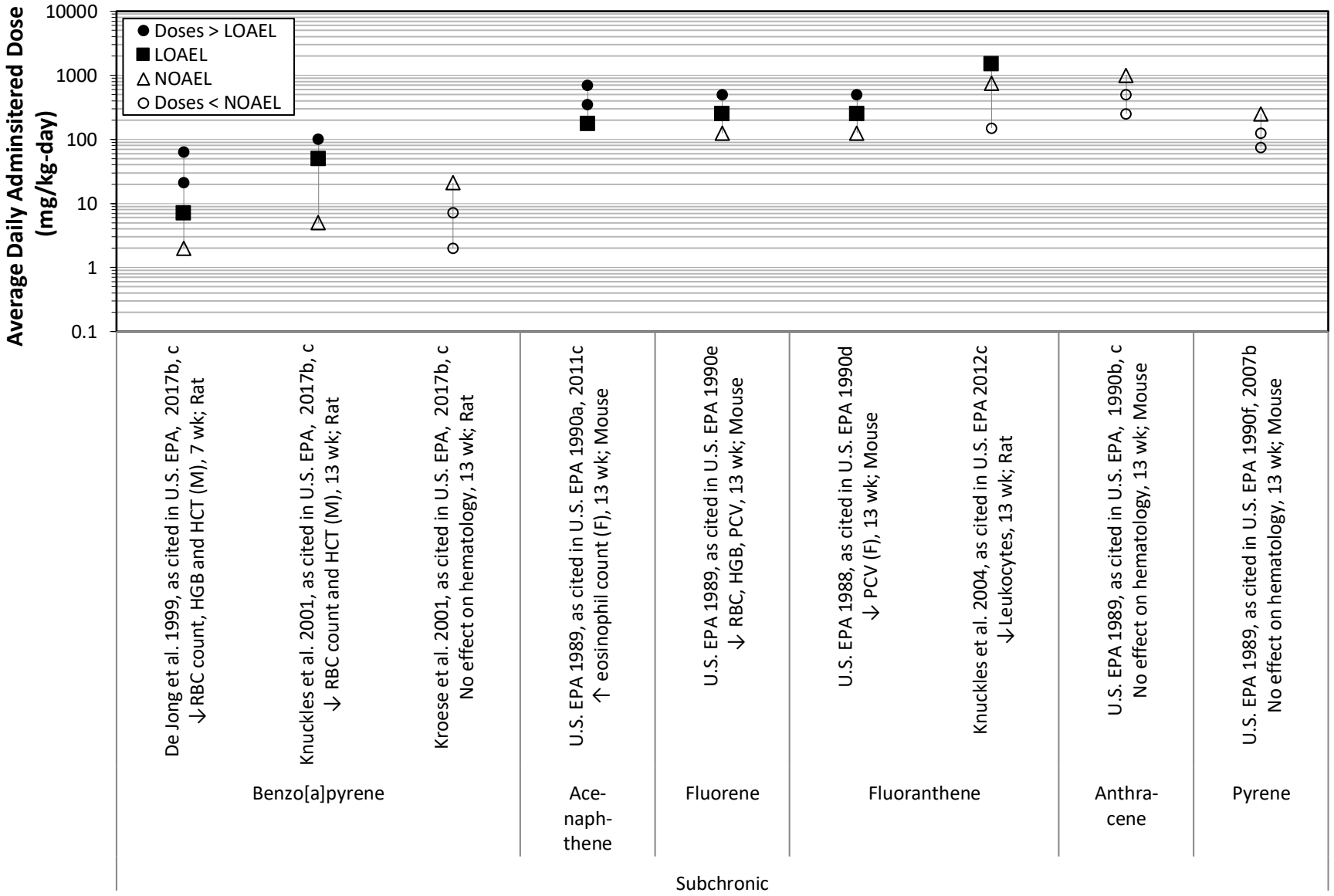


Figure A-1. Hematological Effects Following Oral Exposure to Candidate Analogues of Benzo[e]pyrene (CASRN 192-97-2)

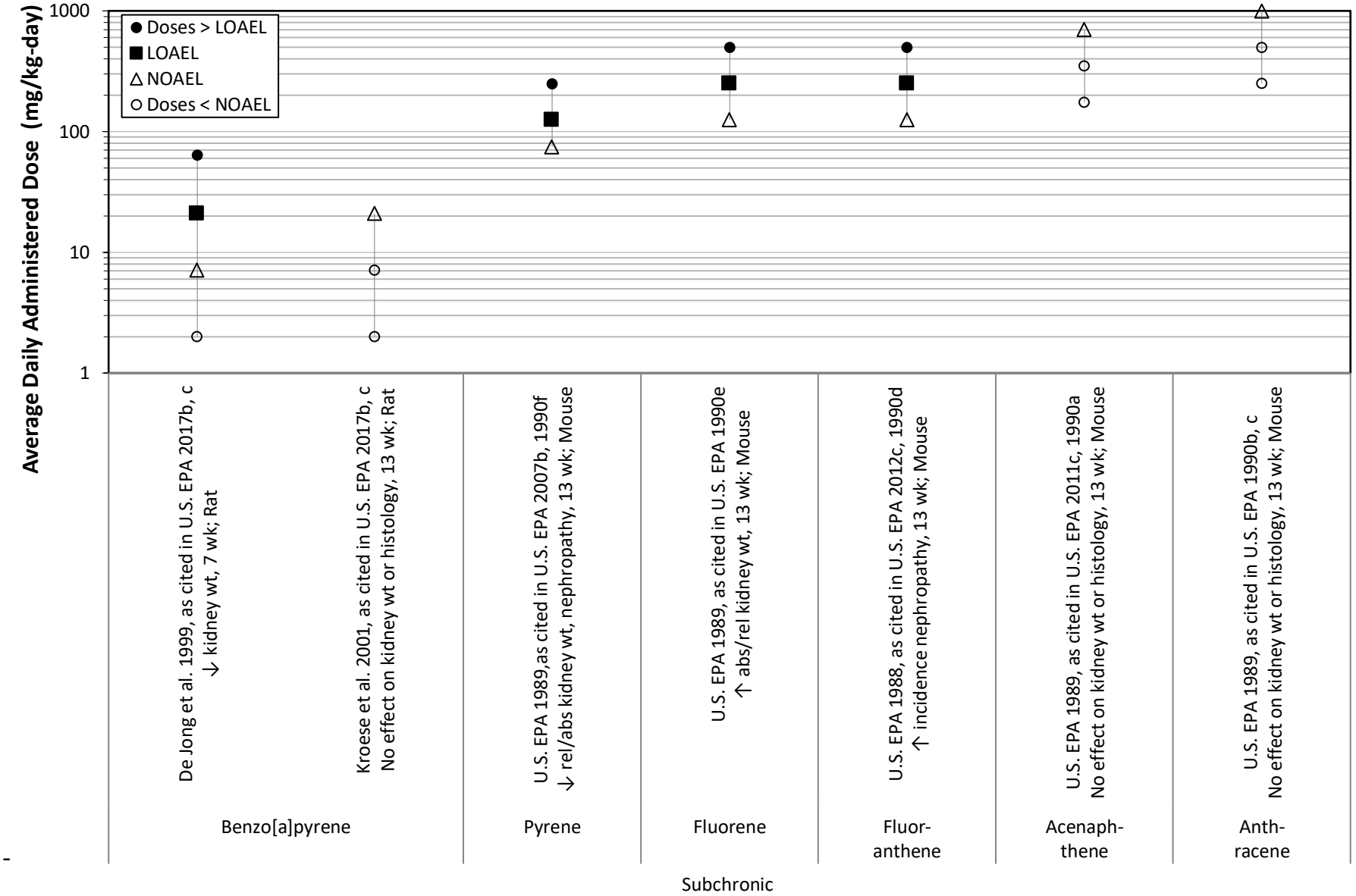


Figure A-2. Renal Effects Following Oral Exposure to Candidate Analogues of Benzo[e]pyrene (CASRN 192-97-2)

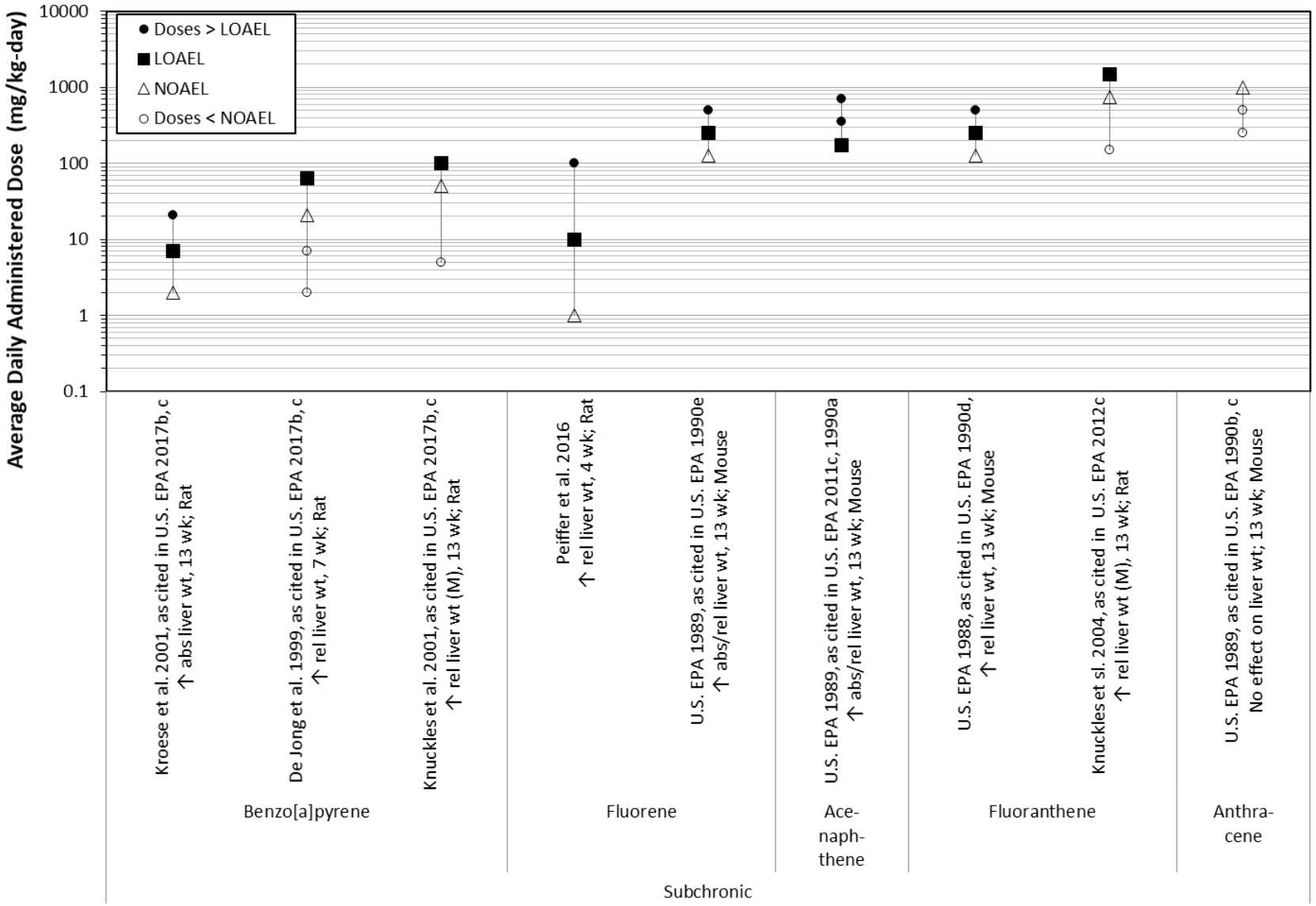


Figure A-3. Liver Weight Following Oral Exposure to Candidate Analogues of Benzo[e]pyrene (CASRN 192-97-2)

