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Provisional Peer-Reviewed Toxicity Values for

The Aliphatic Low Carbon Range Total Petroleum Hydrocarbon (TPH) Fraction (various CASRNs)

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

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Center for Public Health and Environmental Assessment
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

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

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR THE ALIPHATIC LOW CARBON RANGE TOTAL PETROLEUM HYDROCARBON (TPH) FRACTION

BACKGROUND

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

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

Currently available PPRTV assessments can be accessed on the U.S. EPA's PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing adverse human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA's eComments Chemical Safety web page (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 development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP)*. As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

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

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

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

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

QUESTIONS REGARDING PPRTVS

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

1. INTRODUCTION

This Provisional Peer-Reviewed Toxicity Value (PPRTV) assessment supports a fraction-based approach to risk assessment for mixtures of petroleum hydrocarbons (U.S. EPA, 2022, 2009c). In this approach, total petroleum hydrocarbon (TPH) fractions are defined based on expected transport in the environment and analytical methods used to quantify environmental contamination by TPH mixtures. TPH components were first classified into aliphatics and aromatics, and each of these two major fractions were further separated into low, medium, and high carbon range fractions. This PPRTV assessment describes the derivation of toxicity values for the aliphatic low carbon range fraction of TPH. The toxicity values described herein are used in the assessment of Complex Mixtures of Petroleum Hydrocarbons that is intended to replace current approaches used at TPH-contaminated sites (U.S. EPA, 2022, 2009c).

1.1. DEFINITION OF THE ALIPHATIC LOW CARBON RANGE FRACTION

The aliphatic low carbon range fraction includes aliphatic hydrocarbons with a carbon (C) range of C5−C8 (contains between 5 and 8 carbons, inclusive) and an equivalent carbon (EC) number[1] index range of EC5−EC8[2] that occur in, or co-occur with, petroleum contamination. The EC index is equivalent to the retention time of the compound on a boiling-point gas chromatography (GC) column (nonpolar capillary column), normalized to the *n*-alkanes (NJ DEP, 2010). EC numbers are the physical characteristic that underpin analytical separation of petroleum components. EC numbers are useful because they are more closely related to environmental mobility than carbon number. For instance, two chemicals with similar carbon numbers but different structures (e.g., aliphatic vs. aromatic) could partition differently into environmental media and, ultimately, have different environmental fates. Grouping based on EC numbers provides a consistent basis for logically placing petroleum hydrocarbon compounds into fractions because EC measures correlate with physicochemical properties such as water solubility, vapor pressure, Henry's law constant, and soil absorption coefficient (log K_{oc}). For example, cyclohexane, a C6 aliphatic compound, has an EC of 6.59 because its boiling point and GC retention time are approximately halfway between those of *n*-hexane (C6 [EC6]) and *n*-heptane (C7 [EC7]). Individual compounds in this fraction may include linear and branched alkanes, alkenes, and alicyclic compounds. The selection of relevant compounds and mixture is described in Section 2 and Appendix A.

1.2. OVERVIEW OF PHYSICOCHEMICAL PROPERTIES AND ENVIRONMENTAL FATE

The physicochemical properties for members of the aliphatic low carbon range fraction that have toxicity values are provided in Table 1. Section 2 details how the fraction members with toxicity values were identified. As Table 1 shows, the seven chemicals with toxicity values include representatives from the entire carbon range (C5−C8), and include compounds with linear, branched, cyclic, and unsaturated structures. All seven compounds are liquids at room temperature, with moderate water solubility and high vapor pressure. Some members of this fraction are expected to have high mobility in soil, indicating the potential for some members of this fraction to leach to groundwater. Measured biodegradation data for several members of the

[1]Based on an empirical relationship, the EC value can be estimated from a compound's boiling point (BP; °C) using the following equation: $EC = 4.12 + 0.02\ (BP) + 6.5 \times 10^{-5}\ (BP)^2$; see Gustafson et al. (1997).
[2]This range reflects EC values rounded to the nearest whole number. For instance, cyclohexene (EC = 6.74) is included in this fraction because its EC value rounds to 7.

aliphatic low carbon range fraction have been reported. In Japanese Ministry of International Trade and Industry (MITI) ready biodegradation tests, *n*-pentane, *n*-hexane, and *n*-heptane biodegraded an estimated 96, 100, and ~100%, respectively, within 4 weeks (J-CHECK, 2010a, b, c). However, limited biodegradation of methylcyclopentane occurred under aerobic or anaerobic conditions in pure culture studies, and slow biodegradation was reported for 2,4,4-trimethylpentene, cyclohexane, and cyclohexene under aerobic conditions. Volatilization is expected to be the predominant fate process for the fraction members in the environment, based on available Henry's law constant values. The aliphatic low carbon range hydrocarbons do not contain hydrolysable functional groups; therefore, the rate of hydrolysis is expected to be negligible for all members. In the atmosphere, photochemical degradation is expected to be slow for the saturated category members. The three unsaturated category members (cyclohexene and the two isomers of 2,4,4-trimethylpentene) are expected to have a moderate rate of photochemical degradation (NLM, 2021).

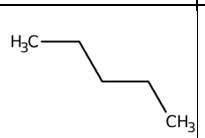
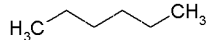
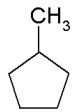
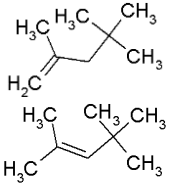
Table 1. Physicochemical Properties of Aliphatic Low Carbon Range Hydrocarbons with Toxicity Values[a]							
Chemical	***n*-Pentane**	***n*-Hexane**	**Methyl-cyclopentane**	**Cyclohexane**	**Cyclohexene**	***n*-Heptane**	**2,4,4-Trimethyl-pentene**
Structure							
CASRN	109-66-0	110-54-3	96-37-7	110-82-7	110-83-8	142-82-5	25167-70-8 (mixture of two isomers, 107-39-1 and 107-40-4)
Molecular formula	C_5H_{12}	C_6H_{14}	C_6H_{12}	C_6H_{12}	C_6H_{10}	C_7H_{16}	C_8H_{16}
EC number[b]	5.00	6.00	6.27	6.59	6.74	7.00	6.8
Molecular weight (g/mol)	72.151	86.178	84.162	84.162	82.146	100.205	112.22
Melting point (°C)	−130	−99.1	−90.9	6.43	−104[c]	−90.8	<−50[i]
Boiling point (°C)	36.0	68.6	71.6	80.7	83.3[c]	98.2	101.4−103.6[i]
Vapor pressure (mm Hg at 25°C)	514	151	137	96.9	89.0	46.0	43.4[i]
Henry's law constant (atm-m^3/mole at 25°C)	1.25	1.8[d]	0.36[e]	0.150	0.0455	1.8[f]	0.75−0.88 (estimated)[g]
Water solubility (mol/L)	5.93×10^{-4}	1.27×10^{-4}	5.01×10^{-4}	7.26×10^{-4}	2.58×10^{-3}	3.25×10^{-5}	1.8 mg/L at 20°C[i]
Log K_{ow}	3.39	3.90	3.37	3.41	2.86	4.66	5.0[i]
Log K_{oa}	1.96	2,40	3.11*	2.74	2.83	2.95	6.64–6.71 (estimated)[h]

Table 1. Physicochemical Properties of Aliphatic Low Carbon Range Hydrocarbons with Toxicity Values[a]

Chemical	*n*-Pentane	*n*-Hexane	Methyl-cyclopentane	Cyclohexane	Cyclohexene	*n*-Heptane	2,4,4-Trimethyl-pentene
Log K_{oc}	455*	1.29 × 103*	467*	531*	196*	5.69 × 103*	2.75[i]

[a]Data were gathered from the U.S. EPA CompTox Chemicals Dashboard unless otherwise specified; https://comptox.epa.gov/dashboard.
[b]EC number was developed by the TPHCWG and is proportional to the BP of a chemical. EC number is analogous to an *n*-paraffin retention time index and can be estimated using $EC = 4.12 + 0.02\ (BP) + 6.5 \times 10^{-5}\ (BP)^2$ (NIST, 2020; Edwards et al., 1997; Gustafson et al., 1997).
[c]OECD (2002).
[d]U.S. EPA (2012a); HLC calculated based on measured VP/WS with user-entered inputs for WS = 9.5 mg/L and VP = 153 mm Hg.
[e]U.S. EPA (2012a); HLC calculated based on measured VP/WS with user-entered inputs for WS = 42 mg/L and VP = 138 mm Hg.
[f]U.S. EPA (2012a); HLC calculated based on measured VP/WS with user-entered inputs for WS = 3.4 mg/L and VP = 46 mm Hg.
[g]U.S. EPA (2012a); EPI Suite™ estimate with no user-entered inputs (Bond method); representative SMILES C(=CC(C)(C)C)(C)C and C(=C)(CC(C)(C)C)C.
[h]Calculated from listed values for log K_{ow} and HLC.
[i]OECD (2008).
*Predicted value.

BP = boiling point; C = carbon; EC = equivalent carbon; EPI Suite™ = Estimation Programs Interface Suite; HLC = Henry's law constant; K_{ow} = octanol-water partition coefficient; K_{oa} = octanol-air partition coefficient; K_{oc} = soil adsorption coefficient; SMILES = simplified molecular input line entry system; TPHCWG = Total Petroleum Hydrocarbon Criteria Working Group; U.S. EPA = U.S. Environmental Protection Agency; VP = vapor pressure; WS = water solubility.

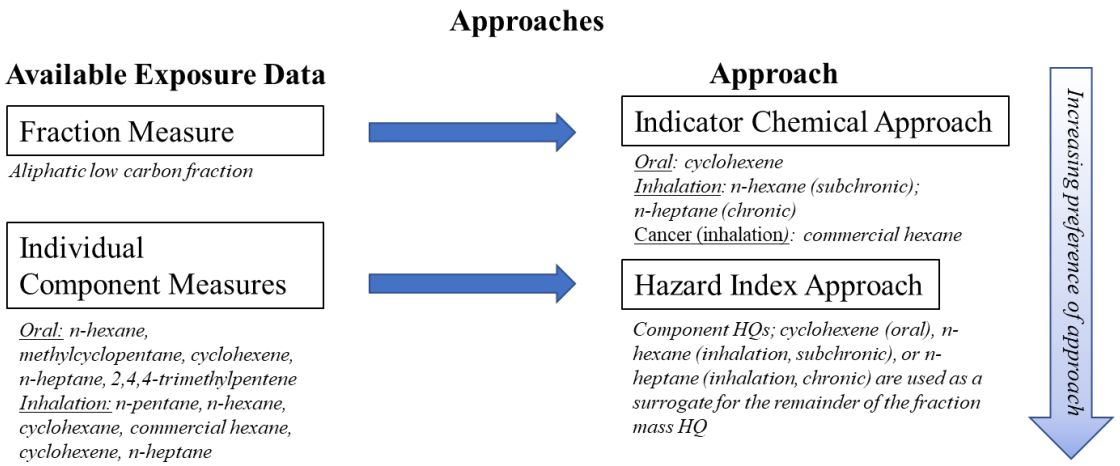
1.3. OVERVIEW OF MIXTURE ASSESSMENT METHODS

A number of different approaches have been developed and used to estimate risks and hazards posed by exposures to chemical mixtures encountered in the environment. Among the simplest of these approaches to implement is the indicator chemical approach (ATSDR, 2018). The indicator chemical approach estimates the risk or hazards of a mixture by evaluating the dose-response assessment developed for a component of the mixture to the exposure rate of the entire mixture. While it has greater uncertainty than the hazard index (HI) approach, the other approach that will be addressed in this PPRTV assessment, the indicator chemical approach, is used when there are only measures of the concentrations of this fraction (i.e., no information is available on the concentrations of individual chemicals in this fraction).

