

# Provisional Peer-Reviewed Toxicity Values for 2,3-Benzofluorene (CASRN 243-17-4)



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2,3-Benzofluorene  
(CASRN 243-17-4)

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

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

## PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2,3-BENZOFLUORENE (CASRN 243-17-4)

### 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 toxicologically relevant human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA eComments Chemical Safety website at <https://ecomments.epa.gov/chemicalsafety/>.

### 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 assessment 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 assessment 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 toxicologically relevant 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.

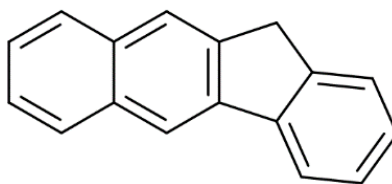
## **QUESTIONS REGARDING PPRTVS**

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

2,3-Benzofluorene, CASRN 243-17-4, also known as benzo[*b*]fluorene, is a tetracyclic compound and is classified as a polycyclic aromatic hydrocarbon (PAH). Its structure has four attached rings consisting of only carbon and hydrogen. 2,3-Benzofluorene is not produced commercially; as a PAH, it is produced during the incomplete combustion or pyrolysis of organic matter such as wood and fuel. PAHs are generated as combustion byproducts from various natural and anthropogenic sources including forest fires, volcanoes, vehicle exhaust and gas-burning engines, wood-burning stoves and furnaces, tobacco smoke, and burning of fossil fuels. PAHs are also generated as byproducts of industrial processes such as coal and petroleum refining, and production of iron, steel, and aluminum ([NLM, 2022](#); [WHO, 1998](#); [ATSDR, 1995](#)). 2,3-Benzofluorene is listed on the Toxic Substance Control Act (TSCA) public inventory ([U.S. EPA, 2024c](#)), in the European Chemicals (EC) inventory, and is preregistered with Europe's Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) program. There are no commercial uses reported for 2,3-benzofluorene.

The empirical formula for 2,3-benzofluorene is C<sub>17</sub>H<sub>12</sub>. The chemical structure is shown in Figure 1. Table 1 provides the physicochemical properties of 2,3-benzofluorene. 2,3-Benzofluorene is a solid at room temperature, has low vapor pressure, and is practically insoluble in water ([U.S. EPA, 2022a](#); [U.S. Pharmacopeia, 2008](#)). At ambient temperatures, the potential for volatilization from water surfaces or moist soil surfaces is expected to be moderate based on an estimated Henry's law constant of  $1.10 \times 10^{-5}$  atm m<sup>3</sup>/mole ([U.S. EPA, 2012a](#)). Given its vapor pressure, it is unlikely to volatilize from dry soil surfaces and will exist as a particulate in air. As a particulate, 2,3-benzofluorene can be removed from the atmosphere through wet or dry deposition. Its negligible water solubility and high estimated soil adsorption coefficient indicate that there is minimal potential for leaching into groundwater due to sorption to soils and sediments. 2,3-Benzofluorene does not contain functional groups that are likely to hydrolyze under environmental conditions; therefore, hydrolysis is not expected to be an important fate process. The potential for 2,3-benzofluorene to persist in the environment is moderate based on an estimated biodegradation half-life of 57.5 days ([U.S. EPA, 2022a](#)).



**Figure 1. 2,3-Benzofluorene (CASRN 243-17-4) Structure**

<b>Table 1. Physical Chemical Properties for 2,3-Benzofluorene (CASRN 243-17-4)<sup>a</sup></b>	
<b>Property (unit)</b>	<b>Value<sup>a</sup></b>
Physical state	Solid
Boiling point (°C)	381 (predicted)
Melting point (°C)	211
Density (g/cm <sup>3</sup> )	1.17 (predicted)
Vapor pressure (mm Hg at 25°C)	$5.50 \times 10^{-8}$ (extrapolated)
pH (unitless)	NA
Acid dissociation constant (pKa) (unitless)	NA
Solubility in water (mol/L)	$1.24 \times 10^{-8}$
Octanol-water partition coefficient (log K <sub>ow</sub> )	5.77
Henry's law constant (atm-m <sup>3</sup> /mol)	$1.10 \times 10^{-5}$ (predicted)
Soil adsorption coefficient (K <sub>oc</sub> ) (L/kg)	$1.10 \times 10^5$ (predicted)
Atmospheric OH rate constant (cm <sup>3</sup> /molecule-sec)	$4.07 \times 10^{-11}$ (predicted)
Atmospheric half-life (h)	3.2 (calculated assuming 12-h day and $1.5 \times 10^6$ OH/cm <sup>3</sup> )
Relative vapor density (air = 1) <sup>b</sup>	NA
Molecular weight (g/mol)	216.283
Flash point (°C)	183 (predicted)

<sup>a</sup>Unless otherwise noted, data were extracted from the U.S. EPA CompTox Chemicals Dashboard, accessed February 13, 2024 (2,3-Benzofluorene [CASRN 243-17-4] <https://comptox.epa.gov/dashboard/chemical/properties/DTXSID1022477>). All values are experimental averages unless otherwise specified.

<sup>b</sup>Vapor density is the relative weight of a gas or vapor compared to air and does not typically apply to solids.

NA = not applicable; U.S. EPA = U.S. Environmental Protection Agency.

A summary of available toxicity values for 2,3-benzofluorene from the U.S. EPA and other agencies/organizations is provided in Table 2.

<b>Table 2. Summary of Available Toxicity Values and Qualitative Descriptors of Carcinogenicity for 2,3-Benzofluorene (CASRN 243-17-4)</b>			
<b>Source/Parameter<sup>a,b</sup></b>	<b>Value (applicability)</b>	<b>Notes</b>	<b>Reference<sup>c</sup></b>
<b>Noncancer</b>			
IRIS	NV	NA	<a href="#">U.S. EPA (2024b)</a>
HEAST	NV	NA	<a href="#">U.S. EPA (1997)</a>
DWSHA	NV	NA	<a href="#">U.S. EPA (2018a)</a>
ATSDR	NV	NA	<a href="#">ATSDR (2022)</a>
WHO	NV	NA	<a href="#">IPCS (2020)</a>
CalEPA	NV	NA	<a href="#">CalEPA (2022)</a> ; <a href="#">CalEPA (2020)</a>
OSHA	NV	NA	<a href="#">OSHA (2020)</a> ; <a href="#">OSHA (2017a)</a> ; <a href="#">OSHA (2017b)</a>
NIOSH	NV	NA	<a href="#">NIOSH (2018)</a>
ACGIH	NV	NA	<a href="#">ACGIH (2022)</a>
SWCAA (ASIL)	0.00048 µg/m <sup>3</sup>	No details provided	<a href="#">SWCAA (2019)</a>
<b>Cancer</b>			
IRIS	NV	NA	<a href="#">U.S. EPA (2024b)</a>
HEAST	NV	NA	<a href="#">U.S. EPA (1997)</a>
DWSHA	NV	NA	<a href="#">U.S. EPA (2018a)</a>
NTP	NV	NA	<a href="#">NTP (2021)</a>
IARC (WOE)	Group 3, not classifiable as to its carcinogenicity to humans	Data inadequate to permit an evaluation of the carcinogenicity of benzo[ <i>b</i> ]fluorene in experimental animals	<a href="#">IARC (2010)</a>
CalEPA	NV	NA	<a href="#">CalEPA (2022)</a> ; <a href="#">CalEPA (2020)</a>
ACGIH	NV	NA	<a href="#">ACGIH (2022)</a>

<sup>a</sup>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; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; SWCAA = Southwest Clean Air Agency; WHO = World Health Organization.

<sup>b</sup>Parameters: ASIL = Acceptable Source Impact Level; WOE = weight of evidence.

<sup>c</sup>Reference date is the publication date for the database and not the date the source was accessed.

NA = not applicable; NV = not available.

Non-date-limited literature searches were conducted in June 2019 and updated most recently in June 2024 for studies relevant to the derivation of provisional toxicity values for 2,3-benzofluorene. Searches were conducted using the U.S. EPA's Health and Environmental Research Online (HERO; [www.hero.epa.gov](http://www.hero.epa.gov)) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE<sup>1</sup> (including TSCATS1), Scopus, and Web of Science. The following resources were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists (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), the U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, the U.S. EPA Integrated Risk Information System (IRIS), the U.S. EPA Health Effects Assessment Summary Tables (HEAST), the U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), the U.S. EPA TSCATS2/TSCATS8e, the U.S. EPA High Production Volume (HPV), Chemicals via International Programme on Chemical Safety (IPCS) INCHEM, Japan Existing Chemical Data Base (JECDB), Organisation for Economic Co-operation 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).

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<sup>1</sup>TOXLINE was retired in December 2019. Searches of this database were conducted through June 2019.

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

As summarized in Tables 3A and 3B, no subchronic, chronic, or reproductive/developmental toxicity studies of 2,3-Benzofluorene in humans or animals exposed by oral or inhalation routes adequate for deriving provisional toxicity values have been identified. The phrase “statistical significance,” used throughout the document, indicates a *p*-value of < 0.05 unless otherwise specified.

<b>Table 3A. Summary of Potentially Relevant Noncancer Data for 2,3-Benzofluorene (CASRN 243-17-4)</b>							
<b>Category</b>	<b>Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration</b>	<b>Dosimetry</b>	<b>Critical Effects</b>	<b>NOAEL</b>	<b>LOAEL</b>	<b>Reference (comments)</b>	<b>Notes</b>
<b>Human</b>							
<b>1. Oral (mg/kg-d)</b>							
ND							
<b>2. Inhalation (mg/m<sup>3</sup>)</b>							
ND							
<b>Animal</b>							
<b>1. Oral (mg/kg-d)</b>							
ND							
<b>2. Inhalation (mg/m<sup>3</sup>)</b>							
ND							

LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level.

<b>Table 3B. Summary of Potentially Relevant Cancer Data for 2,3-Benzofluorene (CASRN 243-17-4)</b>					
<b>Category</b>	<b>Number of Male/Female, Strain, Species, Study Type, Reported Doses, Duration</b>	<b>Dosimetry</b>	<b>Critical Effects</b>	<b>Reference</b>	<b>Notes</b>
<b>Human</b>					
<b>1. Oral (mg/kg-d)</b>					
ND					
<b>2. Inhalation (mg/m<sup>3</sup>)</b>					
ND					
<b>Animal</b>					
<b>1. Oral (mg/kg-d)</b>					
ND					
<b>2. Inhalation (mg/m<sup>3</sup>)</b>					
ND					

ND = no data.

**2.1. HUMAN STUDIES**

No studies were located regarding the toxicity or carcinogenicity of 2,3-benzofluorene in humans after oral or inhalation exposure.

**2.2. ANIMAL STUDIES**

No subchronic or chronic studies were located regarding cancer or noncancer effects of 2,3-benzofluorene in animals after oral or inhalation exposure.

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

A 5-day, repeated-dose oral rodent study that was designed as an in vivo transcriptomic study with the assessment of some toxicological endpoints ([NIEHS, 2023](#)) and a dermal tumor initiating assay ([LaVoie et al., 1981a](#)) were identified. These supporting animal studies are summarized in Table 4A. Table 4B provides an overview of genotoxicity studies of 2,3-benzofluorene. The genotoxicity studies, supporting animal studies, and a metabolism/toxicokinetic study are discussed below.

Table 4A. Summary of 2,3-Benzofluorene Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results Without Activation <sup>a</sup>	Results With Activation <sup>a</sup>	Comments	References
<b>Genotoxicity studies in prokaryotic organisms</b>						
Reverse mutation	<i>Salmonella typhimurium</i> TA100, TA98	NR	NDr	+	Plate incorporation method. The highest number of revertants observed was reportedly more than 3 times higher than controls. No details were provided regarding cytotoxicity or compound solubility in the test medium.	<a href="#">Bos et al. (1988)</a>
Reverse mutation	<i>S. typhimurium</i> TA100, TA98	NR	NDr	-	There was no evidence of mutagenicity in the “taped plate” assay, an Ames assay modified to detect only volatile mutagens. No details were provided regarding cytotoxicity or compound solubility in the test medium. The study authors considered the negative result to reflect lack of volatility of the test compound, not lack of mutagenicity.	<a href="#">Bos et al. (1988)</a>
Reverse mutation	<i>S. typhimurium</i> TA100	0, 15, 50 µg/plate	NDr	+	Modified <i>Salmonella</i> /microsome prescreen that included rat and hamster S9 at concentrations of 50–400 µL/plate. Rat S9 was effective over the whole range of S9 concentrations tested and at both concentrations of 2,3-benzofluorene. Hamster S9 was less effective over a shorter range of S9 concentrations and only at a 2,3-benzofluorene concentration of 15 µg/plate. Details on compound solubility in the test medium were cited to previous publications.	<a href="#">Carver et al. (1986)</a>
Reverse mutation	<i>S. typhimurium</i> TA100	NR	NDr	+	Plate incorporation method. Limited details were provided and none regarding cytotoxicity or compound solubility in the test medium.	<a href="#">Epler et al. (1978)</a>
Reverse mutation	<i>S. typhimurium</i> TA100, TA98	0, 10, 20, 50, 100, 200 µg/plate	-	+ (TA98)  - (TA100)	Revertants were increased ~twofold at 20 µg/plate and ~threefold at 50–200 µg/plate in TA98 with activation. There was no evidence of mutagenicity at up to 200 µg/plate in TA98 without activation or in TA100 with or without activation. No cytotoxicity was observed at the tested concentrations.	<a href="#">LaVoie et al. (1981b)</a> ; <a href="#">LaVoie et al. (1980)</a>

Table 4A. Summary of 2,3-Benzofluorene Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results Without Activation <sup>a</sup>	Results With Activation <sup>a</sup>	Comments	References
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	0.1–1,000 µg/plate	–	–	Plate incorporation method. Revertants were increased <twofold in all strains with or without metabolic activation. 2,3-Benzofluorene-specific data on cell survival or compound solubility in the test medium were not reported, but (when observed) the study authors discussed cytotoxicity and solubility issues observed with other tested compounds.	<a href="#">Salamone et al. (1979)</a>
Forward mutation	<i>S. typhimurium</i> TM677	25 µM	NDr	+	8-Azaguanine resistance assay. 2,3-Benzofluorene was mutagenic at the 8AG <sup>s</sup> /8AG <sup>f</sup> locus at ≥25 µM. No cytotoxicity was observed. No details were provided regarding compound solubility in the test medium; however, concentrations presumably did not reach the limit of solubility, based on data for other tested compounds.	<a href="#">Kaden et al. (1979)</a>
<b>Genotoxicity studies in mammalian cells—in vitro</b>						
Forward mutation	Human B-lymphoblastoid (h1A1v2) cells	1–10,000 ng/mL	–	NA	The cell line used constitutively expressed cytochrome P4501A1. 2,3-Benzofluorene was not mutagenic at the tk locus. No cytotoxicity was observed at the tested concentrations. No information on compound solubility in the test medium was reported.	<a href="#">Durant et al. (1996)</a>

Table 4A. Summary of 2,3-Benzofluorene Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results Without Activation <sup>a</sup>	Results With Activation <sup>a</sup>	Comments	References
DNA damage (comet assay)	Chinese hamster V79 lung fibroblasts	0, 10, 20, 50, 100 µM	+	NDr	The cell line used did not have endogenous metabolic activity. Under white fluorescent light, tail moment was increased by >twofold relative to controls at ≥20 µM; statistical significance ( $p < 0.01$ ) was reported at 100 µM (but was not indicated at other concentrations). 2,3-Benzofluorene presumably did not cause DNA damage in the dark and/or when yellow fluorescent lamps were used. Data for assays performed under these conditions were not shown for 2,3-benzofluorene; however, the study authors noted that 11 PAHs (including 2,3-benzofluorene) caused DNA strand breaks in white fluorescent light without external activation, and that the same 11 PAHs showed no damage in assays performed in the dark or under yellow light, suggesting that DNA damage was caused by the photo-activation of these PAHs. No cytotoxicity and no information on compound solubility in the test medium were reported.	<a href="#">Platt et al. (2008)</a>
<b>Genotoxicity studies in subcellular systems</b>						
DNA adducts/ binding	Rat liver microsomes	NR	–	NA	<sup>32</sup> P-postlabeling analysis. No calf thymus DNA adducts were detected. No details were provided regarding compound solubility in the test medium.	<a href="#">Koganti et al. (2000)</a>
<b>Genotoxicity studies—in vivo</b>						
DNA adducts/ binding	Female CD-1 mice exposed via the diet for 14 d	60 mg/kg	+	NA	<sup>32</sup> P-postlabeling analysis. Adducts were detected in mouse lung.	<a href="#">Koganti et al. (2000)</a>

<sup>a</sup>+ = positive; ± = weakly positive; – = negative.

DNA = deoxyribonucleic acid; NA = not applicable; NDr = not determined; NR = not reported; PAH = polycyclic aromatic hydrocarbon; tk = thymidine kinase.

Table 4B. Other Studies				
Test	Materials and Methods	Results	Conclusions	Reference
<b>Supporting evidence—noncancer effects in animals following oral exposure</b>				
Short-term	<p>Hsd:Sprague Dawley rats (5 M/5 F per treatment group; 10 M/10 F controls) were administered 2,3-benzofluorene via gavage (corn oil) at 0, 0.15, 0.5, 1.4, 4, 12, 37, 111, 333, and 1,000 mg/kg-d for 5 d.</p> <p>Apical endpoints evaluated included mortality, clinical signs of toxicity, body weights, hematology and clinical chemistry parameters (including thyroid hormone measurements), select organ weights (heart, liver, and kidneys), and gross pathology.</p>	<p>Decreased reticulocyte counts were seen in males and increased TSH (by 61%) and increased relative and absolute liver weight (both by 16%) were seen in females at <math>\geq 37</math> mg/kg-d.</p> <p>Effects observed at higher doses included decreased total T<sub>4</sub>, increased cholesterol, and absolute liver weight at <math>\geq 333</math> mg/kg-d in males and increased ALP at <math>\geq 111</math> mg/kg-d in females.</p>	The NOAEL was 12 mg/kg-d and the LOAEL was 37 mg/kg-d based on decreased reticulocyte count in males and increased TSH and relative and absolute liver weights in females.	<a href="#">NIEHS (2023)</a>
<b>Supporting evidence—cancer effects in animals following dermal exposure</b>				
Dermal tumor initiation study	<p>Swiss Albino (Ha/ICR) mice (20 F per group) received 10 dermal applications of 0.1% 2,3-benzofluorene in acetone on alternate days to a total dose of 1.0 mg. Animals in the control group animals received acetone; 10 d after the last dermal application, 2.5 <math>\mu</math>g of tetradecanoyl phorbol acetate in acetone was applied 3 times/wk for 20 wk.</p> <p>The incidence of skin tumors and the number of tumors per tumor-bearing animal were evaluated.</p>	Four mice (20%) treated with 2,3-benzofluorene and one mouse (5%) treated with solvent control were tumor-bearing. The numbers of tumors per animal were 0.35 and 0.05, respectively.	2,3-Benzofluorene was not considered a tumor initiator by the study authors.	<a href="#">LaVoie et al. (1981b)</a>

ALP = alkaline phosphatase; F = female(s); LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; T<sub>4</sub> = thyroxine; TSH = thyroid-stimulating hormone.

### 2.3.1. Supporting Animal Studies

In a dermal tumor initiation assay ([LaVoie et al., 1981a](#)), a 0.1% solution of 2,3-benzofluorene in acetone was applied to the shaved backs of female Swiss Albino (Ha/ICR) mice (20/group) 10 times on alternate days to achieve a total dose of 1.0 mg. Acetone was applied to the shaved backs of controls. Ten days following the last dose, 2.5 µg tetradecanoyl phorbol acetate (TPA) in acetone was applied as a promotor 3 times/week for 20 weeks. Four of 20 mice (20%) treated with 2,3-benzofluorene were tumor-bearing compared to 1/20 (5%) controls; the numbers of tumors per animal were 0.35 and 0.05, respectively. No additional details were provided. Under the study conditions, 2,3-benzofluorene was not considered a tumor initiator by the study authors.

A 5-day, repeated-dose oral study was conducted on Hsd:Sprague Dawley rats by the Division of Translational Toxicology (DTT) ([NIEHS, 2023](#)). The study was designed as a short-term transcriptomic dose-response study to derive transcriptomic reference values and included evaluation of some apical endpoints. Male and female rats (five males and five females/group, 6–7 weeks old) were randomized into nine dose groups, which received 2,3-benzofluorene in corn oil by daily gavage at dose levels 0.15, 0.5, 1.4, 4, 12, 37, 111, 333, or 1,000 mg/kg-day for 5 days. The selection of doses was guided by a median lethal dose (LD<sub>50</sub>) of 2,250 mg/kg-day predicted by the Open (Quantitative) Structure-activity/property Relationship Application (OPERA) and nominal dosing concentrations were verified by ultra-high pressure liquid chromatography/ultra-violet (UPLC/UV) analysis ([Mansouri et al., 2018](#)). Vehicle control group animals (10 per sex) received only corn oil. Treatment or solvent control was administered at the same dosage volume of 5 mL/g body weight.