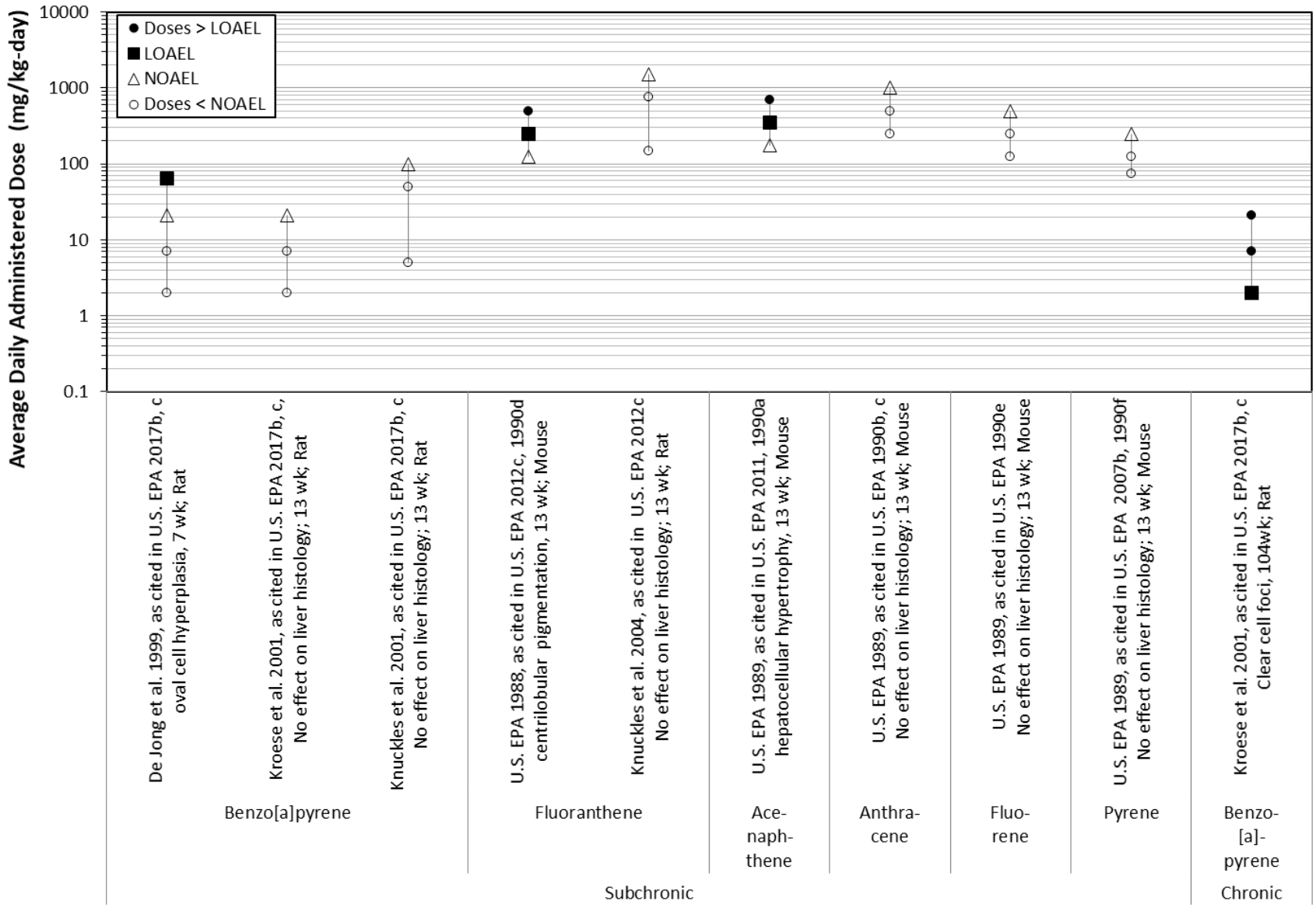


Figure A-4. Liver Histopathology Following Oral Exposure to Candidate Analogues of Benzo[e]pyrene (CASRN 192-97-2)

Table A-4. Comparison of Available Inhalation Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

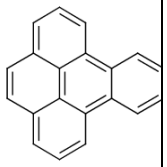
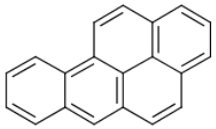
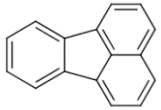
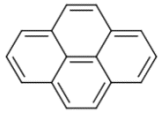
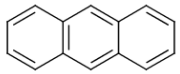
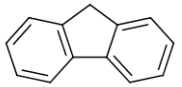
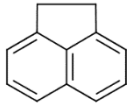
Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Structure							
CASRN	192-97-2	50-32-8	206-44-0	129-00-0	120-12-7	86-73-7	83-32-9
Subchronic inhalation toxicity values							
POD (mg/m^3)	ND	ND *Note: The POD for the chronic RfC may also be used as a subchronic POD because it is based on a developmental study (see below)*	ND	ND	ND	ND	ND
POD type	ND	ND	ND	ND	ND	ND	ND
Subchronic UF_C	ND	ND	ND	ND	ND	ND	ND
Subchronic p-RfC/MRL (mg/m^3)	ND	ND	ND	ND	ND	ND	ND
Critical effects	ND	ND	ND	ND	ND	ND	ND
Species	ND	ND	ND	ND	ND	ND	ND
Duration	ND	ND	ND	ND	ND	ND	ND
Route (method)	ND	ND	ND	ND	ND	ND	ND
Source	NA	U.S. EPA (2017c) (IRIS)	U.S. EPA (2012) (PPRTV)	U.S. EPA (2007) (PPRTV)	U.S. EPA (2009) (PPRTV)	ATSDR (1995) (intermediate MRL)	U.S. EPA (2011c) (PPRTV)

Table A-4. Comparison of Available Inhalation Toxicity Data for BeP (CASRN 192-97-2) and Candidate Structural Analogues

Type of Data	BeP	BaP	Fluoranthene	Pyrene	Anthracene	Fluorene	Acenaphthene
Chronic inhalation toxicity values							
POD (mg/m^3)	ND	0.0046	ND	ND	ND	ND	ND
POD type	ND	LOAEL	ND	ND	ND	ND	ND
Chronic UF_C	ND	3,000 (UF_A, UF_H, UF_L, UF_D)	ND	ND	ND	ND	ND
Chronic p-RfC/MRL (mg/m^3)	ND	2×10^{-6}	ND	ND	ND	ND	ND
Critical effects	ND	Decreased embryo/fetal survival	ND	ND	ND	ND	ND
Species	ND	Rat	ND	ND	ND	ND	ND
Duration	ND	GDs 11−20	ND	ND	ND	ND	ND
Route (method)	ND	Inhalation (nose only)	ND	ND	ND	ND	ND
Source	NA	U.S. EPA (2017c) (IRIS)	U.S. EPA (2012) (PPRTV)	U.S. EPA (2007) (PPRTV)	U.S. EPA (2009) (PPRTV)	ATSDR (1995) (intermediate MRL)	U.S. EPA (2011c) (PPRTV)
Acute inhalation lethality data							
LC_{50} (mg/m^3)	ND	ND	170 mg/m^3	ND	ND	ND	ND
Toxicity at LC_{50}	ND	ND	Behavioral effects (excitement, muscle contraction or spasticity); eye irritation	ND	ND	ND	ND
Source	NLM (2019e)	U.S. EPA (2017c); NLM (2019d)	U.S. EPA (1990d) (IRIS); NLM (2019f)	U.S. EPA (1990f) (IRIS); NLM (2019h)	U.S. EPA (1990b) (IRIS); NLM (2019c)	U.S. EPA (1990e) (IRIS); NLM (2019g)	U.S. EPA (2011c) (PPRTV); NLM (2019b)

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; GD = gestation day; IRIS = Integrated Risk Information System; LC_{50} = median lethal concentration; LOAEL = lowest-observed-adverse-effect level; MRL = minimal risk level; NA = not applicable; ND = no data; NOAEL = no-observed-adverse-effect level; POD = point of departure; (p-)RfC = (provisional) reference concentration; PPRTV = provisional peer-reviewed toxicity value; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor.

Weight-of-Evidence Approach

To select the best analogue chemical based on all of the information from the three analogue types, the following considerations are used in a WOE approach: (1) lines of evidence from U.S. EPA assessments are preferred; (2) biological and toxicokinetic data are preferred over the structural similarity scores; (3) lines of evidence that indicate pertinence to humans are preferred; (4) chronic studies are preferred over subchronic studies when selecting an analogue for a chronic value; (5) chemicals with more sensitive toxicity values are of potential concern; and (6) if there are no clear indications as to the best analogue chemical based on the other considerations, then the candidate analogue with the highest structural similarity scores may be preferred.

Oral

The WOE approach used to select the analogue compound for BeP oral exposure is based on structural similarity, comparable physicochemical properties, and similar oxidative metabolism to reactive intermediates. BaP is the preferred structural analogue for BeP due its physicochemical properties, which closely resemble BeP. BaP is also the preferred metabolic analogue for BeP due to the potential for BeP to form reactive dihydrodiol and diol epoxide metabolites, acknowledging that the generation of these reactive metabolites is estimated to be much lower for BeP compared to BaP. Although in vivo toxicological similarity cannot be assessed due to the absence of data for BeP, metabolism data suggest that the candidate analogue with bioactivity most similar to BeP is BaP. Furthermore, the neurodevelopmental effects of BaP provide the most sensitive measure of toxicity among the candidate analogue compounds. Based on available data, BaP was selected as the most appropriate analogue compound for both subchronic and chronic effects.

Inhalation

As discussed above for oral exposure, BaP is the preferred structural and metabolic analogue for BeP. Regarding toxicity, BaP was the only candidate analogue compound with repeated-dose inhalation toxicity data. Therefore, BaP was selected as the analogue compound for both subchronic and chronic inhalation exposure.

ORAL NONCANCER TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, BaP is selected as the analogue for BeP for derivation of screening subchronic and chronic p-RfDs. The study used for the U.S. EPA screening subchronic and chronic p-RfD values for BeP is an early postnatal gavage study of BaP in rats. The *Toxicological Review of Benzo[a]pyrene (CASRN 50-32-8): Supplemental Information* (U.S. EPA, 2017b) provided the following summary:

> *Chen et al. (2012) treated male and female neonatal Sprague-Dawley rats (10/sex/group) with BaP (unspecified purity) dissolved in peanut oil by gavage daily on PNDs 5−11, at doses of 0.02, 0.2, or 2 mg/kg in 3 mL vehicle/kg body weight, determined individually based upon daily measurements. This time period was described as representing the brain growth spurt in rodents, analogous to brain developmental occurring from the third trimester to 2 years of age in human infants. Breeding was performed by pairs of 9-week-old rats, with delivery designated as PND 0. Litters were culled to eight pups/dam (four males and four*

females, when possible) and randomly redistributed at PND 1 among the nursing dams; dams themselves were rotated every 2−3 days to control for caretaking differences, and cage-side observations of maternal behavior were made daily. One male and female from each litter were assigned per treatment group, and the following physical maturation landmarks were assessed daily in all treatment groups until weaning at PND 21: incisor eruption, eye opening, development of fur, testis decent, and vaginal opening.

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

Sensory and motor developmental tests, including the surface righting reflex test, negative geotaxis test, and cliff aversion test, were performed only once, while the forelimb grip strength test was assessed during three 60-second trials on PND 12. Rat movements during the open-field test were recorded by camera, and two blinded investigators scored movement and rearing separately during a 5-minute evaluation period. Blinded investigators directly observed video monitoring of rat movements during the elevated plus maze, and after a 5-minute free exploration period, recorded number of entries into the closed and open arms, time spent in the open arms, and latency to the first arm entry. Assessment of the Morris water maze was slightly different, in that the rats were habituated to the testing pool by a 60-second swim without a platform on the day prior to testing. The rats were then tested during a 60-second swim with a hidden platform present at a constant position each day for 4 days; on the 5th day, the rats were evaluated during a 60-second probe swim without a platform. The number of times each animal crossed the original platform location and the duration of time spent in the platform quadrant were recorded during this final evaluation. One pup/sex/litter were assigned for behavioral testing to each of four tracks: Track 1, surface righting reflex test, cliff aversion test, and open-field test (PNDs 12−18); Track 2, negative geotaxis test, forelimb grip strength test, and open-field test (PNDs 12−20); Track 3, elevated plus maze, Morris water maze, and open-field test (PNDs 34−36); and Track 4, elevated plus maze, Morris water maze, and open-field test (PNDs 69−71). All results were presented in graphic form only.