The U.S. Environmental Protection Agency (U.S. EPA) *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000, 1986) describes the following two broad categories of approaches for assessing human health risks and health hazards associated with environmental exposures to chemical mixtures: component methods and whole mixture methods. Component-based approaches, which involve analyzing the toxicity of a mixture's individual components, have more uncertainty and are recommended when appropriate toxicity data on a complex mixture of concern, or on a sufficiently similar mixture (discussed below), are unavailable (U.S. EPA, 2000, 1986). In this PPRTV assessment, a component approach, the HI approach, is described for assessing noncancer hazards posed by exposures to the aliphatic low carbon range fraction.

Chemical mixture assessments are conducted most appropriately with quantitative dose-response information resulting from comparable exposures to the mixture of concern. If the dose-response data are insufficient to develop a health reference value for the specific mixture of concern in the environment, the second option that the U.S. EPA *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000, 1986) recommended is a "sufficient similarity" approach that uses a health reference value from a characterized surrogate mixture to estimate the hazard or risk associated with exposures to the mixture of concern. This method requires chemistry and toxicity data on both the potential surrogate mixture and the mixture of concern (e.g., a key event that is related to the apical endpoint observed in an epidemiological study or whole animal study), and a health reference value (e.g., from an in vivo study) on the surrogate mixture. If the chemistry and toxicity data indicate that the mixtures are "sufficiently similar" to one another, then the health reference value for the surrogate mixture can be used as a proxy for the mixture of concern. No data were identified that were suitable to implement a whole mixture approach.

The choice of a chemical mixtures risk assessment method is driven by the available data. Starting with the method requiring the least information and then discussing the method requiring more information, the following subsections summarize the indicator chemical approach and the HI approach. Figure 1 summarizes the two approaches and the preference for using each approach.



Two approaches are available to estimate the noncancer hazards associated with exposure to the aliphatic low range fraction. Approach selection should be driven by the available exposure data. Increased analytical characterization of fraction components allows for more refined risk estimates with less inherent uncertainty. Approach preference is inversely correlated with approach uncertainty.

HQ = hazard quotient.

Figure 1. Provisional Peer-Reviewed Toxicity Approaches for the Aliphatic Low Carbon Range TPH Fraction Assessment

1.3.1. Indicator Chemical Approach

When the chemical composition of a mixture or a mixture fraction is not known, or toxicity measures are only available for a few individual chemicals in a mixture, the toxicity of an individual chemical can be used as an indicator for the toxicity of a mixture or a mixture fraction (ATSDR, 2018). ATSDR (2018) describes an indicator chemical as "a chemical . . . selected to represent the toxicity of a mixture because it is characteristic of other components in the mixture and has adequate dose-response data." Indicator chemical approaches are typically implemented to assess health risks in a health-protective manner; the chemical chosen as an indicator is among the best characterized toxicologically and likely among the most potent components of the mixture. The indicator chemical needs to have adequate dose-response data to indicate hazard potential or a dose-response relationship for noncancer outcomes, depending on the purpose of the assessment. The health risk value of the indicator chemical is integrated with exposure estimates for the mixture or mixture fraction to estimate health hazards associated with the fraction (i.e., calculate fraction-specific HI for a specific exposure pathway or a fraction-specific cancer risk estimate for a specific exposure pathway). This approach does not scale for potency of individual constituents; instead, it assumes that toxicity of all measured members of the fraction can be adequately estimated, given the purpose of the risk assessment, by the indicator chemical.

1.3.2. Hazard Index Approach

The HI approach combines estimated population exposures with toxicity information to characterize the potential for toxicological effects. The HI is not a risk estimate, in that it is not expressed as a probability, nor is it an estimate of a toxicity measure (e.g., percentage decrement in enzyme activity). Instead, the HI is an indicator of potential hazard. In the HI approach, a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a health hazard

reference value (RfV) for each mixture component chemical (*i*) (U.S. EPA, 1986). These HQs are summed to yield the HI for the mixture. In health risk assessments, the U.S. EPA's preferred RfVs are the reference dose (RfD) for the oral exposure route, and the reference concentration (RfC) for the inhalation exposure route.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

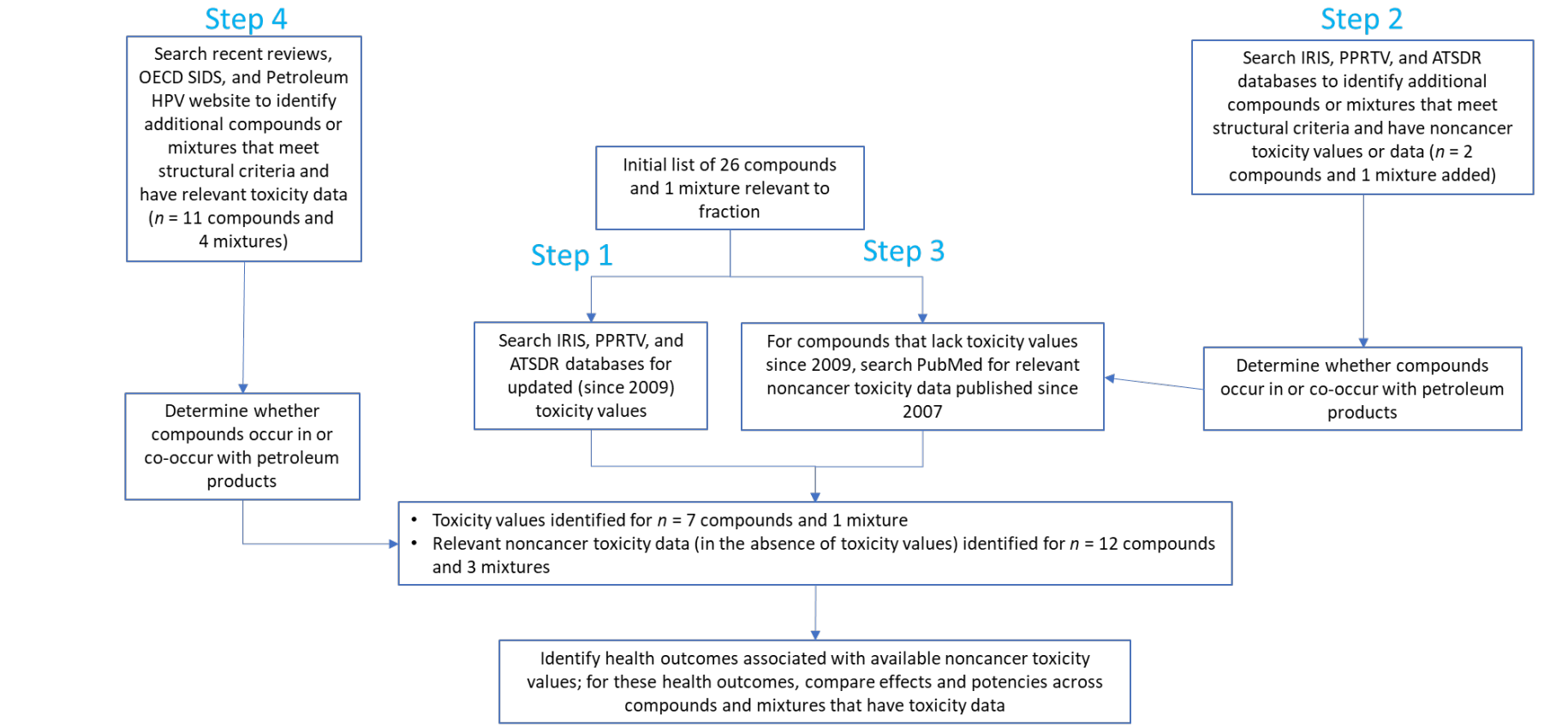
The HI is based on dose addition (U.S. EPA, 2000; Svendsgaard and Hertzberg, 1994); the hazard is evaluated as the potency-weighted sum of the component exposures. The HI is dimensionless, so *E* and the RfV must be in the same units.

2. SUMMARY OF TOXICITY AND DOSE-RESPONSE ASSESSMENT APPROACH

Toxicity and dose-response assessment for the aliphatic low carbon range fraction depends upon selection of an indicator chemical from among the component chemicals and mixtures with existing toxicity values and entailed the four basic steps outlined here and described in more detail below. Mixtures and compounds that met structural criteria (see definition of the fraction, above) and had available toxicity values from designated sources were identified.

In Step 1 and Step 2 of the assessment, literature searches were performed for the mixtures and compounds with toxicity values and for other mixtures and compounds that are relevant to the fraction. These literature searches were conducted in February 2018 and updated most recently in August 2021, and were date-limited to identify assessments published after 2009. The searches were designed for two purposes: first, to determine whether new information suggested that toxicity values for mixtures or compounds relevant to the fraction should be updated from those identified in the U.S. EPA (2009c) PPRTV assessment for complex mixtures of aliphatic and aromatic hydrocarbons; and second, to determine whether new noncancer toxicity values or data on other mixtures or compounds meeting the structural criteria of the fraction might alter the overall understanding of the toxicity of the fraction. The third step in the assessment involved searching PubMed for new noncancer toxicity data on compounds and mixtures lacking either Integrated Risk Information System (IRIS) oral or inhalation toxicity values. These literature searches were conducted in February 2018 and were date-limited to studies published from 2007 forward, in order to capture studies that were published since the searches performed in U.S. EPA (2009c). The fourth step in the assessment involved searching of recent comprehensive reviews on the toxicity of petroleum components or classes of compounds relevant to the fraction, as well as Organisation for Economic Co-operation and Development (OECD) Screening Information Data Set (SIDS) assessments[3] and the Petroleum High Production Volume (HPV) Testing Group website, to identify other mixtures or compounds within this carbon range with existing toxicity data that may inform hazard identification for the fraction. Toxicity data criteria included human studies of any duration by oral, inhalation, and dermal exposure, and animal studies of oral or inhalation exposure lasting at least 28 days (or any duration of gestational exposure). Mixture toxicity data were considered relevant only if the mixture composition under study was quantitatively defined to enable assessment of relevance to the fraction. Figure 2 shows a schematic depiction of the process, and further detail is provided below.