Body weights were recorded on the first day of dosing and on the day of necropsy, which was performed the day following the last day of dosing. At necropsy, the hearts, livers, and kidneys were removed and weighed, and blood was collected for hematology and clinical chemistry analysis. Hematology parameters that were measured included erythrocyte count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell count and differential, reticulocyte count, platelet count, and nucleated erythrocyte count. Clinical chemistry analysis included alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bile acids, total bilirubin, direct bilirubin, cholesterol, creatine kinase, creatinine, glucose, sorbitol dehydrogenase (SDH), total protein, triglycerides, and urea nitrogen. Serum concentrations of thyroid stimulating hormone (TSH), free thyroxine (free T<sub>4</sub>), total thyroxine (total T<sub>4</sub>), and total triiodothyronine (total T<sub>3</sub>) were also determined. Statistical analysis included pairwise comparisons using Williams' and Dunnett's test (body and organ weight data) and Shirley's and Dunn's tests (clinical pathology data), and the Jonckheere test for dose-response trends.

To determine gene sets that were sensitive to 2,3-benzofluorene exposure, liver and kidney samples were processed for transcriptomic analysis using the TempO-Seq assay. Sample sequencing data were aligned, normalized, and processed as described by [NIEHS \(2023\)](#). Gene ontology gene set and individual gene analyses were performed. Benchmark dose (BMD) modeling of transcriptomic data was performed according to [NTP \(2018\)](#). Transcriptomics methods are described in detail in [NIEHS \(2023\)](#).

All male and female rats survived to the end of the study. No clinical observations were noted except soft stool on the first day of dosing in one male rat from the 12-mg/kg-day group

and one male rat from the 333-mg/kg-day group. On the day of necropsy, the mean body weights of animals exposed to 2,3-benzofluorene were either reduced by no more than 4% or increased by no more than 6% compared to the vehicle control groups. These differences were not statistically significant.

Statistically significant positive trends were found for both absolute and relative liver weights in male and female rats. Absolute liver weights were statistically significantly increased in males at  $\geq 333$  mg/kg-day (22%) and in females at  $\geq 37$  mg/kg-day (16%). Similarly, relative liver weights were statistically significantly increased in males at  $\geq 111$  mg/kg-day (9%) and in females at  $\geq 37$  mg/kg-day (16% increase). Male rats showed a significant positive trend in globulin concentration, with a statistically significant increase in the groups receiving  $\geq 333$  mg/kg-day (7%). There was also a significant positive trend in globulin concentration for female rats, with a statistically significant decrease observed in the lowest dose group of 0.15 mg/kg-day (14%), which was attributed to biological variability by the study authors. Blood cholesterol concentration in male rats exhibited a significant positive trend, with statistically significant increases of 14 and 42% in the 333- and 1,000-mg/kg-day groups, respectively. Female rats also showed a significant positive trend for cholesterol and a statistically significant increase of 55% in the 1,000-mg/kg-day group. ALP activity exhibited a significant positive trend only in female rats. Pairwise comparison revealed a statistically significant increase of 19% in the 111-mg/kg-day dose group and statistically significant increases were observed in all higher dose groups.

In both male and female rats, reticulocyte count showed a significant negative trend. Statistically significant decreases were observed at  $\geq 37$  mg/kg-day (31%) and  $\geq 111$  mg/kg-day (22%) for males and females, respectively. Platelet count exhibited a significant positive trend only in male rats, with a statistically significant increase of 36% in the 1,000-mg/kg-day dose group. Eosinophil counts demonstrated significant negative trends in male and female rats, with statistically significant differences between vehicle controls observed starting at  $\geq 333$  mg/kg-day (50% for males and 63% for females).

Concentrations of total T<sub>4</sub> showed a significant negative trend in male rats. Pairwise comparisons revealed a statistically significant decrease of 41% at 333 mg/kg-day. TSH exhibited a significant positive trend in both male and female rats. In male rats, TSH was significantly increased in the 111-mg/kg-day dose group (138%) and all higher dose groups. In female rats, TSH was significantly increased in the 37-mg/kg-day group (61%) and all higher dose groups.

In the transcriptomic analysis, “Regulation of ossification” was identified as the most sensitive liver Gene Ontology Biological Process (GOBP) gene set in male rats, and “DNA conformation change” was identified as the most sensitive GOBP gene set in the livers of female rats. “Brain development” was found to be the most sensitive GOBP gene set in the kidneys of male rats, while “Regulation of fibroblast proliferation” was the most sensitive GOBP gene set in the kidneys of female rats. The transcriptomic portion of this study was designed to determine toxicological potency from gene expression data rather than to provide hazard or mechanistic information. As a result, the study authors cautioned that the gene set results should be interpreted with care regarding underlying biological mechanisms.

### 2.3.2. Genotoxicity

The database for the genotoxicity of 2,3-benzofluorene is limited to several in vitro studies and one in vivo study that evaluated deoxyribonucleic acid (DNA) adduct formation. Available studies are summarized in Table 4B.

#### *Mutagenicity*

In two studies, 2,3-benzofluorene did not induce reverse mutations in the absence of activation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 ([LaVoie et al., 1981b, 1980](#); [Salamone et al., 1979](#)). However, results were predominantly positive in the presence of activation. In several studies, 2,3-benzofluorene induced reverse mutations in *S. typhimurium* strains TA98 and/or TA100 in the presence of activation ([Bos et al., 1988](#); [Carver et al., 1986](#); [LaVoie et al., 1981b, 1980](#); [Epler et al., 1978](#)); 2,3-benzofluorene also induced forward mutations in *S. typhimurium* strain TM677 in the presence of activation ([Kaden et al., 1979](#)). In another study, 2,3-benzofluorene was negative for reverse mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 in the presence of activation ([Salamone et al., 1979](#)).

In mammalian cells (human Blymphoblastoid cells), 2,3-benzofluorene did not induce forward mutations at the thymidine kinase locus ([Durant et al., 1996](#)).

#### *DNA Damage and DNA Adducts*

Findings were positive in a single in vitro assay of DNA damage performed under white fluorescent light. 2,3-Benzofluorene induced DNA strand breaks in cultured Chinese hamster V79 lung fibroblasts that cannot metabolically activate PAHs. The DNA damage occurred without external metabolic activation, suggesting that it was caused by photoactivated 2,3-benzofluorene under the conditions of standard laboratory illumination. DNA strand breaks were not induced when the assay was performed in the dark ([Platt et al., 2008](#)).

2,3-Benzofluorene did not form DNA adducts with calf thymus DNA in rat liver microsomes ([Koganti et al., 2000](#)). However, DNA adducts were identified in the mouse lung following ad libitum administration of 2,3-benzofluorene to mice in the diet at concentration of 60 mg/kg-day for 14 days ([Koganti et al., 2000](#)).

### 2.3.3. Metabolism/Toxicokinetic Studies

Neonatal Holtzman rats (3–5 days old) administered a single topical dose of 2,3-benzofluorene at 100 mg/kg 24 hours prior to sacrifice showed approximately 5 times higher induction of the drug-metabolizing enzymes, aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase, in the skin and liver relative to vehicle-only (acetone) controls ([Mukhtar et al., 1982](#)). The responsiveness of the liver to treatment suggests that there was absorption of 2,3-benzofluorene through the skin.

[NIEHS \(2023\)](#) conducted a screening-level internal dose assessment to determine whether 2,3-benzofluorene bioaccumulates in exposed animals. Satellite groups of male and female Hsd:Sprague Dawley rats (three per sex per group) were administered either 4 or 37 mg/kg-day 2,3-benzofluorene in corn oil by gavage for 5 consecutive days. Blood was collected at 2 and 24 hours following the administration of the final dose. At the 2-hour time point, male rats displayed average 2,3-benzofluorene plasma concentrations of 8.52 and 49.3 ng/mL after receiving doses of 4 and 37 mg/kg-day, respectively. Twenty-four hours after

the last dosing, the average plasma concentrations for male rats were 4.67 and 1.53 ng/mL in the 4- and 37-mg/kg-day dose groups, respectively. Reported average 2,3-benzofluorene plasma concentrations for female rats at the 2-hour time point were 3.62 and 15.5 ng/mL for the 4- and 37-mg/kg-day dose groups, respectively; plasma concentrations were below the limit of detection (1.8 ng/mL) in females at both dose groups at the 24-hour time point.

The lower plasma concentrations of 2,3-benzofluorene in female rats compared to male rats, when both were dosed at the same levels, indicate sex-based differences in the absorption, distribution, metabolism, and excretion (ADME) processes. Additionally, the results at 2 hours revealed that a ninefold increase in the dose of 2,3-benzofluorene led to only six- and fourfold increases in its blood concentrations in males and females, respectively, suggesting dose-dependent variations in ADME processes. At 24 hours, the plasma concentrations of 2,3-benzofluorene decreased to values near or below the analytical limit of detection in all male and female groups. The half-life values for male rats were reported as 25.3 and 4.4 hours for the dose groups of 4 and 37 mg/kg-day, respectively. Half-lives were not reported for females.

### 3. DERIVATION OF PROVISIONAL REFERENCE DOSES

No adequate studies were located regarding the toxicity of 2,3-benzofluorene to humans following repeated-dose oral exposure. Aside from a 14-day dietary study in female mice that only analyzed chemical-DNA adduct formation in the lung (Koganti et al., 2000), the oral toxicity database for 2,3-benzofluorene is limited to a 5-day gavage experiment in rats, designed as an in vivo transcriptomic study with the assessment of limited toxicological endpoints (NIEHS, 2023). Due to the lack of toxicity data generated from subchronic, chronic, or developmental exposure to 2,3-benzofluorene via the oral route, subchronic and chronic provisional reference doses (p-RfDs) were not derived directly. Instead, screening subchronic and chronic p-RfDs are derived in Appendix A using an alternative analogue approach.

#### 3.1. DERIVATION OF PROVISIONAL REFERENCE CONCENTRATIONS

No studies were located regarding toxicity of 2,3-benzofluorene to humans or animals via inhalation exposure. Due to the lack of inhalation toxicity data for 2,3-benzofluorene, subchronic and chronic provisional reference concentrations (p-RfCs) were not derived. An alternative analogue approach to derivation of inhalation toxicity values was attempted, but a suitable analogue was not identified (see Appendix A).

#### 3.2. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

The noncancer screening provisional reference values for 2,3-benzofluorene are summarized in Table 5.

Table 5. Summary of Noncancer Reference Values for 2,3-Benzofluorene (CASRN 243174)							
Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED/HEC)	UF <sub>C</sub>	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Increased relative liver weight	$8 \times 10^{-4}$	NOAEL	0.24 (based on analogue POD) <sup>a</sup>	300	Peiffer et al. (2016) as cited in <a href="#">U.S. EPA (2021)</a>
Chronic p-RfD (mg/kg-d)	Rat/F	Decreased RBC, packed cell volume, and hemoglobin	$5 \times 10^{-3}$	NOAEL	16.3 (based on analogue POD) <sup>a</sup>	3,000	TRL (1989) as cited in <a href="#">U.S. EPA (1990c)</a>
Subchronic p-RfC (mg/m <sup>3</sup> )	NDr						
Chronic p-RfC (mg/m <sup>3</sup> )	NDr						

<sup>a</sup>Fluorene was selected as a suitable source analogue of 2,3-benzofluorene for the oral route of exposure as described in Appendix A.

F = female(s); HEC = human equivalent concentration; HED = human equivalent dose; M = male(s); NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure p-RfC = provisional reference concentration; p-RfD = provisional reference dose; RBC = red blood cell; UF<sub>C</sub> = composite uncertainty factor.

### 3.3. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Although the scientific literature provides some (limited) information on the mutagenicity and genotoxicity of 2,3-benzofluorene, no oral or inhalation studies have been conducted to assess its carcinogenicity. Under the U.S. EPA Cancer Guidelines ([U.S. EPA, 2005](#)), there is “*Inadequate Information to Assess the Carcinogenic Potential*” of 2,3-benzofluorene by oral or inhalation exposure (see Table 6).

<b>Table 6. Cancer WOE Descriptor for 2,3-Benzofluorene (CASRN 243-17-4)</b>			
<b>Possible WOE Descriptor</b>	<b>Designation</b>	<b>Route of Entry (oral, inhalation, or both)</b>	<b>Comments</b>
“ <i>Carcinogenic to humans</i> ”	NS	NA	The available data do not support this descriptor.
“ <i>Likely to be carcinogenic to humans</i> ”	NS	NA	The available data do not support this descriptor.
“ <i>Suggestive evidence of carcinogenic potential</i> ”	NS	NA	The available data do not support this descriptor.
“ <i>Inadequate information to assess carcinogenic potential</i> ”	<b>Selected</b>	<b>Both</b>	<b>No adequate information is available to assess the carcinogenic potential of 2,3-benzofluorene by the inhalation or oral routes of exposure.</b>
“ <i>Not likely to be carcinogenic to humans</i> ”	NS	NA	The available data do not support this descriptor.

NA = not applicable; NS = not selected; WOE = weight-of-evidence.

### 3.4. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of data indicating a tumorigenic effect precludes development of cancer risk estimates for 2,3-benzofluorene (see Table 7).

<b>Table 7. Summary of Cancer Risk Estimates for 2,3-Benzofluorene (CASRN 243-17-4)</b>				
<b>Toxicity Type</b>	<b>Species/Sex</b>	<b>Tumor Type</b>	<b>Cancer Value</b>	<b>Principal Study</b>
p-OSF (mg/kg-d) <sup>-1</sup>	NDr			
p-IUR (mg/m <sup>3</sup> ) <sup>-1</sup>	NDr			

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

## APPENDIX A. NONCANCER SCREENING PROVISIONAL VALUES

Due to the lack of evidence described in the main Provisional Peer Reviewed Toxicity Value (PPRTV) assessment, it is inappropriate to derive provisional toxicity values for 2,3-benzofluorene. However, some information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited 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 deriving 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 (METHODS)

The analogue approach allows for the use of data from related compounds to calculate screening values when data for the target chemical are limited or unavailable. Details regarding searches and methods for analogue analysis are adapted from [Wang et al. \(2012\)](#) and [Lizarraga et al. \(2023\)](#) and chemical-specific parameters of read-across tools can be found in Appendix B. Candidate analogues are identified on the basis of three similarity categories (structure, toxicokinetics [metabolism], and toxicodynamics [toxicity and mode of action; MOA]) to facilitate the final source analogue selection. The analogue approach may or may not be route-specific or applicable to multiple routes of exposure. All information is considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable source analogue.

In this assessment, an expanded analogue identification approach was utilized to collect an augmented set of candidate analogues for the target chemical. As described below, this approach applies a variety of tools and methods for identifying candidate analogues that are similar to the target chemical based on structural features; metabolic relationships; or related toxic effects and mechanisms of action. The application of a variety of different tools and methods to identify candidate analogues minimizes the impact of limitations of any individual tool or method on the pool of chemicals included, chemical fragments considered, and methods for assessing similarity. Further, the inclusion of techniques to identify analogues based on metabolism and toxicity or bioactivity expands the pool of candidates beyond those based exclusively on structural similarity. The specific tools described below used for the expanded analogue searches were selected because they are publicly available, supported by U.S. and Organisation for Economic Co-operation and Development (OECD) agencies, updated regularly, and widely used.

To identify structurally related compounds, an initial pool of analogues is identified using automated tools, including ChemIDplus<sup>2</sup> ([NLM, 2020](#)), the CompTox Chemicals Dashboard<sup>3</sup> ([U.S. EPA, 2022a](#)), and the OECD Quantitative Structure-Activity Relationship (QSAR) Toolbox<sup>4</sup> ([OECD, 2020](#)). Additional analogues identified as ChemIDplus-related substances, mixtures, and CompTox “related substances”<sup>5</sup> are also considered. CompTox General Read-Across (GenRA)<sup>6</sup> analogues are collected using the methods deployed on the publicly available GenRA Beta version, which may include Morgan fingerprints, Torsion fingerprints, ToxPrints, and the use of ToxCast, Tox21, and ToxRef data ([Patlewicz and Shah, 2023](#)). For compounds that have very few analogues identified by structural similarity using a similarity threshold of 0.8 or 80%, substructure searches may be performed in the QSAR Toolbox, or similarity searches may be rerun using a reduced similarity threshold (e.g., <80%). Structural analogues are clustered using the Chemical Assessment Clustering Engine (ChemACE)<sup>7</sup> ([U.S. EPA, 2011b](#)) based on chemical fragments to support expert-driven refinement of the candidate pool. The ChemACE output is reviewed by an experienced chemist, who narrows the list of structural analogues based on expert judgment of multiple lines of evidence including known or expected structure-activity relationships, reactivity, and known or expected metabolic pathways. Initially, candidate analogues are screened for structural and chemical similarity to confirm that the analogues have the same reactive functional groups and similar overall size and structural features as the target chemical. Chemicals lacking key functionality or bearing additional functionality relative to the target are less desirable as analogues and are not selected as structural analogues. The selection may be expanded to include chemicals expected to be part of a metabolic series (either as metabolic precursors or as metabolites) of the target chemical.

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<sup>2</sup>ChemIDplus is a free, web search system that provides access to the structure and nomenclature authority files used for the identification of chemical substances cited in National Library of Medicine (NLM) databases, including the TOXNET system. The database contains over 350,000 chemical records, of which over 80,000 include chemical structures and allows users to draw a chemical structure to search for similar substances using PubChem Substructure fingerprints ([NLM, 2009](#); [Liwanag et al., 2000](#)). NLM retired ChemIDplus in December, 2022.

<sup>3</sup>The U.S. EPA’s CompTox Chemicals Dashboard provides publicly-accessible chemistry, toxicity, and exposure information for over one million chemicals ([Williams et al., 2017](#)). Using EPAM’s Bingo fingerprints, the “Similar Compounds” tab provides a list of chemicals that are similar in structure to the selected chemical, based on the Tanimoto similarity search metric with a minimum similarity factor threshold of 0.8 ([EPAM, 2024](#)).

<sup>4</sup>The OECD QSAR Toolbox is a software application intended to be used by government, industry and other stakeholders to fill gaps in data needed for assessing the hazards of chemicals. The application allows users to search for analogues based on structure similarity criteria and input similarity thresholds ([OECD, 2017](#)). It also contains metabolism simulators which are simplified versions of the simulators in CATALOGIC and TIMES and consist of hierarchically ordered molecular transformations ([Yordanova et al., 2019](#)).

<sup>5</sup>The CompTox Chemicals Dashboard “Related Substances” tab provides a chemical list of all chemicals related to the queried chemical through mapped relationships underlying the database. Relationships include searched chemical (self-relationship), salt form, monomer, polymer, predecessor component, component, Markush parent, Markush child, transformation parent, and transformation product ([Williams et al., 2021](#)).

<sup>6</sup>Operationalized within the CompTox Chemicals Dashboard, GenRA is an algorithmic approach that makes read-across predictions on the basis of a similarity weighted activity of source analogues (nearest neighbors). GenRA gives users the ability to identify candidate analogues based on structural and bioactivity information ([U.S. EPA, 2022b](#)).

<sup>7</sup>ChemACE clusters chemicals into groups based on structural features and a reasonable presumption that toxicity may be influenced by such structural characteristics (e.g., structural alerts, toxicophores). ChemACE identifies structural diversity in a large chemical inventory and highlights analogous clusters for potential read across. In the expanded analogue approach, clustering with ChemACE supports expert refinement of the candidate analogue pool. The ChemACE methodology is based on logic implemented in the Analog Identification Methodology (AIM) tool (<http://aim.epa.gov>) that identifies analogues based on the presence of common fragments using a tiered approach ([U.S. EPA, 2011a](#)).

Chemicals that produce metabolites in common with the target may also be selected if the metabolite is known or suspected to be part of the mechanism of action. All candidate analogues are then screened for structural features that can influence their activity relative to the target. Examples of such features include steric influences of bulky substituent groups, branching, rigidity, presence of blocking groups on a functional group, and differing substitution patterns on aromatic rings. Finally, key physical and chemical properties of the candidate analogues are compared with the target to confirm that they can be expected to have similar bioavailability, similar transport, and similar abiotic transformation properties.

Toxicokinetic studies tagged as potentially relevant supplemental material during screening are used to identify metabolic analogues (metabolites and metabolic precursors). Metabolites are also identified from two OECD QSAR Toolbox metabolism simulators (in vivo rat metabolism simulator and rat liver S9 metabolism simulator). Targeted PubMed searches are conducted to identify metabolic precursors and other compounds that share any of the observed or predicted metabolites identified for the target chemical.