No significant effects on pup body weight were observed during the 7-day treatment period (PNDs 5−11). Three-way ANOVA (time × BaP treatment × sex) indicated that effects of BaP were not sex-dependent throughout the 71-day experiment, so both sexes were pooled together. From this pooled analysis, pups in the 2 mg/kg-day treatment group gained significantly less weight at both

PND 36 and 71. There were no differences among treatment groups in incisor eruption, eye opening, development of fur, testis decent, or vaginal opening.

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

The elevated plus maze performance was only evaluated in adolescent and adult rats. Unlike the previous tests, 3-way ANOVA revealed a statistically significant interaction between neonatal BaP treatment and sex, so male and female performance was analyzed independently. No significant differences in PND 35 males were observed, and the only significant observation in PND 35 females was increased time spent in the open maze arms by the 2 mg/kg-day treatment group. Significantly decreased latency time to first open arm entry was observed in PND 70 males and females in both 0.2 and 2 mg/kg-day treatment groups; these groups also spent significantly more time in open maze arms, along with the 0.02 mg/kg-day female group. At PND 70, the 2 mg/kg-day males, along with the 0.2 and 2 mg/kg-day females, entered more frequently into open arms and less frequently into closed arms than the vehicle controls. In the Morris water maze, escape latency (time to reach the platform during each of the four testing days) was consistently increased in the 2 mg/kg-day treatment group of both sexes, in both adolescent and adult animals. These increases were statistically significant in both males and females treated with 2 mg/kg-day BaP at both PNDs 39 and 74, and were also significantly elevated in 0.2 mg/kg-day animals of both sexes at PND 74. Likewise, performance during the 5th test day, in the absence of the escape platform, was significantly adversely affected by both metrics (decreased time spent in the target quadrant and decreased number of attempts to cross the platform location) in 2 mg/kg-day rats of both sexes at both PNDs 40 and 75. PND 75 females treated with 0.2 mg/kg-day BaP also showed significant decreases in both performance metrics, while PND 75 0.2 mg/kg-day males only demonstrated significant differences in 'time spent in target quadrant.' Swim speed was also assessed, but there were no differences among any treatment group at either age evaluated.

The $BMDL_{1SD}$ of 0.092 mg/kg-day was identified as the point of departure (POD) for BaP based on the induction of neurobehavioral changes during a susceptible life stage (U.S. EPA, 2017c). This POD is selected from among a suite of available endpoints because it represents multiple neurobehavioral endpoints from several behavioral tests. Similar effects were replicated among numerous additional studies. The U.S. EPA (2017c) did not convert the POD into a human equivalent dose (HED) using $BW^{3/4}$ because the critical study evaluated developmental toxicity in early postnatal animals directly exposed to BaP. $BW^{3/4}$ scaling was determined to be inappropriate because (1) it is unknown whether allometric scaling derived from adult animals is appropriate for extrapolating doses in neonates in the absence of quantitative toxicokinetic and toxicodynamic differences and (2) differences in temporal patterns of development across species results in complications for interspecies dose extrapolation.

The RfD for BaP is derived using a composite uncertainty factor (UF_C) of 300, reflecting 10-fold uncertainty factors for interspecies extrapolation and intraspecies variability and a 3-fold uncertainty factor for database uncertainties (UF_A, UF_H, and UF_D, respectively) (U.S. EPA, 2017c). Wang et al. (2012) indicated that the uncertainty factors typically applied in deriving a toxicity value for the chemical of concern are the same as those applied to the analogue unless additional information is available. To derive the screening subchronic p-RfD for BeP from the BaP data, the UF_D of 3 is increased to 10 to account for the absence of adequate repeated-dose oral toxicity data for BeP.

Screening Subchronic p-RfD	**=**	Analogue POD ÷ UF_C
	=	0.092 mg/kg-day ÷ 1,000
	=	**9×10^{-5} mg/kg-day**

Table A-5 summarizes the uncertainty factors for the screening subchronic p-RfD for BeP.

Table A-5. Uncertainty Factors for the Screening Subchronic p-RfD for BeP (CASRN 192-97-2)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty associated with extrapolating from animals to humans when no cross-species dosimetric adjustment (HED calculation) is performed.
UF_D	10	A UF_D of 10 is applied to reflect database limitations for the BaP analogue and the absence of toxicity data for the target chemical (BeP).
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of BeP in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a developmental study was selected as the principal study. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_C	1,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

BaP is also selected as the analogue for BeP for deriving a screening chronic p-RfD. The key study and calculation of the POD are described above for the subchronic p-RfD. In deriving the screening chronic p-RfD for BeP, the same uncertainty factors used for the screening subchronic p-RfD (UF_A of 10, UF_H of 10, and UF_D of 10) are applied. An additional uncertainty factor for study duration is not applied because a developmental study is used as the principal study.

Screening Chronic p-RfD = Analogue POD ÷ UF_C
= 0.092 mg/kg-day ÷ 1,000
= **9×10^{-5} mg/kg-day**

Table A-6 summarizes the uncertainty factors for the screening chronic p-RfD for BeP.

Table A-6. Uncertainty Factors for the Screening Chronic p-RfD for BeP (CASRN 192-97-2)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty associated with extrapolating from animals to humans when no cross-species dosimetric adjustment (HED calculation) is performed.
UF_D	10	A UF_D of 10 is applied to reflect database limitations for the BaP analogue and the absence of toxicity data for BeP.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of BeP in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a developmental study is selected as the principal study. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). The database also contains chronic-duration exposure information, which did not characterize a more sensitive endpoint.
UF_C	1,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BeP = benzo[e]pyrene; BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

INHALATION NONCANCER TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Concentration

Based on the overall analogue approach presented in this PPRTV assessment, BaP is selected as the analogue for BeP for deriving the screening subchronic and chronic p-RfCs. The study used for the U.S. EPA screening subchronic and chronic p-RfC values for BeP is a prenatal inhalation study of BaP in rats [Archibong et al. (2002) as cited in U.S. EPA (2017b, 2017c)]. The supplemental information for the toxicological review of BaP (U.S. EPA, 2017b) provided the following summary:

> *Archibong et al. (2002) evaluated the effect of exposure to inhaled benzo[a]pyrene on fetal survival and luteal maintenance in timed-pregnant F344 rats. Prior to exposure on GD 8, laparotomy was performed to determine the number of implantation sites, and confirmed pregnant rats were divided into three groups, consisting of rats that had four to six, seven to nine, or more than nine conceptuses in utero. Rats in these groups were then assigned randomly to the treatment groups or control groups to ensure a similar distribution of litter sizes. Animals (10/group) were exposed to benzo[a]pyrene:carbon black aerosols at concentrations of 25, 75, or 100 μg/m^3 via nose-only inhalation, 4 hours/day on GDs 11−20. Control animals were either sham-exposed to carbon black or remained entirely unexposed. Results of particle size analysis of generated aerosols were reported by several other reports from this laboratory (Inyang et al., 2003; Ramesh et al., 2001a; Hood et al., 2000). Aerosols showed a trimodal distribution (average of cumulative mass, diameter) <95%, 15.85 μm; 89%,*

<10 μm; 55%, <2.5 μm; and 38%, <1 μm (Inyang et al., 2003). Ramesh et al. (2001a) reported that the MMAD (± geometric SD) for the 55% mass fraction with diameters <2.5 μm was 1.7 ± 0.085. Progesterone, estradiol-17β, and prolactin concentrations were determined in plasma collected on GDs 15 and 17. Fetal survival was calculated as the total number of pups divided by the number of all implantation sites determined on GD 8. Individual pup weights and crown-rump length per litter per treatment were determined on PND 4 (PND 0 = day of parturition).

Archibong et al. (2002) reported that exposure of rats to benzo[a]pyrene caused biologically and statistically significant ($p \leq 0.05$) reductions in fetal survival compared with the two control groups; fetal survival rates were 78.3, 38.0, and 33.8% per litter at 25, 75, and 100 $\mu g/m^3$, respectively, and 96.7% with carbon black or 98.8% per litter in untreated controls (see Table D-30). Consequently, the number of pups per litter was also decreased in a concentration-dependent manner. The decrease was ~50% at 75 $\mu g/m^3$ and ~65% at 100 $\mu g/m^3$, compared with sham-exposed and unexposed control groups. No effects on hormone levels were observed on GDs 15 or 17 at the low dose. Biologically significant decreases in mean pup weights (expressed as g per litter) of >5% relative to the untreated control group were observed at doses ≥75 $\mu g/m^3$ (14 and 16% decreases at 75 and 100 $\mu g/m^3$, respectively, $p < 0.05$). There were no statistically significant differences from the control groups in crown-rump length (see Table D-30).

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

The lowest-observed-adverse-effect level (LOAEL) of 25 µg/m^3 for decreased embryo/fetal survival was selected as the POD for BaP (U.S. EPA, 2017c). The POD was converted into a LOAEL (HEC) of 4.6 µg/m^3 (0.0046 mg/m^3) by the U.S. EPA (2017c). The quotation from U.S. EPA (2017c) continues:

> *By definition, the RfC is intended to apply to continuous lifetime exposures for humans (U.S. EPA, 1994a). EPA recommends that adjusted continuous exposures be used for developmental toxicity studies by the inhalation route as well as for inhalation studies of longer durations (U.S. EPA, 2002). The PODs were adjusted to account for the discontinuous daily exposure as follows:*
>
> $$POD_{ADJ} = POD \times \text{hours exposed per day/24 hours}$$
> $$= LOAEL \times (\text{duration of exposure/24 hours})$$
> $$= POD_{ADJ}$$
>
> *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, 1994a). The observed developmental effects are considered systemic in nature (i.e., extrarespiratory) and the normalizing factor for extrarespiratory effects of particles is body weight (i.e., the equivalent dose across species is mass deposited in the entire respiratory tract per unit body weight). The $RDDR_{ER}$ was calculated as follows:*
>
> $$RDDR_{ER} = (BW_H/BW_A) \times ([V_E]_A/[V_E]_H) \times ([F_{TOT}]_A/[F_{TOT}]_H)$$
>
> *where:*
>
> *BW = body weight (kg);*
> *VE = ventilation rate (L/minute); and*
> *F_{TOT} = total fractional deposition.*
>
> *The total fractional deposition includes particle deposition in the nasal-pharyngeal, tracheobronchial, and pulmonary regions. F_{TOT} for both animals and humans was calculated using the Multi-Path Particle Dosimetry (MPPD) model, a computational model used for estimating human and rat airway particle deposition (MPPD; Version 2.0 © 2006, as accessed through the former Hamner Institute; now publicly available through Applied Research Associates). 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 after adjusting for inhalability. 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; VE, 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); VE, 0.18 L/minute; breathing frequency, 102 per minute; tidal volume, 1.8 mL; functional residual capacity, 4 mL; and upper respiratory tract volume, 0.42 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:

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

The RfC for BaP is derived from the $LOAEL_{HEC}$ of 0.0046 mg/m^3 using a UF_C of 3,000, reflecting a 10-fold UF_L, UF_H, and UF_D and a 3-fold UF_A (U.S. EPA, 2017c). Wang et al. (2012) indicated that the uncertainty factors typically applied in deriving a toxicity value for the chemical of concern are the same as those applied to the analogue unless additional information is available. Given the limitations of the current database, the uncertainty factors for BaP were adopted for BeP unless otherwise noted below in Tables A-7 and A-8.

Screening Subchronic p-RfC = Analogue POD ÷ UF_C
= 0.0046 mg/m^3 ÷ 3,000
= **2×10^{-6} mg/m^3**

Table A-7 summarizes the uncertainty factors for the screening subchronic p-RfC for BeP.

Table A-7. Uncertainty Factors for the Screening Subchronic p-RfC for BeP (CASRN 192-97-2)

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for uncertainty associated with extrapolating from animals to humans when a cross-species dosimetric adjustment (HEC calculation) is performed.
UF_D	10	A UF_D of 10 is applied to reflect the database limitations for the BaP analogue and absence of toxicity data for BeP.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of BeP in humans.
UF_L	10	A UF_L of 10 is applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 is applied because a developmental study is selected as the principal study. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Concentration

BaP is also selected as the analogue for BeP for deriving a screening chronic p-RfC. The key study and calculation of the POD are described above for the subchronic p-RfC. In deriving the screening chronic p-RfC for BeP, the same uncertainty factors used for the screening subchronic p-RfC (UF_A of 3, UF_H of 10, UF_D of 10, UF_L of 10) are applied. An additional uncertainty factor for study duration is not applied because a developmental study is used as the principal study.

$$\begin{aligned} \textbf{Screening Chronic p-RfC} &= \text{Analogue POD} \div UF_C \\ &= 0.0046\ \text{mg/m}^3 \div 3{,}000 \\ &= \mathbf{2 \times 10^{-6}\ mg/m^3} \end{aligned}$$

Table A-8 summarizes the uncertainty factors for the screening chronic p-RfC for BeP.