[3]The OECD Existing Chemicals Database (https://hpvchemicals.oecd.org) was reviewed for relevant categories, and dossiers for the following categories were screened: alpha-olefins, higher olefins, C5 aliphatic hydrocarbon solvents, C7−C9 aliphatic hydrocarbon solvents, and methyl- and ethylcyclohexane. A category of C6 aliphatic hydrocarbon solvents is under assessment, but dossiers and hazard characterization for this category were not available at the time of the search (October 2018).



ATSDR = Agency for Toxic Substances and Disease Registry; C = carbon; EC = equivalent carbon; HPV = High Production Volume: IRIS = Integrated Risk Information System; OECD = Organisation for Economic Co-operation and Development; PPRTV = Provisional Peer-Reviewed Toxicity Value; RfC = reference concentration; RfD = reference dose; SIDS = Screening Information Data Set.

Figure 2. Selection of Compounds and Mixtures for Aliphatic Low Carbon Range Fraction Hazard Identification and Dose-Response Assessment

2.1. IDENTIFICATION OF RELEVANT MIXTURES AND COMPOUNDS WITH TOXICITY VALUES

The first step (see Figure 2) in assessment of the toxicity for the aliphatic low carbon range fraction was to identify constituents of the fraction that have existing toxicity values from any of the sources considered for the U.S. EPA (2009c) PPRTV assessment for complex mixtures of aliphatic and aromatic hydrocarbons (these included IRIS, PPRTVs, Agency for Toxic Substances and Disease Registry [ATSDR] Minimal Risk Levels [MRLs], Massachusetts Department of Environmental Protection [MassDEP], Total Petroleum Hydrocarbon Criteria Working Group [TPHCWG], and Health Effects Assessment Summary Tables [HEAST]). Of these sources, only IRIS, PPRTVs, and ATSDR MRLs have been updated since 2009, so only these sources were consulted for toxicity values. Based on the U.S. EPA's previous assessments and assessment activities as well as those relevant chemicals reviewed by the MassDEP (MassDEP, 2003) or TPHCWG (Edwards et al., 1997), the U.S. EPA compiled an initial list of 26 chemicals and 1 mixture (commercial hexane) considered relevant to the fraction [see full list in Appendix A and description of approach and results in Wang et al. (2012)]. Published toxicity values were identified from the IRIS, PPRTV, and ATSDR MRL databases. At least one subchronic or chronic oral or inhalation reference value or cancer toxicity value was available for six chemicals or mixtures: *n*-pentane, *n*-hexane, methylcyclopentane, cyclohexane, commercial hexane, and *n*-heptane. Comprehensive toxicity assessments for 2,2,4-trimethylpentane (U.S. EPA, 2007) and methylcyclohexane (U.S. EPA, 2013) were available, but did not result in the derivation of noncancer or cancer toxicity values due to inadequate data.

In the second step (see Figure 2), all existing chemicals in the IRIS, PPRTV, and ATSDR MRL databases were searched to determine whether any other compounds or mixtures (not on the initial list) meeting the structural criteria for inclusion (C5−C8 and EC5−EC8 aliphatics) were available. Searches of the IRIS and ATSDR databases did not identify any additional compounds, but review of the PPRTV database identified two additional compounds that had toxicity values and met structural criteria for inclusion: 2,4,4-trimethylpentene and cyclohexene. To evaluate whether these compounds occur in, or co-occur with, petroleum contamination, the compounds were compared against the list of petroleum mixture constituents in the TPHCWG's (1998) *Selection of Representative TPH Fractions Based on Fate and Transport Considerations* (Volume 3). In that compendium, cyclohexene was identified as a constituent of gasoline (Gustafson et al., 1997). In contrast, 2,4,4-trimethylpentene was not identified as a constituent of petroleum mixtures (Gustafson et al., 1997). However, other information indicates that 2,4,4-trimethylpentene may be added to gasoline as a fuel additive, antioxidant, or octane booster (Rankovic et al., 2015; EU, 2008; Calamur et al., 2003; Gomez and Basil, 1998). Thus, while not a natural component of petroleum, 2,4,4-trimethylpentene may co-occur with petroleum contaminants and was therefore considered relevant to the fraction. Including cyclohexene and 2,4,4-trimethylpentene brought the number of compounds or mixtures with toxicity values to eight (seven chemicals and the commercial hexane mixture). Table 2 shows the toxicity values available for these compounds.

Table 2. Summary of Available Toxicity Values for Mixtures and Constituents of Aliphatic Low Carbon Range Fraction (C5−C8, EC5−EC8)[a]

CASRN	Name	C	EC	Oral Reference Dose (mg/kg-d)		Inhalation Reference Concentration (mg/m^3)		Inhalation Unit Risk $(mg/m^3)^{-1}$	Oral Slope Factor $(mg/kg\text{-}d)^{-1}$
				Subchronic	Chronic	Subchronic	Chronic		
109-66-0	*n*-Pentane	5	5	–	–	10	1	–	–
110-54-3	*n*-Hexane	6	6	0.3	–	2	0.7 (IRIS)	–	–
96-37-7	Methylcyclopentane	6	6.27	0.4	–	–	–	–	–
110-82-7	Cyclohexane	6	6.59	–	–	18	6 (IRIS)	–	–
Various	Commercial hexane	6	NA	–	–	27	0.6	*0.0002*	–
110-83-8	Cyclohexene	6	6.74	0.05	0.005	–	*1*	–	–
142-82-5	*n*-Heptane	7	7	*0.003*	*0.0003*	4	0.4	–	–
25167-70-8	2,4,4-Trimethylpentene	8	6.8	*0.1*	*0.01*	–	–	–	–

[a]Except where indicated, all toxicity values are from PPRTVs. Where more than one source reported a toxicity value, the values were selected based on the following hierarchy: IRIS > PPRTV > ATSDR > HEAST > MassDEP > TPHCWG.
[b]Values in *italics* are screening provisional values obtained from an existing PPRTV assessment. Screening values are not assigned confidence statements; however, confidence in these values is presumed to be low. Screening provisional values are derived when the available data do not meet the requirements for deriving a provisional toxicity value.

ATSDR = Agency for Toxic Substances and Disease Registry; C = carbon; EC = equivalent carbon; HEAST = Health Effects Assessment Summary Tables; IRIS = Integrated Risk Information System; MassDEP = Massachusetts Department of Environmental Protection; NA = not applicable; PPRTV = Provisional Peer-Reviewed Toxicity Value; TPHCWG = Total Petroleum Hydrocarbon Criteria Working Group.

2.2. IDENTIFICATION OF OTHER RELEVANT TOXICITY DATA

Among the 28 compounds and 1 mixture identified (26 chemicals and 1 mixture on the initial list determined relevant, plus 2,4,4-trimethylpentene and cyclohexene identified through additional searches), there were 7 compounds and 1 mixture with toxicity values. Of the 29 fraction members, 2 (*n*-heptane and 2,4,4-trimethylpentene) had toxicity assessments published within the last 5 years (2016 and 2015, respectively). In Step 3 (see Figure 2), literature searches were conducted in PubMed to identify any new studies that could fill data gaps for the remaining 27 fraction members. The literature searches were conducted in February 2018, were updated in August 2021, and were date-limited to studies published from 2007 forward, in order to capture studies that were published since the searches performed for the 2009 PPRTV assessment for complex TPH mixtures. A summary of the literature search strategy is provided in Appendix A. As detailed in the appendix, studies considered relevant to hazard identification included animal studies using inhalation or oral exposure routes, in which exposures continued for at least 28 days (or any duration of gestational exposure), at least one health outcome was assessed, and an untreated or vehicle control group was included. Human studies of any duration in which exposure was known or presumed to be through oral, inhalation, or dermal routes and at least one health outcome was assessed were also considered relevant.

Results of the updated literature search are as follows. Ten human studies of occupational exposure to *n*-hexane were identified (Jiménez-Garza et al., 2018; Beckman et al., 2016; Hassani et al., 2014; Jia et al., 2014; Wang et al., 2014; Neghab et al., 2012; Kutlu et al., 2009; Elci et al., 2007; Prieto-Castelló et al., 2007; Puri et al., 2007). Acute human studies evaluated effects of cyclohexane following inhalation exposure (Lammers et al., 2009) or *n*-octane after dermal exposure (Schliemann et al., 2013) in volunteers. Animal studies of oral exposure include 8-week (Wang et al., 2017) and 24-week (Yin et al., 2014) studies of *n*-hexane in rats. Animal studies of inhalation exposure included a 5-week study of *n*-hexane in mice (Liu et al., 2012), a 30-day study of cyclohexane in mice (Campos-Ordonez et al., 2015), a 4-week study of 3-methylpentane in rats (Chung et al., 2016), 13-week studies of *n*-pentane (Kim et al., 2012) and *n*-octane (Sung et al., 2010) in rats, and two developmental studies of *n*-hexane in rats (Li et al., 2015; Li et al., 2014).

In Step 4 (see Figure 2), to determine whether additional relevant compounds or mixtures had been tested for repeat-dose and/or reproductive/developmental toxicity since 2007, recent reviews of petroleum toxicity (Mckee et al., 2015; Baxter, 2012; Carreón and Herrick, 2012; Saavedra et al., 2007), OECD SIDS dossiers (OECD, 2010, 2004, 2000), and the Petroleum High Production Volume (HPV) Testing Group website were searched. Mixtures considered relevant to the fraction met the following criteria:

1. at least 90% of the mixture consisted of identified compounds within the C5−C8 and/or EC5−EC8 ranges.
2. 99% of the mixture consisted of aliphatic compounds (≤1% aromatic).
3. the mixture has been tested in animals in at least one repeat-dose (≥28 days) or reproductive/developmental toxicity study using inhalation or oral exposure routes and included an untreated or vehicle control group.
4. human mixture studies of any duration by oral, inhalation, and dermal exposure, and animal studies of oral or inhalation exposure lasting at least 28 days (or any duration of gestational exposure).