In vivo toxicity data for the target chemical (if available) are evaluated to determine whether characteristic effects associated with a particular mechanism of toxicity are observed (e.g., cholinesterase inhibition, inhibition of oxidative phosphorylation). In addition, in vitro mechanistic data tagged as potentially relevant supplemental material during screening or obtained from tools including GenRA, ToxCast/Tox21<sup>8</sup>, and Comparative Toxicogenomics Database (CTD)<sup>9</sup> ([CTD, 2022](#)) are also evaluated for this purpose. ToxCast/Tox21 data available from the CompTox Chemicals Dashboard are collected for the target chemical to determine bioactivity in in vitro assays that may indicate potential mechanism(s) of action. The GenRA tool is used to search for analogues using Morgan, Torsion and ToxPrints fingerprint similarities and activity in ToxCast/Tox21 in vitro assays or ToxRef data (10 analogues collected from each neighbors data set). Using the ToxCast/Tox21 bioactivity data, nearest neighbors identified may be considered potential candidate analogues. The CTD is searched to identify compounds with gene interactions similar to those induced by the target chemical; compounds with gene interactions similar to the target chemical (similarity index >0.5) may be considered potential candidate analogues.

Candidate analogues identified on the basis of the structural, metabolic, and toxicodynamic similarity contexts are interrogated through the CompTox Chemicals Dashboard, where QSAR-ready simplified molecular-input line-entry system (SMILES) are collected and toxicity value availability is determined (e.g., from the Agency for Toxic Substances and Disease Registry [ATSDR], California Environmental Protection Agency [CalEPA] Office of Environmental Health Hazard Assessment [OEHHA], the U.S. EPA Integrated Risk Information System [IRIS], PPRTVs). Analogues that have subchronic or chronic toxicity data or toxicity values available from other public health agencies are flagged for potential consideration as supportive evidence.

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<sup>8</sup>ToxCast and Tox21 are publicly available databases containing high-throughput assay endpoints covering a range of high-level cell responses ([Thomas et al., 2018](#); [U.S. EPA, 2018b](#)).

<sup>9</sup>The CTD is a publicly available database that provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships. The CTD allows users to identify chemicals that induce gene interactions similar to those induced by the target chemical ([Davis et al., 2021](#)).

## Analogue Search Results for 2,3-Benzofluorene

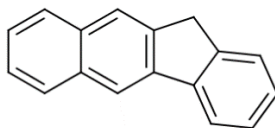
Candidate analogues for 2,3-benzofluorene were identified based on structural, metabolic, and toxicity/mechanisms/mode-of-action (MOA) relationships. For candidates identified through these approaches, the U.S. EPA (IRIS and PPRTV), ATSDR, and CalEPA sources were searched for subchronic, intermediate, and chronic inhalation toxicity values. Details are provided below.

### *Identification of Structural Analogues with Established Toxicity Values*

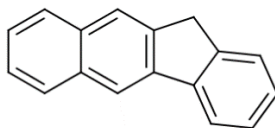
2,3-Benzofluorene is not a member of an existing OECD or New Chemical category. Candidate structural analogues for 2,3-benzofluorene were identified using similarity searches in the OECD Toolbox ([OECD, 2020](#)), the U.S. EPA CompTox Chemicals Dashboard ([U.S. EPA, 2022a](#)), and ChemIDplus tools ([NLM, 2020](#)). A total of 255 unique structural analogues were identified for 2,3-benzofluorene in the Dashboard, GenRA, OECD Toolbox, ChemIDplus, using an 80% similarity threshold, and including the nearest 10 analogues from each GenRA neighbor's data set. The QSAR Toolbox tool analysis was performed twice for 2,3-benzofluorene because different analogue results were generated depending on whether the location of the hydrogen on the compound was specified.

After eliminating deuterated compounds, the remaining list of analogues was reviewed by a chemist with expertise in read-across. Criteria for including candidates, to be consistent with the structure of 2,3-benzofluorene, were as follows: (1) includes one and only one five-membered ring to maintain a key structural feature of the molecule, which can be critical for preserving similar biological activity; (2) includes no fewer than two and no more than three benzene rings to ensure that the aromatic character is retained, while not deviating too far from the original structure in size and complexity, which may result in steric effects; and (3) does not include any ring substitutions. Substitutions would introduce steric hinderance, modify the solubility and the octanol-water partition coefficient ( $\log K_{ow}$ ) of the compound, and alter metabolic transformations relative to unsubstituted analogues. Substituents can also block sites of attack on the rings and can become targets of oxidation, leading to products that differ from those of the target.

Using these criteria, a total of nine unique candidate structural analogues for 2,3-benzofluorene were identified, as shown in Table A-1. For two analogues (benzo[*a*]fluorene, CASRN 30777-18-5 and benzo[*c*]fluorene, CASRN 30777-20-9), systematic names indicating the location of the hydrogen could not be verified with readily available sources. However, for completeness, the names and CASRNs were included in searches for toxicity values (no toxicity values were identified for either of the compounds).

**Table A-1. Candidate Structural Analogues Identified for 2,3-Benzofluorene**

Tool (method) <sup>a</sup>	Analogue (CASRNs) Selected for Toxicity Value Searches <sup>b</sup>	Structure
Dashboard (Tanimoto) <i>and</i> OECD Toolbox (Dice) <i>and</i> ChemIDplus (method not described)	<b>9H-Fluorene (86-73-7)</b>	
	7H-Benzo[ <i>c</i> ]fluorene (205-12-9)	
Dashboard (Tanimoto) <i>and</i> ChemIDplus (method not described)	11H-Benzo[ <i>a</i> ]fluorene (238-84-6)	
OECD Toolbox (Dice) only	1H-Fluorene (244-36-0)	
	1H-Benzo[ <i>b</i> ]fluorene (14458-76-5)	
ChemIDplus (method not described) only	Benzo[ <i>a</i> ]fluorene (30777-18-5)	
	Benzo[ <i>c</i> ]fluorene (30777-20-9)	
	<b>Fluoranthene (206-44-0)</b>	
Dashboard (Tanimoto) only	1H-Benzo[ <i>a</i> ]fluorene (238-82-4)	

**Table A-1. Candidate Structural Analogues Identified for 2,3-Benzofluorene**

Tool (method) <sup>a</sup>	Analogue (CASRN) Selected for Toxicity Value Searches <sup>b</sup>	Structure
Professional judgment based on chemistry expertise in read-across <sup>c</sup>	<b>Acenaphthene (83-32-9)</b>	

<sup>a</sup>All software tools set to 80% similarity threshold for analogue identification.

<sup>b</sup>**Bold** shows compounds with oral toxicity values (see Table A-6). None of the candidate structural analogues have an inhalation toxicity value.

<sup>c</sup>Based on the criteria defined by expertise in structure-activity relationships as follows: (1) includes one and only one five-membered ring; (2) includes no fewer than two and no more than three aromatic rings; (3) does not include any ring substitutions; and (4) has an oral toxicity value.

OECD = Organisation for Economic Co-operation and Development.

No inhalation toxicity values were identified for any of the candidate structural analogues; oral toxicity values were identified for fluorene and fluoranthene only.

Because only two structural analogues with toxicity values were identified using the methods reported above, additional searches of health assessment databases were conducted. The IRIS, PPRTV, ATSDR MRL, and OEHHA databases were searched for unsubstituted, nonheterocyclic polycyclic aromatic hydrocarbons with toxicity values. Those that met the first three structural criteria outlined above were selected irrespective of similarity threshold for additional consideration. This approach identified one additional candidate analogue, acenaphthene, which has an available oral toxicity value (see Table A-1).

#### ***Identification of Toxicokinetic Precursors or Metabolites with Established Toxicity Values***

No metabolites or metabolic precursors were reported for 2,3-benzofluorene in the scientific literature. PubMed searches (searching “2,3-benzofluorene” or “243-17-4” and “metabolite”) were conducted to identify metabolic precursors to 2,3-benzofluorene. No metabolic precursors were identified. Predicted metabolites were collected from the OECD QSAR Toolbox (OECD, 2020). Of the 42 predicted metabolites, 15 were too unstable to be suitable analogues, leaving 27 metabolites as candidate analogues. PubMed was also searched to identify other compounds that are metabolized to one of the 27 predicted metabolites of 2,3-benzofluorene (searching the metabolite name [none of the metabolites had CASRN] and “metabolite”). No compounds that share at least one metabolite with 2,3-benzofluorene were identified.

Table A-2 summarizes the 27 candidate metabolic analogues for 2,3-benzofluorene. Searches for relevant toxicity values for the candidate metabolic analogues of 2,3-benzofluorene did not identify toxicity values for any of the predicted metabolites.

**Table A-2. Candidate Metabolic Analogues of 2,3-Benzofluorene**

Relationship to 2,3-Benzofluorene	Compound <sup>a</sup>
Metabolic precursor	None identified
Metabolite	1H-benzo[ <i>b</i> ]fluoren-1-ol
	1H,6H,7H-dihydrobenzo[ <i>b</i> ]fluorene-6,7-diol
	1H,8H,9H-dihydrobenzo[ <i>b</i> ]fluorene-8,9-diol
	1H-benzo[ <i>b</i> ]fluoren-6-ol
	1H-benzo[ <i>b</i> ]fluoren-9-ol
	1H,2H,3H-dihydrobenzo[ <i>b</i> ]fluorene-2,3-diol
	1H,4H,4aH-dihydrobenzo[ <i>b</i> ]fluorene-4,4a-diol
	1H,2H,3H-dihydrobenzo[ <i>b</i> ]fluorene-2,3-diol 11,11a-oxidel
	1H,11H,11aH-dihydrobenzo[ <i>b</i> ]fluorene-11,11a-diol
	1H-benzo[ <i>b</i> ]fluorene-1,5-diol
	1H-benzo[ <i>b</i> ]fluorene-1,10-diol
	1H-benzo[ <i>b</i> ]fluoren-10-ol
	1H-benzo[ <i>b</i> ]fluorene-9,10-diol
	1H-benzo[ <i>b</i> ]fluorene-5,6-diol
	1H-benzo[ <i>b</i> ]fluoren-5-ol
	1H-benzo[ <i>b</i> ]fluorene-5,9-diol
	1H-benzo[ <i>b</i> ]fluorene-6,10-diol
	1H-benzo[ <i>b</i> ]fluorene-8,9-diol
	1H-benzo[ <i>b</i> ]fluorene-6,7-diol
	1H-Benzo[ <i>b</i> ]fluoren-7-ol
	1H-Benzo[ <i>b</i> ]fluoren-8-ol
	1H-benzo[ <i>b</i> ]fluorene-1,9-diol
	1H-benzo[ <i>b</i> ]fluorene-1,8-diol
1H-benzo[ <i>b</i> ]fluorene-1,7-diol	
1H-benzo[ <i>b</i> ]fluorene-1,6-diol	
1H,6H,7H-dihydrobenzo[ <i>b</i> ]fluorene-1,6,7-triol	
1H,8H,9H-dihydrobenzo[ <i>b</i> ]fluorene-1,8,9-triol	
Shares common metabolite(s)	None identified

<sup>a</sup>No CASRNs are available for these metabolites; consequently, chemical structures are not provided.

***Identification of Analogues on the Basis of Toxicity/Mechanistic/MOA Information and Established Toxicity Values***

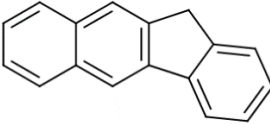
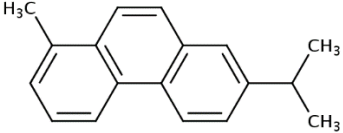
Available toxicity and mechanistic data for 2,3-benzofluorene were evaluated to determine whether there were in vivo toxicity data suggesting characteristic effects associated with a particular MOA (e.g., cholinesterase inhibition, inhibition of oxidative phosphorylation) that could potentially be used to identify candidate analogues. However, data for 2,3-benzofluorene are inadequate for these purposes. Although [NIEHS \(2023\)](#) identified

sensitive gene sets in male and female liver and kidney tissues via transcriptomic analysis, this study did not pinpoint mechanisms of toxicity of 2,3-benzofluorene. The study authors noted that the gene set results should be “interpreted with caution from the standpoint of the underlying biological mechanism” and that the transcriptomic data generated by the study “primarily should be considered a metric of potency for chemical-induced transcriptional changes.”

2,3-Benzofluorene was queried for bioactivity in assays reported in the U.S. EPA CompTox Chemicals Dashboard ([U.S. EPA, 2024a](#)). There were no PubChem assays in which 2,3-benzofluorene was active (zero out of nine assays) (accessed on August 23, 2024). The GenRA option within the U.S. EPA CompTox Chemicals Dashboard enables a search for analogues based on similarities in activity in ToxCast in vitro assays; however, there were no such bioactivity data for 2,3-benzofluorene in the Dashboard (invitrodb version 4.1; accessed on August 23, 2024). No candidate analogues were identified from bioactivity data on the basis of toxicodynamic similarity.

The CTD identified 20 compounds with gene interactions similar to interactions induced by 2,3-benzofluorene ([Davis et al., 2021](#)). In the CTD, similarity is measured by the Jaccard index, calculated as the size of the intersection of interacting genes for chemical A and chemical B divided by the size of the union of those genes (range 0 [no similarity] to 1 [complete similarity]). Among the compounds with gene interactions similar to 2,3-benzofluorene, the numbers of common gene interactions ranged from 4 to 32 and similarity indices ranged from 0.1 to 0.78. The only compound with a similarity index over 0.5 was retene (CASRN 483-65-8; similarity index of 0.78, 32 common gene interactions); this compound was selected as a candidate mechanistic analogue for 2,3-benzofluorene (see Table A-3).

There were no U.S. EPA, ATSDR, or CalEPA toxicity values for retene.

<b>Table A-3. Candidate Mechanistic Analogue Identified for 2,3-Benzofluorene</b>			
			
<b>Chemical Name</b>	<b>Structure</b>	<b>Gene Interaction Similarity Index</b>	<b>Number of Common Interacting Genes</b>
Retene (1-methyl-7-[1-methylethyl]phenanthrene; CASRN 483-65-8)		0.78	32

#### ***Candidate Analogues Moving Forward for Evaluation***

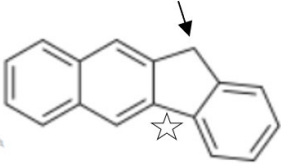
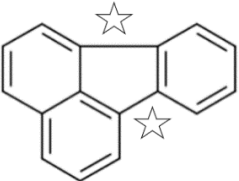
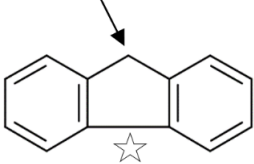
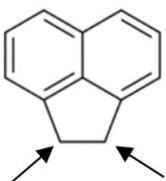
Searches for metabolic, structural, and toxicity/mechanistic analogues for 2,3-benzofluorene yielded a total of 38 unique candidate analogues: 27 metabolites, 10 structural analogues, and 1 mechanistic/mode of action analogue. None of the candidate analogues have

inhalation toxicity values from the U.S. EPA, ATSDR, or CalEPA, and only fluorene, fluoranthene, and acenaphthene have oral toxicity values.

**Structural Analogues**

The candidate analogues to 2,3-benzofluorene that have oral toxicity values are fluorene, fluoranthene, and acenaphthene, all identified on the basis of structural similarity. Table A-4 summarizes structural and physicochemical properties for 2,3-benzofluorene and candidate analogues.

**Table A-4. Physicochemical Properties of 2,3-Benzofluorene (CASRN 243-17-4) and Its Candidate Analogues<sup>a</sup>**

Chemical	Target Chemical	Candidate Analogues		
	2,3-Benzofluorene	Fluoranthene	9H-Fluorene	Acenaphthene
Structure <sup>b</sup>				
CASRN	243-17-4	206-44-0	86-73-7	83-32-9
Molecular weight (g/mol)	216.283	202.256	166.223	154.212
Melting point (°C)	211	108	115	93.9
Boiling point (°C)	381 (predicted)	380	295	279
Vapor pressure (mm Hg at 25°C)	$5.50 \times 10^{-8}$ (extrapolated)	$9.22 \times 10^{-6}$	$6.00 \times 10^{-4}$	$2.15 \times 10^{-3}$
Henry's law constant (atm-m <sup>3</sup> /mole)	$1.10 \times 10^{-5}$ (predicted)	$8.86 \times 10^{-6}$	$9.62 \times 10^{-5}$	$1.84 \times 10^{-4}$
Water solubility (mol/L)	$1.24 \times 10^{-8}$	$1.24 \times 10^{-6}$	$1.15 \times 10^{-5}$	$4.64 \times 10^{-5}$
Octanol-water partition coefficient (log K <sub>ow</sub> )	5.77	5.16	4.18	3.92

<sup>a</sup>Data are measured values from the U.S EPA CompTox Chemicals Dashboard, accessed September 11, 2024 (2,3-benzofluorene [CASRN 243-17-4]

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID1022477>; fluoranthene [CASRN 206-44-0]

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID3024104>; 9H-fluorene [CASRN 86-73-7]

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID8024105>; acenaphthene [CASRN 83-32-9]

<https://comptox.epa.gov/dashboard/chemical/details/DTXSID3021774>), unless otherwise specified.

<sup>b</sup>Open benzylic positions are indicated with arrows; bay-like pockets are indicated with stars.

U.S. EPA = U.S. Environmental Protection Agency.

Candidate analogues have similar, but lower, molecular weights than the target compound (range = 154–202 g/mol compared to 216 g/mol for 2,3-benzofluorene). Each candidate analogue is somewhat more water soluble and more volatile than 2,3-benzofluorene. Based on solubilities, the candidate analogues may be somewhat more bioavailable than the target chemical. Based on the similarity in their octanol-water partition coefficient ( $\log K_{ow}$ ) values (ranging from 3.92 to 5.77), 2,3-benzofluorene and all candidate analogues are lipophilic, with 2,3-benzofluorene being somewhat more lipophilic than the candidate analogues.

2,3-Benzofluorene and its candidate analogues contain one five-membered ring and two to three benzene rings, and do not include any ring substitutions. A key structural difference among the analogues relates to the presence and number of open benzylic positions, which may influence metabolism (see Metabolic Analogues section). 2,3-Benzofluorene and fluorene each have one open benzylic position, acenaphthene has two, and fluoranthene has none (see Table A-4). Neither 2,3-benzofluorene nor the candidate analogues have a true bay region representing a structural motif formed by three angularly arranged benzene rings that can influence metabolism and mutagenicity. However, 2,3-benzofluorene has one bay-like region, which is represented by angularly arranged benzene rings on a central five-membered ring, in which the bond angles are slightly different due to the five-membered ring in the middle of the pocket. Fluorene has one bay-like region, fluoranthene has two, and acenaphthene has none.

Of the candidate analogues, fluorene is the most similar to 2,3-benzofluorene on the basis of structural properties (both compounds have one open benzylic position and one bay-like region). Based on physicochemical properties, all candidate analogues are reasonably similar to 2,3-benzofluorene.

Relevant structural alerts and toxicity predictions for noncancer health effects were identified using computational tools from the [OECD \(2020\)](#) QSAR Toolbox profilers, and [OCHEM \(2022\)](#) ToxAlerts. The model results for 2,3-benzofluorene and candidate analogues are shown in Figure A-1. Structural alerts identified common concerns for hepatic and renal toxicity, estrogen receptor binding, and cytochrome P450 (CYP450)-mediated metabolism for 2,3-benzofluorene and all candidate analogues.

Structural Category	Compounds (CASRN)				Source
	Target Chemical	Candidate Analogues			
	2,3-Benzofluorene (243-17-4)	Fluoranthene (206-44-0)	Fluorene (86-73-7)	Acenaphthene (83-32-9)	
<b>Hepatotoxicity</b>					
Hepatotoxicity (based on 3-methylcholanthrene and alpha-naphthyl-isothiocyanate alerts)—Hazard Evaluation Support System (HESS)					OECD QSAR Toolbox
Hepatotoxicity (based on carbamazepine alert)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on 2-acetylaminofluorene, imipramine, and <i>N</i> -hydroxy-2-acetylaminofluorene alerts)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on diclofenac alert)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on beta-naphthylisothiocyanate alert)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on mefenamic acid and phenytoin alerts)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on amineptine alert)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on tamoxifen alerts)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on bromfenac alerts)—HESS					OECD QSAR Toolbox
Hepatotoxicity (based on ticlopidine alerts)—HESS					OECD QSAR Toolbox
<b>Renal Toxicity</b>					
Renal toxicity (based on 2-amino-4,5-diphenyl thiazole, anthraquinone, carbamazepine and propranolol alerts)—HESS					OECD QSAR Toolbox
Renal toxicity (based on toluene alert)—HESS					OECD QSAR Toolbox
Renal toxicity (based on ticlopidine alert)—HESS					OECD QSAR Toolbox
<b>Endocrine Receptor Binding</b>					
Estrogen receptor binding (based on multicyclic hydrocarbons alert)—Estrogen Receptor Expert System					OECD QSAR Toolbox
Endocrine disruption based on SA18 alert (reference cited but no other details provided)					ToxAlerts
<b>Metabolism/Reactivity</b>					
Liver enzyme induction (based on aromatic hydrocarbons)—HESS					OECD QSAR Toolbox
Cytochrome P450-mediated drug metabolism predicted (based on the presence of sp <sup>3</sup> and sp <sup>2</sup> hybridized carbon atoms)					ToxAlerts
<b>Other</b>					
Idiosyncratic toxicity (based on arenes alert)					ToxAlerts

■ Model results or structural alert indicating concern for noncancer toxicity/endpoint of interest.

■ Model results or structural alert indicating no concern for noncancer toxicity/endpoint of interest.