Table A-8. Uncertainty Factors for the Screening Chronic p-RfC for BeP (CASRN 192-97-2)

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for uncertainty associated with extrapolating from animals to humans when a cross-species dosimetric adjustment (HEC calculation) is performed.
UF_D	10	A UF_D of 10 is applied to reflect the database limitations for the BaP analogue and absence of toxicity data for BeP.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of BeP in humans.
UF_L	10	A UF_L of 10 is applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 is applied because a developmental study is selected as the principal study. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). The database also contains chronic-duration exposure information, which did not characterize a more sensitive endpoint.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

APPENDIX B. BACKGROUND AND METHODOLOGY FOR THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

Due to the lack of evidence described in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, there is inadequate information to assess the carcinogenic potential of benzo[e]pyrene (BeP). However, some information is available for this chemical which, although insufficient to support a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening evaluation of potential carcinogenicity." Appendices receive the same level of internal and external scientific peer review as the provisional cancer assessments in PPRTVs to ensure their appropriateness within the limitations detailed in the document. Users of the information regarding potential carcinogenicity in this appendix should understand that there could be more uncertainty associated with this evaluation than for the cancer WOE descriptors presented in the body of the assessment. Questions or concerns about the appropriate use of the screening evaluation of potential carcinogenicity should be directed to the CPHEA.

The screening evaluation of potential carcinogenicity includes the general steps shown in Figure B-1. The methods for Steps 1 through 8 apply to any target chemical and are described in this appendix. Chemical-specific data for all steps in this process are summarized in Appendix C.

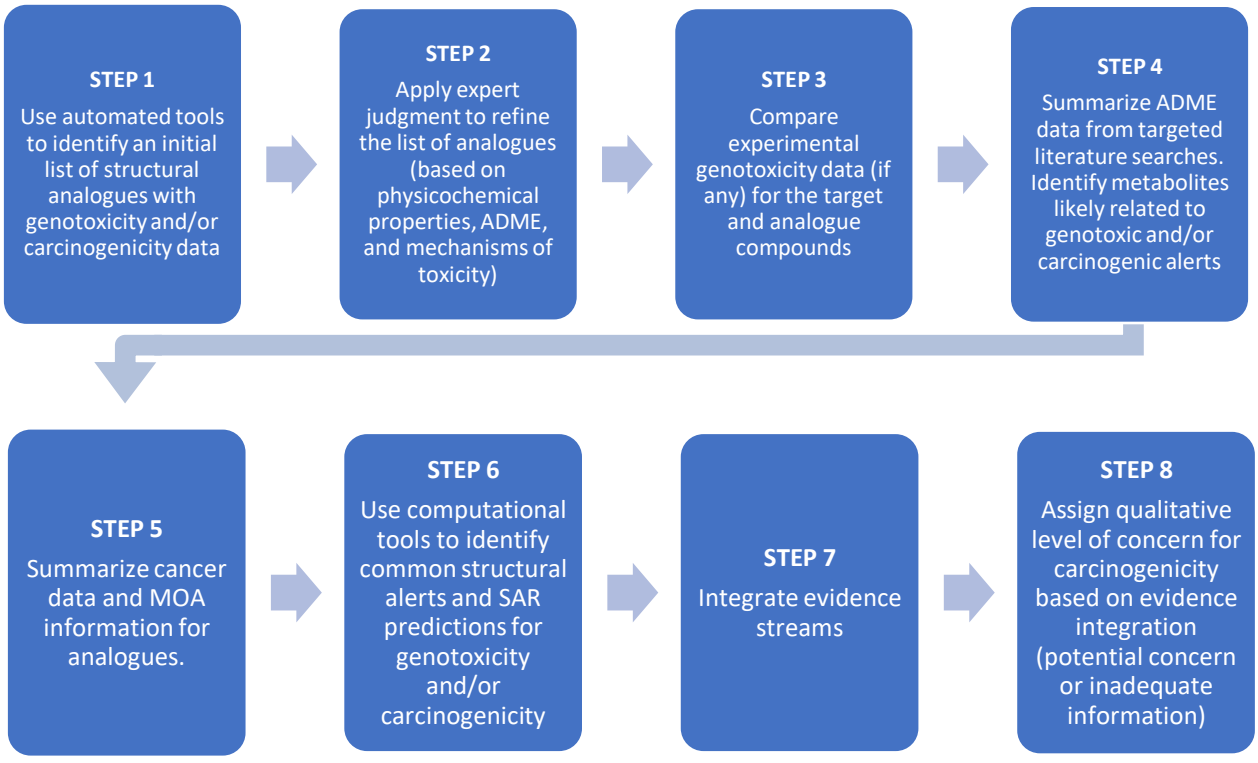


Figure B-1. Steps Used in the Screening Evaluation of Potential Carcinogenicity

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

ChemACE Clustering

The U.S. EPA's Chemical Assessment Clustering Engine [ChemACE; U.S. EPA (2011a)] is an automated tool that groups (or clusters) a user-defined list of chemicals based on chemical structure fragments. The methodology used to develop ChemACE was derived from U.S. EPA's Analog Identification Methodology (AIM) tool, which identifies structural analogues for a chemical based on common structural fragments. ChemACE uses the AIM structural fragment recognition approach for analogue identification and applies advanced queries and user-defined rules to create the chemical clusters. The ChemACE outputs are available in several formats and layouts (i.e., Microsoft Excel, Adobe PDF) to allow rapid evaluation of structures, properties, mechanisms, and other parameters, which are customizable based on an individual user's needs. ChemACE grouping has been successfully used with chemical inventories for identifying trends within a series of structurally similar chemicals, demonstrating structural diversity in a chemical inventory, and detecting structural analogues to fill data gaps and/or perform read-across.

For this project, ChemACE is used to identify potential structural analogues of the target compound that have available carcinogenicity assessments and/or carcinogenicity data. An overview of the ChemACE process is shown in Figure B-2.

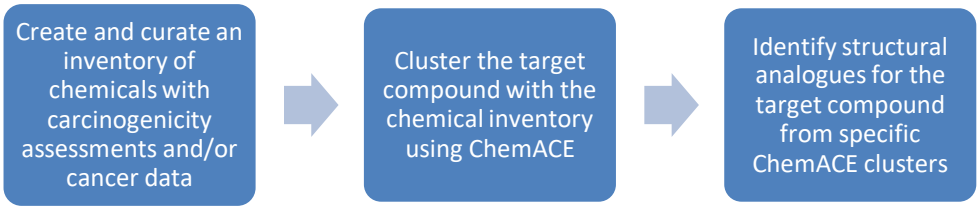


Figure B-2. Overview of ChemACE Clustering Process

The chemical inventory was populated with chemicals from the following databases and lists:

- Carcinogenic Potency Database [CPDB; CPDB (2011)]
- Agents classified by the International Agency for Research on Cancer (IARC) monographs (IARC, 2018)
- National Toxicology Program (NTP) Report on Carcinogens [ROC; NTP (2016)]
- NTP technical reports (NTP, 2017)
- Integrated Risk Information System (IRIS) carcinogens (U.S. EPA, 2017a)
- California prop 65 list (CalEPA, 2017)
- European Chemicals Agency (ECHA) carcinogenicity data available in the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox (OECD, 2019)
- PPRTVs for Superfund (U.S. EPA, 2020b)

In total, 2,189 distinct substances were identified from the sources above. For the purpose of ChemACE clustering, each individual substance needed to meet the following criteria:

1) Substance is not a polymer, metal, inorganic, or complex salt because ChemACE is not designed to accommodate these substances;
2) Substance has a CASRN or unambiguous chemical identification; and
3) A unique Simplified Molecular Input Line Entry System (SMILES) notation (encoded molecular structure format used in ChemACE) for the substance can be identified from one of these sources:
    a. Syracuse Research Corporation (SRC) and Distributed Structure-Searchable Toxicity (DSSTox) lists of known SMILES associated with unique CASRNs (the combined lists contained >200,000 SMILES) or
    b. ChemIDplus, U.S. EPA Chemicals Dashboard, or internet searches.

Of the initial list of 2,189 substances, 254 were removed because they did not meet at least one of the three criteria. The final inventory of substances contained 1,935 unique compounds.

Two separate ChemACE approaches were compared for clustering of the chemical inventory. The restrictive clustering approach, where all compounds in a cluster contain all of the same fragments and no different fragments, resulted in 235 clusters. The less restrictive approach included the following rules for remapping the chemical inventory:

- treat adjacent halogens as equivalent, allowing fluorine (F) to be substituted for chlorine (Cl), Cl for bromine (Br), Br for iodine (I);
- allow methyl, methylene, and methane to be equivalent;
- allow primary, secondary, and tertiary amines to be equivalent; and
- exclude aromatic thiols (removes thiols from consideration).

Clustering using the less restrictive approach (Pass 2) resulted in 253 clusters. ChemACE results for clustering of the target chemical within the clusters of the chemical inventory are described in Appendix C.

Analogue Searches in the OECD QSAR Toolbox (Dice Method)

The OECD QSAR Toolbox (Version 4.1) is used to search for additional structural analogues of the target compound. Several structural similarity score equations are available in the toolbox (Dice, Tanimoto, Kulczynski-2, Ochiai/Cosine, and Yule). Dice is considered the default equation. The specific options that are selected for the performance of this search include a comparison of molecular features (atom-centered fragments) and atom characteristics (atom type, count hydrogens attached, and hybridization). Chemicals identified in these similarity searches are selected if their similarity scores exceeded 50%.

The OECD QSAR Toolbox Profiler is used to identify those structural analogues from the Dice search that have carcinogenicity and/or genotoxicity data. Nine databases in the OECD QSAR Toolbox (Version 4.1) provide data for carcinogenicity or genotoxicity (see Table B-1).

Analogue search results for the target chemical are described in Appendix C.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
CPDB	The CPDB provides access to bioassay literature with qualitative and quantitative analysis of published experiments from the general literature (through 2001) and from the NCI/NTP (through 2004). Reported results include bioassays in rats, mice, hamsters, dogs, and nonhuman primates. A calculated carcinogenic potency (TD_{50}) is provided to standardize quantitative measures for comparison across chemicals. The CPDB contains 1,531 chemicals and 3,501 data points.
ISSCAN	The ISSCAN database provides information on carcinogenicity bioassays in rats and mice reported in sources including NTP, CPDB, CCRIS, and IARC. This database reports a carcinogenicity TD_{50}. There are 1,149 chemicals and 4,518 data points included in the ISSCAN database.
ECHA CHEM	The ECHA CHEM database provides information on chemicals manufactured or imported in Europe from registration dossiers submitted by companies to ECHA to comply with the REACH Regulation framework. The ECHA database includes 9,229 chemicals with almost 430,000 data points for a variety of endpoints including carcinogenicity and genotoxicity. ECHA does not verify the information provided by the submitters.
ECVAM Genotoxicity and Carcinogenicity	The ECVAM Genotoxicity and Carcinogenicity database provides genotoxicity and carcinogenicity data for Ames-positive chemicals in a harmonized format. ECVAM contains in vitro and in vivo bacteria mutagenicity, carcinogenicity, CA, CA/aneuploidy, DNA damage, DNA damage and repair, mammalian culture cell mutagenicity, and rodent gene mutation data for 744 chemicals and 9,186 data points.
ISSCTA	ISSCTA provides results of four types of in vitro cell transformation assays including SHE cells, mouse BALB/c 3T3, mouse C3H/10T1/2, and mouse Bhas 42 assays that inform nongenotoxic carcinogenicity. ISSCTA consists of 352 chemicals and 760 data points.
Bacterial mutagenicity ISSSTY	The ISSSTY database provides data on in vitro *Salmonella typhimurium* Ames test mutagenicity (positive and negative) taken from the CCRIS database in TOXNET. The ISSSTY database provides data for 7,367 chemicals and 41,634 data points.
Genotoxicity OASIS	The Genotoxicity OASIS database provides experimental results for mutagenicity results from "Ames tests (with and without metabolic activation), in vitro chromosomal aberrations and MN and MLA evaluated in vivo and in vitro, respectively." The Genotoxicity OASIS database consists of 7,920 chemicals with 29,940 data points from 7 sources.
Micronucleus OASIS	The Micronucleus OASIS database provides experimental results for in vivo bone marrow and peripheral blood MNT CA studies in blood erythrocytes, bone marrow cells, and polychromatic erythrocytes of humans, mice, rabbits, and rats for 557 chemicals.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
ISSMIC	The ISSMIC database provides data on the results of in vivo MN mutagenicity assay to detect CAs in bone marrow cells, peripheral blood cells, and splenocytes in mice and rats. Sources include TOXNET, NTP, and the Leadscope FDA CRADA toxicity database. The ISSMIC database includes data for 563 chemicals and 1,022 data points.