None of the mixtures described on the Petroleum HPV Testing Group website met these criteria. In addition to commercial hexane (already included), Mckee et al. (2015) described two other mixtures that met these criteria: a C6 mixture without *n*-hexane, tested in an inhalation study by Egan et al. (1980); and practical-grade hexane (≤40% *n*-hexane and not included in the PPRTV assessment for commercial hexane), tested in an oral study by Krasavage et al. (1980). In addition, OECD (2004) described studies of a C5−C7 alkene mixture that met these criteria. Thus, toxicity data for four mixtures were considered potentially relevant to the assessment of the aliphatic low carbon range fraction. Available information on the compositions of these mixtures is provided in Appendix B.

In addition to the two compounds with IRIS or PPRTV assessments that did not yield toxicity value derivations (2,2,4-trimethylpentane and methylcyclohexane), searches of the reviews and OECD assessments identified toxicity data for 10 additional aliphatic low carbon range compounds.[4] Human and animal studies that met criteria outlined above were reviewed to support selection of surrogates for the aliphatic low carbon range fraction toxicity values.

2.3. METHODS FOR INDICATOR CHEMICAL SELECTION

Only compounds or mixtures with at least one U.S. EPA (IRIS or PPRTV) or ATSDR toxicity value (see Table 2) were considered for use as potential indicator chemicals (or indicator mixtures) for derivation of the fraction-specific toxicity values, although toxicity data for other compounds were used for hazard identification and to assess consistency in effects and potency across the components of the fraction. The method for selecting indicator chemicals was adapted from the 2009 complex TPH mixtures document (U.S. EPA, 2009c). First, mixtures consisting of fraction component chemicals were preferred over individual compounds, provided that the mixture study was adequate and the mixture exhibited in vivo toxic effects similar to those exhibited by the individual fraction components. If suitable mixture data were lacking, a representative compound exhibiting in vivo toxic effects and potency similar to those exhibited by other compounds in the fraction was chosen. In the event that components of the fraction varied widely in toxic effects or potency, the toxicity value for the most potent component (i.e., component with lowest toxicity value) was selected as an indicator chemical for the fraction. Finally, if toxicity values were available for many or most of the individual compounds in a fraction, and these compounds are typically monitored at sites of hydrocarbon contamination, then a component approach would be considered.

2.4. DEVELOPMENT OF EXPOSURE-RESPONSE ARRAYS

In order to assess consistency in effects and potency across the components of the fraction, experimental data from compound-specific IRIS and PPRTV documents and primary data sources (identified from literature searches) were used to create exposure-response arrays provided in Appendix C. Data were extracted only from reliable studies (e.g., studies that provided dose-response data enabling the identification of no-observed-adverse-effect levels [NOAELs] and lowest-observed-adverse-effect levels [LOAELs]). Target-organ-specific NOAELs and LOAELs were determined using the following methodology.

[4]The 10 additional aliphatic low carbon range compounds identified in searches of the reviews and OECD assessments are cyclopentane, 2,3-dimethylbutane, 2-methylpentane, 3-methylpentane, 1-hexene, 2-methyl-2-pentene, 2-methylhexane, 2,3-dimethylpentane, ethylcyclohexane, and 1-octene.

5. Whenever possible, NOAELs and LOAELs were identified from existing IRIS or PPRTV assessments. For chemicals in which both types of assessments were available, preference was given to IRIS (in accordance with U.S. EPA Office of Superfund Remediation and Technology Innovation [OSRTI] hierarchy of human health toxicity values for Superfund assessments). In general, these assessments explicitly identified NOAEL and LOAEL values only for the most sensitive target of toxicity, so characterization of additional adverse effect levels allowed for a comprehensive comparison of toxic effects across additional endpoints and tissues.

6. All other target-organ-specific effect levels (i.e., for targets other than the most sensitive target identified in IRIS or PPRTV assessments, and all targets evaluated in newly identified studies) were determined using professional judgment, taking into consideration factors such as statistical significance (at a p-value < 0.05), biological significance (e.g., a greater than or equal to 10% increase in liver weight), magnitude and direction of change, and study quality. In the case of chemicals with existing IRIS or PPRTV assessments, NOAELs and LOAELs could often be identified from existing study summaries.

Dose-response data were presented in exposure-response arrays by health outcome and exposure route (see Appendix C). From left to right, compounds exhibiting an effect are shown before those not exhibiting an effect, to facilitate identification of patterns. Within the group exhibiting an effect, compounds are ordered from lowest LOAEL to highest. For compounds that do not exhibit an effect, NOAELs in the arrays are ordered by EC number (low to high from left to right), with mixtures shown last. Both administered doses and exposure concentrations reported in the arrays and in the text reflect time-weighted average (TWA) exposures to facilitate comparisons across studies and compounds. Consistency across the fraction was evaluated by assessing if comparable outcomes were observed for members of the fraction, and if these effects were observed at similar dose levels.

3. REVIEW OF POTENTIALLY RELEVANT DATA

3.1. NONCANCER EVIDENCE

Compound-specific IRIS and PPRTV documents, supplemented by the literature search findings and recent reviews of petroleum toxicity and OECD SIDS dossiers (described above), were reviewed to evaluate the available noncancer data for the aliphatic low carbon range fraction compounds. Critical effects identified with existing toxicity values include peripheral neuropathy, decreased hearing sensitivity, hepatic toxicity, decreased body weight, nasal lesions, and developmental toxicity (decreased pup weights). Appendix C summarizes the evidence provided by human and experimental animal studies of noncancer health outcomes. Table 3 presents an overview of the human and animal data available to evaluate these primary toxicological endpoints for the fraction (neurological, hepatic, body weight, gastrointestinal [GI], respiratory, and developmental). As Table 3 shows, both oral and inhalation data available to assess consistency in effects across members of the fraction are discrepant across endpoints. Body weight was the only endpoint consistently evaluated across most components and mixtures. Another important data limitation not captured in Table 3 is the lack of chronic systemic toxicity information for all but three members of the fraction. Only cyclohexene, methylcyclohexane, and commercial hexane have been tested in comprehensive systemic toxicity studies in animals exposed for at least 1 year, all by the inhalation route. Furthermore, most of the oral toxicity studies observed in this database are <13 weeks in duration, and few examined comprehensive endpoints, as most were focused on selected neurotoxicity or alpha 2u-globulin (α2u-g)-mediated renal effects in male rats. The latter effects, which if established as acting through this mechanism, are not considered to be relevant to humans (U.S. EPA, 1991), and are not discussed further in this assessment. In addition, few compounds have been tested for systemic toxicity in animals exposed orally or after chronic exposure by inhalation.

Table 3. Overview of Noncancer Human and Animal Data Availability[a, b]

CASRN	Name	C	EC	Neurological	Hepatic	Body Weight	Gastrointestinal	Respiratory	Developmental
109-66-0	***n*-Pentane**	5	5	H, I	I	O, I	O, I	I	O, I
287-92-3	Cyclopentane	5	5.66	I	I	I	I	I	
79-29-8	2,3-Dimethylbutane	6	5.68			O	O		
107-83-5	2-Methylpentane	6	5.72	O, I		O, I	O		
96-14-0	3-Methylpentane	6	5.85	O, I	I	O, I	I	I	
592-41-6	1-Hexene	6	5.9	O, I	O, I	O, I	O	I	O
110-54-3	***n*-Hexane**	6	6	H, O, I	I	O, I	O, I	I	O, I
625-27-4	2-Methyl-2-pentene	6	6.07			O	O		
96-37-7	**Methylcyclopentane**	6	6.27	O, I	I	O, I	O, I	I	
110-82-7	**Cyclohexane**	6	6.59	H, I	H, I	I		I	I
591-76-4	2-Methylhexane	7	6.68			O	O		
565-59-3	2,3-Dimethylpentane	7	6.69			O	O		
110-83-8	**Cyclohexene**	6	6.74		O, I	O, I			O
25167-70-8	**2,4,4-Trimethylpentene**	8	6.8	O	O	O	O	O	O
540-84-1	2,2,4-Trimethylpentane	8	6.98		I	O, I	O		
142-82-5	***n*-Heptane**	7	7	H, I	H	I			
108-87-2	Methylcyclohexane	7	7.22		H, O	I			O
111-66-0	1-Octene	8	7.89			O			
1678-91-7	Ethylcyclohexane	8	7.89		O				
111-65-9	*n*-Octane	8	8			I	I	I	

Table 3. Overview of Noncancer Human and Animal Data Availability[a, b]

CASRN	Name	C	EC	Neurological	Hepatic	Body Weight	Gastrointestinal	Respiratory	Developmental
NA	Practical-grade hexane, 40% *n*-hexane	5−6	NA	O					
NA	C6 Alkane mixture without *n*-hexane	6	NA	I		I			
NA	**Commercial hexane**	6	NA	I	I	I	I	I	I
68526-52-3	C5−C7 Alkene mixture	6−7	NA	O	O	O	O	O	O

[a]Includes human and animal studies meeting inclusion criteria. **Bolded** compounds and mixtures have at least one oral or inhalation toxicity value available (see Table 2).
[b]Compounds are arranged by increasing EC number.

C = carbon; EC = equivalent carbon; H = human data; I = animal inhalation studies; NA = not applicable; O = animal oral studies.

Based on the review of the available data, there is evidence that oral or inhalation exposures to C6 alkanes and *n*-heptane can induce neurological effects; most of the other compounds in the fraction have not been explicitly tested for sensitive measures of peripheral neuropathy or hearing. Thus, consistency in effects and potency across members of the fraction cannot be adequately assessed for neurological endpoints. Information among a wider range of compounds suggests that aliphatic low carbon range fraction compounds and mixtures can induce hepatic effects in the form of increased liver weight, and that potencies are generally comparable in subchronic inhalation studies (LOAELs range from 2,763.3 to 6,294 mg/m^3 in rats and mice), but not in subchronic oral studies (LOAELs range from 50 to 1,000 mg/kg-day in rats). However, the small number of compounds with information on liver toxicity after oral exposure, lack of chronic oral studies, and availability of chronic inhalation studies for only two fraction members limit conclusions that can be drawn for hepatic effects. Data on body-weight effects after oral and inhalation exposure to a variety of aliphatic low carbon range fraction compounds and mixtures indicate that members of the fraction can be expected to induce body-weight reductions at doses ≥400 mg/kg-day or duration-adjusted concentrations ≥1,000 mg/m^3.

The available data are not considered adequate to evaluate consistency in effects or potencies across fraction members for GI endpoints. Respiratory effects have also not been consistently shown to be associated with oral or inhalation exposure to members of the aliphatic low carbon range fraction. Finally, too few members of the fraction have received rigorous testing for developmental effects to assess consistency in effects or potencies for these endpoints.