<sup>a</sup>Models with results are presented in the heat map (models without results indicate that the queried chemical fell outside of the applicability domain and are omitted).

**Figure A-1. Structural Alerts for 2,3-Benzofluorene and its Candidate Analogues<sup>a</sup>**

The OECD QSAR Toolbox showed a concern for hepatotoxicity for 2,3-benzofluorene and all candidate analogues based on a Hazard Evaluation Support System (HESS) alert for 3-methylcholanthrene (inducer of hepatic enzymes) and alpha-naphthyl-isothiocyanate (inducer of cholestasis, hyper-bilirubinemia, and necrotic injury in biliary epithelial cells); 3-methylcholanthrene is more structurally similar to the target chemical and its candidate analogues than alpha-naphthyl-isothiocyanate. Additional alerts for hepatotoxicity for 2,3-benzofluorene and at least one of the candidate analogues were based on carbamazepine, which is associated with vanishing bile duct syndrome (fluorene and fluoranthene); 2-acetylaminofluorene, which is associated with nuclear aneuploidy and oval cell hyperplasia; imipramine, which is associated with immune-mediated idiosyncratic hepatotoxicity; *N*-hydroxy-2-acetylaminofluorene, which is associated with hepatocyte cell death and periportal necrosis (fluorene and acenaphthene); diclofenac, which is also associated with immune-mediated idiosyncratic hepatotoxicity (fluorene); and beta-naphthylisothiocyanate, which is associated with hepatitis (acenaphthene). Other alerts for hepatotoxicity were indicated for candidate analogues of 2,3-benzofluorene but not for the target compound itself.

A HESS alert for renal toxicity for 2,3-benzofluorene, fluorene, and fluoranthene was based on 2-amino-4,5-diphenyl thiazole (renal polycystic disease), anthraquinone (renal degeneration), carbamazepine (associated with renal failure, hyponatremia, and immunologically-mediated acute interstitial nephritis without nephrotic syndrome), and propranolol (which also shows nephrotoxicity; not further specified), with anthraquinone being the most structurally similar to the target chemical and its analogues. Other alerts for renal toxicity were indicated for candidate analogues of 2,3-benzofluorene but not for the target compound itself.

Estrogen receptor binding was predicted for the target and all candidate analogues based on the U.S. EPA Estrogen Receptor Expert System multicyclic hydrocarbon category. The ToxAlerts tool showed the potential for endocrine disruption based on the SA18 alert for 2,3-benzofluorene and candidate analogues, fluorene and fluoranthene.

The OECD QSAR Toolbox HESS model showed potential for liver enzyme induction for 2,3-benzofluorene and all candidate analogues based on the presence of aromatic hydrocarbons. The ToxAlerts tool showed potential for CYP450-mediated drug metabolism for 2,3-benzofluorene and candidate analogues based on the presence of sp<sup>3</sup> and sp<sup>2</sup> hybridized carbon atoms.

The ToxAlerts tool also showed concern for idiosyncratic toxicity for 2,3-benzofluorene and candidate analogues, fluorene and fluoranthene (arenes alert). Idiosyncratic toxicity refers to rare, potentially threatening adverse events in some individuals exposed to chemicals. Their mechanisms likely involve immune responses but are not entirely understood. These events, which often have a delayed onset, cannot be reliably predicted using widely applicable animal models ([Mosedale and Watkins, 2020](#)). The arenes alert is a structural alert for chemicals with potential health concerns based on the presence of aromatic moieties in their structures. Supporting documentation for interpretation of this ToxAlert is limited.

The structural alert profile for 2,3-benzofluorene most closely resembles that of fluorene (10 alerts common to 2,3-benzofluorene), followed by fluoranthene (8 alerts common to 2,3-benzofluorene) and acenaphthene (6 alerts common to 2,3-benzofluorene).

**Metabolic Analogues**

Table A-5 summarizes the available information pertaining to toxicokinetic properties for 2,3-benzofluorene and candidate analogues. Key observations and comparisons are summarized below.

Table A-5. Comparison of ADME Data for 2,3-Benzofluorene and Its Candidate Analogues<sup>a</sup>

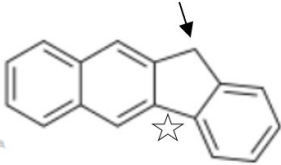
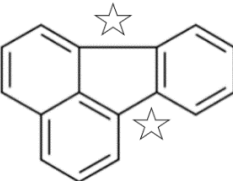
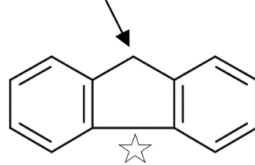
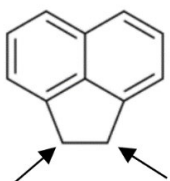
Type of Data	Target Chemical	Candidate Analogues		
	2,3-Benzofluorene	Fluoranthene	Fluorene	Acenaphthene
Structure				
CASRN	243-17-4	206-44-0	86-73-7	83-32-9
<b>Absorption</b>				
Rate and extent of absorption	<p><b>Rats (dermal)</b></p> <ul style="list-style-type: none"> <li>Substantial absorption through the skin based on induction of drug-metabolizing enzymes in the liver of neonatal animals.</li> </ul>	<p><b>Rats (oral):</b></p> <ul style="list-style-type: none"> <li>Peak blood level achieved ~1–2 h after oral dosing.</li> <li>Absorption from GI tract enhanced by administration in lipophilic vehicle (dietary fat).</li> </ul>	<p><b>Humans (all routes):</b></p> <ul style="list-style-type: none"> <li>Rapid absorption based on peak urinary levels of fluorene metabolites 1–10 h postexposure.</li> <li>Highest bioavailability from dietary exposure; bioavailability higher via dermal absorption than inhalation exposure.</li> </ul>	ND
<b>Distribution</b>				
Rate and extent of distribution	ND	<p><b>Rats (oral, i.v.):</b></p> <ul style="list-style-type: none"> <li>Widely distributed throughout the body.</li> <li>Initial rapid uptake into well-perfused tissues (e.g., lung, kidney, liver).</li> <li>Levels in fat and testis were lower than level in lung, kidney, and liver and peaked later.</li> <li>Coadministration with saturated fat extended duration of observed peaks relative to administration with no fat (controls) or unsaturated fat.</li> </ul>	<p><b>Humans (unspecified):</b></p> <ul style="list-style-type: none"> <li>Accumulates preferentially in fatty tissues based on analyses of tissue samples from cadavers.</li> </ul> <p><b>Rats (oral, inhalation, i.p.):</b></p> <ul style="list-style-type: none"> <li>Distributed throughout the body (radiolabel detected in all tissues examined).</li> <li>Highest amounts of radiolabel found in the gut and gut contents (route to GI tract not specified), kidney, and liver 1–8 d after injection (fat not tested).</li> <li>Fluorene and its metabolites (9- and 2-fluoreno) distribute to the brain.</li> </ul>	ND

Table A-5. Comparison of ADME Data for 2,3-Benzofluorene and Its Candidate Analogues<sup>a</sup>

Type of Data	Target Chemical	Candidate Analogues		
	2,3-Benzofluorene	Fluoranthene	Fluorene	Acenaphthene
<b>Metabolism</b>				
Rate; predicted metabolites	<p><b>Rats (dermal):</b></p> <ul style="list-style-type: none"> <li>Drug-metabolizing enzymes, AHH and 7-ethoxycoumarin <i>O</i>-deethylase, were induced in the skin and liver following a single topical exposure.</li> </ul> <p><b>Predicted metabolites<sup>b</sup>:</b></p> <ul style="list-style-type: none"> <li>Metabolites are predicted to be hydroxylation products, epoxides, diols, and triols of 1H-benzo[<i>b</i>]fluorene.</li> </ul>	<p><b>Rats (oral):</b></p> <ul style="list-style-type: none"> <li>Primary reactive metabolites identified were fluoranthene-2,3-dihydrodiol and <i>trans</i>-2,3-dihydroxy-1,10-b-epoxy-1,2,3,10b-tetrahydrofluoranthene; primary nonreactive metabolites identified were 3-hydroxy-fluoranthene and 8-hydroxy-fluoranthene.</li> <li>The relative amounts of these metabolites varied by tissue type. In general, fluoranthene-2,3-dihydrodiol was the more abundant reactive metabolite in plasma and tissue samples; 3-hydroxy-fluoranthene was the predominant metabolite in the urine. 8-Hydroxy fluoranthene was the least abundant metabolite in all tissues.</li> </ul>	<p><b>Laboratory animals (oral, i.p.):</b></p> <ul style="list-style-type: none"> <li>2-, 3-, and 9-Fluorenel were detected in the blood and brain of fluorene-exposed rats. Metabolism is rapid based on peak levels of these metabolites in the urine 1–10 h postexposure.</li> <li>Primary urinary metabolites were 2- and 9-fluorenel (hydroxyfluorene) glucuronide and sulfate conjugates; small amounts of free and/or conjugated 2,9-difluorenel were also detected.</li> <li>The 9-fluorenel glucuronide metabolite predominates in rats, with lesser amounts of 2-fluorenel sulfate; rabbits and guinea pigs showed similar amounts of 9- and 2-fluorenel glucuronides, as well as lesser amounts of 2-fluorenel sulfate.</li> <li>The relative abundance of conjugated metabolites varied by species, ranging from 48 to 64% for glucuronide conjugates and from 17 to 39% for sulfate conjugates.</li> </ul> <p><b>In vitro (rat liver preparations):</b></p> <ul style="list-style-type: none"> <li>In addition to 2-, 3-, and 9-fluorenel, metabolites included 1-fluorenel and 9-fluorenone.</li> <li>Fluorene reacts with oxygen to form hydroperoxides.</li> </ul> <p><b>Predicted metabolites<sup>b</sup>:</b> Metabolites are predicted to be hydroxylation products only.</p>	<p><b>Rats (oral):</b></p> <ul style="list-style-type: none"> <li>Urinary metabolites include naphthalic anhydride (the anhydride of naphthalene-1,8-dicarboxylic acid), indicating fission of the five-carbon ring.</li> </ul> <p><b>In vitro:</b></p> <ul style="list-style-type: none"> <li>Oxidized in vitro to mono- and dioxygenated products; 1-acenaphthenol was identified as a major product.</li> </ul> <p><b>Predicted metabolites<sup>b</sup>:</b></p> <ul style="list-style-type: none"> <li>Metabolites are predicted to be oxygenated products (including acids and anhydrides).</li> </ul>

Table A-5. Comparison of ADME Data for 2,3-Benzofluorene and Its Candidate Analogues<sup>a</sup>

Type of Data	Target Chemical	Candidate Analogues		
	2,3-Benzofluorene	Fluoranthene	Fluorene	Acenaphthene
<b>Excretion</b>				
Elimination half-time; route of excretion	<b>Rodents (oral):</b> <ul style="list-style-type: none"> <li>Half-lives in plasma were reported as 25.3 and 4.4 h for male rats receiving 4 and 37 mg/kg, respectively, via gavage.</li> </ul>	<b>Rodents (oral, i.v.):</b> <ul style="list-style-type: none"> <li>Elimination is rapid.</li> <li>Primary reactive and nonreactive metabolites were detected in the urine and feces in similar relative amounts.</li> <li>Coadministration with saturated fat increased elimination half-time relative to administration with unsaturated fat.</li> <li>Biological half-life of fluoranthene orally administered to male F344 rats depended on dosing vehicle and ranged from 0.7 to 3.0 h.</li> </ul>	<b>Humans (oral, dermal):</b> <ul style="list-style-type: none"> <li>Elimination is rapid.</li> <li>Maximum concentrations of mono-hydroxylated metabolites were detected in the urine 3.8–3.9 h postdietary exposure.</li> <li>Half-lives of elimination for 2-, 3-, and 9-fluorenol ranged from 3.1 to 9.3 h.</li> </ul> <b>Laboratory animals (oral, i.p.):</b> <ul style="list-style-type: none"> <li>Elimination is rapid.</li> <li>Eliminated predominantly in the urine (39–82% of the administered i.p. dose within 48 h) as conjugated metabolites; lower amounts detected in the feces (1–16% of radiolabeled dose within 48 h; form not specified).</li> </ul>	<b>Rats (oral):</b> <ul style="list-style-type: none"> <li>The anhydride of naphthalene-1,8-dicarboxylic acid is excreted in the urine of dosed animals.</li> </ul>
<b>References</b>	<a href="#">NIEHS (2023)</a> ; <a href="#">Mukhtar et al. (1982)</a>	<a href="#">Harris et al. (2008)</a> ; <a href="#">Walker et al. (2007)</a> ; <a href="#">Lipniak and Brandys (1993)</a> ; <a href="#">Mukhtar et al. (1982)</a>	<a href="#">Rossbach et al. (2020)</a> ; <a href="#">Fent et al. (2019)</a> ; <a href="#">Pastor-Belda et al. (2019)</a> ; <a href="#">Peiffer et al. (2016)</a> ; <a href="#">Peiffer et al. (2013)</a> ; <a href="#">Li et al. (2012)</a> ; <a href="#">IARC (1983)</a> ; <a href="#">LaVoie et al. (1981a)</a> ; <a href="#">Grantham (1963)</a> ; <a href="#">Neish (1948)</a>	<a href="#">Shimada et al. (2015)</a> ; <a href="#">Chang and Young (1943)</a>

<sup>a</sup>Open benzylic positions are indicated with arrows in the chemical structures; bay-like pockets are indicated with stars.

<sup>b</sup>Based on in silico predictions from the OECD QSAR Toolbox (the rat liver S9 metabolism [version 3.7] and in vivo rat metabolism [version 3.5] simulators).

ADME = absorption, distribution, metabolism, and excretion; AHH = aryl hydrocarbon hydroxylase; GI = gastrointestinal; i.p. = intraperitoneal; i.v. = intravenous; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship.

The only relevant study identified on the absorption of 2,3-benzofluorene indicates that there was substantial absorption through the skin, based on the induction of drug-metabolizing enzymes in the liver of neonatal rats following a single topical application ([Mukhtar et al., 1982](#)). No data on absorption are available for acenaphthene. Limited data for fluoranthene indicate that the peak blood level occurs within 1–2 hours of oral dosing; absorption is enhanced using a lipophilic vehicle (e.g., dietary fat). Fluorene is absorbed rapidly via all routes, as evidenced by observations of peak urinary levels of fluorene metabolites 1–10 hours postexposure. In general, polycyclic aromatic hydrocarbons (PAHs) are readily absorbed via all routes of exposure. Having fewer rings and lower molecular weight facilitates absorption ([ATSDR, 1995](#)).

Distribution data are not available for 2,3-benzofluorene or acenaphthene. Data for fluoranthene and fluorene indicate that these compounds are distributed throughout the body, preferentially to highly perfused tissues such as the lung, gut, liver, and/or kidney. There is evidence that fluorene accumulates preferentially in the fatty tissues of humans. Animal studies have shown that fluorene and its mono-hydroxylated metabolites distribute to the brain. Based on log  $K_{ow}$  values  $\geq 4$ , 2,3-benzofluorene, and to a lesser extent, fluoranthene and acenaphthene, are also expected to accumulate in fatty tissues. In general, PAHs are widely distributed throughout the body, with some accumulation in adipose tissue ([ATSDR, 1995](#)).

There are no experimental data on the metabolites of 2,3-benzofluorene. The only study identified relevant to 2,3-benzofluorene showed that the drug-metabolizing enzymes, aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase, were induced in the skin and liver of neonatal rats following a single topical application ([Mukhtar et al., 1982](#)). In silico predictions from the OECD QSAR Toolbox include oxidation products of 1H-benzo[*b*]fluorene, such as hydroxylation products, epoxides, diols, and triols. Oxidative metabolism is expected to occur preferentially at open benzylic positions. This prediction is supported by experimental data for fluorene (one open benzylic position) and acenaphthene (two open benzylic positions), which identified 9-fluorenol and 1-acenaphthenol, respectively, as primary metabolites. Metabolism at the open benzylic positions of acenaphthene contributes to fission of the five-membered ring, which is required to generate the observed naphthalic anhydride metabolite (the anhydride of naphthalene-1,8-dicarboxylic acid). Like fluorene, 2,3-benzofluorene has one open benzylic position; fluoranthene has none.

In addition to 9-fluorenol, other mono-hydroxylated metabolites of fluorene include 2- and 3-fluorenol. Fluorene metabolism is rapid, based on observations of peak levels of these metabolites in the urine 1–10 hours postexposure. Levels of 9-fluorenol, 2-fluorenol, and 3-fluorenol in biological matrices have been suggested as biomarkers of fluorene exposure. 1-Fluorenol and 9-fluorenone were identified as metabolites of fluorene in vitro; fluorene also reacts with oxygen to form hydroperoxides ([Alhamdow et al., 2020](#); [Rossella et al., 2009](#); [Toriba et al., 2003](#)).

Of the metabolites identified for candidate analogues, available data show that highly reactive epoxide metabolites and their hydrolysis products were detected only for fluoranthene. No epoxide metabolites have been identified in experimental studies of fluorene or acenaphthene, and no epoxide metabolites are predicted for these compounds using the OECD QSAR Toolbox. For 2,3-benzofluorene, predicted epoxide metabolites are formed from oxidation of 1H-benzo[*b*]fluorene.

Oxidative metabolism occurs in the bay region of PAHs to generate reactive diol epoxide intermediates ([ATSDR, 1995](#)). Epoxide metabolites involving the bay-like region have been predicted for 2,3-benzofluorene (which has one bay-like region) and observed experimentally for fluoranthene (which has two bay-like regions). However, the predicted epoxide metabolites of 2,3-benzofluorene do not contain a diol motif. Additionally, the positions of the epoxide moieties in the metabolites of 2,3-benzofluorene and fluoranthene differ from the epoxide positions in the metabolites of PAHs with a true bay region. No epoxide metabolites were observed or predicted for fluorene, which has one bay-like region, or for acenaphthene, which has no bay-like regions. Based on analogy to other PAHs, oxidative metabolism is expected to be mediated by CYP450s.

Excretion data are limited for 2,3-benzofluorene, with a 5-day study in rats reporting plasma half-lives of 4.4–25.3 hours after gavage administration of 4 and 37 mg/kg-day 2,3-benzofluorene ([NIEHS, 2023](#)). Available data for candidate analogues indicate that elimination is rapid (i.e., typically <1 day). The primary route of elimination for fluorene is the urine; fluoranthene metabolites were found in both the urine and the feces. Limited data for acenaphthene identified a metabolite in the urine of dosed animals. PAHs and/or their metabolites (frequently conjugated with sulfate, glutathione, or glucuronic acid) are excreted in the urine and/or feces ([ATSDR, 1995](#)).

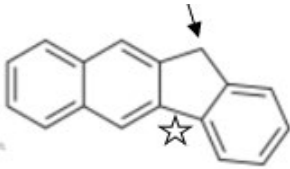
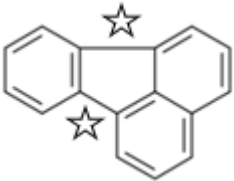
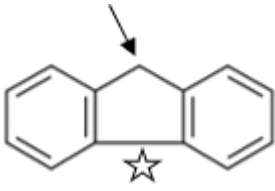
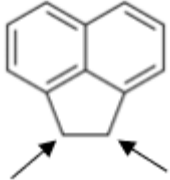
In summary, only limited toxicokinetic data for 2,3-benzofluorene are available. Limited absorption, distribution, metabolism, and excretion (ADME) data are also available for the candidate analogues. The available experimental data indicate that 2,3-benzofluorene is absorbed through the skin, fluorene and fluoranthene are readily absorbed following oral exposure, fluoranthene and fluorene are widely distributed throughout the body, and the candidate analogues and/or their metabolites (frequently conjugated with sulfate, glutathione, or glucuronic acid) are eliminated rapidly via the urine and/or feces. There are apparent differences in the metabolism of the candidate analogues based on the presence and number of open benzylic positions. An anhydride of dicarboxylic acid was detected as a metabolite of acenaphthene only, indicative of oxidative metabolism at the open benzylic positions and fission of the five-membered ring. 2,3-Benzofluorene and fluorene each have a single open benzylic position and predicted (2,3-benzofluorene) or predicted and observed (fluorene) metabolites do not include carboxylic acids. Fluoranthene has no open benzylic positions and there is no indication that carboxylic acid metabolites are formed. Of the candidate analogues, only fluoranthene has been shown to be metabolized to epoxide intermediates, which are among the more reactive products predicted or observed. Available experimental data do not identify epoxide metabolites of fluorene or acenaphthene, and no epoxide metabolites are predicted for these compounds by the OECD QSAR Toolbox. Epoxide metabolites involving the bay-like region are predicted for 2,3-benzofluorene; however, unlike PAHs with a true bay region, these epoxides do not have a diol group and are not found in a position analogous to the metabolites for PAHs with a true bay region. This indicates that there are differences between the epoxide metabolites of 2,3-benzofluorene and epoxide metabolites of PAHs containing a true-bay region. The latter form dihydrodiol epoxides, known as their ultimate carcinogenic metabolites.