[a]Descriptions were obtained from the OECD QSAR Toolbox documentation (Version 4.1).

CA = chromosomal aberration; CCRIS = Chemical Carcinogenesis Research Information System; CPDB = Carcinogenic Potency Database; CRADA = cooperative research and development agreement; DNA = deoxyribonucleic acid; ECHA = European Chemicals Agency; ECVAM = European Centre for the Validation of Alternative Methods; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; ISSCAN = Istituto Superiore di Sanità Chemical Carcinogen; ISSCTA = Istituto Superiore di Sanità Cell Transformation Assay; ISSMIC = Istituto Superiore di Sanità Micronucleus; ISSSTY = Istituto Superiore di Sanità *Salmonella typhimurium*; MLA = mouse lymphoma gene mutation assay; MN = micronuclei; MNT = micronucleus test; NCI = National Cancer Institute; NTP = National Toxicology Program; OECD = Organization for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; REACH = Registration, Evaluation, Authorization and Restriction of Chemicals; SHE = Syrian hamster embryo; TD_{50} = median toxic dose.

STEPS 2−5. ANALOGUE REFINEMENT AND SUMMARY OF EXPERIMENTAL DATA FOR GENOTOXICITY, TOXICOKINETICS, CARCINOGENICITY, AND MODE OF ACTION

The outcome of the Step 1 analogue identification process using ChemACE and the OECD QSAR Toolbox is an initial list of structural analogues with genotoxicity and/or carcinogenicity data. Expert judgment is applied in Step 2 to refine the list of analogues based on physicochemical properties; absorption, distribution, metabolism, and excretion (ADME); and mechanisms of toxicity. The analogue refinement process is chemical specific and is described in Appendix C. Steps 3, 4, and 5 (summary of experimental data for genotoxicity, toxicokinetics, carcinogenicity, and mode of action [MOA]) are also chemical specific (see Appendix C for further details).

STEP 6. STRUCTURAL ALERTS AND STRUCTURE-ACTIVITY RELATIONSHIP PREDICTIONS FOR BEP AND ANALOGUES

Structural alerts and predictions for genotoxicity and carcinogenicity are identified using six freely available structure-based tools (described in Table B-2).

Table B-2. Tools Used to Identify SAs and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
OECD QSAR Toolbox (Version 4.1)	Seven OECD QSAR Toolbox profiling methods were used, including: • Carcinogenicity (genotox and nongenotox) alerts by ISS (Version 2.3); updated version of the module originally implemented in Toxtree. Toxtree is a decision tree for estimating carcinogenicity based on 55 SAs (35 from the Toxtree module and 20 newly derived). • DNA alerts for Ames by OASIS (Version 1.4); based on the Ames mutagenicity TIMES model; uses 85 SAs responsible for interaction of chemicals with DNA. • DNA alerts for CA and MNT by OASIS (Version 1.1); based on the DNA reactivity of the CA TIMES model; uses 85 SAs for interaction of chemicals with DNA. • In vitro mutagenicity (Ames test) alerts by ISS (Version 2.3); based on the Mutagenicity module in Toxtree. ISS is a decision tree for estimating in vitro (Ames test) mutagenicity, based on a list of 43 SAs relevant for the investigation of chemical genotoxicity via DNA adduct formation. • In vivo mutagenicity (MN) alerts by ISS (Version 2.3); based on the ToxMic rulebase in Toxtree. The rulebase has 35 SAs for in vivo MN assays in rodents. • OncoLogic Primary Classification (Version 4.0); "developed by LMC and OECD to mimic the structural criteria of chemical classes of potential carcinogens covered by the U.S. EPA's OncoLogic Cancer Expert System for Predicting the Carcinogenicity Potential" for categorization purposes only, not for predicting carcinogenicity. This tool is applicable to organic chemicals with at least one of the 48 alerts specified. • Protein-binding alerts for CAs by OASIS (Version 1.3); based on 33 SAs for interactions with specific proteins including topoisomerases, cellular protein adducts, etc.
OncoLogic (Version 7)	OncoLogic is a tool for predicting the potential carcinogenicity of chemicals based on the application of rules for SAR analysis, developed by experts. Results may range from "low" to "high" concern level.

ToxAlerts	ToxAlerts is a platform for screening chemical compounds against SAs, developed as an extension to the OCHEM system (https://ochem.eu). Only "approved alerts" were selected, which means a moderator approved the submitted data. A list of the ToxAlerts found for the chemicals screened in the preliminary batch is below:
	• Genotoxic carcinogenicity, mutagenicity:
	  ○ Aliphatic halide (general)
	  ○ Aliphatic halide (specific)
	  ○ Aliphatic halogens
	  ○ Aromatic amine (general)
	  ○ Aromatic amine (specific)
	  ○ Aromatic amines
	  ○ Aromatic and aliphatic substituted primary alkyl halides
	  ○ Aromatic nitro (general)
	  ○ Aromatic nitro (specific)
	  ○ Aromatic nitro groups
	  ○ Nitroarenes
	  ○ Nitro-aromatic
	  ○ Primary and secondary aromatic amines
	  ○ Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group
	• Nongenotoxic carcinogenicity
	  ○ Aliphatic halogens

Table B-2. Tools Used to Identify SAs and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
ToxRead (Version 0.9)	ToxRead is a tool designed to assist in making read-across evaluations reproducible. SAs for mutagenicity are extracted from similar molecules with available experimental data in its database. Five similar compounds were selected for this project. The rule sets included: • Benigni/Bossa as available in Toxtree (Version 1) • SARpy rules extracted by Politecnico di Milano, with the automatic tool SARpy • IRFMN rules extracted by human experts • CRS4 rules extracted by CRS4 Institute with automatic tools
Toxtree (Version 2.6.13)	Toxtree estimates toxic hazard by applying a decision tree approach. Chemicals were queried in Toxtree using the Benigni/Bossa rulebase for mutagenicity and carcinogenicity. If a potential carcinogenic alert based on any QSAR model or if any SA for genotoxic and nongenotoxic carcinogenicity was reported, then the prediction was recorded as a positive carcinogenicity prediction for the test chemical. The output definitions from the tool manual are listed below: • SA for genotoxic carcinogenicity (recognizes the presence of one or more SAs and specifies a genotoxic mechanism) • SA for nongenotoxic carcinogenicity (recognizes the presence of one or more SAs and specifies a nongenotoxic mechanism) • Potential *Salmonella typhimurium* TA100 mutagen based on QSAR • Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR • Potential carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Unlikely to be a carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Negative for genotoxic carcinogenicity (no alert for genotoxic carcinogenicity) • Negative for nongenotoxic carcinogenicity (no alert for nongenotoxic carcinogenicity)

Table B-2. Tools Used to Identify SAs and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
VEGA	VEGA applies several QSARs to a given chemical, as described below: • Mutagenicity (Ames test) CONSENSUS model: a consensus assessment is performed based on predictions of the VEGA mutagenicity models (CAESAR, SARpy, ISS, and *k*-NN) • Mutagenicity (Ames test) model (CAESAR): integrates two models, one is a trained SVM classifier, and the other is for FN removal based on SAs matching • Mutagenicity (Ames test) model (SARpy/IRFMN): rule-based approach with 112 rules for mutagenicity and 93 for nonmutagenicity, extracted with SARpy software from the original training set from the CAESAR model; includes rules for both mutagenicity and nonmutagenicity • Mutagenicity (Ames test) model (ISS): rule-based approach based on the work of Benigni and Bossa (ISS) as implemented in the software Toxtree (Version 2.6) • Mutagenicity (Ames test) model (*k*-NN/read-across): performs a read-across and provides a qualitative prediction of mutagenicity on *S. typhimurium* (Ames test) • Carcinogenicity model (CAESAR): counter-propagation artificial neural network developed using data for carcinogenicity in rats extracted from the CPDB • Carcinogenicity model (ISS): built implementing the same alerts Benigni and Bossa (ISS) implemented in the software Toxtree (Version 2.6) • Carcinogenicity model (IRFMN/ANTARES): a set of rules (127 SAs), extracted with the SARpy software from a data set of 1,543 chemicals obtained from the carcinogenicity database of EU-funded project ANTARES • Carcinogenicity model (IRFMN/ISSCAN-CGX): based on a set of rules (43 SAs) extracted with the SARpy software from a data set of 986 compounds; the data set of carcinogenicity of different species was provided by Kirkland et al. (2005)

[a]There is some overlap between the tools. For example, OncoLogic classification is provided by the QSAR Toolbox but the prediction is available only through OncoLogic, and alerts or decision trees were used or adapted in several models (e.g., Benigni and Bossa alerts and Toxtree decision tree).

ANTARES = Alternative Non-Testing Methods Assessed for REACH Substances; CA = chromosomal aberration; CAESAR = Computer-Assisted Evaluation of industrial chemical Substances According to Regulations; CONSENSUS = consensus assessment based on multiple models (CAESAR, SARpy, ISS, and *k*-NN); CPDB = Carcinogenic Potency Database; CRS4 = Center for Advanced Studies, Research and Development in Sardinia; DNA = deoxyribonucleic acid; EU = European Union; FN = false negative; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; LMC = Laboratory for Mathematical Chemistry; MN = micronucleus; MNT = micronucleus test; OCHEM = Online Chemical Monitoring Environment; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; SA = structural alert; SAR = structure-activity relationship; SVM = support vector machine; TIMES = The Integrated MARKEL-EFOM System; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

The tool results for the target and analogue compounds are provided in Appendix C.

STEP 7. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF CARCINOGENICITY

Available data across multiple lines of evidence from Steps 1−6 (outlined above) are integrated to determine the qualitative level of concern for the potential carcinogenicity of the target compound (Step 8). In the absence of information supporting carcinogenic portal-of-entry effects, the qualitative level of concern for the target chemical should be considered applicable to all routes of exposure.

Evidence integration for the target compound is provided in Appendix C.

APPENDIX C. RESULTS OF THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

U.S. EPA's Chemical Assessment Clustering Engine (ChemACE) clustering was performed as described in Appendix B. Using the most restrictive clustering rules (where ChemACE assigns a unique definition for each fragment, ensuring that each chemical submitted for clustering appears in only one ChemACE cluster), benzo[e]pyrene (BeP) appeared in Cluster 51, which contained seven additional compounds (benzo[g]chrysene, perylene, picene, benzo[b]chrysene, dibenz[a,c]anthracene, dibenzo[a,j]anthracene, and benzo[a]pyrene [BaP]). Using the less restrictive clustering option did not change the result (BeP found in a single cluster with seven other compounds).

The Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox Profiler was used to identify structural analogues from the Dice analogue search with carcinogenicity and/or genotoxicity data (see Step 1 methods in Appendix B). The Dice analogue search identified >1,500 chemicals with ≥50% similarity to BeP; therefore, the similarity threshold was raised to 80%. The Dice analogue search identified 107 compounds with carcinogenicity and/or genotoxicity data and ≥80% similarity to BeP, including five of the seven compounds identified by ChemACE. Refinement of the selection of final analogues is described below.

STEP 2. ANALOGUE REFINEMENT USING EXPERT JUDGMENT

Expert judgment was applied to refine the initial list of 109 potential analogues based on physicochemical properties; absorption, distribution, metabolism, and excretion (ADME); and mechanisms of toxicity.