In summary, there is evidence to suggest consistency in body-weight changes and hepatic effects of some aliphatic low carbon range fraction members. However, there is not enough information to assess consistency across the entire fraction. Data limitations (most notably, a lack of testing for sensitive measures of peripheral neuropathy or hearing) preclude an assessment of consistency in neurological effects and potencies for fraction members. There is little evidence to indicate respiratory tract effects for compounds other than commercial hexane and *n*-hexane. The available data are not adequate to provide confidence in an assessment of the consistency in effects for GI tract and developmental toxicity endpoints. Finally, new studies suggest that *n*-hexane may elicit adverse effects on the developing female reproductive tract, but no other information is available to support this finding or to assess this endpoint for other compounds.

3.2. CANCER EVIDENCE

3.2.1. Human Studies

No relationship was found between exposure to *n*-hexane and the occurrence of intracranial tumors in petrochemical plant workers (U.S. EPA, 2005). No other studies of carcinogenicity in humans exposed to aliphatic low carbon range compounds have been identified.

3.2.2. Animal Studies—Oral

No carcinogenicity studies of animals exposed orally to compounds or mixtures in the aliphatic low carbon range fraction have been identified.

3.2.3. Animal Studies—Inhalation

Statistically significant increases in the incidences of liver tumors (adenomas and carcinomas) and pituitary tumors (adenomas and adenocarcinomas) were observed in female mice exposed to commercial hexane at duration-adjusted concentrations ≥366 mg/m^3 (U.S. EPA, 2009b). There were no increases in tumor incidences among male mice or rats of either sex. The findings in female mice were the basis for characterizing the weight of evidence (WOE) as "*Suggestive Evidence of Carcinogenic Potential*" for commercial hexane (U.S. EPA, 2009b). A screening provisional inhalation unit risk (p-IUR) of 2×10^{-4} per mg/m^3 was derived based on benchmark dose (BMD) modeling of the combined pituitary adenomas and adenocarcinomas (U.S. EPA, 2009b).

In 2-year carcinogenicity studies of rats and mice exposed to cyclohexene by inhalation, there was a statistically significant dose-related trend for increased incidence of combined hepatocellular adenomas and carcinomas at the highest dose in male rats, but not in female rats or in mice of either sex (U.S. EPA, 2012b). However, these data were not considered adequate to assess the carcinogenic potential of cyclohexene given the small increase in incidence and lack of dose-response relationship (U.S. EPA, 2012b).

In rats exposed to methylcyclohexane via inhalation (268 or 1,339 mg/m^3) for 1 year, a statistically significant increase in testicular tumors was observed at the low exposure level (5/10 compared with 0/11 in controls) but not at the high exposure level (2/11) (U.S. EPA, 2013). No information on tumor histology was reported. Given the lack of dose-response relationship, small group sizes, and abbreviated duration of exposure, U.S. EPA (2013) did not consider these data adequate for assessment of carcinogenic potential for methylcyclohexane.

In a study examining the potential for 2,2,4-trimethylpentane to promote renal cell tumor formation, rats were exposed to 234 mg/m^3 by inhalation for up to 61 weeks (U.S. EPA, 2007). Study groups included an initiation-only group (pre-exposed to *N*-ethyl-*N*-hydroxyethyl-nitrosamine in drinking water for 2 weeks), a promoter-only group (2,2,4-trimethylpentane only, 6 hours/day and 5 days/week), and an initiation-promotion group. No renal cell tumors were observed in rats exposed only to 2,2,4-trimethylpentane, and the incidence in the initiation-promotion group was not significantly different from the incidence in the initiation-only group (U.S. EPA, 2007). These data were not considered adequate for the assessment of 2,2,4-trimethylpentane carcinogenicity (U.S. EPA, 2007).

3.2.4. Cancer Evidence Summary

Few data with which to assess the carcinogenic potential of compounds and mixtures in the aliphatic low carbon range fraction are available. No human or animal studies examining carcinogenicity were located for any compound or mixture other than commercial hexane, *n*-hexane, cyclohexene, methylcyclohexane, and 2,2,4-trimethylpentane. In addition, only the inhalation data for commercial hexane were considered adequate to assess carcinogenic potential, resulting in a WOE descriptor of "*Suggestive Evidence of Carcinogenic Potential*."

4. TOXICOKINETIC CONSIDERATIONS

The available toxicokinetic information on compounds and mixtures in the aliphatic low carbon range fraction has been reviewed extensively (Mckee et al., 2015; Baxter, 2012; Carreón and Herrick, 2012). In general, these compounds and mixtures are absorbed by both inhalation and oral routes and are distributed widely in the body with some preference for adipose tissue and kidney. Metabolism of alkane compounds is predominantly via hydroxylation to alcohols, which are further hydroxylated or dehydrogenated to hydroxy and/or ketone derivatives. Alkenes are metabolized via epoxide intermediates to glycols. Elimination of aliphatic low carbon range fractions occurs via exhaled air (as carbon dioxide [CO_2]) and urine.

Oral absorption of compounds in the aliphatic low carbon range fraction is high. Estimates of the absorbed fraction of orally-administered doses are 86% for 2,2,4-trimethylpentane (U.S. EPA, 2007) and 90% for cyclohexane (Mckee et al., 2015). Oral absorption of aliphatic hydrocarbons was inversely proportional to molecular weight and independent of structure (linear, branched, or alicyclic) in a rat study examining a wide range of aliphatic compounds [reviewed by Mckee et al. (2015)]. Based on conclusions from Mckee et al. (2015), oral absorption of the remaining compounds in the aliphatic low carbon range fraction is expected to be in the range of 80−90%.

Absorption of inhaled aliphatic low carbon range hydrocarbons is high and increases with molecular weight and boiling point (Mckee et al., 2015), as suggested by existing blood-gas partition coefficients. For example, relatively little *n*-pentane is absorbed into the bloodstream after inhalation exposure, because it partitions preferentially into the gas phase (Perbellini et al., 1985). Blood-gas partition coefficients reported in comprehensive toxicity assessments for fraction members, or in publications cited by these assessments (Gargas et al., 1989; Perbellini et al., 1985) are shown in Table 4. As the table indicates, partition coefficients in humans are higher for compounds with higher EC (which is linearly correlated to boiling point) and molecular weight.

Table 4. Blood-Gas Partition Coefficients for Aliphatic Low Carbon Compounds

Compound	C	EC	Molecular Weight (g/mol)	Human	Rat
n-Pentane	5	5	72.15	0.38[a]	1.48[b]
2,2-Dimethylbutane	6	5.68	86.18	0.26[a]	–
2-Methylpentane	6	5.72	86.18	0.41[a]	–
3-Methylpentane	6	5.85	86.18	0.43[a]	–
n-Hexane	6	6	86.18	0.80[a]	2.29[c]
Methylcyclopentane	6	6.27	84.16	0.86[a]	–
Cyclohexane	6	6.58	84.16	1.4[c]	1.39[c]
3-Methylhexane	7	6.76	100.21	1.3[a]	–
2,2,4-Trimethylpentane	8	6.98	114.23	1.60[c]	1.77[c]
n-Heptane	7	7	100.21	2.85[c]	4.75[c]

[a]Perbellini et al. (1985).
[b]Meulenberg and Vijverberg (2000) as cited in U.S. EPA (2009e).
[c]Gargas et al. (1989).

C = carbon; EC = equivalent carbon.

Compounds in the aliphatic low carbon range are widely distributed in the body after inhalation or oral exposure. In rats exposed by inhalation, *n*-pentane was distributed primarily to liver, kidney, and small intestine (Mckee et al., 2015). The highest deposition of cyclohexane in rats exposed orally was in adipose tissue (Mckee et al., 2015). After oral exposure, radioactivity from labeled 2,2,4-trimethylpentane was primarily distributed to kidneys in male rats, with significantly higher levels in the kidneys of male rats compared with female rats (U.S. EPA, 2007). Other deposition sites (primarily peritoneal fat and liver) contained lower amounts of radioactivity with little difference between the sexes (U.S. EPA, 2007). Alpha-olefins (those having a double bond at the first carbon) in the C2−C10 range are primarily distributed to the brain, liver, kidneys, and peritoneal fat (OECD, 2004). In vitro air-tissue partitioning studies show that many aliphatic low carbon range compounds partition into adipose tissue (coefficients range from 39.6 to 443) and to a lesser extent into liver, brain, and kidney (coefficients ≤18.8) (Gargas et al., 1989; Perbellini et al., 1985).

Metabolism of aliphatic low carbon range compounds is largely dependent on structure (linear, branched, or cyclic; alkane or alkene). Available information indicates that alkanes are oxidatively metabolized in the liver to alcohols, ketones, carboxylic acids, dihydrodiols, and diketones, and are subsequently conjugated to glucuronide or sulfate (Mckee et al., 2015; ATSDR, 1999). OECD (2004) reported that short-chain *n*-alkenes are predominantly metabolized to epoxide intermediates that are subsequently converted to glycols or conjugated with glutathione and excreted as mercapturic acids. Table 5 shows the urinary metabolites identified after in vivo exposure to members of the fraction. Few in vivo data on metabolism of alkenes were identified. An in vitro study using rat and human liver microsomes exposed to 1-hexene identified two metabolites: 1-hexen-3-ol and hexen-3-one (Carreón and Herrick, 2012). Little is known about the dose dependence of aliphatic low carbon range compound metabolism;

uptake and metabolism of cyclopentane was concentration-dependent, with greater amounts exhaled (and less absorbed or metabolized) at higher concentrations (20% exhaled as unmetabolized parent compound at 100 ppm, but 88% at 1,000 ppm) (Galvin and Marashi, 1999).