### **Toxicity-Like Analogues**

Toxicity values for candidate analogues are presented in Table A-6. Available toxicity values are based on hematological, hepatic, and renal effects in subchronic studies. To compare the available in vivo data on these organ systems across candidate analogues, exposure-response arrays were generated for hematological (Figure A-2), hepatic (Figure A-3), and renal effects

(Figure A-4). Key points and information on potential mechanisms of toxicity, organized by target organ, are summarized below.

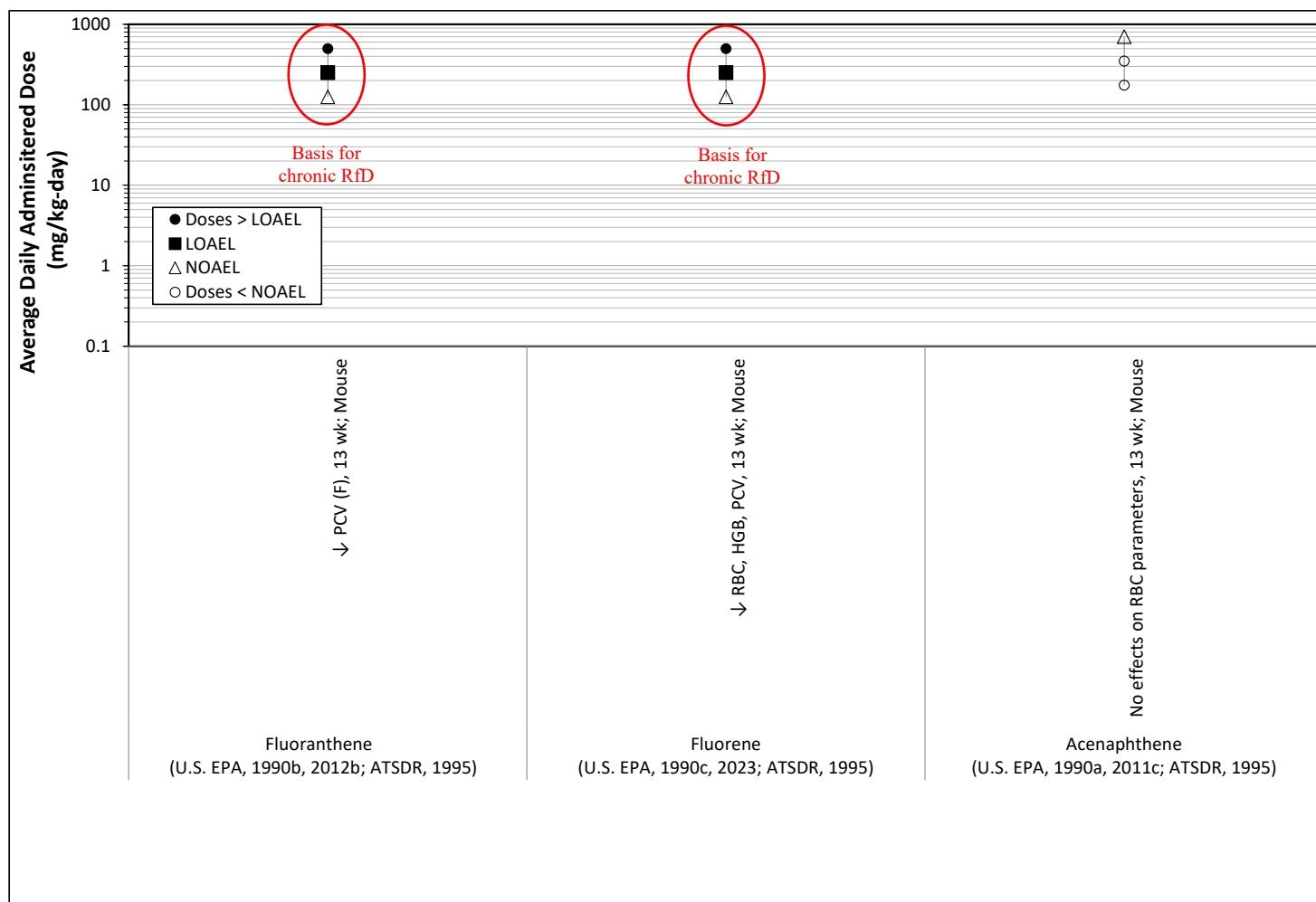
**Table A-6. Comparison of Available Oral Toxicity Data for 2,3-Benzofluorene (CASRN 243-17-4) and Its Candidate Analogues**

Type of Data	Target Chemical	Candidate Analogues					
	2,3-Benzofluorene	Fluoranthene		Fluorene		Acenaphthene	
Structure							
CASRN	243-17-4	206-44-0		86-73-7		83-32-9	
<b>Subchronic oral toxicity values</b>							
POD (mg/kg-d)	ND	125	124	125	0.24	175	161
POD type	ND	LOAEL	BMDL <sub>10</sub>	LOAEL	NOAEL (HED)	LOAEL	BMDL <sub>10</sub>
Subchronic UFc	ND	300 (UF <sub>A</sub> , UF <sub>H</sub> , UF <sub>L</sub> )	1,000 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> )	300 (UF <sub>A</sub> , UF <sub>H</sub> , UF <sub>L</sub> )	300 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> )	300 (UF <sub>A</sub> , UF <sub>H</sub> , UF <sub>L</sub> )	1,000 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> )
Subchronic p-RfD/MRL (mg/kg-d)	ND	4 × 10 <sup>-1</sup>	1 × 10 <sup>-1</sup>	4 × 10 <sup>-1</sup>	8 × 10 <sup>-4</sup>	6 × 10 <sup>-1</sup>	2 × 10 <sup>-1</sup>
Critical effects	ND	Increased relative liver weight (M)	Nephropathy	Increased relative liver weight		Increased relative liver weight	Increased relative liver weight (F)
Species	ND	Mouse		Mouse	Rat	Mouse	
Duration	ND	13 wk		13 wk	60 d	13 wk	
Route (method)	ND	Oral (gavage)		Oral (gavage)		Oral (gavage)	
Source	NA	<a href="#">ATSDR (1995)</a>	<a href="#">U.S. EPA (2012b)</a>	<a href="#">ATSDR (1995)</a>	<a href="#">U.S. EPA (2023)</a>	<a href="#">ATSDR (1995)</a>	<a href="#">U.S. EPA (2011c)</a>

**Table A-6. Comparison of Available Oral Toxicity Data for 2,3-Benzofluorene (CASRN 243-17-4) and Its Candidate Analogues**

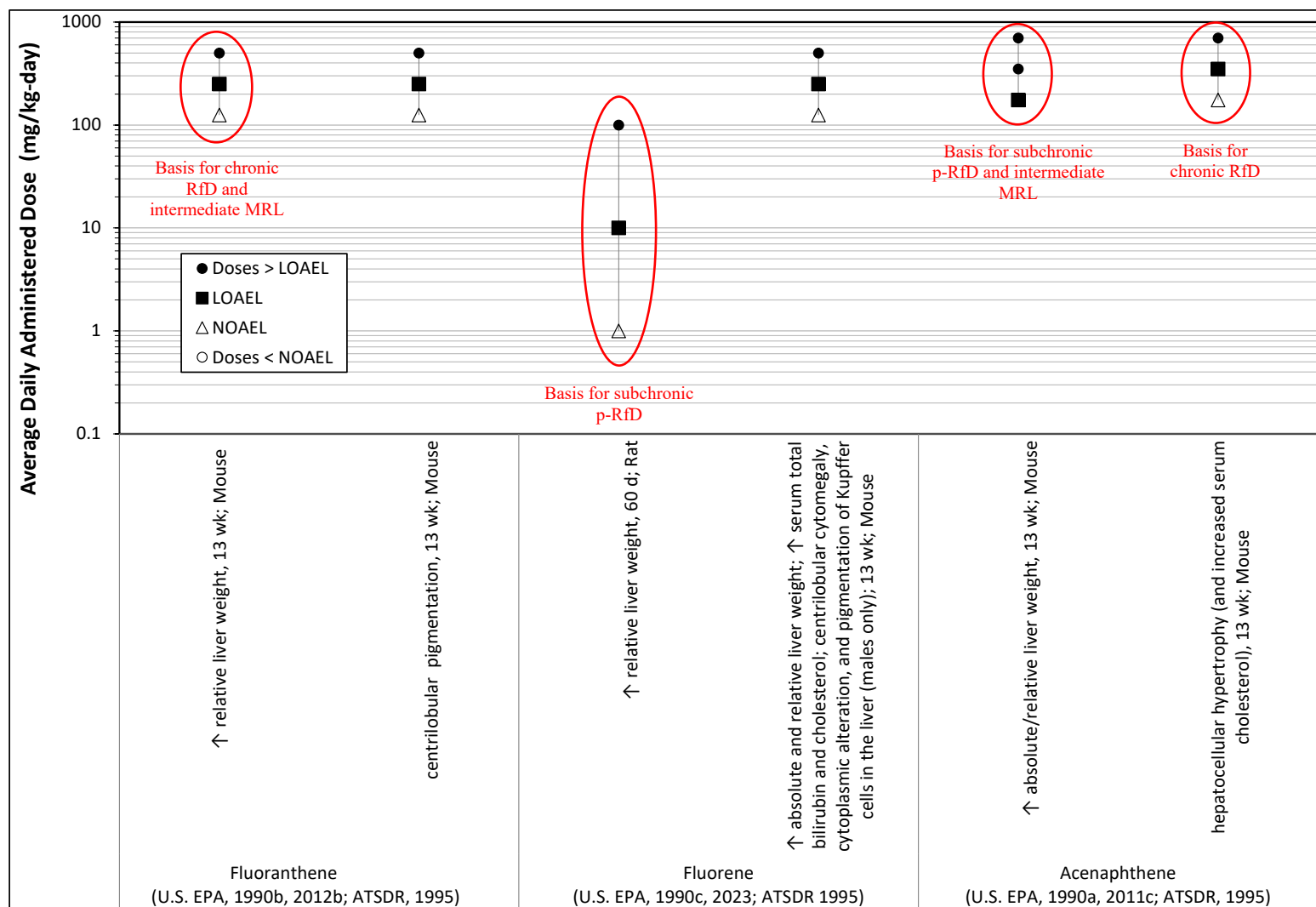
Type of Data	Target Chemical	Candidate Analogues		
	2,3-Benzofluorene	Fluoranthene	Fluorene	Acenaphthene
<b>Chronic oral toxicity values</b>				
POD	ND	125	125	175
POD type	ND	NOAEL	NOAEL	NOAEL
Chronic UF <sub>c</sub>	ND	3,000 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> , UF <sub>S</sub> )	3,000 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> , UF <sub>S</sub> )	3,000 (UF <sub>A</sub> , UF <sub>D</sub> , UF <sub>H</sub> , UF <sub>S</sub> )
Chronic p-RfD	ND	4 × 10 <sup>-2</sup>	4 × 10 <sup>-2</sup>	6 × 10 <sup>-2</sup>
Critical effects	ND	Nephropathy, increased liver weights, hematological alterations, and clinical effects	Decreased RBC, packed cell volume, and hemoglobin	Hepatotoxicity
Species	ND	Mouse	Mouse	Mouse
Duration	ND	13 wk	13 wk	13 wk
Route (method)	ND	Oral (gavage)	Oral (gavage)	Oral (gavage)
Source	NA	<a href="#">U.S. EPA (1990b)</a>	<a href="#">U.S. EPA (1990c)</a>	<a href="#">U.S. EPA (1990a)</a>

BMDL<sub>10</sub> = 10% benchmark dose lower confidence limit; F = female(s); HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; M = males(s); NA = not applicable; ND = no data; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; RBC = red blood cell; UF<sub>A</sub> = interspecies uncertainty factor; UF<sub>C</sub> = composite uncertainty factor; UF<sub>D</sub> = database uncertainty factor; UF<sub>H</sub> = intraspecies uncertainty factor; UF<sub>L</sub> = LOAEL-to-NOAEL uncertainty factor; UF<sub>S</sub> = subchronic-to-chronic uncertainty factor.



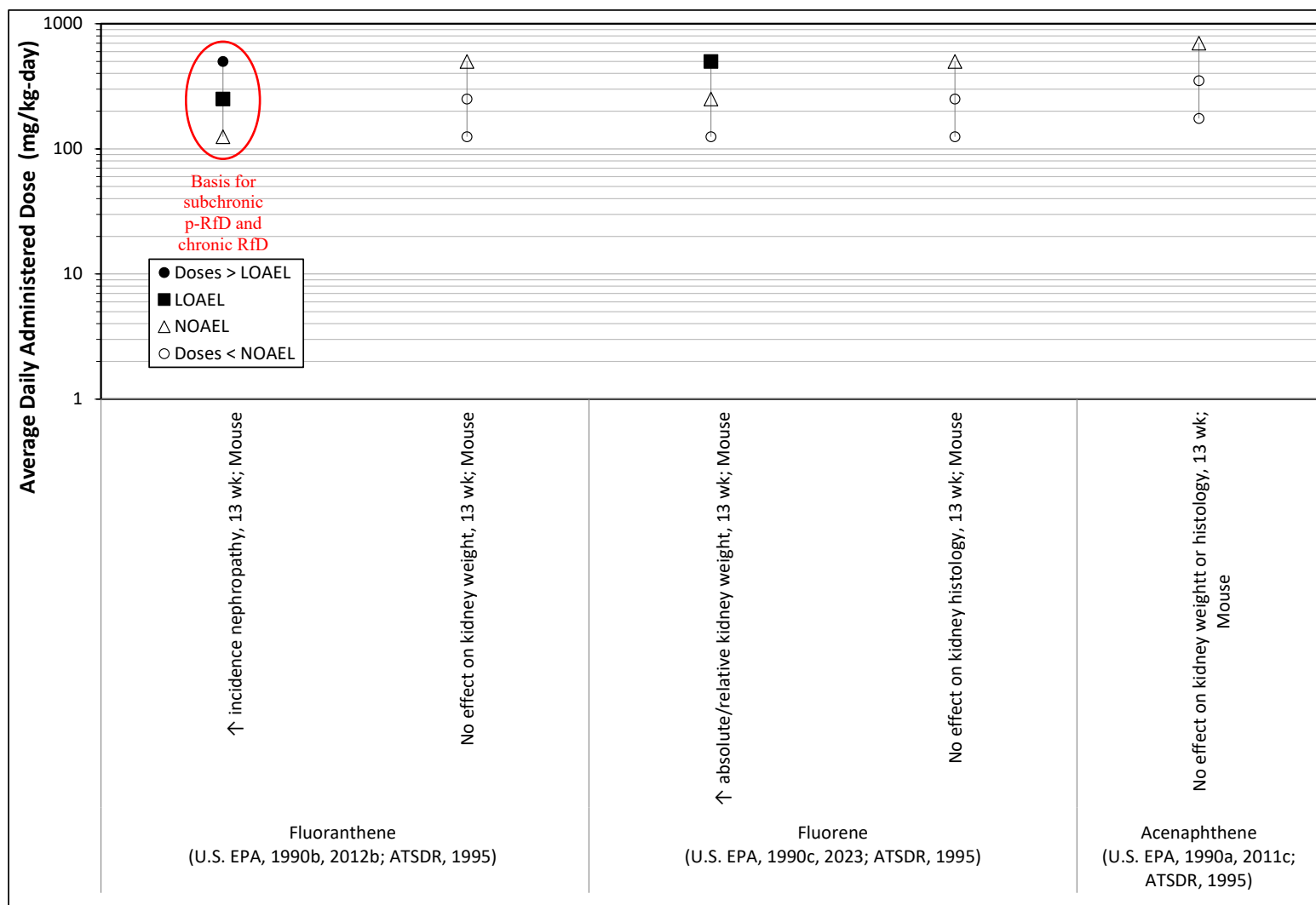
<sup>a</sup>Data for the 5-day oral rat study of 2,3-benzofluorene ([NIEHS, 2023](#)) were not included owing to the short duration of this study relative to the other studies.

**Figure A-2. Red Blood Cell-Related Effects Induced by Candidate Analogues (Circled Endpoints are the Basis for Subchronic and/or Chronic Toxicity Values)<sup>a</sup>**



<sup>a</sup>Data for the 5-day oral rat study of 2,3-benzofluorene ([NIEHS, 2023](#)) were not included owing to the short duration of this study relative to the other studies.

**Figure A-3. Hepatic Effects Induced by Candidate Analogues (Circled Endpoints are the Basis for Subchronic, Intermediate, and/or Chronic Toxicity Values)<sup>a</sup>**



<sup>a</sup>Data for the 5-day oral rat study of 2,3-benzofluorene ([NIEHS, 2023](#)) were not included owing to the short duration of this study relative to the other studies.

**Figure A-4. Renal Effects Induced by Candidate Analogues (Circled Endpoints are the Basis for Subchronic and/or Chronic Toxicity Values)<sup>a</sup>**

### ***Red Blood Cell (RBC)-Related Effects***

A 5-day oral rat study of 2,3-benzofluorene ([NIEHS, 2023](#)) found decreased reticulocyte counts at 37 and 111 mg/kg-day in male and female rats, respectively. RBC-related effects are common to candidate analogues, fluoranthene and fluorene, following subchronic oral exposure (Figure A-2).

RBC-related effects (decreased packed cell volume [PCV] in females) were observed at  $\geq 250$  mg fluoranthene/kg-day in a 13-week study in mice ([U.S. EPA, 2012b](#); [ATSDR, 1995](#); [U.S. EPA, 1990b](#)). Although RBC-related effects were also evaluated in a separate 13-week study in rats ([Knuckles et al., 2004](#)), no toxicologically relevant effects could be definitively identified, owing to uncertainties associated with the data. The data presented graphically showed a high degree of variation with no clear dose-related trends; therefore, no effect levels were identified. Hematological alterations [not further specified in [U.S. EPA \(1990b\)](#)], along with clinical effects, nephropathy, and increased liver weight in mice were the basis for the U.S. EPA chronic oral reference dose (RfD) for fluoranthene ( $4 \times 10^{-2}$  mg/kg-day) ([U.S. EPA, 1990b](#)).

With respect to fluorene, reductions in RBC count, hemoglobin, and/or PCV were observed at  $\geq 250$  mg/kg-day in a 13-week study in male and female mice ([U.S. EPA, 2023](#); [ATSDR, 1995](#); [U.S. EPA, 1990c](#)). Decreased RBCs, hemoglobin, and PCV were the basis for the U.S. EPA chronic oral RfD ( $4 \times 10^{-2}$  mg/kg-day) ([U.S. EPA, 1990c](#)).

No significant effects on RBC parameters were observed following 13 weeks of oral exposure to acenaphthene in mice at up to 700 mg/kg-day ([U.S. EPA, 2011c](#); [ATSDR, 1995](#); [U.S. EPA, 1990a](#)).

Mechanisms of hematological toxicity for the candidate analogues are not known.

### ***Liver Effects***

A 5-day oral rat study of 2,3-benzofluorene ([NIEHS, 2023](#)) found statistically significantly increased absolute liver weights at 333 mg/kg-day (22%) and 37 mg/kg-day (16%) in male and female rats, respectively. Relative liver weights were statistically significantly increased at 111 mg/kg-day (9%) and 37 mg/kg-day (16%) in male and female rats. Observed increases in thyroid-stimulating hormone (TSH) concentration at 37 mg/kg-day in female rats and 111 mg/kg-day in male rats and a decrease in total thyroxine (T<sub>4</sub>) concentrations at 333 mg/kg-day in male rats may be related to hepatic effects through hepatic enzyme induction followed by increased metabolism of T<sub>4</sub> and elevation of TSH via negative feedback on the hypothalamic-pituitary-thyroid axis. Hepatic enzyme induction was confirmed in the livers of rats after dermal exposure to 2,3-benzofluorene, leading to the induction of AHH and 7-ethoxycoumarin *O*-deethylase ([Mukhtar et al., 1982](#)). Statistically significant increases in cholesterol concentrations were observed at  $\geq 333$  mg/kg-day (14–42%) in male rats and at 1,000 mg/kg-day (55%) in female rats. Statistically significant increases in alkaline phosphatase (ALP) activity were observed at  $\geq 111$  mg/kg-day (19–35%) in female rats.

The liver is a common target of toxicity for all candidate analogues following subchronic oral exposure (Figure A-3).

For fluoranthene, a small (<10%) increase in relative liver weight was observed in males at 125 mg/kg-day in a 13-week study in mice. Statistically significant increases in alanine aminotransferase (ALT) and absolute and relative liver weights in both sexes, accompanied by microscopic liver lesions (increased pigmentation), were observed at higher doses ( $\geq 250$  mg/kg-day) ([U.S. EPA, 2012b](#); [ATSDR, 1995](#); [U.S. EPA, 1990b](#)). Increased relative liver weight in males was the basis for the ATSDR intermediate Minimal Risk Level (MRL) for fluoranthene ( $4 \times 10^{-1}$  mg/kg-day) ([ATSDR, 1995](#)). The U.S. EPA reported biologically significant increases in relative liver weights in both sexes at  $\geq 250$  mg/kg-day in the same 13-week study in mice. Increased incidences of centrilobular pigmentation were also noted at  $\geq 250$  mg/kg-day; however, the composition of the brown pigment, primarily contained within Kupffer cells, was not determined. Increased liver weight, along with nephropathy, hematological alterations, and clinical effects were the basis for the U.S. EPA chronic oral RfD for fluoranthene ( $4 \times 10^{-2}$  mg/kg-day) using the lowest-observed-adverse-effect level (LOAEL) of 250 mg/kg-day ([U.S. EPA, 1990b](#)).