BeP contains five unsubstituted benzene rings. Therefore, compounds were only considered as potential analogues if they were unsubstituted and contained five benzene rings. Of the 109 chemicals identified as potential analogues by ChemACE clustering and the OECD QSAR Toolbox analogue selection tool (Dice), 101 were not selected for further review, including:

- Polycyclic aromatic hydrocarbon (PAH) compounds with benzene ring number ≠ 5
- Compounds containing nonaromatic ring structures (e.g., benzo[b]fluoranthene)
- Substituted PAHs (e.g., alkyl, amino/nitro/nitroso or halogenated substituents)
- Hydroxyl PAH metabolites

Each of these attributes introduce significant differences in bioavailability, reactivity, and applicable metabolic pathways relative to BeP.

The remaining eight possible analogues for BeP are listed in Table C-1. The existence of a cancer toxicity value and/or a weight-of-evidence (WOE) determination for cancer is indicated for each analogue.

Table C-1. Summary of Cancer Assessment Information for Analogues of BeP (CASRN 192-97-2)

Analogue Name (CASRN)[a]	Cancer Risk Estimates (if available)	WOE Determinations
BaP[a, b] (50-32-8)	U.S. EPA (2017c)—OSF, IUR CalEPA (2011)—OSF, IUR	U.S. EPA (2017c)—*"Carcinogenic to Humans"* IARC (2010)—*carcinogenic to humans (Group 1)*
BbC[a, b] (214-17-5)	None	IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*
BgC[a] (196-78-1)	None	IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*
DBacA[a] (215-58-7)	None	IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*
DBahA[b] (53-70-3)	CalEPA (1992), CalEPA (2019)—OSF, IUR	U.S. EPA (1990c)—*probable human carcinogen (Group B2)* IARC (2010)—*probably carcinogenic to humans (Group 2A)*
DBajA[a, b] (224-41-9)	None	IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*
Perylene[a, b] (198-55-0)	None	U.S. EPA (2007)—*"Inadequate Information to Assess Carcinogenic Potential"* IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*
Picene[a, b] (213-46-7)	None	IARC (2010)—*not classifiable as to their carcinogenicity to humans (Group 3)*

[a]Found by ChemACE.
[b]Found by Dice.

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenzo[a,j]anthracene; IUR = inhalation unit risk; OSF = oral slope factor; WOE = weight of evidence.

STEP 3. COMPARISON OF THE EXPERIMENTAL GENOTOXICITY DATA FOR BEP AND ANALOGUES

The genotoxicity data available for BeP are described in the "Other Data" section in the main body of this report. Available data indicate that BeP is weakly mutagenic following metabolic activation but not mutagenic without activation. Most studies indicate that BeP is not clastogenic and does not cause deoxyribonucleic acid (DNA) damage; however, BeP (or a metabolite) is capable of forming DNA adducts, and there is inconsistent evidence from in vivo studies indicating that BeP is capable of causing chromosomal damage under certain conditions. Genotoxicity data for the analogue compounds have been extensively reviewed. A summary of the genotoxicity data for analogue PAHs is provided in Table C-2. Overall data indicate that these analogues are mutagenic and clastogenic, and capable of binding DNA and causing DNA damage. Numerous studies have reported a weak genotoxic potential of BeP in vitro compared with other PAHs, including analogues BaP, dibenz[a,c]anthracene (DBacA), dibenz[a,h]anthracene (DBahA), dibenz[a,j]anthracene (DBajA), and, in some assays, perylene (Durant et al., 1996; Mersch-Sundermann et al., 1993; Mersch-Sundermann et al., 1992; De

Flora et al., 1984; Haas et al., 1981; Kaden et al., 1979; Simmon, 1979; Andrews et al., 1978; McCann et al., 1975).

Table C-2. Comparison of Available Genotoxicity Data for BeP (CASRN 192-97-2) and Analogues

Chemical (CASRN)	Mutagenicity	Clastogenicity	DNA Damage and Adducts	Cell Transformation	Reference
BeP	• Weakly mutagenic in *Salmonella typhimurium* with metabolic activation; not mutagenic without activation • Not mutagenic in *Escherichia coli* • Mutagenic in mammalian cells with metabolic activation only at cytotoxic concentrations	• Predominantly negative in mammalian cells in vitro • Mixed results in vivo (induced CAs in rats but not hamsters, induced SCEs in hamsters, negative for MN in mice)	• Predominantly negative for DNA damage/repair assays in vitro and in vivo • DNA adducts measured in mammalian cells and isolated human DNA	• Weak induction of cell transformation in mammalian cells in vitro	See Table 4A
BaP (50-32-8)	• Mutagenic in *S. typhimurium* and *E. coli* • Mutagenic in mammalian cells in vitro • Induced somatic and sex-linked recessive mutations in *Drosophila melanogaster* • Mutagenic in dominant lethal and transgenic mouse studies • *K-ras* and *p53* mutations in human tumor tissues	• Induced SCEs, CAs, and MNs in mammalian cells in vitro and in vivo	• DNA damage/repair in *E. coli* • Induced DNA damage/repair in mammalian cells in vitro and in vivo • DNA adducts measured in mammalian cells	• Induced neoplastic transformation in mammalian cells	De Flora et al. (1984); U.S. EPA (2017c); U.S. EPA (2017b)
BbC (214-17-5)	ND	ND	ND	ND	NA
BgC (196-78-1)	• BgC 11,12-diol-13,14-oxides mutagenic in *S. typhimurium* and *E. coli* • BgC 11,12-diol-13,14-oxides mutagenic in mammalian cells in vitro	ND	• DNA adducts of BgC 11,12-diols and BgC 11,12-diol-13,14-oxides in mouse skin • CYP-activated BgC 11,12-diols cause DNA damage in cDNA-based recombinant (*E. coli* or *Trichoplusia ni*) systems	ND	IARC (2010)

Table C-2. Comparison of Available Genotoxicity Data for BeP (CASRN 192-97-2) and Analogues

Chemical (CASRN)	Mutagenicity	Clastogenicity	DNA Damage and Adducts	Cell Transformation	Reference
DBacA (215-58-7)	• Mutagenic in *S. typhimurium* • Mutagenic in mammalian cells in vitro • DBacA 10,11-dihydrodiol mutagenic in bacteria	• Negative for SCE in mammalian cells in vitro (single study)	• DNA damage in *Bacillus subtilis* • DNA damage in mammalian cells in vitro • DNA adducts in mammalian cells in vitro	• Cell transformation in mammalian cells in vitro	IARC (1983)
DBahA (53-70-3)	• DBahA, DBahA, 3,4-diol, and DBahA 3,4:10,11-bisdiol mutagenic in *S. typhimurium* • DBahA mutagenic in mammalian cells in vitro	• Induced MN in mammalian cells in vivo and SCEs and MN in mammalian cells in vitro	• DNA adducts of DBahA 3,4-diol-1,2-oxide and DBahA 3,4:10,11-bisdiol in vitro and/or in vivo	• DBahA 3,4-diol and DBahA 3,4-diol-1,2-oxide induced morphological cell transformation in mammalian cells	IARC (2010)
DBajA (224-41-9)	• Mutagenic in *S. typhimurium* • Mutagenic in human lymphoblastoid cells • DBajA 3,4-diol-1,2-oxide mutagenic in *E. coli*	ND	• DNA adducts of DBajA diols, bisdiols, and oxides in vivo	ND	IARC (2010)
Perylene (198-55-0)	• Mutagenic in *S. typhimurium* • Not mutagenic in human lymphoblastoid cells	• CAs in mammalian cells in vitro	• Negative for DNA damage/repair in *E. coli*	ND	De Flora et al. (1984); IARC (1983)
Picene (213-46-7)	• Not mutagenic in *S. typhimurium* • Not mutagenic in human lymphoblastoid cells	ND	ND	ND	Durant et al. (1996); Kaden et al. (1979)

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; CA = chromosomal aberration; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; DNA = deoxyribonucleic acid; MN = micronuclei; ND = no data; SCE = sister chromatid exchange.

STEP 4. TOXICOKINETICS OF BEP AND ANALOGUES

The toxicokinetics of BeP and potential analogues are briefly described in Table C-3.

No data on absorption or distribution are available for BeP or any analogue with the exception of BaP. BaP absorption and distribution are extensively reviewed by U.S. EPA (2017b), IARC (2010), and ATSDR (1995). BaP is absorbed via inhalation, oral, and dermal routes. The rate of inhalation absorption is dependent on numerous factors, including particle size, region of deposition, and rate of dissolution or desorption from particles, with rapid absorption occurring through thin alveolar epithelium and slower absorption in thicker regions of the airways. Inhaled BaP is also transported to the gut via the mucociliary escalator. Gastrointestinal absorption of BaP ranges from 10 to 60%, with enhanced absorption in the presence of lipophilic compounds. Dermal absorption is rapid and near complete when administered in crude oil or acetone, with decreased absorption if administered in high-viscosity oil vehicles or in soils with high organic carbon content. Following absorption, BaP shows rapid, widespread systemic distribution followed by accumulation and retention in fat. Because absorption and distribution of a chemical in the body are determined largely by physical and chemical properties related to its molecular size and general structure (e.g., lipophilicity, vapor pressure, etc.), it is reasonable to assume that BeP and other PAH analogues will be absorbed and distributed similarly to BaP based on similar size, structure, and physicochemical properties.

In general, PAHs (including BeP and analogues) are oxidized by cytochrome P450 (CYP450) in multiple tissues in the body to more soluble metabolites, including dihydrodiols, phenols, quinones, and epoxides, which then form conjugates with glucuronide, glutathione (GSH), or sulfate (U.S. EPA, 2017b; IARC, 2010; ATSDR, 1995). While PAHs share similar metabolic pathways, there are differences in the primary metabolic products that can affect carcinogenic potential (see “Metabolism/Toxicokinetic Studies” section and Table C-3 for more details). For example, in contrast to BaP (the most well-studied carcinogenic PAH), there is minimal evidence for bay-region activation of BeP in rodent model systems and generation of the 9,10-dihydrodiol metabolite, and subsequent formation of the DNA reactive epoxide is described as limited within this model system (Jacob et al., 1985; Jacob et al., 1983; Wood et al., 1979). Data support the notion that production of the 9,10-dihydrodiol metabolite is increased in human model systems, but the extent and consequence of production of the DNA reactive epoxide metabolite in humans remains uncertain.

No excretion data are available for BeP or any analogue, with the exception of BaP (see Table C-3). BaP excretion has been extensively reviewed by U.S. EPA (2017b), IARC (2010), and ATSDR (1995). Excretion of BaP, primarily as conjugated metabolites, is rapid and occurs primarily via biliary excretion to feces, with small amounts excreted in urine. Because PAHs share common metabolic profiles, it is reasonable to assume that conjugated metabolites of BeP and other PAH analogues will be excreted similarly to BaP.