Table 5. Urinary Metabolites Identified for Aliphatic Low Carbon Compounds

Compound	Route	Species	Urinary Metabolites	Reference
n-Pentane (C5 [EC5])	Inhalation (5% in air for 1 h)	Mouse	2-Pentanol, 3-pentanol, 2-pentanone	U.S. EPA (2009e)
2-Methylpentane (C6 [EC5.72])	Inhalation 1,500 ppm for 14 wk)	Rat	2-Methyl-2-pentanol	Frontali et al. (1981)
3-Methylpentane (C6 [EC5.85])	Inhalation (1,500 ppm for 14 wk)	Rat	3-Methyl-3-pentanol, 3-methyl-2-pentanol	Frontali et al. (1981)
n-Hexane (C6 [EC6])	Inhalation (1,000 ppm for 8 h)	Rat	2-Hexanol, 2,5-hexanedione, 3-hexanol, 1-hexanol, 2-hexanone	U.S. EPA (2005)
Cyclohexane (C6 [EC6.58])	Oral (0.3−400 mg/kg once)	Rabbit	Cyclohexanol, *trans*-1,2-cyclohexane-diol	Mckee et al. (2015)
Cyclohexene (C6 [EC6.74])	Oral (3 mmol/kg once)	Rat	3-Hydroxycyclohexyl mercapturic acid, 2-hydroxycyclohexylmercapturic acid	U.S. EPA (2012b)
n-Heptane (C7 [EC7])	Inhalation (1,800 ppm for 6 h)	Rat	2-Heptanol, 3-heptanol, gamma-valerolactone, 2-heptanone, 3-heptanone, 4-heptanone, 2,5-heptanedione	U.S. EPA (2016)
Methylcyclohexane (C7 [EC7.22])	Oral (2−2.5 mmol/kg once)	Rabbit	*trans*-4-Methylcyclohexane	Mckee et al. (2015)
	Oral (800 mg/kg on alternate days for 2 wk	Rat	2-*trans*-Hydroxy-4-*cis*-methylcyclohexanol, 2-*cis*-hydroxy-4-*trans*-methylcyclohexanol, *trans*-3-methylcyclohexanol, 2-*cis*-hydroxy-4-*cis*-methylcyclohexanol, *trans*-4-methylcyclohexanol, cyclohexylmethanol	Carreón and Herrick (2012)
n-Octane (C8 [EC8])	Oral (1,400 mg/kg every other day for 14 d)	Rat	2-Octanol, 3-octanol, 5-oxohexanoic acid, 6-oxohexanoic acid	Mckee et al. (2015)
2-Methylheptane (C8 [EC7.71])	Oral	Rat	2-Methyl-2,5-heptanediol, 2-methyl-5-heptanoloactone, 2-methylheptanoic acid, 2-methyl-1,2-heptanediol	Mckee et al. (2015)
2,2,4-Trimethylpentane (C8 [EC6.98])	Oral (4.4 mmol/kg once)	Rat	2,4,4-Trimethyl-2-pentanol, 2,4,4-trimethyl-1-pentanol, 2,4,4-trimethylpentanoic acid, 2,4,4-trimethyl-5-hydroxypentanoic acid, 2,2,4-trimethyl-1-pentanol, 2,2,4-trimethylpentanoic acid, 2,2,4-trimethyl-5-hydroxypentanoic acid	U.S. EPA (2007)

C = carbon; EC = equivalent carbon.

Excretion of aliphatic low carbon compounds is predominantly via exhaled air (either as parent or as CO_2) and urine, with little excreted in feces. In rats exposed orally to cyclohexane, 60−80% (depending on dose) of the administered compound was eliminated in exhaled air (parent and metabolite compositions were not reported) and the rest was excreted via urine (Mckee et al., 2015). After oral exposure to radiolabeled 2,2,4-trimethylpentane, excretion of radioactivity occurred primarily via urine (50−67%) and exhaled air (43−49%); after inhalation exposure, urinary excretion accounted for 60−70% of the absorbed compound (U.S. EPA, 2007). Elimination of the aliphatic low carbon compounds is generally rapid; elimination half-lives of 0.13 hours for *n*-pentane and 14−18 hours for cyclohexane have been reported in rats and humans exposed by inhalation (Mckee et al., 2015). After inhalation exposure to *n*-octane, 50% of the absorbed dose was eliminated as exhaled CO_2 within 10 hours after exposure (Mckee et al., 2015).

5. MECHANISTIC CONSIDERATIONS AND GENOTOXICITY

Of the health effects induced by aliphatic low carbon range compounds, mechanistic information is available to inform mode of action only for peripheral nervous system effects. Peripheral neuropathy after exposure to *n*-hexane has been previously established to result from production of a γ-diketone metabolite, 2,5-hexanedione (U.S. EPA, 2005). Metabolism of *n*-hexane yields relatively high levels of the diketone (U.S. EPA, 2005). Available metabolic data (see Table 5) show only two compounds (*n*-hexane and *n*-heptane) for which γ-diketone formation has been demonstrated; however, few data are available to assess whether other compounds in the fraction may be metabolized to γ-diketone intermediates. Compared to *n*-hexane, metabolism of *n*-heptane yields much smaller amounts of γ-diketone (U.S. EPA, 2016).

Among the compounds and mixtures with any genotoxicity data summarized in comprehensive U.S. EPA toxicity assessments (commercial hexane, *n*-pentane, methylcyclopentane, cyclohexane, cyclohexene, *n*-hexane, *n*-heptane, 2,2,4-trimethylpentane, and 2,4,4-trimethylpentene), genotoxicity data were largely negative. Positive findings were reported for *n*-hexane (minimal mutagenic activity in *Saccharomyces cerevisiae* and slightly increased incidences of chromosomal aberrations [CAs] in rat bone marrow after in vivo exposure) (U.S. EPA, 2005).

6. DERIVATION OF PROVISIONAL VALUES

6.1. DERIVATION OF ORAL REFERENCE DOSES

Subchronic provisional RfDs (p-RfDs) are available for five constituents of the fraction. The critical effects for these subchronic p-RfDs are peripheral nervous system effects (*n*-hexane), body-weight changes (methylcyclopentane), hepatic changes (2,4,4-trimethyl-pentene, cyclohexene), and forestomach lesions (*n*-heptane based on read-across analogue analysis). There are three available chronic RfDs for constituent compounds (cyclohexene, *n*-heptane, and 2,4,4-trimethylpentene); all of these are based on the same studies and points of departure (PODs) as the corresponding subchronic RfDs. Table 6 summarizes the subchronic and chronic RfDs for constituent compounds and mixtures, with PODs, uncertainty factors, critical effects, and associated confidence descriptors.

Table 6. Available RfD Values for Aliphatic Low Carbon Range Fraction (C5−C8 [EC5−EC8])[a]

Indicator Chemical or Components	POD (mg/kg-d)	POD Type	UF_C	UF Components	RfD or p-RfD (mg/kg-d)	Confidence in RfD or p-RfD	Critical Effect(s)	Species, Mode, and Duration	Reference
Subchronic									
n-Hexane (C6 [EC6])	785	LOAEL	3,000	UF_A, UF_D, UF_H, UF_L	0.3	Low	Reductions in motor nerve conduction velocity (nervous)	Rat, gavage, 8 wk	U.S. EPA (2009a); Ono et al. (1981)
Methylcyclopentane (C6 [EC6.27])	357	NOAEL	1,000	UF_A, UF_D, UF_H	0.4	Low	Reduced body weight (body weight)	Rat, gavage, 5 d/wk for 4 wk	U.S. EPA (2009d); Halder et al. (1985)
Cyclohexene (C6 [EC6.74])	**4.81**	**$BMDL_{1SD}$ (HED)**	**100**	**UF_A, UF_D, UF_H**	**0.05**	**Low**	**Increased total serum bilirubin (hepatic)**	**Rat, gavage, one-generation**	**MHLW (2001) as cited in U.S. EPA (2012b)**
n-Heptane (C7 [EC7])	3.13	$BMDL_{10}$	1,000	UF_A, UF_D, UF_H	*0.003*[b]	Low	Based on *n*-nonane as analogue; forestomach histopathology (GI)	Mouse, gavage, 13 wk	Dodd et al. (2003) as cited in U.S. EPA (2016)
2,4,4-Trimethylpentene (C8 [EC6.8])	41.5	$BMDL_{10}$ (HED)	300	UF_A, UF_D, UF_H	*0.1*[b]	Low	Increased relative liver weight (hepatic)	Rat, gavage, one-generation	Huntingdon Life Sciences (1997a) as cited in U.S. EPA (2015)

Table 6. Available RfD Values for Aliphatic Low Carbon Range Fraction (C5−C8 [EC5−EC8])[a]

Indicator Chemical or Components	POD (mg/kg-d)	POD Type	UF_C	UF Components	RfD or p-RfD (mg/kg-d)	Confidence in RfD or p-RfD	Critical Effect(s)	Species, Mode, and Duration	Reference
Chronic									
Cyclohexene (C6 [EC6.74])	**4.81**	**$BMDL_{1SD}$ (HED)**	**1,000**	**UF_A, UF_D, UF_H, UF_S**	**0.005**	**Low**	**Increased total serum bilirubin (hepatic)**	**Rat, gavage, one-generation**	**MHLW (2001) as cited in U.S. EPA (2012b)**
n-Heptane (C7 [EC7])	3.13	$BMDL_{10}$	10,000	UF_A, UF_D, UF_H, UF_S	*0.0003*[b]	Low	Based on *n*-nonane as analogue; forestomach histopathology (GI)	Mouse, gavage, 13 wk	Dodd et al. (2003) as cited in U.S. EPA (2016)
2,4,4-Trimethylpentene (C8 [EC6.8])	41.5	$BMDL_{10}$ (HED)	3,000	UF_A, UF_D, UF_H, UF_S	*0.01*[b]	Low	Increased relative liver weight (hepatic)	Rat, gavage, one-generation	Huntingdon Life Sciences (1997a) as cited in U.S. EPA (2015)

[a]**Bolded** row shows the compound and toxicity value selected as the indicator chemical for the fraction if analytical chemistry data do not identify concentrations of individual chemicals composing this fraction.
[b]Toxicity values are provisional values obtained from an existing PPRTV assessment. Values in *italics* are screening provisional values obtained from an existing PPRTV assessment. Screening values are not assigned confidence statements; however, confidence in these values is presumed to be low. Screening provisional values are derived when the available data do not meet the requirements for deriving a provisional toxicity value.

BMDL = benchmark dose lower confidence limit; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; C = carbon; EC = equivalent carbon; GI = gastrointestinal; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; PPRTV = Provisional Peer-Reviewed Toxicity Value; p-RfD = provisional reference dose; RfD = oral reference dose; SD = standard deviation; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

As suggested by the disparity in critical effects and values of RfDs for fraction members and discussed in Appendix C, the available oral toxicity data for aliphatic low carbon range compounds do not demonstrate significant consistency across fraction members in terms of toxicological effects or potencies. Thus, there is no basis to identify an indicator chemical or mixture that is representative of the effects and potency of the fraction as a whole. Therefore, the most potent component compounds and mixtures were considered as the basis for indicator chemical selection, as outlined in the methodology (see Section 2.3).