The most sensitive hepatic effect observed following short-term or subchronic oral exposure to fluorene was increased relative liver weight ([U.S. EPA, 2023](#); [ATSDR, 1995](#); [U.S. EPA, 1990c](#)). A no-observed-adverse-effect level (NOAEL) of 1 mg/kg-day (NOAEL human equivalent dose [NOAEL<sub>HED</sub>] = 0.24 mg/kg-day) for increased relative liver weight was the basis for the derivation of the subchronic p-RfD ([U.S. EPA, 2023](#)) of  $8 \times 10^{-4}$  mg/kg-day. This toxicity value was based on a 60-day oral rat study ([Peiffer et al., 2016](#)) in which statistically significant and dose-related increases in relative liver weight were observed at all doses. Body-weight gain was significantly reduced only at the highest tested dose (100 mg/kg-day). Absolute liver weights were not reported, and histopathology was not performed. Based on analyses of the data presented graphically in the study report using GRABIT software, relative liver weights were statistically significantly and biologically significantly (>10%) increased relative to controls at  $\geq 10$  mg/kg-day; the NOAEL was 1 mg/kg-day. Increased relative liver weight at  $\geq 125$  mg/kg-day was the basis for the derivation of the intermediate [ATSDR \(1995\)](#) MRL of  $4 \times 10^{-1}$  mg/kg-day. The toxicity value was based on a 13-week oral mouse study in which relative liver weights were statistically significantly increased at all doses. At the lowest dose (125 mg/kg-day), relative liver weights in male and female mice were increased <10% relative to controls. At higher doses, changes in relative liver weight were accompanied by increased absolute liver weight and/or histopathological changes (increased hemosiderin in Kupffer cells and hepatocellular hypertrophy in males).

The most sensitive hepatic effect following acenaphthene exposure was increased absolute and relative liver weight in a 13-week study in mice (increased in females by >10% at  $\geq 175$  mg/kg-day) ([U.S. EPA, 2011c](#); [ATSDR, 1995](#); [U.S. EPA, 1990a](#)). Increased liver weight in females was the basis of the U.S. EPA subchronic oral reference value for acenaphthene ( $2 \times 10^{-1}$  mg/kg-day) ([U.S. EPA, 2011c](#)). Increased relative liver weight in both sexes and increased absolute liver weight in females at 175 mg/kg-day were the basis of the ATSDR intermediate MRL for acenaphthene ( $6 \times 10^{-1}$  mg/kg-day) ([ATSDR, 1995](#)). ATSDR noted that increased liver weights were accompanied by microscopic alterations (cellular hypertrophy) and/or increased cholesterol at higher doses. For the U.S. EPA chronic oral assessment on IRIS ([U.S. EPA, 1990a](#)), based on the same study, increased liver weight without accompanying microscopic alterations or increased cholesterol at 175 mg/kg-day was considered adaptive (rather than toxicologically relevant). Hepatotoxicity in males and females, indicated by the combination of increased liver weight, increased serum cholesterol and increased incidence of

hepatocellular hypertrophy, was observed at  $\geq 350$  mg/kg-day and was the basis of the U.S. EPA chronic oral RfD for acenaphthene ( $6 \times 10^{-2}$  mg/kg-day) ([U.S. EPA, 1990a](#)).

Mechanisms of hepatic toxicity for the candidate analogues are not known.

### ***Kidney Effects***

No kidney effects were found in a 5-day oral rat study of 2,3-benzofluorene that measured absolute and relative kidney weight, urea nitrogen, and creatinine ([NIEHS, 2023](#)). The kidney is a target organ of toxicity for candidate analogues, fluoranthene and fluorene, following subchronic oral exposure (Figure A-4).

The most sensitive renal effect following oral fluoranthene exposure was an increased incidence of nephropathy at  $\geq 250$  mg/kg-day in mice for 13 weeks ([U.S. EPA, 2012b](#); [ATSDR, 1995](#); [U.S. EPA, 1990b](#)). No changes in kidney weights were seen in the study. Nephropathy was the basis for the U.S. EPA subchronic oral RfD for fluoranthene ( $1 \times 10^{-1}$  mg/kg-day) ([U.S. EPA, 2012b](#)), and one of the effects (with increased liver weights, hematological alterations, and clinical effects) upon which the U.S. EPA chronic oral RfD ( $4 \times 10^{-2}$  mg/kg-day) was based ([U.S. EPA, 1990b](#)).

For fluorene, the most sensitive renal effect observed after 13 weeks of oral exposure was increased absolute and relative kidney weights in male mice at 500 mg/kg-day ([U.S. EPA, 2023](#); [ATSDR, 1995](#); [U.S. EPA, 1990c](#)). No kidney histopathology changes were observed at this dose (highest dose tested).

No effects on kidney weight or histology were observed in mice exposed orally to acenaphthene at doses up to 700 mg/kg-day ([U.S. EPA, 2011c](#); [ATSDR, 1995](#); [U.S. EPA, 1990a](#)).

Mechanisms of renal toxicity for the candidate analogue are not known.

### ***Summary***

A 5-day oral toxicity study of 2,3-benzofluorene in rats, designed primarily as an in vivo transcriptomic study with the assessment of some toxicological endpoints, identified effects on erythrocytes, liver, and thyroid hormone homeostasis. Available toxicity data for the candidate analogues identified effects on erythrocytes, liver, and kidney following subchronic oral exposure to fluoranthene and fluorene, and liver effects for acenaphthene. Alerts were indicated by the OECD QSAR Toolbox for hepatic and renal toxicity for 2,3-benzofluorene and all candidate analogues.

### **Weight-of-Evidence Approach**

A WOE approach is used to evaluate information available for candidate analogues as described by [Wang et al. \(2012\)](#) and [Lizarraga et al. \(2023\)](#). Similarities between candidate analogues and the target chemical are identified across three major categories of evidence: structural/physicochemical properties; toxicokinetics (ADME) and toxicodynamics (toxicity or MOA). Evidence of toxicological and/or toxicokinetic similarity is prioritized over evidence of similarity in structural/physicochemical properties. Candidate analogues are excluded if they demonstrate substantial differences from the pool of candidate analogues as a whole and/or the target chemical in any of the three categories of evidence. From the remaining pool of candidate

analogues, the most suitable analogue (i.e., the analogue that displays the closest biological or toxicological similarity to the target chemical) with the greatest structural similarity and/or most health-protective point-of-departure is selected. Additional considerations include preference for evidence from existing U.S. EPA assessments and suitability of study duration (i.e., chronic studies are preferred over subchronic studies when selecting an analogue for the derivation of a chronic value.)

### ***Oral Noncancer***

All candidate analogues with oral toxicity values exhibit similar structural and physicochemical properties.

A key structural difference among the analogues relates to the presence and number of open benzylic positions, which is expected to influence metabolism. 2,3-Benzofluorene has one such position, as does fluorene, while acenaphthene has two, and fluoranthene has none. Acenaphthene is the only candidate analogue for which the anhydride of a carboxylic acid was detected as a metabolite (from oxidation at the two open benzylic positions and fission of the five-membered ring). Owing to data limitations, the toxicological significance of this metabolite is unclear. Although 2,3-benzofluorene and the candidate analogues do not have a true bay region, 2,3-benzofluorene has one bay-like region with a five-membered ring in the middle of the pocket. The candidate analogues have two bay-like regions (fluoranthene), one bay-like region (fluorene), or no bay-like regions (acenaphthene). The structural alert profile for 2,3-benzofluorene most closely resembles that of fluorene (10 alerts common to 2,3-benzofluorene) followed by fluoranthene (8 alerts common to 2,3-benzofluorene), and acenaphthene (6 alerts common to 2,3-benzofluorene).

Available toxicokinetic data identify highly reactive epoxide metabolites and their hydrolysis products for fluoranthene only. No epoxide metabolites have been identified in experimental studies of fluorene or acenaphthene, and no epoxide metabolites are predicted for these compounds. For 2,3-benzofluorene, epoxide metabolites are predicted from oxidation of 1H-benzo[*b*]fluorene. The relationship between epoxide formation and resultant toxicity is not clear from the available data. Epoxide metabolites involving the bay-like region are predicted for 2,3-benzofluorene and observed experimentally for fluoranthene; however, these epoxides are not accompanied by a diol (in the case of 2,3-benzofluorene) and/or are not found in a position analogous to the metabolites for PAHs with a true bay region (in the case of both 2,3-benzofluorene and fluoranthene). No epoxide metabolites were observed or predicted for fluorene, which has one bay-like region, or for acenaphthene, which has no bay-like regions. Bay-region diol epoxides have been implicated in mutagenesis and carcinogenesis; however, the relevance of bay-like region epoxide formation to toxicity (especially noncancer effects) is uncertain from the available data.

Differences in metabolism related to the presence and number of open benzylic positions and epoxide formation were identified from data for the candidate analogues. Based on the same number of open benzylic positions and bay-like regions (as well as structural similarity scores), fluorene is the candidate analogue most similar to 2,3-benzofluorene. Based on the potential for epoxide metabolite formation, fluoranthene is the candidate analogue most similar to 2,3-benzofluorene. However, epoxides formed at the bay-like regions of 2,3-benzofluorene and fluoranthene are not accompanied by a diol and/or are not formed in the same position as PAHs

with true bay regions. The toxicological implications of these structural and metabolic differences are not clear based on the data available.

Owing to limitations in data for 2,3-benzofluorene, it is not possible to select the most suitable candidate analogue based on toxicodynamic similarities. In a 5-day gavage study that evaluated limited toxicological endpoints, 2,3-benzofluorene exhibited hepatic effects and effects on erythrocytes/erythropoiesis. All three candidate analogues exhibit hepatic effects, and both fluorene and fluoranthene also showed erythrocyte toxicity and kidney effects.

Points of departure (PODs) for subchronic oral toxicity values for two of the three candidate analogues are similar, with slightly lower values for fluoranthene (124–125 mg/kg-day) compared with acenaphthene (161–175 mg/kg-day) (see Table A-6). The NOAEL for fluorene based on increased relative liver weight (1 mg/kg-day) is 2 orders of magnitude lower than the NOAELs identified for the other candidate analogues.

With respect to chronic oral toxicity values, PODs for the three candidate analogues are similar (range = 125–175 mg/kg-day). Compared to these chronic values, the subchronic POD for fluorene (0.24 mg/kg-day) is 2 orders of magnitude lower, reflecting newer data that identified effects at lower dose levels.

In summary, all of the candidate analogues are considered suitable based on the WOE considerations outlined above. However, fluorene is the most appropriate analogue based on structural considerations and provides the most health-protective POD. Like the target chemical, fluorene has one open benzylic position and one bay-like region, and a similar structural alert profile. Limited data demonstrate that liver and RBC effects are observed following short-term oral exposure to 2,3-benzofluorene in rats, and critical effects of fluorene that were selected as the basis for its subchronic p-RfD, intermediate MRL, and chronic RfD values were increased relative liver weight and decreased RBC, packed cell volume, and hemoglobin. Therefore, fluorene is selected as the source analogue for the derivation of oral toxicity values for the target chemical, 2,3-benzofluorene.

### ***Inhalation Noncancer***

None of the candidate analogues had an inhalation toxicity value, precluding derivation of screening reference concentrations for 2,3-benzofluorene using the alternative analogue approach.

## **ORAL NONCANCER TOXICITY VALUES**

### **Derivation of a Screening Subchronic Provisional Reference Dose**

Based on the overall analogue approach presented in this PPRTV assessment, fluorene is selected as the source analogue for 2,3-benzofluorene for derivation of screening subchronic and chronic p-RfDs. The subchronic p-RfD for fluorene is based on a 60-day gavage study in rats ([Peiffer et al., 2016](#)). The *Provisional Peer-Reviewed Toxicity Values for Fluorene* (CASRN 86-73-7) ([U.S. EPA, 2023](#)) provided the following summary:

*In a published, peer-reviewed study, Wistar rats (eight males/group, aged 8–9 weeks) were administered fluorene (98% pure) in vegetable oil (a mixture of sunflower, rapeseed, and grape seed oils with no PAH contamination) via gavage at 0 (vehicle control), 1, 10, or 100 mg/kg-day for 60 days. Body weights were*

recorded on Study Days 2, 7, 14, 21, and 28. Four behavioral tests, initiated on Study Day 28 and performed through Study Day 60, were conducted 30 minutes after daily gavage administration (during the dark phase of the circadian cycle). Tests included an elevated-plus maze test on Study Day 28 to evaluate anxiety, an openfield test on Study Day 29 to evaluate motor and exploratory activity, an eight-arm maze on Study Days 30–44 (7 days of food restriction, 3 days familiarization, and 5 days of testing) to evaluate spatial learning and memory, and an aversive light stimulus avoidance test (Test d'Évitement d'un Stimulus Lumieux Aversif, or TESLA) on Study Days 45–53 (7 days acclimatization, 1 day habituation, and 1 day of recall) to evaluate learning and memory. At sacrifice on Study Day 60, blood samples were collected, and brain and liver weights were recorded.

*The outcomes measured in the elevated-plus maze (a raised maze consisting of three main areas: two open arms [ledges without enclosure] and two closed arms [ledges enclosed by vertical surrounding walls] intersecting at a central area to form a “plus” shape) included number of arm entries (total, open, and closed), time spent in each area (open arms, closed arms, and central area), total head dipping, percent head dipping in open arms, total rearing, and percent rearing in closed arms during a 5-minute period. Decreased open arm entries, decreased time spent in open arms, and increased occurrences of head dipping and rearing were considered indicative of anxiety.*

*In the open-field test, levels of activity on a platform containing 32 equivalent sections and three concentric zones (central, intermediate, and peripheral) were observed for 5 minutes and quantified by recording the numbers of squares crossed, number of rears, and amount of time spent in each zone.*

*The eight-arm maze, an enclosed maze consisting of eight arms (containing food pellets during the 5-day testing phase) joined in a central circular area, tested learning and spatial memory by measuring the ability of rats to position themselves within the maze using external visual cues located within the testing room. Parameters evaluated included total time to complete the maze (time taken to visit each arm with a cutoff value at 15 minutes), total arm entries, arm entries before the first error, and number of arms visited per minute.*

*The TESLA evaluated reference memory. Rats were placed in a box with high-intensity lighting and two pedals: an active lever that turned off the light for a 30-second period and an inactive lever that did not turn the light off. The active lever is deactivated while the light is turned off and regains its active status after the light has been off for 30 seconds; cumulative lever presses of the deactivated active lever do not result in longer periods of light reduction. After 1 day of habituation, rats were observed for 20 minutes and reference memory was evaluated based on discrimination of the active lever, discrimination of the active (light) period, discrimination of the lever and the active period, and total number of lever presses.*

Based on data presented for eight animals/group, it was presumed that no mortality occurred at any of the dosing levels (Peiffer et al., 2016). Body-weight data were not provided; however, percent body-weight gain (compared to the first day of treatment) was presented graphically with indicators of statistical significance. No statistically significant changes in bodyweight gain measured on Study Days 2, 7, 14, 21, and 28 were observed for rats in the first two dose groups (1 and 10 mg/kg-day) relative to controls. Rats treated at the high dose of 100 mg/kg-day lost approximately 3 and 6% of their body weight by Study Days 2 and 7, respectively (based on analysis of the graphical data using the MATLAB tool, GRABIT<sup>10</sup>; see Table B-1), but then gained weight thereafter. Overall, rats in this group showed decreased percent weight gain relative to controls through Study Day 28 ( $p < 0.01$  at each time point). The study authors reported that rats treated at the high dose exhibited lower average body weights throughout the 60-day study (data not shown). No treatment-related, toxicologically relevant effects on anxiety were observed based on the results of the elevated-plus maze; rats treated at the low dose (but not other doses) showed reduced anxiety (as evidenced by significantly decreased time spent in the closed arms and significantly increased time spent in the central area relative to controls) (Peiffer et al., 2016). Motor activity was unaffected by treatment (open-field test data not shown). In the eight-arm maze to evaluate learning and memory, a trend for decreased time to visit all arms ( $p = 0.074$ ) as well as a statistically significant trend for the increase in the number of arms visited per minute ( $p < 0.01$ ) were observed in all groups (including controls) based on the time of testing (i.e., all rats became more efficient at the maze as testing progressed). Rats treated at the high dose had fewer arm entries before the first error (i.e., performed worse in this behavioral test) compared to the other groups regardless of time of testing ( $p = 0.098$ ). In addition to this overall effect, the only statistically significant interaction between time of testing and treatment group was for reduction in the number of arm entries before the first error ( $p < 0.05$ ), which was particularly evident at the high dose (reduced by 24% on Study Day 5 relative to Study Day 1 compared to an 8% reduction in the control group and increases [i.e., improved performance] in the other dose groups). In the TESLA, another test that evaluated learning and memory, ability to discriminate between active (light) and inactive (dark) periods was significantly improved based on the time of testing for rats from all dosing groups (i.e., during testing relative to habituation;  $p < 0.05$ ). The total number of lever presses increased based on treatment group (i.e., higher-dose groups typically had a higher number of lever presses;  $p = 0.073$ ) and significantly decreased based on time of testing (i.e., there were fewer lever presses during testing than habituation;  $p < 0.01$ ). There were no statistically significant interactions between treatment group and time of testing for this effect or any of the other TESLA endpoints. Based on overall results from the behavioral battery, the study authors concluded that learning and memory were not affected by treatment.

<sup>10</sup>“GRABIT (<https://www.mathworks.com/matlabcentral/fileexchange/7173-grabit>) is an application of MATLAB and extracts data points from an image file using a graphical user interface.”

*Absolute brain and liver weights were not reported (Peiffer et al., 2016). Relative brain and liver weight data were presented graphically by dose group, with indicators of statistical significance for treatment groups relative to the control group. Nonsignificant increases in relative brain weight were observed at the mid and high dose (4 and 8% higher than controls, respectively). Relative liver weight showed statistically significant increases ( $p < 0.05$ ) at all doses (by approximately 6, 17, and 37% relative to controls at 1, 10, and 100 mg/kg-day, respectively, based on analyses using the MATLAB tool, GRABIT; see Table B-1). Increases in relative liver weight at the mid and high doses (10 and 100 mg/kg-day) were considered to be biologically significant.*

*Limitations of the study include the lack of data reported for biological endpoints of interest, including absolute brain and liver weights at study termination. Data were presented graphically, but not reported numerically, for relative brain and liver weights as well as body weights measured on Study Days 2, 7, 14, 21, and 28. No clinical chemistry evaluations or histological examinations were performed. Despite these limitations, a NOAEL of 1 mg/kg-day is identified from these data based on biologically significant (>10%) increases in relative liver weight in male rats at 10 and 100 mg/kg-day, which were also reported to be statistically significant ( $p < 0.01$ ). The administered doses of 0, 1, 10, and 100 mg/kg-day correspond to human equivalent doses (HEDs) of 0, 0.2, 2.4, and 23.6 mg/kg-day, respectively<sup>11</sup>.*

The NOAEL of 1 mg/kg-day for increased relative liver weight in male rats was selected as the POD for fluorene (U.S. EPA, 2023). The relative liver weight data from Peiffer et al. (2016) were not suitable for benchmark dose (BMD) modeling because no variance was shown for the control group (based on the data presented graphically). The POD was converted to a NOAEL<sub>HED</sub> of 0.24 mg/kg-day (U.S. EPA, 2023) by multiplying by a dosimetric adjustment factor (DAF):

$$\text{DAF} = (\text{BW}_a^{1/4} \div \text{BW}_h^{1/4})$$

where:

BW<sub>a</sub> = animal body weight

BW<sub>h</sub> = human body weight

In the absence of data for study-specific time-weighted average (TWA) animal body weights, the reference value for the body weight of male Wistar rats in a subchronic study of 0.217 kg was used. For humans, the reference value of 70 kg was used for body weight, as recommended by U.S. EPA (1988). Using the above equation, the DAF was calculated to be 0.24.

<sup>11</sup>“Administered doses were converted to HEDs by multiplying by a dosimetric adjustment factor (DAF) of 0.236 calculated as follows:  $\text{DAF} = (\text{BW}_a^{1/4} \div \text{BW}_h^{1/4})$ , where BW<sub>a</sub> = animal body weight, and BW<sub>h</sub> = human body weight. In the absence of data for study-specific time-weighted average (TWA) animal body weights, the reference value for the body weight of male Wistar rats in a subchronic study of 0.217 kg was used (U.S. EPA, 1988). For humans, the reference value of 70 kg was used for body weight, as recommended by U.S. EPA (1988).”