Table C-3. Summary of Toxicokinetic Data for BeP (CASRN 192-97-2) and Analogues

Compound (CASRN)	Absorption, Distribution, Excretion	Metabolism	References
BeP (192-97-2)	ND	• Metabolism is rapid (complete within 48 h) • Oxidized via CYP450 to the k-region 4,5-dihydrodiol, phenols and quinones • Oxidative metabolism can be induced by CYP450 inducers • Bay-region 9,10-dihydrodiol is formed in small amounts and then preferentially oxidized at the 4,5-position, rather than forming the 9,10-expoxide • Oxidative metabolites conjugated with glucuronic acid	See "Other Data" section of this document.
BaP (50-32-8)	**Absorption:** • Absorbed by oral, inhalation, and dermal exposure • Rate and extent of absorption is variable, depending on exposure medium (e.g., oral and dermal absorption enhanced in presence of oils and fats; dermal absorption decreased in presence of soils with high organic carbon content) • Significant mucociliary clearance of inhaled particulate to gut • Absorption from gut depends on presence of bile in intestinal lumen **Distribution:** • Widely distributed throughout the body • Initial rapid uptake into well-perfused tissues (e.g., lung, kidney, liver) • Subsequent accumulation, retention, and slow release from fat • High levels in gut (from any route) due to mucociliary clearance from respiratory tract and hepatobiliary excretion of metabolites • Limited placental transfer **Excretion:** • Excretion is rapid, with half-times of 22−30 h • Primary route is biliary excretion to feces; urine is secondary route • Excreted mainly as conjugated metabolites • Small amounts excreted in milk	• Metabolism is rapid and occurs in many tissues throughout the body • Oxidized via CYP450; primary metabolites are 9,10-, 7,8-, 4,5-, and 2,3-dihydrodiols and epoxides, as well as various phenols, quinones, and derivatives. • Oxidative metabolism can be induced by CYP450 inducers • Oxidative metabolites conjugated with GSH, glucuronic acid, and sulfate esters	ATSDR (1995); IARC (2010); U.S. EPA (2017b); U.S. EPA (2017c)

Table C-3. Summary of Toxicokinetic Data for BeP (CASRN 192-97-2) and Analogues

Compound (CASRN)	Absorption, Distribution, Excretion	Metabolism	References
BbC (214-17-5)	ND	ND	NA
BgC (196-78-1)	ND	• Fjord region oxidation to form 11,12-diols • Further metabolism via intermediary 11,12-diol-13,14-oxides to form diastereomeric tetrols	IARC (2010)
DBacA (215-58-7)	ND	• Oxidation to 10,11-dihydrodiol (major metabolite) • Further oxidation to 10,11-diol-12,13-epoxide • Minor metabolites include 1,2- and 3,4-diols	IARC (1983)
DBahA (53-70-3)	ND	• Metabolic activation via the diol epoxide mechanism to diols, oxides, catechols, phenols, and hexols	IARC (2010)
DBajA (224-41-9)	ND	• Metabolic activation via the diol epoxide mechanism to diols, bisdiols, and oxides • Glucuronidation of diols	IARC (2010)
Perylene (198-55-0)	ND	ND	NA
Picene (213-46-7)	ND	• Primary metabolites formed via oxidation include dihydrodiols, phenols, and quinones	Platt et al. (1988)

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; CYP450 = cytochrome P450; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; GSH = glutathione; NA = not applicable; ND = no data.

STEP 5. CARCINOGENICITY OF BEP ANALOGUES AND MOA DISCUSSION

U.S. EPA cancer WOE descriptors for BeP and analogue compounds are shown in Tables C-4 (oral) and C-5 (inhalation). As noted in the PPRTV document, there is inadequate information to assess the carcinogenic potential of BeP by oral or inhalation exposure. Only two of the potential analogue compounds are characterized as having evidence of carcinogenic potential. Additionally, several potential analogues have been identified as unmeasured components of complex chemical mixtures that are known to be carcinogenic in humans. BaP was characterized as *"Carcinogenic to Humans"* (U.S. EPA, 2017c) under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) and DBahA was considered a *probable human carcinogen* (U.S. EPA, 1993) under the 1986 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986). Reported oral slope factor (OSF) and inhalation unit risk (IUR) values were two- to threefold higher for DBahA compared with BaP.

Table C-4. Comparison of Available Oral Carcinogenicity Toxicity Data for BeP (CASRN 192-97-2) and Analogues

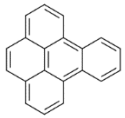
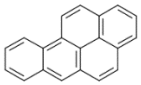
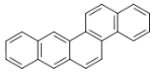
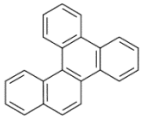
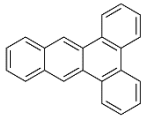
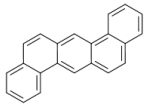
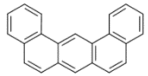
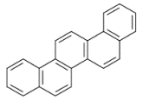
Type of Data	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
Structure									
CASRN	192-97-2	50-32-8	214-17-5	196-78-1	215-58-7	53-70-3	224-41-9	198-55-0	213-46-7
U.S. EPA WOE characterization	*"Inadequate Information to Assess Carcinogenic Potential"*	*"Carcinogenic to Humans"*	ND	ND	ND	*Probable human carcinogen (Group B2)*	ND	*"Inadequate Information to Assess Carcinogenic Potential"*	ND
OSF $(mg/kg\text{-}d)^{-1}$	NDr	1.4	NDr	NDr	NDr	4.12	NDr	NDr	NDr
Data set(s) used for slope factor derivation	ND	Alimentary tract tumors (multiple sites) in female B6C3F1 mice	ND	ND	ND	Pulmonary (alveolar carcinomas)	ND	ND	ND
Other tumors observed in animal oral bioassays	ND	Auditory canal, kidney, liver, skin, mammary gland	ND	ND	ND	Mammary, forestomach, hemangio-endothelioma	ND	ND	ND
Study doses (mg/kg-d)	ND	0, 0.7, 3.3, 16.5	ND	ND	ND	NS	ND	ND	ND
Route (method)	ND	Oral (diet)	ND	ND	ND	NS	ND	ND	ND
Duration	ND	2 yr	ND	ND	ND	NS	ND	ND	ND

Table C-4. Comparison of Available Oral Carcinogenicity Toxicity Data for BeP (CASRN 192-97-2) and Analogues

Type of Data	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
POD type	ND	$BMDL_{10}$ 0.071 mg/kg-d	ND	ND	ND	NS	ND	ND	ND
Source	NA	U.S. EPA (2017b); U.S. EPA (2017c)	NA	NA	NA	U.S. EPA (1990c); CalEPA (2019); CalEPA (1992)	NA	NA	NA

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; BMDL = benchmark dose lower confidence limit; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; NA = not applicable; ND = no data; NDr = not determined; NS = not specified; OSF = oral slope factor; POD = point of departure; WOE = weight of evidence.

Table C-5. Comparison of Available Inhalation Carcinogenicity Toxicity Data for BeP (CASRN 192-97-2) and Analogues

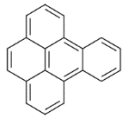
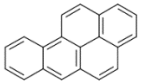
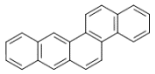
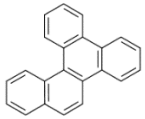
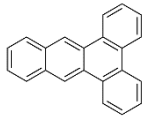
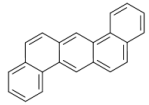
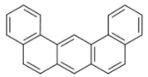
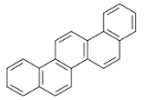
Type of Data	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
Structure									
CASRN	192-97-2	50-32-8	214-17-5	196-78-1	215-58-7	53-70-3	224-41-9	198-55-0	213-46-7
U.S. EPA WOE characterization	*"Inadequate Information to Assess Carcinogenic Potential"*	*"Carcinogenic to Humans"*	ND	ND	ND	*Probable human carcinogen (Group B2)*	ND	*"Inadequate Information to Assess Carcinogenic Potential"*	ND
IUR $(\mu g/m^3)^{-1}$	NDr	0.0006	NDr	NDr	NDr	0.00122	NDr	NDr	NDr
Data set(s) used for unit risk derivation	ND	Squamous cell neoplasia in upper respiratory tract (multiple sites), esophagus, and forestomach of male Syrian golden hamster	ND	ND	ND	NS	ND	ND	ND
Other tumors observed in animal inhalation bioassays	ND	ND	ND	ND	ND	ND	ND	ND	ND
Study concentrations (mg/m^3)	ND	0, 2.2, 9.5, 46.5	ND	ND	ND	NS	ND	ND	ND
Route (method)	ND	Inhalation (nose only)	ND	ND	ND	NS	ND	ND	ND
Duration	ND	Up to 130 wk	ND	ND	ND	NS	ND	ND	ND

Table C-5. Comparison of Available Inhalation Carcinogenicity Toxicity Data for BeP (CASRN 192-97-2) and Analogues

Type of Data	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
POD type	ND	$BMCL_{10}$ 0.163 mg/m^3	ND	ND	ND	NS	ND	ND	ND
Source	NA	U.S. EPA (2017b); U.S. EPA (2017c)	NA	NA	NA	U.S. EPA (1990c); CalEPA (2019)	NA	NA	NA

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; BMCL = benchmark concentration lower confidence limit; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; IUR = inhalation unit risk; NA = not applicable; ND = no data; NDr = not determined; NS = not specified; POD = point of departure; WOE = weight of evidence.

As summarized in Tables C-4 (oral) and C-5 (inhalation), BaP is a multisite carcinogen in laboratory animals following oral and inhalation exposure and DBahA is a multisite carcinogen in laboratory animals following oral exposure. No oral or inhalation carcinogenicity data are available for BeP or analogues other than BaP and DBahA. However, data from other routes (e.g., dermal, injection, implantation) are available for all compounds except benzo[g]chrysene (BgC) (see Table C-6). For BeP, evidence of carcinogenicity from other route studies is limited to a positive finding for skin tumor initiation in one of six dermal initiation-promotion studies; BeP was not carcinogenic in complete dermal, injection, or implantation studies. When metabolites were tested directly, 9,10-dihydrobenzo[e]pyrene was a skin tumor initiator and the 9,10-dihydrodiol and a diol epoxide induced hepatic tumors in neonatal injection studies (metabolite studies not included in Table C-6). For analogues, BaP and DBahA are the best studied and demonstrate consistent carcinogenic potential across exposure routes. Most other PAH analogues (benzo[b]chrysene, DBacA, DBajA, and picene) show evidence of carcinogenicity in a limited number of studies; however, perylene shows negative or equivocal findings in dermal and injection studies.

The U.S. EPA (2017c) proposed a mutagenic MOA for BaP carcinogenicity via all routes of exposure. Proposed key events include (1) metabolism of BaP into DNA-reactive metabolites (diol epoxides, radical cations, and *o*-quinone and reactive oxygen species [ROS]), (2) direct DNA damage via DNA adducts and ROS-mediated damage, (3) DNA mutation, and (4) clonal expansion of mutated cells. Alternative MOAs that may contribute to tumor promotion include aryl hydrocarbon receptor (AhR) affinity, immune suppression, cytotoxicity and inflammation, and inhibition of gap junctional intercellular communication (U.S. EPA, 2017c). IARC (2010) also proposed that the formation of DNA-reactive diol epoxides during metabolism of PAHs underlies carcinogenic potential. A mutagenic MOA is plausible for all analogues, as well as BeP, especially those that have demonstrated mutagenic potential and DNA adduct formation (BeP, BgC, DBacA, DBahA, and DBajA) (see Table C-2).

Table C-6. Summary of Carcinogenicity Studies Using Other Exposure Routes for BeP (CASRN 192-97-2) Analogues

Exposure Route (species)	BeP[a] 192-97-2	BaP[b] 50-32-0	BbC[b] 214-17-5	BgC[b] 196-78-1	DBacA[b] 215-58-7	DBahA[b] 53-70-3	DBajA[b] 224-41-9	Perylene[b] 198-55-0	Picene[b] 213-46-7
Dermal (mouse)		Sk			Sk	Sk	Sk		Sk
Dermal initiation promotion (mouse)	Sk	Sk	Sk		Sk	Sk	Sk	(Sk)	Sk
i.v. Injection (mouse)						Lu			
i.p. Injection (mouse)		Lu, Lv, St			(Lv)	Lu			
i.p. Injection (rat)		Ms							
s.c. Injection (mouse)		IS, Lu				IS, Lu	(IS)		IS, Lu
s.c. Injection (rat)		IS				IS			
Intratracheal (hamster)		Lu, OR				OR			
Intratracheal (rat or mouse)		Lu							
Intrapulmonary (rat)		Lu				Lu			
Intrapulmonary (mouse)						Lu			
Buccal pouch application (hamster)		BP, St							

Table C-6. Summary of Carcinogenicity Studies Using Other Exposure Routes for BeP (CASRN 192-97-2) Analogues

Exposure Route (species)	BeP[a] 192-97-2	BaP[b] 50-32-0	BbC[b] 214-17-5	BgC[b] 196-78-1	DBacA[b] 215-58-7	DBahA[b] 53-70-3	DBajA[b] 224-41-9	Perylene[b] 198-55-0	Picene[b] 213-46-7
Intramammary, intramamillary (rat)									
		Mm							

Color Key:

Experiments in this test system	1 to 2	3 to 5	≥6
Experiments reporting statistically significant increase	1 to 2	3 to 5	≥6
Experiments reporting equivocal result	(+/−)		

Tumor Site Key:

BP = buccal pouch; IS = injection site; Lu = lung; Lv = liver; Mm = mammary; Ms = mesothelioma, abdominal; OR = other respiratory tract; Sk = skin; St = forestomach, stomach.