6.1.1. Oral Noncancer Assessment Using the Indicator Chemical Method for the Aliphatic Low Carbon Range Fraction

If available analytical chemistry data do not identify concentrations of individual chemicals composing this fraction, the subchronic and chronic p-RfDs (0.05 and 0.005 mg/kg-day, respectively) for cyclohexene are recommended as the indicator chemical for the aliphatic low carbon range fraction (U.S. EPA, 2012b). The p-RfDs for cyclohexene are based on hepatic toxicity, and available data generally support the liver as a target of aliphatic low carbon compounds. Although the RfDs for cyclohexene are not the lowest available, the subchronic and chronic p-RfD values for *n*-heptane (0.003 and 0.0003 mg/kg-day, respectively) are not recommended, for the following three reasons. First, the *n*-heptane p-RfDs are screening values based on a read-across analysis and therefore carry additional uncertainty associated with the analogue approach. Second, the analogue upon which the values are based (*n*-nonane) is outside (C9 [EC9]) the carbon range of the fraction. Third, the chronic p-RfD for *n*-heptane is highly uncertain, derived with a composite uncertainty factor (UF_C) of 10,000. Evaluation of available data as discussed in Appendix C suggests that use of the cyclohexene p-RfD values is reasonably anticipated to be protective for effects associated with exposures to other constituents of the fraction. Users of the indicator chemical method should understand that there could be more uncertainty associated with the application of this toxicity value to the aliphatic low carbon range fraction than for its derivation in U.S. EPA (2012b).

The cyclohexene PPRTV assessment cited Ministry of Health, Labour, and Welfare (MHLW, 2001a, b as cited in U.S. EPA, 2012b) as the principal studies for the subchronic and chronic p-RfDs:

> *MHLW (2001a) conducted a subchronic oral toxicity study that also examined reproductive and developmental effects that will be discussed separately (MHLW, 2001b). This study appears to be proprietary (may have been part of a Japanese toxicity assessment conducted by MHLW) and is in Japanese. OECD SIDS (2002) peer-reviewed and summarized the study (cited as MHLW, 2002) and EPA subsequently had the document translated. The internal and external peer reviewers of this PPRTV document also concurred that the MHLW (2001a) study was suitable for deriving a provisional toxicity value. This study was conducted as a combined repeated dose toxicity study with reproduction/ developmental toxicity screening according to OECD test guideline 422 and was stated by OECD to be GLP compliant (no GLP statement was provided in the study report).*

Crj:CD(SD)IGS rats (12 animals/sex/treatment group) were administered 0, 50, 150, or 500 mg/kg-day of cyclohexene (98.6% pure) in corn oil via gavage. Dose formulations were tested for concentration and stability. Males were dosed for 48 days and females for 43−53 days beginning 14 days before mating, throughout the mating and gestational period, to Day 4 of lactation. Animals were observed for clinical signs of toxicity daily. Body weight and food consumption were measured weekly and at necropsy. Urinalysis was conducted on 5 males/ treatment group at 43−48 days of treatment. At sacrifice (on Day 49 for males and 5 days after delivery for females), blood was collected for hematology and clinical chemistry in all animals. The brain, liver, kidney, spleen, adrenal glands, thymus, testis, and epididymis were weighed. Tissues and organs were examined histologically in at least the control and high-dose group. Statistical analyses performed included Bartlett's test for homogeneity of variance, Dunnett's multiple comparison test (if equal variance), and Steel's test for unequal variances. The $\chi 2$ and Fisher's exact probability tests were also used where appropriate.

Salivation was observed at 150 (for about 5 minutes in 3/12 males and 2/12 females) and 500 mg/kg-day (all animals for 30−60 minutes in males and 6 hours in females). Lacrimation was observed in 2/12 males at 500 mg/kg-day and females at ≥150 mg/kg-day (1/12 for each dose group). There were some small—but statistically significant—hematological changes at 500 mg/kg-day. Increased were the number of reticulocytes and bilirubin in males and prothrombin time and total bile acids in females. Decreased was the level of APTT in males. There were no treatment-related significant changes in body weight, or food consumption, in either sex or in the urinalysis findings for males (females not measured). There was a dose-dependent decrease in triglyceride in males (see Table B.1). Even though triglyceride in the 500 mg/kg-day group males was 43% lower than in the controls, the results were not statistically significant nor was this effect noted in the females. There was an increase in total bilirubin in both sexes; reanalysis of the data indicates that there are statistically significant increases at all doses in males and in high-dose females. Total bile acid was increased by >10% in all dose groups. However, the results were highly variable and not dose dependent. Only the 150-mg/kg-day males and the 50- and 500-mg/kg-day females showed statistically significant changes above the controls. High-dose males had a statistically significant increase in relative kidney weight that was not accompanied by any histopathological changes and did not reach 1SD (standard deviation) above the control (see Table B.2). OECD SIDS (2002) reported a NOAEL of 50 mg/kg-day for the repeated dose toxicity portion of the test based on transient salivation observed in both sexes at 150 mg/kg-day. Transient salivation is not considered sufficiently adverse to identify as a critical effect. Although the bile acid increase was not dose dependent and was variable, the data taken together may indicate bile duct blockage. Bile duct blockage is also consistent with the statistically significant increase in alkaline phosphatase in rats noted by Laham (1976) following inhalation exposure. Based on the statistically significant increase in total bile acid in females and total bilirubin in males at the lowest dose, no NOAEL can be determined, and the LOAEL is 50 mg/kg-day.

The selected critical effect of total bilirubin in male rats was BMD modeled. The resultant benchmark dose lower confidence limit with one standard deviation ($BMDL_{1SD}$) of 19.71 mg/kg-day was subsequently converted to a human equivalent dose (HED) of 4.81 mg/kg-day (see Table 6). As reported in U.S. EPA (2012b), confidence in the principal study was medium. Although the study was described as being conducted according to OECD Test Guideline (TG) 422 and was subsequently translated by U.S. EPA, the OECD (2002) SIDS report is a secondary data source. As reported in U.S. EPA (2012b), confidence in the database was low, because only one oral repeated-dose study was available. Therefore, confidence in the subchronic and chronic p-RfDs was also low.

6.1.2. Alternative Oral Noncancer Assessment Using the Hazard Index Method for the Aliphatic Low Carbon Range Fraction

If the available analytical chemistry data quantify the concentrations of *n*-hexane, methylcyclopentane, cyclohexene, *n*-heptane, or 2,4,4-trimethylpentene separately from the remainder of the low carbon fraction, it is recommended that HQs for the individual chemicals with analytical data be calculated and an HI for the mixture be developed using the calculated HQs.

For subchronic oral exposures, the following subchronic p-RfDs can be used as the denominator in the HQ equations: *n*-hexane (0.3 mg/kg-day), methylcyclopentane (0.4 mg/kg-day), cyclohexene (0.05 mg/kg-day), *n*-heptane (0.003 mg/kg-day), and 2,4,4-trimethylpentene (0.1 mg/kg-day). In this alternative approach, the subchronic p-RfD (0.05 mg/kg-day) for cyclohexene is recommended for use with the remainder of the fraction, including any other fraction members analyzed individually (see Table 6).

For chronic oral exposures, the following chronic p-RfDs can be used in the denominator of the HQ equations: cyclohexene (0.005 mg/kg-day), *n*-heptane (0.0003 mg/kg-day), and 2,4,4-trimethylpentene (0.01 mg/kg-day). In this alternative approach, the chronic p-RfD (0.005 mg/kg-day) for cyclohexene is recommended for use with the remainder of the fraction, including any other fraction members analyzed individually (see Table 6).

6.2. DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

The available subchronic and chronic RfC values, with PODs, uncertainty factors, critical effects, and confidence descriptors are presented in Table 7. As shown in the table, there are subchronic and chronic RfCs or provisional RfCs (p-RfCs) for one mixture (commercial hexane) and four individual compounds (*n*-pentane, *n*-hexane, cyclohexane, and *n*-heptane) relevant to the aliphatic low carbon range fraction. In addition, there is a chronic p-RfC for cyclohexene. The critical effects for the subchronic RfCs include peripheral nervous system injury (*n*-hexane), diminished hearing sensitivity (*n*-heptane), decreased body weight and nervous system effects (commercial hexane), and developmental toxicity (decreased pup weight; cyclohexane). The critical effects for the chronic RfCs include peripheral nervous system injury (*n*-hexane), diminished hearing sensitivity (*n*-heptane), liver pathology (spongiosis hepatis; cyclohexene), nasal lesions (hyperplasia; commercial hexane), and developmental toxicity (decreased pup weight; cyclohexane).

Table 7. Available RfC Values for Aliphatic Low Carbon Range Fraction (C5−C8 [EC5−EC8])[a]

Indicator Chemical or Components	POD (mg/m^3)	POD Type (all are HECs)	UF_C	UF Components	RfC or p-RfC (mg/m^3)	Confidence in RfC or p-RfC	Critical Effect(s)	Species, Mode, and Duration	Reference
Subchronic									
n-Pentane (C5 [EC5])	3,658	NOAEL	300	UF_A, UF_D, UF_H	10	Low	No treatment-related effects	Rat, 6 h/d, 5 d/wk for 13 wk	McKee and Frank (1998) as cited in U.S. EPA (2009e)
Commercial hexane (C6)	804	NOAEL	30	UF_A, UF_H	27	Medium	Abnormal gait; decreased body weight; mild atrophy of sciatic and/or tibial nerve and skeletal muscle (nervous and body weight)	Rat, 22 h/d, 7 d/wk for 6 mo	IRDC (1992) as cited in U.S. EPA (2009b)
_n_-Hexane (C6 [EC6])	**215**	**$BMCL_{1SD}$**	**100**	**UF_A, UF_D, UF_H**	**2**	**Low**	**Peripheral neuropathy (nervous)**	**Rat, 12 h/d, 7 d/wk for 16 wk**	**Huang (1989) as cited in U.S. EPA (2009a)**
Cyclohexane (C6 [EC6.58])	1,822	$BMCL_{1SD}$	100	UF_A, UF_D, UF_H	18	Moderate	Reduced pup weight (developmental)	Rat, 6 h/d, 5 d/wk, two-generation	Kreckmann (2000) and Dupont HLR (1997a), both as cited in U.S. EPA (2010)
n-Heptane (C7 [EC7])	1,170	$BMCL_{1SD}$	300	UF_A, UF_D, UF_H	4	Low	Loss of hearing sensitivity (nervous)	Rat, 6 h/d, 7 d/wk for 28 d	Simonsen and Lund (1995) as cited in U.S. EPA (2016)

Table 7. Available RfC Values for Aliphatic Low Carbon Range Fraction (C5−C8 [EC5−EC8])[a]