From this, the NOAEL<sub>HED</sub> was calculated as follows:

$$\begin{aligned}\text{NOAEL}_{\text{HED}} &= \text{NOAEL} \times \text{DAF} \\ &= 1 \text{ mg/kg-day} \times 0.24 \\ &= 0.24 \text{ mg/kg-day}\end{aligned}$$

The subchronic p-RfD for fluorene was derived from the NOAEL<sub>HED</sub> of 0.24 mg/kg-day using a composite uncertainty factor (UF<sub>C</sub>) of 300, reflecting a interspecies uncertainty factor (UF<sub>A</sub>) of 3, a database uncertainty factor (UF<sub>D</sub>) of 10, and an intraspecies variability uncertainty factor (UF<sub>H</sub>) of 10 ([U.S. EPA, 2023](#)). [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 2,3-benzofluorene from the fluorene data, the same uncertainty factors are applied as those applied to fluorene.

$$\begin{aligned}\text{Screening Subchronic p-RfD} &= \text{Analogue POD} \div \text{UF}_C \\ &= 0.24 \text{ mg/kg-day} \div 300 \\ &= \mathbf{8 \times 10^{-4} \text{ mg/kg-day}}\end{aligned}$$

Table A-7 summarizes the uncertainty factors for the screening subchronic p-RfD for 2,3-benzofluorene.

**Table A-7. Uncertainty Factors for the Screening Subchronic p-RfD for 2,3-Benzofluorene (CASRN 243-17-4)**

UF	Value	Justification
UF <sub>A</sub>	3	A UF <sub>A</sub> of 3 (10 <sup>0.5</sup> ) is applied to account for remaining uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed.
UF <sub>D</sub>	10	A UF <sub>D</sub> of 10 is applied to reflect database limitations for the fluorene analogue, absence of toxicity data for 2,3-benzofluorene, and application of a read across-based analogue assessment. The oral database for fluorene includes an unpublished 13-wk gavage study in male and female mice evaluating a comprehensive set of toxicological endpoints (TRL, 1989); a 60-d gavage study in male rats that evaluated limited standard toxicological endpoints (body, brain, and liver weights) and performed an extensive neurobehavioral test battery evaluating anxiety, motor activity, and learning and memory (Peiffer et al., 2016); and two subchronic to chronic studies in rats with reporting deficiencies (Morris et al., 1960; Wilson et al., 1947). No studies of reproductive or developmental toxicity were located for fluorene; however, for PAHs with larger databases, reproductive and/or developmental effects have been reported (U.S. EPA, 2017; EC, 2002; ATSDR, 1995). The oral database for 2,3-benzofluorene is limited to a 14-d dietary study in female mice that only analyzed chemical-DNA adduct formation in the lung (Koganti et al., 2000) and a 5-d gavage experiment in rats, designed as an in vivo transcriptomic study with the assessment of limited toxicological endpoints (NIEHS, 2023). No studies investigating subchronic, chronic, or developmental exposure to 2,3-benzofluorene via the oral route are available.
UF <sub>H</sub>	10	A UF <sub>H</sub> of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of 2,3-benzofluorene in humans.
UF <sub>L</sub>	1	A UF <sub>L</sub> of 1 is applied because the POD is a NOAEL.
UF <sub>S</sub>	1	A UF <sub>S</sub> of 1 is applied because the POD was derived from a study of suitable duration (60 days) for a subchronic value.
UF <sub>C</sub>	300	Composite UF = UF <sub>A</sub> × UF <sub>H</sub> × UF <sub>D</sub> × UF <sub>L</sub> × UF <sub>S</sub> .

DNA = deoxyribonucleic acid; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PAH = polycyclic aromatic hydrocarbon; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF<sub>A</sub> = interspecies uncertainty factor; UF<sub>C</sub> = composite uncertainty factor; UF<sub>D</sub> = database uncertainty factor; UF<sub>H</sub> = intraspecies uncertainty factor; UF<sub>L</sub> = LOAEL-to-NOAEL uncertainty factor; UF<sub>S</sub> = subchronic-to-chronic uncertainty factor.

### Derivation of a Screening Chronic Provisional Reference Dose

Fluorene is also selected as the source analogue for 2,3-benzofluorene for derivation of a screening chronic p-RfD. The IRIS Program (U.S. EPA, 1990c) derived a chronic RfD for fluorene based on a 13-week gavage study of fluorene in mice. U.S. EPA (1990c) provided the following study summary [TRL (1989) as cited in U.S. EPA (1990c)]:<sup>12</sup>

*CD-1 mice (25/sex/group) were exposed to 0, 125, 250, or 500 mg/kg/day fluorene suspended in corn oil by gavage for 13 weeks. Parameters used to assess toxicity included food intake, body weight, clinical observations, hematology and serum chemistry and gross and histopathological examinations. Increased salivation, hypoactivity, and urine-wet abdomens in males were observed in all treated animals. The percentage of mice exhibiting hypoactivity was dose-related.*

<sup>12</sup>TRL. (1989). 13-Week mouse oral subchronic toxicity study. Toxicity Research Laboratory. TRL Study No. #42-010. Dynamac Corporation, Rockville, MD [as cited in U.S. EPA (1990c)].

*In mice exposed at 500 mg/kg/day, labored respiration, ptosis (drooping eyelids), and unkempt appearance were also observed. A significant decrease in red blood cell count and packed cell volume were observed in females treated with 250 mg/kg/day fluorene and in males and females treated with 500 mg/kg/day. Decreased hemoglobin concentration and increased total serum bilirubin levels were also observed in the 500 mg/kg/day group. Decreases in erythrocyte count, packed cell volume, and hemoglobin concentration were all observed at 125 mg/kg; however, these effects, although apparently dose-dependent, were not statistically significant. A significant decreasing trend in BUN and a significant increasing trend in total serum bilirubin were observed in both high-dose males and females. A dose-related increase in relative liver weight was observed in treated mice; a significant increase in absolute liver weight was also observed in the mice treated with 250 and 500 mg/kg/day fluorene. A significant increase in absolute and relative spleen and kidney weight was observed in males and females exposed to 500 mg/kg/day and males at 250 mg/kg/day. Increases in the absolute and relative liver and spleen weights in the high-dose males and females were accompanied by histopathological increases in the amounts of hemosiderin in the spleen and in the Kupffer cells of the liver. No other histopathological lesions were observed. The LOAEL is 250 mg/kg/day based on hematological effects; the NOAEL is 125 mg/kg/day.*

The NOAEL of 125 mg/kg-day was selected as the POD for derivation of the chronic RfD for fluorene ([U.S. EPA, 1990c](#)) and was adopted as the POD for derivation of the screening chronic p-RfD for 2,3-benzofluorene. This aligns with the U.S. EPA's tiered approach hierarchy of human health toxicity values for Superfund risk assessments, which gives preference to toxicity reference values available in IRIS (Tier 1), as these values have undergone extensive review and validation ([U.S. EPA, 2003](#)).

The POD was converted to a NOAEL<sub>HED</sub> of 16.3 mg/kg-day by multiplying by a DAF:

$$\text{DAF} = (\text{BW}_a^{1/4} \div \text{BW}_h^{1/4})$$

where:

BW<sub>a</sub> = animal body weight

BW<sub>h</sub> = human body weight

In the absence of study-specific data for TWA animal body weights, the reference value of 0.0220 kg for the body weight of female mice (inbred unspecified strain) in a subchronic study was used. For humans, the reference value of 70 kg was used for body weight, as recommended by [U.S. EPA \(1988\)](#). Using the above equation, the DAF was calculated to be 0.13.

From this, the NOAEL<sub>HED</sub> was calculated as follows:

$$\begin{aligned} \text{NOAEL}_{\text{HED}} &= \text{NOAEL} \times \text{DAF} \\ &= 125 \text{ mg/kg-day} \times 0.13 \\ &= 16.3 \text{ mg/kg-day} \end{aligned}$$

To derive the screening chronic p-RfD for 2,3-benzofluorene, a  $UF_C$  of 3,000 was applied, reflecting a  $UF_A$  of 3, a  $UF_D$  of 10, a  $UF_H$  of 10, and a subchronic-to-chronic uncertainty factor ( $UF_S$ ) of 10. [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.

$$\begin{aligned}\text{Screening Chronic p-RfD} &= \text{Analogue POD} \div UF_C \\ &= 16.3 \text{ mg/kg-day} \div 3,000 \\ &= 5 \times 10^{-3} \text{ mg/kg-day}\end{aligned}$$

Table A-8 summarizes the uncertainty factors for the screening chronic p-RfD for 2,3-benzofluorene.

<b>Table A-8. Uncertainty Factors for the Screening Chronic p-RfD for 2,3-benzofluorene (CASRN 243-17-4)</b>		
<b>UF</b>	<b>Value</b>	<b>Justification</b>
$UF_A$	3	A $UF_A$ of 3 ( $10^{0.5}$ ) is applied to account for remaining uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed.
$UF_D$	10	A $UF_D$ of 10 is applied to reflect database limitations for the fluorene analogue, absence of toxicity data for 2,3-benzofluorene, and application of a read across-based analogue assessment. The oral database for fluorene includes an unpublished 13-wk gavage study in male and female mice evaluating a comprehensive set of toxicological endpoints ( <a href="#">TRL, 1989</a> ); a 60-d gavage study in male rats that evaluated limited standard toxicological endpoints (body, brain, and liver weights) and performed an extensive neurobehavioral test battery evaluating anxiety, motor activity, and learning and memory ( <a href="#">Peiffer et al., 2016</a> ); and two subchronic to chronic studies in rats with reporting deficiencies ( <a href="#">Morris et al., 1960</a> ; <a href="#">Wilson et al., 1947</a> ). No studies of reproductive or developmental toxicity were located for fluorene; however, for PAHs with larger databases, reproductive and/or developmental effects have been reported ( <a href="#">U.S. EPA, 2017</a> ; <a href="#">EC, 2002</a> ; <a href="#">ATSDR, 1995</a> ). The oral database for 2,3-benzofluorene is limited to a 14-d dietary study in female mice that only analyzed chemical-DNA adduct formation in the lung ( <a href="#">Koganti et al., 2000</a> ) and a 5-d gavage experiment in rats, designed as an in vivo transcriptomic study with the assessment of limited toxicological endpoints ( <a href="#">NIEHS, 2023</a> ). No studies investigating subchronic, chronic, or developmental exposure to 2,3-benzofluorene via the oral route are available
$UF_H$	10	A $UF_H$ of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of 2,3-benzofluorene in humans.
$UF_L$	1	A $UF_L$ of 1 is applied because the POD is a NOAEL.
$UF_S$	10	A $UF_S$ of 10 is applied because the principal study is less-than-chronic duration.
$UF_C$	3,000	Composite $UF = UF_A \times UF_H \times UF_D \times UF_L \times UF_S$ .

DNA = deoxyribonucleic acid; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PAH = polycyclic aromatic hydrocarbon; 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.

## APPENDIX B. PARAMETERS OF TOOLS USED FOR READ-ACROSS

<b>Table B-1. Parameters of Tools Used for Read-Across Evaluation of 2,3-Benzofluorene</b>			
<b>Similarity Context [number of analogues identified]<sup>a</sup></b>	<b>Tool Name [number identified]</b>	<b>Settings/Parameters</b>	<b>Searched by (date)</b>
Structural [255]	The U.S. EPA CompTox Chemicals Dashboard [177]	Tanimoto similarity threshold of 0.80 and related substances	CASRN (November 2020)
	ChemIDplus [62]	ChemIDplus similarity search (default method) with $\geq 80\%$ threshold and related substances, parent (or exact structure match), salts, and mixtures <sup>b</sup>	
	GenRA Beta version (in the U.S. EPA CompTox Chemicals Dashboard) [68]	Collect 10 nearest neighbors by each similarity setting and combination available: <ul style="list-style-type: none"> <li>• Morgan Fingerprints</li> <li>• Torsion Fingerprints</li> <li>• ToxPrints</li> <li>• Morg2Tor1Bio1</li> <li>• CT1:Bio3</li> </ul> Using each of the following data sources: ToxCast, Tox 21, and ToxRef	
	OECD QSAR Toolbox [40]	Similarity search with $\geq 80\%$ similarity threshold using default settings: <ul style="list-style-type: none"> <li>• Dice similarity</li> <li>• Atom centered fragments</li> <li>• Hologram calculation</li> <li>• All features combined</li> <li>• Atom characteristics: atom type, count H attached, and hybridization</li> </ul>	
	QSAR Toolbox Profilers <sup>c</sup>	No settings or parameters; results obtained from: <ul style="list-style-type: none"> <li>• HESS model</li> <li>• Estrogen Receptor Expert System, the U.S. EPA</li> <li>• Estrogen Receptor Binding</li> <li>• in vitro mutagenicity (Ames test) alerts by ISS</li> <li>• in vivo mutagenicity (Micronucleus) alerts by ISS</li> </ul>	SMILES <sup>d</sup> (October 2020)

**Table B-1. Parameters of Tools Used for Read-Across Evaluation of 2,3-Benzofluorene**

Similarity Context [number of analogues identified] <sup>a</sup>	Tool Name [number identified]	Settings/Parameters	Searched by (date)
		<ul style="list-style-type: none"> <li>• Carcinogenicity (genotox and nongenotox) alerts by ISS</li> <li>• Skin sensitization for DASS</li> </ul>	
	ToxAlerts <sup>c</sup>	No settings or parameters; structural alerts obtained from: <ul style="list-style-type: none"> <li>• Genotoxic carcinogenicity, mutagenicity (based on polycyclic aromatic system alert)</li> <li>• Idiosyncratic toxicity (based on Arenes alert)</li> <li>• Cytochrome P450-mediated drug metabolism predicted (based on the presence of sp<sup>3</sup> and sp<sup>2</sup> hybridized carbon atoms)</li> <li>• Endocrine disruption based on SA18 alert (reference cited but no other details provided)</li> </ul>	SMILES (October 2020)
Metabolic [27]	OECD QSAR Toolbox Metabolism Simulators [42]	No settings or parameters; results obtained from: <ul style="list-style-type: none"> <li>• Rat liver S9 metabolism simulator version 3.7</li> <li>• in vivo rat metabolism simulator version 3.5</li> </ul>	CASRN/SMILES <sup>c</sup> (October 2020)
Toxicity/mechanistic [1]	GenRA beta version (in the U.S. EPA CompTox Chemicals Dashboard) [0]	Collect 10 nearest neighbors using the ToxCast similarity settings: Nearest neighbors with a similarity index $\geq 0.5$ considered for use as analogue	CASRN (January 2021; August 2024)
	Comparative Toxicogenomics Database (CTD) [20]	Identify compounds with gene interactions similar to those induced by 2,3-benzofluorene: <ul style="list-style-type: none"> <li>• Used the interacting genes comparison search</li> <li>• A similarity index of <math>\geq 0.5</math> is considered for use as a mechanistic analogue</li> </ul>	(January 2021)

<sup>a</sup>Unique analogues identified using analogue identification search tools.

<sup>b</sup>For more information, see [https://www.nlm.nih.gov/pubs/techbull/ma06/ma06\\_technote.html](https://www.nlm.nih.gov/pubs/techbull/ma06/ma06_technote.html).

<sup>c</sup>Tool used for candidate analogue evaluation.

<sup>d</sup>SMILES collected from the U.S. EPA CompTox Chemicals Dashboard batch search of CASRNs of the structural analogues and QSAR Toolbox.

<sup>e</sup>2,3-Benzofluorene SMILES: C1C2=CC=CC=C2C2=CC3=CC=CC=C3C=C12 and C1C=CC=C2C1=Cc1cc3ccccc3cc12, different location of double bonds (CASRN 243-17-4).

DART = developmental and reproductive toxicity; DASS = defined approaches for skin sensitization; GenRA = General Read-Across; HESS = Hazard Evaluation Support System; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SMILES = Simplified Molecular Input Line Entry System; U.S. EPA = U.S. Environmental Protection Agency.

## APPENDIX C. REFERENCES

- ACGIH. (2022). 2022 TLVs and BEIs: Based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. In 2022 TLVs and BEIs: Based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. Cincinnati, OH.
- Alhamdow, A; Essig, YJ; Krais, AM; Gustavsson, P; Tinnerberg, H; Lindh, CH; Hagberg, J; Graff, P; Albin, M; Broberg, K. (2020). Fluorene exposure among PAH-exposed workers is associated with epigenetic markers related to lung cancer. *Occup Environ Med* 77: 488-495. <http://dx.doi.org/10.1136/oemed-2020-106413>
- ATSDR. (1995). Toxicological profile for polycyclic aromatic hydrocarbons - Update [ATSDR Tox Profile]. (CIS/97/00215). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. <https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=25>
- ATSDR. (2022). Toxic substances portal: Toxicological profiles [Database]. Atlanta, GA. Retrieved from <https://www.atsdr.cdc.gov/toxprofiledocs/index.html>
- Bos, RP; Theuws, JLG; Jongeneelen, FJ; Henderson, PT. (1988). Mutagenicity of bi-, tri- and tetra-cyclic aromatic hydrocarbons in the “taped-plate assay” and in the conventional Salmonella mutagenicity assay. *Mutat Res Genet Toxicol* 204: 203-206. [http://dx.doi.org/10.1016/0165-1218\(88\)90090-0](http://dx.doi.org/10.1016/0165-1218(88)90090-0)
- CalEPA. (2020). Consolidated table of OEHHA/CARB approved risk assessment health values. Sacramento, California. <https://ww2.arb.ca.gov/resources/documents/consolidated-table-oehha-carb-approved-risk-assessment-health-values>
- CalEPA. (2022). OEHHA chemical database [Database]. Sacramento, CA: Office of Environmental Health Hazard Assessment. Retrieved from <https://oehha.ca.gov/chemicals>
- Carver, JH; Machado, ML; Macgregor, JA. (1986). Application of modified Salmonella/microsome prescreen to petroleum-derived complex mixtures and polynuclear aromatic hydrocarbons (PAH). *Mutat Res* 247: 247-253. [http://dx.doi.org/10.1016/0165-7992\(86\)90042-4](http://dx.doi.org/10.1016/0165-7992(86)90042-4)
- Chang, LH; Young, L. (1943). The metabolism of acenaphthene in the rat. *J Biol Chem* 151: 87-91.
- CTD. (2022). Comparative toxicogenomics database [Database]: MDI Biological Laboratory. Retrieved from <http://ctdbase.org/>
- Davis, AP; Grondin, CJ; Johnson, RJ; Sciaky, D; Wieggers, J; Wieggers, TC; Mattingly, CJ. (2021). The comparative toxicogenomics database (CTD): update 2021 [Database]. Raleigh, NC: MDI Biological Laboratory. North Carolina State University. Retrieved from <http://ctdbase.org/>
- Durant, JL; Busby, WF, Jr; Lafleur, AL; Penman, BW; Crespi, CL. (1996). Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutat Res DNA Repair* 371: 123-157. [http://dx.doi.org/10.1016/S0165-1218\(96\)90103-2](http://dx.doi.org/10.1016/S0165-1218(96)90103-2)
- EC. (2002). Opinion of the scientific committee on food on the risks to human health of polycyclic aromatic hydrocarbons in food. (SCF/CS/CNTM/PAH/29 Final). Brussels, Belgium: European Commission, Scientific Committee on Food. [http://ec.europa.eu/food/fs/sc/scf/out153\\_en.pdf](http://ec.europa.eu/food/fs/sc/scf/out153_en.pdf)