[a]See Table 3B in main document for BeP references.
[b]Data obtained from IARC (2010).

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; DBacA =dibenz[a,c]anthracene ; DBahA = dibenz[a,h]anthracene; DBajA = dibenzo[a,j]anthracene; i.p. = intraperitoneal; s.c. = subcutaneous.

STEP 6. STRUCTURAL ALERTS AND SAR PREDICTIONS FOR BEP AND ANALOGUES

Structural alerts and predictions for genotoxicity and carcinogenicity were identified using computational tools as described in Appendix B. The model results for BeP and its analogue compounds are shown in Table C-7. Concerns for carcinogenicity and/or mutagenicity of BeP and its analogues were indicated by several models within each predictive tool (see Table C-7). Table C-8 provides a list of the specific structural and mechanistic alerts that underlie the findings of a concern for carcinogenicity or mutagenicity in Table C-7. These include a structural alert for PAHs and mechanistic alerts for DNA alkylation and intercalation.

Table C-7. Heat Map Illustrating the Structural Alert and SAR Prediction Results for BeP (CASRN 192-97-2) and Analogues[a]

Tool	Model[b]	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
Mutagenicity/genotoxicity alerts										
OECD QSAR Toolbox	DNA alerts for Ames by OASIS									
	DNA alerts for CA and MNT by OASIS									
	In vitro mutagenicity (Ames test) alerts by ISS									
	In vivo mutagenicity (MN) alerts by ISS									
	Protein binding alerts for chromosomal aberration by OASIS									
ToxRead	ToxRead (mutagenicity)									
VEGA	Mutagenicity (Ames test) CONSENSUS model—assessment									
	Mutagenicity (Ames test) model (CAESAR)—assessment									
	Mutagenicity (Ames test) model (SARpy/IRFMN)—assessment									
	Mutagenicity (Ames test) model (ISS)—assessment									
	Mutagenicity (Ames test) model (*k*-NN/read-across)—assessment									
Toxtree	Potential *Salmonella typhimurium* TA100 mutagen based on QSAR									
	Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR									
Carcinogenicity alerts										
OECD QSAR Toolbox	Carcinogenicity (genotoxicity and nongenotoxicity) alerts by ISS									
OncoLogic	OncoLogic (prediction of the carcinogenic potential of the chemical)									
VEGA	Carcinogenicity model (CAESAR)—assessment									
	Carcinogenicity model (ISS)—assessment									
	Carcinogenicity model (IRFMN/ANTARES)—assessment									
	Carcinogenicity model (IRFMN/ISSCAN-CGX)—assessment									
	Carcinogenicity oral classification model (IRFMN)									
	Carcinogenicity inhalation classification model (IRFMN)									

Table C-7. Heat Map Illustrating the Structural Alert and SAR Prediction Results for BeP (CASRN 192-97-2) and Analogues[a]

Tool	Model[b]	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
ToxAlerts	Polycyclic aromatic hydrocarbons (for nongenotoxic carcinogenicity)									
Toxtree	Potential carcinogen based on QSAR									
	Unlikely to be a carcinogen based on QSAR									
	Nongenotoxic carcinogenicity									
Combined alerts										
ToxAlerts	Polycyclic aromatic hydrocarbons (for genotoxic carcinogenicity)									
Toxtree	Structural alert for genotoxic carcinogenicity									

	[b]Model results or alerts indicating no concern for carcinogenicity/mutagenicity.
	[b]Model results outside the applicability domain for carcinogenicity/mutagenicity.
	[b]Model results or alerts indicating concern for carcinogenicity/mutagenicity.

[a]All tools and models described in Appendix B were used. Models with results are presented in the heat map (models without results were omitted).

ANTARES = Alternative Non-Testing Methods Assessed for REACH Substances; BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; CA = chromosomal aberration; CAESAR = Computer-Assisted Evaluation of industrial chemical Substances According to Regulations; CONSENSUS = consensus assessment based on multiple models (CAESAR, SARpy, ISS, and *k*-NN); DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; DNA = deoxyribonucleic acid; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; MN = micronucleus; MNT = micronucleus test; OECD = Organisation for Economic Co-operation and Development; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; SAR = structure-activity relationship; QSAR = quantitative structure-analysis relationship; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

Table C-8. Structural Alerts and Chemical Mechanisms for BeP (CASRN 192-97-2) and Analogues

Structural Alert	**Tools**	**Compounds**
PAHs	OECD QSAR Toolbox	BeP, BaP, BbC, BgC, DBacA, DBahA, DBajA, perylene, picene
	ToxAlerts	
	Toxtree	
	OncoLogic	BaP, BgC, DBajA, picene
Mechanistic Alert	**Tools**	**Compounds**
DNA intercalation	OECD QSAR Toolbox	BbC, BgC, DBacA, DBahA, DBajA, picene
Alkylation after metabolically formed carbenium ion species		
Alkylation, direct acting epoxides and related after CYP450-mediated metabolic activation		

BaP = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; CYP450 = cytochrome P450; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene DBajA = dibenz[a,j]anthracene; DNA = deoxyribonucleic acid; OECD = Organisation for Economic Co-operation and Development; PAH = polycyclic aromatic hydrocarbon; QSAR = quantitative structure-activity relationship.

STEP 7. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF BEP CARCINOGENICITY

Table C-9 presents the data for multiple lines of evidence pertinent to the screening evaluation of the carcinogenic potential of BeP.

Table C-9. Integration of Evidence for BeP (CASRN 192-97-2) and Analogues

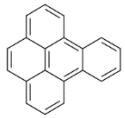
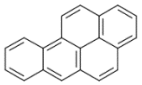
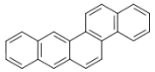
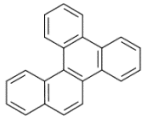
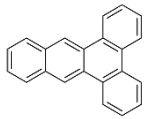
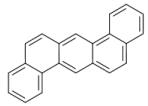
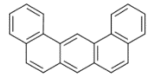
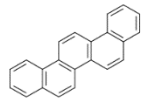
Evidence Streams	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
Structure									
CASRN	192-97-2	50-32-8	214-17-5	196-78-1	215-58-7	53-70-3	224-41-9	198-55-0	213-46-7
Analogue selection and evaluation (see Steps 1 and 2)	Target compound: PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings	PAH, five unsubstituted benzene rings
Experimental genotoxicity data (see Step 3)	Weak mutagen with metabolic activation; predominately negative for clastogenicity and DNA damage/repair; forms DNA adducts; weak inducer of cell transformation	Mutagenic; clastogenic; DNA damaging; forms DNA adducts; induces cell transformation	ND	Mutagenic; DNA damaging; forms DNA adducts	Mutagenic; not clastogenic; DNA damaging; forms DNA adducts; induces cell transformation	Mutagenic; clastogenic; forms DNA adducts; induces cell transformation	Mutagenic; forms DNA adducts	Mutagenic in bacteria; not mutagenic in mammalian cells; clastogenic; does not cause DNA damage in bacteria	Not mutagenic

Table C-9. Integration of Evidence for BeP (CASRN 192-97-2) and Analogues

Evidence Streams	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
ADME evaluation (see Step 4)	Common metabolic pathways with other PAHs (oxidation and glucuronide conjugation) Metabolites: Major: k-region 4,5-dihydrodiol, phenols, and quinones Minor: bay-region 9,10-di-hydrodiol, 4,5,9,10-tetra-hydroxy-4,5,9,10-tetra-hydro-benzo[e]pyrene	Common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: 9,10-, 7,8-, 4,5-, and 2,3-di-hydrodiols and epoxides, as well as various phenols, quinones, and derivatives	Presumed common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: ND	Common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: fjord region 11,12-diols, 11,12-diol-12,14-oxides, diastereomeric tetrols	Common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: Major: 10,11-di-hydrodiol, 10,11-diol-12,13-epoxide Minor: 1,2- and 3,4-diols	Common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: diols, oxides, catechols, phenols, and hexols	Common metabolic pathways with other PAHs (oxidation and glucuronide conjugation) Metabolites: diols, bisdiols, oxides	Presumed common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: ND	Common metabolic pathways with other PAHs (oxidation and glucuronide, GSH, or sulfate conjugation) Metabolites: dihydrodiols, phenols, and quinones

Table C-9. Integration of Evidence for BeP (CASRN 192-97-2) and Analogues

Evidence Streams	BeP	BaP	BbC	BgC	DBacA	DBahA	DBajA	Perylene	Picene
Cancer data and MOA (see Step 5)	No oral or inhalation data Predominately negative in other route studies: tumor initiator in 1/6 studies, 1/5 metabolites was a dermal initiator, 2/7 metabolites induced tumors in injection studies Inferred mutagenic MOA (based on BaP)	Multisite carcinogen in rodents following oral or inhalation exposure Carcinogenic in other route studies Proposed mutagenic MOA (U.S. EPA, 2017c)	No oral or inhalation data Skin tumor promotion in dermal assays Inferred mutagenic MOA (based on BaP)	ND	No oral or inhalation data Skin tumors in dermal assays; limited data evidence for liver tumors in injection studies Inferred mutagenic MOA (based on BaP)	Multisite carcinogen in rodents following oral exposure Carcinogenic in other route studies Inferred mutagenic MOA (based on BaP)	No oral or inhalation data Skin tumors in dermal assays; limited evidence for injection site tumors Inferred mutagenic MOA (based on BaP)	No oral or inhalation data Equivocal evidence for skin tumors in dermal assays; negative in injection studies Inferred mutagenic MOA (based on BaP)	No oral or inhalation data Skin tumors in dermal assays; lung and injection site tumors in injection studies Unknown MOA (negative in mutation assay)

Table C-9. Integration of Evidence for BeP (CASRN 192-97-2) and Analogues									
Evidence Streams	**BeP**	**BaP**	**BbC**	**BgC**	**DBacA**	**DBahA**	**DBajA**	**Perylene**	**Picene**
Common structural alerts and SAR predictions (see Step 6)	**Alerts:** • PAHs **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models	**Alerts:** • PAHs • DNA intercalation • Alkylation (metabolites) **SAR prediction:** Concerns for mutagenicity and carcino-genicity in most models

ADME = absorption, distribution, metabolism, and excretion; BaP. = benzo[a]pyrene; BbC = benzo[b]chrysene; BeP = benzo[e]pyrene; BgC = benzo[g]chrysene; DBacA = dibenz[a,c]anthracene; DBahA = dibenz[a,h]anthracene; DBajA = dibenz[a,j]anthracene; DNA = deoxyribonucleic acid; GSH = glutathione; MOA = mode of action; ND = no data; PAH = polycyclic aromatic hydrocarbon; SAR = structure-activity relationship.

STEP 8. QUALITATIVE LEVEL OF CONCERN FOR BEP POTENTIAL CARCINOGENICITY

Table C-10 identifies the qualitative level of *concern for potential carcinogenicity* of BeP based on the multiple lines of evidence described above. Because the proposed mutagenic MOA for BaP is relevant for all exposure routes, a qualitative level of concern for BeP potential carcinogenicity is applicable to both oral and inhalation exposures.

Table C-10. Qualitative Level of Concern for Carcinogenicity of BeP (CASRN 192-97-2)

Level of Concern	Designation	Comments
Concern for potential carcinogenicity	**Selected**	**Concern is based on (1) limited evidence of skin tumor initiation by BeP (at highest concentration tested), (2) limited evidence of skin tumor initiation by BeP metabolite 9,10-dihydrobenzo[e]pyrene, (3) limited evidence of tumor induction following neonatal i.p. exposure to specific BeP dihydrodiol and expected epoxide metabolites, (4) evidence of oral and/or inhalation carcinogenicity in animal studies of analogues BaP and DBahA, and (5) plausibility of a mutagenic MOA (potential, though limited, formation of reactive dihydrodiols and epoxides; evidence of mutagenicity with metabolic activation; formation of DNA adducts).**
Inadequate information for assigning qualitative level of concern	NS	NA

BaP = benzo[a]pyrene; BeP = benzo[e]pyrene; DBahA = dibenz[a,h]anthracene; DNA = deoxyribonucleic acid; i.p. = intraperitoneal; MOA = mode of action; NA = not applicable; NS = not selected.

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