- EPAM. (2024). Resources. Available online at <https://lifescience.opensource.epam.com/resources.html>
- Epler, JL; Larimer, FW; Rao, TK; Nix, CE; Ho, T. (1978). Energy-related pollutants in the environment: Use of short-term tests for mutagenicity in the isolation and identification of biohazards. Environ Health Perspect 27: 11-20. <http://dx.doi.org/10.1289/ehp.782711>
- Fent, KW; Toennis, C; Sammons, D; Robertson, S; Bertke, S; Calafat, AM; Pleil, JD; Wallace, MAG; Kerber, S; Smith, D; Horn, GP. (2019). Firefighters' absorption of PAHs and VOCs during controlled residential fires by job assignment and fire attack tactic. J Expo Sci Environ Epidemiol 30: 338-349. <http://dx.doi.org/10.1038/s41370-019-0145-2>
- Grantham, PH. (1963). Metabolism of fluorene-9-C14 in rats, guinea pigs, and rabbits. Biochemistry 2: 610-616. <http://dx.doi.org/10.1021/bi00903a038>
- Harris, DL; Hood, DB; Ramesh, A. (2008). Vehicle-dependent disposition kinetics of fluoranthene in Fisher-344 rats. Int J Environ Res Public Health 5: 41-48. <http://dx.doi.org/10.3390/ijerph5010041>
- IARC. (1983). Fluorene [IARC Monograph]. In Polynuclear aromatic compounds, part 1: Chemical, environmental and experimental data (pp. 365-371). Lyon, France. <https://publications.iarc.fr/50>
- IARC. (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures [IARC Monograph]. Lyon, France. <http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf>
- IPCS. (2020). INCHEM: Chemical safety information from intergovernmental organizations [Database]. Geneva, Switzerland: World Health Organization, Canadian Centre for Occupational Health and Safety. Inter-Organization Programme for the Sound Management of Chemicals. Retrieved from <http://www.inchem.org/>
- Kaden, DA; Hites, RA; Thilly, WG. (1979). Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to Salmonella typhimurium. Cancer Res 39: 4152-4159.
- Knuckles, ME; F, I; Ramesh, A. (2004). Acute and subchronic oral toxicity of fluoranthene in F-344 rats. Ecotoxicol Environ Saf 59: 102-108. [http://dx.doi.org/10.1016/S0147-6513\(03\)00110-6](http://dx.doi.org/10.1016/S0147-6513(03)00110-6)
- Koganti, A; Singh, R; Rozett, K; Modi, N; Goldstein, LS; Roy, TA; Zhang, FJ; Harvey, RG; Weyand, EH. (2000). 7H-benzo[c]fluorene: a major DNA adduct-forming component of coal tar. Carcinogenesis 21: 1601-1609. <http://dx.doi.org/10.1093/carcin/21.8.1601>
- LaVoie, EJ; Tulley-Freiler, L; Bedenko, V; Girach, Z; Hoffmann, D. (1981a). Comparative studies on the tumor initiating activity and metabolism of methylfluorenes and methylbenzofluorenes. In Polynuclear aromatic hydrocarbons: Chemistry and biological effects. Columbus, OH: Battelle Press.
- LaVoie, EJ; Tulley, L; Bedenko, V; Hoffmann, D. (1980). Mutagenicity, tumor initiating activity, and metabolism of tricyclic polynuclear aromatic hydrocarbons. In Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects. Columbus, OH: Battelle Press.
- LaVoie, EJ; Tulley, L; Bedenko, V; Hoffmann, D. (1981b). Mutagenicity of methylated fluorenes and benzofluorenes. Mutat Res 91: 167-176. [http://dx.doi.org/10.1016/0165-7992\(81\)90027-0](http://dx.doi.org/10.1016/0165-7992(81)90027-0)
- Li, Z; Romanoff, L; Bartell, S; Pittman, EN; Trinidad, DA; Mcclean, M; Webster, TF; Sjödin, A. (2012). Excretion profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. Chem Res Toxicol 25: 1452-1461. <http://dx.doi.org/10.1021/tx300108e>

- [Lipniak, M; Brandys, J. \(1993\)](#). Toxicokinetics of fluoranthene, pyrene and benz(a)anthracene in the rat. *Polycycl Aromat Compd* 3: 111-119.  
<http://dx.doi.org/10.1080/10406639308047863>
- [Liwanag, PM; Hudson, VW; Hazard, GF, Jr. \(2000\)](#). ChemIDplus: A web-based chemical search system. *NLM Tech Bull* March-April: e3.
- [Lizarraga, LE; Suter, GW; Lambert, JC; Patlewicz, G; Zhao, JQ; Dean, JL; Kaiser, P. \(2023\)](#). Advancing the science of a read-across framework for evaluation of data-poor chemicals incorporating systematic and new approach methods. *Regul Toxicol Pharmacol* 137: 105293. <http://dx.doi.org/10.1016/j.yrtph.2022.105293>
- [Mansouri, K; Grulke, CM; Judson, RS; Williams, AJ. \(2018\)](#). OPERA models for predicting physicochemical properties and environmental fate endpoints. *J Cheminform* 10: 10. <http://dx.doi.org/10.1186/s13321-018-0263-1>
- [Morris, HP; Velat, CA; Wagner, BP; Dahlgard, M; Ray, FE. \(1960\)](#). Studies of carcinogenicity in the rate of derivatives of aromatic amines related to N-2-fluorenylacetylamide. *J Natl Cancer Inst* 24: 149-180.
- [Mosedale, M; Watkins, PB. \(2020\)](#). Understanding Idiosyncratic Toxicity: Lessons Learned from Drug-Induced Liver Injury. *J Med Chem* 63: 6436-6461.  
<http://dx.doi.org/10.1021/acs.jmedchem.9b01297>
- [Mukhtar, H; Link, CM; Cherniack, E; Kushner, DM; Bickers, DR. \(1982\)](#). Effect of topical application of defined constituents of coal tar on skin and liver aryl hydrocarbon hydroxylase and 7-ethoxycoumarin deethylase activities. *Toxicol Appl Pharmacol* 64: 541-549. [http://dx.doi.org/10.1016/0041-008X\(82\)90251-4](http://dx.doi.org/10.1016/0041-008X(82)90251-4)
- [Neish, WJ. \(1948\)](#). Metabolism of fluorene in the rabbit. *Biochem J* 43: 533-537.  
<http://dx.doi.org/10.1042/bj0430533>
- [NIEHS. \(2023\)](#). NIEHS report on their in vivo repeat dose biological potency study of 2,3-benzofluorene (CASRN 243-17-4) in Sprague Dawley (HSD:Sprague Dawley® SD®) rats (gavage studies). (NIEHS Report 09). Research Triangle Park (NC).  
[https://www.ncbi.nlm.nih.gov/books/NBK589973/pdf/Bookshelf\\_NBK589973.pdf](https://www.ncbi.nlm.nih.gov/books/NBK589973/pdf/Bookshelf_NBK589973.pdf)
- [NIOSH. \(2018\)](#). NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Atlanta, GA. <http://www.cdc.gov/niosh/npg/npgdcas.html>
- [NLM. \(2009\)](#). PubChem substructure fingerprint.  
[https://ftp.ncbi.nlm.nih.gov/pubchem/specifications/pubchem\\_fingerprints.pdf](https://ftp.ncbi.nlm.nih.gov/pubchem/specifications/pubchem_fingerprints.pdf)
- [NLM. \(2020\)](#). ChemIDplus advanced. Available online at <https://chem.nlm.nih.gov/chemidplus/>
- [NLM. \(2022\)](#). PubChem: 2,3-Benzofluorene (CASRN 243-17-4) (Version 5/8/2014) [Database]. Bethesda, MD: National Institute of Health, U.S. National Library of Medicine. Retrieved from <https://pubchem.ncbi.nlm.nih.gov/compound/9201>
- [NTP. \(2018\)](#). NTP research report on national toxicology program approach to genomic dose-response modeling.  
[https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05\\_508.pdf?utm\\_source=direct&utm\\_medium=prod&utm\\_campaign=ntpgolinks&utm\\_term=rr05](https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=rr05)
- [NTP. \(2021\)](#). Report on carcinogens, fifteenth edition. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.  
<http://dx.doi.org/10.22427/NTP-OTHER-1003>
- [OCHEM. \(2022\)](#). ToxAlerts (Version 4.2.151) [Database]: Online Chemical Database with Modeling Environment. Helmholtz Zentrum Muenchen - Deutsches Forschungszentrum fuer Gesundheit und Umwelt. Retrieved from <https://ochem.eu/alerts/home.do>

- [OECD. \(2017\)](https://www.oecd.org/chemicalsafety/risk-assessment/TB4_Application_manual_F1.compressed.pdf). Application manual of OECD QSAR toolbox v.4. [https://www.oecd.org/chemicalsafety/risk-assessment/TB4\\_Application\\_manual\\_F1.compressed.pdf](https://www.oecd.org/chemicalsafety/risk-assessment/TB4_Application_manual_F1.compressed.pdf)
- [OECD. \(2020\)](http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm). The OECD QSAR toolbox [version 4.4] [Database]. Retrieved from <http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>
- [OSHA. \(2017a\)](https://www.govinfo.gov/content/pkg/CFR-2017-title29-vol6/pdf/CFR-2017-title29-vol6-sec1910-1000.pdf). Table Z-1: Limits for air contaminants. Occupational safety and health standards, subpart Z, toxic and hazardous substances. (29 CFR 1910.1000). Washington, DC. <https://www.govinfo.gov/content/pkg/CFR-2017-title29-vol6/pdf/CFR-2017-title29-vol6-sec1910-1000.pdf>
- [OSHA. \(2017b\)](https://www.govinfo.gov/content/pkg/CFR-2017-title29-vol7/pdf/CFR-2017-title29-vol7-sec1915-1000.pdf). Table Z: Shipyards. Occupational safety and health standards for shipyard employment. Subpart Z, toxic and hazardous substances. Air contaminants. (29 CFR 1915.1000). Washington, DC. <https://www.govinfo.gov/content/pkg/CFR-2017-title29-vol7/pdf/CFR-2017-title29-vol7-sec1915-1000.pdf>
- [OSHA. \(2020\)](https://www.govinfo.gov/content/pkg/CFR-2020-title29-vol8/pdf/CFR-2020-title29-vol8-sec1926-55.pdf). Table 1: Permissible exposure limits for airborne contaminants. Safety and health regulations for construction. Gases, vapors, fumes, dusts, and mists. (29 CFR 1926.55). Washington, DC. <https://www.govinfo.gov/content/pkg/CFR-2020-title29-vol8/pdf/CFR-2020-title29-vol8-sec1926-55.pdf>
- [Pastor-Belda, M; Campillo, N; Arroyo-Manzanares, N; Torres, C; Pérez-Cárceles, MD; Hernández-Córdoba, M; Viñas, P. \(2019\)](http://dx.doi.org/10.1021/acs.chemrestox.9b00213). Bioaccumulation of polycyclic aromatic hydrocarbons for forensic assessment using gas chromatography-mass spectrometry. *Chem Res Toxicol* 32: 1680-1688. <http://dx.doi.org/10.1021/acs.chemrestox.9b00213>
- [Patlewicz, G; Shah, I. \(2023\)](http://dx.doi.org/10.1016/j.comtox.2022.100258). Towards systematic read-across using Generalised Read-Across (GenRA). *Computational Toxicology* 25: 100258. <http://dx.doi.org/10.1016/j.comtox.2022.100258>
- [Peiffer, J; Cosnier, F; Grova, N; Nunge, H; Salquère, G; Decret, MJ; Cossec, B; Rychen, G; Appenzeller, BM; Schroeder, H. \(2013\)](http://dx.doi.org/10.1371/journal.pone.0071413). Neurobehavioral toxicity of a repeated exposure (14 days) to the airborne polycyclic aromatic hydrocarbon fluorene in adult Wistar male rats. *PLoS ONE* 8: e71413. <http://dx.doi.org/10.1371/journal.pone.0071413>
- [Peiffer, J; Grova, N; Hidalgo, S; Salquère, G; Rychen, G; Bisson, JF; Appenzeller, BM; Schroeder, H. \(2016\)](http://dx.doi.org/10.1016/j.neuro.2015.11.006). Behavioral toxicity and physiological changes from repeated exposure to fluorene administered orally or intraperitoneally to adult male Wistar rats: A dose-response study. *Neurotoxicology* 53: 321-333. <http://dx.doi.org/10.1016/j.neuro.2015.11.006>
- [Platt, KL; Aderhold, S; Kulpe, K; Fickler, M. \(2008\)](http://dx.doi.org/10.1016/j.mrgentox.2007.09.011). Unexpected DNA damage caused by polycyclic aromatic hydrocarbons under standard laboratory conditions. *Mutat Res* 650: 96-103. <http://dx.doi.org/10.1016/j.mrgentox.2007.09.011>
- [Rossbach, B; Wollschläger, D; Letzel, S; Gottschalk, W; Muttray, A. \(2020\)](http://dx.doi.org/10.1016/j.toxlet.2020.05.024). Internal exposure of firefighting instructors to polycyclic aromatic hydrocarbons (PAH) during live fire training. *Toxicol Lett* 331: 102-111. <http://dx.doi.org/10.1016/j.toxlet.2020.05.024>
- [Rossella, F; Campo, L; Pavanello, S; Kapka, L; Siwinska, E; Fustinoni, S. \(2009\)](http://dx.doi.org/10.1136/oem.2008.042796). Urinary polycyclic aromatic hydrocarbons and monohydroxy metabolites as biomarkers of exposure in coke oven workers. *Occup Environ Med* 66: 509-516. <http://dx.doi.org/10.1136/oem.2008.042796>
- [Salamone, MF; Heddle, JA; Katz, M. \(1979\)](http://dx.doi.org/10.1016/0160-4120(79)90092-8). The mutagenic activity of thirty polycyclic aromatic hydrocarbons (PAH) and oxides in urban airborne particulates. *Environ Int* 2: 37-43. [http://dx.doi.org/10.1016/0160-4120\(79\)90092-8](http://dx.doi.org/10.1016/0160-4120(79)90092-8)

- Shimada, T; Takenaka, S; Murayama, N; Yamazaki, H; Kim, J; Kim, D; Yoshimoto, FK; Guengerich, FP; Komori, M. (2015). Oxidation of Acenaphthene and Acenaphthylene by Human Cytochrome P450 Enzymes. *Chem Res Toxicol* 28: 268-278.  
<http://dx.doi.org/10.1021/tx500505y>
- SWCAA. (2019). SWCAA pollutant details: Benzo[b]fluorene. Available online at <http://www.swcleanair.org/epages/PollutantDetail.asp?id=3207> (accessed July 1, 2019).
- Thomas, RS; Paules, RS; Simeonov, A; Fitzpatrick, SC; Crofton, KM; Casey, WM; Mendrick, DL. (2018). The US Federal Tox21 Program: A strategic and operational plan for continued leadership. *ALTEX* 35: 163-168. <http://dx.doi.org/10.14573/altex.1803011>
- Toriba, A; Chetiyankornkul, T; Kizu, R; Hayakawa, K. (2003). Quantification of 2-hydroxyfluorene in human urine by column-switching high performance liquid chromatography with fluorescence detection. *Analyst* 128: 605-610.  
<http://dx.doi.org/10.1039/b212738e>
- TRL. (1989). 13-Week mouse oral subchronic toxicity study. (TRL Study No. #42-010). Rockville, MD: Dynamac Corporation.
- U.S. EPA. (1988). Recommendations for and documentation of biological values for use in risk assessment [EPA Report]. (EPA600687008). Cincinnati, OH.  
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855>
- U.S. EPA. (1990a). Integrated Risk Information System (IRIS) chemical assessment summary for acenaphthene (CASRN 83-32-9). Washington, DC: National Center for Environmental Assessment, Integrated Risk Information System.  
[https://cfpub.epa.gov/ncea/iris/iris\\_documents/documents/subst/0442\\_summary.pdf](https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0442_summary.pdf)
- U.S. EPA. (1990b). Integrated Risk Information System (IRIS) chemical assessment summary for fluoranthene (CASRN 206-44-0). Washington, DC: National Center for Environmental Assessment, Integrated Risk Information System.  
[https://cfpub.epa.gov/ncea/iris/iris\\_documents/documents/subst/0444\\_summary.pdf](https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0444_summary.pdf)
- U.S. EPA. (1990c). Integrated Risk Information System (IRIS) chemical assessment summary for fluorene (CASRN 86-73-7). Washington, DC: National Center for Environmental Assessment, Integrated Risk Information System.  
[https://cfpub.epa.gov/ncea/iris/iris\\_documents/documents/subst/0435\\_summary.pdf](https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0435_summary.pdf)
- U.S. EPA. (1997). Health effects assessment summary tables: FY 1997 update [EPA Report]. (EPA540R97036). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response.  
<http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000O0GZ.txt>
- U.S. EPA. (2003). Human health toxicity values in superfund risk assessments. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.  
<https://rais.ornl.gov/documents/hhmemo.pdf>
- U.S. EPA. (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. [https://www.epa.gov/sites/production/files/2013-09/documents/cancer\\_guidelines\\_final\\_3-25-05.pdf](https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf)
- U.S. EPA. (2011a). ChemACE users manual [EPA Report]. <https://www.epa.gov/tsca-screening-tools/chemical-assessment-clustering-engine-chemace-user-tutorial>
- U.S. EPA. (2011b). Chemical assessment clustering engine (ChemACE). Retrieved from <https://www.epa.gov/tsca-screening-tools/chemical-assessment-clustering-engine-chemace>

- [U.S. EPA. \(2011c\)](#). Provisional peer-reviewed toxicity values for acenaphthene (CASRN 83-32-9) [EPA Report]. Cincinnati, OH.  
<https://cfpub.epa.gov/ncea/pprtv/documents/Acenaphthene.pdf>
- [U.S. EPA. \(2012a\)](#). Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11 [Computer Program]. Washington, DC. Retrieved from <https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface>
- [U.S. EPA. \(2012b\)](#). Provisional peer-reviewed toxicity values for fluoranthene (CASRN 206-44-0) [EPA Report]. Cincinnati, OH.  
<https://cfpub.epa.gov/ncea/pprtv/documents/Fluoranthene.pdf>
- [U.S. EPA. \(2017\)](#). Integrated Risk Information System (IRIS) Chemical Assessment Summary for Benzo[a]pyrene (BaP), CASRN 50-32-8 [EPA Report]. Washington, DC: U.S. Environmental Protection Agency (USEPA), Office of Research and Development.  
[https://iris.epa.gov/ChemicalLanding/&substance\\_nmbr=136](https://iris.epa.gov/ChemicalLanding/&substance_nmbr=136)
- [U.S. EPA. \(2018a\)](#). 2018 Edition of the drinking water standards and health advisories tables [EPA Report]. (EPA822F18001). Washington, DC: U.S. Environmental Protection Agency, Office of Water. <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockkey=P100U7U8.txt>
- [U.S. EPA. \(2018b\)](#). ToxCast owner's manual- Guidance for exploring data. Available online at <https://www.epa.gov/sites/default/files/2018-04/documents/toxcastownermanual4252018.pdf>
- [U.S. EPA. \(2021\)](#). Draft provisional peer-reviewed toxicity values for fluorene (casrn 86-73-7) [EPA Report]. Cincinnati, OH.
- [U.S. EPA. \(2022a\)](#). Comptox chemicals dashboard: 2,3-Benzofluorene (Version 2.0.1). Retrieved from <https://comptox.epa.gov/dashboard/chemical/details/DTXSID1022477>
- [U.S. EPA. \(2022b\)](#). Generalized read-across (GenRA) manual [EPA Report].  
<https://www.epa.gov/comptox-tools/generalized-read-across-genra-manual>
- [U.S. EPA. \(2023\)](#). Provisional peer-reviewed toxicity values for fluorene. (EPA/690/R-23/006F). Washington, DC.  
[https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p\\_download\\_id=548072](https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=548072)
- [U.S. EPA. \(2024a\)](#). CompTox chemicals dashboard: 2,3-Benzofluorene (Version 2.4.1). Washington, DC. Retrieved from <https://comptox.epa.gov/dashboard>
- [U.S. EPA. \(2024b\)](#). Integrated risk information system (IRIS) database. Available online at <http://www.epa.gov/iris/>
- [U.S. EPA. \(2024c\)](#). The Toxic Substances Control Act's public inventory (TSCA inventory). Updated May 2024 [Database]. Washington, DC: U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. Retrieved from <https://www.epa.gov/tsca-inventory/how-access-tsca-inventory>
- [U.S. Pharmacopeia. \(2008\)](#). 2,3-Benzofluorene. In The National formulary. Rockville, MD: United States Pharmacopeial.
- [Walker, SA; Addai, AB; Mathis, M; Ramesh, A. \(2007\)](#). Effect of dietary fat on metabolism and DNA adduct formation after acute oral exposure of F-344 rats to fluoranthene. J Nutr Biochem 18: 236-249. <http://dx.doi.org/10.1016/j.jnutbio.2006.04.001>
- [Wang, NC; Zhao, QJ; Wesselkamper, SC; Lambert, JC; Petersen, D; Hess-Wilson, JK. \(2012\)](#). Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach. Regul Toxicol Pharmacol 63: 10-19.  
<http://dx.doi.org/10.1016/j.yrtph.2012.02.006>

- WHO. (1998). Selected non-heterocyclic polycyclic aromatic hydrocarbons [WHO EHC]. (Environmental Health Criteria 202). Geneva, Switzerland: International Programme on Chemical Safety. World Health Organization.  
<http://www.inchem.org/documents/ehc/ehc/ehc202.htm>
- Williams, AJ; Grulke, CM; Edwards, J; Mceachran, AD; Mansouri, K; Baker, NC; Patlewicz, G; Shah, I; Wambaugh, JF; Judson, RS; Richard, AM. (2017). The CompTox chemistry dashboard: A community data resource for environmental chemistry. J Cheminform 9: 61. <http://dx.doi.org/10.1186/s13321-017-0247-6>
- Williams, AJ; Lambert, JC; Thayer, K; Dorne, J. (2021). Sourcing data on chemical properties and hazard data from the US-EPA CompTox Chemicals Dashboard: A practical guide for human risk assessment [Review]. Environ Int 154: 106566.  
<http://dx.doi.org/10.1016/j.envint.2021.106566>
- Wilson, RH; DeEds, F; Cox, AJ. (1947). The carcinogenic activity of 2-acetaminofluorene. IV. Action of related compounds. Cancer Res 7: 453-458.
- Yordanova, D; Kuseva, C; Tankova, K; Pavlov, T; Chankov, G; Chapkanov, A; Gissi, A; Sobanski, T; Schultz, TW; Mekenyan, OG. (2019). Using metabolic information for categorization and read-across in the OECD QSAR Toolbox. Computational Toxicology 12: 100102. <http://dx.doi.org/10.1016/j.comtox.2019.100102>