Indicator Chemical or Components	POD (mg/m^3)	POD Type (all are HECs)	UF_C	UF Components	RfC or p-RfC (mg/m^3)	Confidence in RfC or p-RfC	Critical Effect(s)	Species, Mode, and Duration	Reference
Chronic									
n-Pentane (C5 [EC5])	3,658	NOAEL	3,000	UF_A, UF_D, UF_H, UF_S	1	Low	No treatment-related effects	Rat, 6 h/d, 5 d/wk for 13 wk	McKee et al. (1998) as cited in U.S. EPA (2009e)
Commercial hexane (C6)	17.59	$BMCL_{10}$	30	UF_A, UF_H	0.6	Medium	Nasal epithelial cell hyperplasia (respiratory)	Rat, 6 h/d, 5 d/wk for 2 yr	Daughtrey et al. (1999) and Biodynamics (1993), both as cited in U.S. EPA (2009b)
n-Hexane (C6 [EC6])	215	$BMCL_{1SD}$	300	UF_A, UF_D, UF_H, UF_S	0.7	Medium	Peripheral neuropathy (nervous)	Rat, 12 h/d, 7 d/wk for 16 wk	Huang et al. (1989) as cited in U.S. EPA (2005)
Cyclohexane (C6 [EC6.58])	1,822	$BMCL_{1SD}$	300	UF_A, UF_D, UF_H	6	Low-moderate	Reduced pup weight (developmental)	Rat, 6 h/d, 5 d/wk, two-generation	Kreckmann et al. (2000) and DuPont HLR (1997a) as cited in U.S. EPA (2010)
Cyclohexene (C6 [EC6.74])	360	NOAEL	300	UF_A, UF_D, UF_H	*1*[b]	Low	Spongiosis hepatis (hepatic)	Rat, 6 h/d, 5 d/wk for 104 wk	MHLW (2003) as cited in U.S. EPA (2012b)
***n*-Heptane (C7 [EC7])**	**1,170**	**$BMCL_{1SD}$**	**3,000**	**UF_A, UF_D, UF_H, UF_S**	**0.4**	**Low**	**Loss of hearing sensitivity (nervous)**	**Rat, 6 h/d, 7 d/wk for 28 d**	**Simonsen and Lund (1995) as cited in U.S. EPA (2016)**

[a]**Bolded** row shows the compounds and toxicity value selected as the indicator chemical for the fraction if analytical chemistry data do not identify concentrations of individual chemicals composing this fraction.
[b]Toxicity values are provisional values obtained from an existing PPRTV assessment. Values in *italics* are screening provisional values obtained from an existing PPRTV assessment. Screening provisional values are not assigned confidence statements; however, confidence in these values is presumed to be low. Screening provisional values are derived when the available data do not meet the requirements for deriving a provisional toxicity value.

BMCL = benchmark concentration lower confidence limit; $BMCL_{10}$ = 10% benchmark concentration lower confidence limit; C = carbon; EC = equivalent carbon; HEC = human equivalent concentration; NOAEL = no-observed-adverse-effect level; POD = point of departure; PPRTV = Provisional Peer-Reviewed Toxicity Value; p-RfC = provisional reference concentration; RfC = inhalation reference concentration; SD = standard deviation; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

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As suggested by the disparity in critical effects and values of RfCs for fraction members and discussed in Appendix C, the available inhalation toxicity data for aliphatic low carbon range compounds do not demonstrate significant consistency across fraction members in terms of toxicological effects or potencies. There is no basis to identify an indicator chemical or mixture that is representative of the effects and potency of the fraction as a whole. Therefore, the most potent component compounds and mixtures were considered as the basis for indicator chemical selection, as outlined in the methodology (see Section 2.3).

6.2.1. Inhalation Noncancer Assessment Using the Indicator Chemical Method for the Aliphatic Low Carbon Range Fraction

If available analytical chemistry data do not identify concentrations of individual chemicals composing this fraction, the lowest subchronic and chronic p-RfCs among the compounds in this fraction, for *n*-hexane and *n*-heptane, respectively [(U.S. EPA, 2016, 2009a); see Table 7] are recommended as indicator chemicals for the aliphatic low carbon range fraction. Use of these values is anticipated to be protective for exposure to other constituents based on available information (see Appendix C). However, users of the indicator chemical method should understand that there could be more uncertainty associated with the application of this toxicity value to the aliphatic low carbon range fraction than for its derivation in (U.S. EPA, 2016, 2009a).

The U.S. EPA (2009a) *n*-hexane PPRTV assessment cited Huang et al. (1989) Huang et al. (1989) as cited in U.S. EPA (2009a) as the principal study for the subchronic p-RfC:

> *Male Wistar rats (eight/group) were exposed to 0, 500, 1200, or 3000 ppm (0, 1762, 4230, 10,574 mg/m3) n-hexane (>99% pure) for 12 hours/day, 7 days/week for 16 weeks (Huang et al., 1989). The authors measured motor nerve conduction velocity (MCV) in the tail nerve along with body weight before exposure and after 4, 8, 12, and 16 weeks of exposure to n-hexane. One animal from each group was sacrificed at 16 weeks exposure for histopathological evaluation of the nerve fibers in the tail. In addition, Huang et al. (1989) measured the levels of neuron-specific enolase and beta-S-100. These nervous system-specific proteins are a family of calcium binding proteins that are involved in processes such as cell-to-cell communication, cell growth, intracellular signal transduction, and development and maintenance of the central nervous system. A dose-dependent, statistically significant reduction in body weight gain was observed in the mid- (at 12 weeks) and high-dose (at 8 weeks) rats. Additionally, there were some neurological deficits in mid- and high-dose rats, including a reduction in grip strength and a comparative slowness of motion from week 12 of exposure. However, no hindlimb paralysis was observed by the termination of the experiment. Rats exposed to the mid and high doses of n-hexane showed a reduction in MCV. This reduction was statistically significant during weeks 8–16 of the exposure period compared with controls. Increased incidence of paranodal swellings, along with some evidence of demyelination and remyelination, was present in the peripheral nerves at both mid and high doses. However, these histopathological findings were more severe in the high dose group. Among biochemical changes, there were dose-dependent reductions in nervous system specific proteins, particularly the beta-S-100 proteins from tail nerve fibers, which were significantly reduced by approximately 75% at all dose levels. The*

neurophysiological deficits and histopathological effects that were evident in mid- and high-dose rats indicate a NOAEL of 500 ppm.

The Huang et al. (1989) study as cited in U.S. EPA (2009a) provided adequate dose-response data for BMD modeling with an estimated POD (benchmark concentration lower confidence limit [BMCL] human equivalent concentration [HEC]) of 215 mg/m^3 (see Table 7). As reported in U.S. EPA (2009a), confidence in the principal study was medium. The study used a low, but acceptable, number of animals per group (8/sex); data enabled identification of NOAEL and LOAEL values for neurological effects. As reported in U.S. EPA (2009a), confidence in the database was low due to the lack of a multigenerational developmental and reproductive toxicity study. Therefore, confidence in the subchronic p-RfC was also low.

The U.S. EPA (2009a) *n*-heptane PPRTV assessment cited Simonsen and Lund (1995) as the principal study for the chronic p-RfC:

In this neurotoxicity study, groups of male Long-Evans rats (9−10/group) were placed in whole-body chambers and exposed to n-heptane (99.5% pure) vapors at reported mean concentrations of 0, 801 ± 79, or 4,006 ± 242 ppm, 6 hours/day for 28 days. The study was aimed at evaluating potential effects of n-heptane on the central auditory system, given that exposure to organic solvents has been associated with hearing loss in rats and humans (Simonsen and Lund, 1995). Feed and water were available ad libitum except during exposure periods. Six weeks prior to exposure, screw electrodes were mounted in the skull of the rats for measurement of auditory brainstem responses. The amplitudes and latencies of Components Ia and IV of the auditory brainstem responses elicited at frequencies 4, 8, 16, or 32 kHz and intensities 25−95 dB were measured in anaesthetized rats 2 months after cessation of exposure using both implanted electrodes and needle electrodes. Body weight was monitored throughout the study. No other systemic endpoints were assessed.

Body-weight gain during the first 2 weeks postexposure was significantly decreased by 53% in the 4,006-ppm group. However, body weights were similar in all three exposure groups during the course of treatment. The peak amplitudes of the Ia and IV components of the auditory brainstem responses were reduced in rats exposed to 4,006 ppm at all frequencies and intensities, compared with control (0-ppm treatment group), but not at 801 ppm. Statistically significant reductions were reported for Component IV, most prominently at higher frequencies and intensities (see Table B-4). Decreases in amplitude of Component Ia displayed a similar pattern to IV; however statistical analyses for this component were not provided. No exposure-related changes were observed in the latencies or interpeak latencies of the Ia and IV components. The reduction in the peak amplitudes corresponded to an approximate 10-dB increase in the auditory threshold. The difference in auditory threshold between the control and the 4,006-ppm group was observed at all frequencies, although statistical significance was only reached at 8 and 16 kHz (see Table B-5; data have been digitally extracted using GrabIt! Software).

A NOAEL of 801 ppm and a LOAEL of 4,006 ppm is identified for abnormal auditory brainstem responses and increased auditory threshold that suggest a loss of hearing sensitivity in rats. Concentrations of 801 and 4,006 ppm are converted to human equivalent concentrations (HECs) of 821 and 4,105 mg/m3 for extrarespiratory effects by treating n-heptane as a Category 3 gas (generally water insoluble and unreactive in the extrathoracic or tracheobronchial regions) and using the following equation (U.S. EPA, 1994a): HECEXRESP = (ppm × molecular weight [MW] ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × ratio of blood-gas partition coefficient (animal:human). For n-heptane, the blood-air partition coefficient for rats is greater than that for humans (Gargas et al., 1989); thus, a default ratio of 1 is applied (U.S. EPA, 1994a).

BMD analyses were performed to model central auditory effects (all frequencies) in rats exposed to *n*-heptane. Only data sets at frequencies of 16 and 32 Hz provided an adequate fit. The lowest benchmark concentration lower confidence limit with one standard deviation ($BMCL_{1SD}$) (HEC) of 1,170 mg/m^3 was selected as the POD (see Table 7). As reported in U.S. EPA (2016), confidence in the study was medium. Although the study was peer-reviewed, used adequate methodology, and provided identification of NOAEL and LOAEL values for auditory effects, it was a short-term (28 days) study in male rats only, and a limited number of endpoints were evaluated. As reported in U.S. EPA (2016), confidence in the database was low, because no developmental or multigeneration studies were available; the chronic study did not provide organ-weight data or perform thorough histopathological examinations. Therefore, confidence in the chronic p-RfC was also low.

6.2.2. Alternative Inhalation Noncancer Assessment Using the Hazard Index Method for the Aliphatic Low Carbon Range Fraction

If the available analytical chemistry data quantify the concentrations of *n*-pentane, *n*-hexane, cyclohexane, cyclohexene, or *n*-heptane separately from the remainder of the low carbon fraction, it is recommended that HQs for the individual chemicals with analytical data be calculated and an HI for the mixture be developed using the calculated HQs.

For subchronic inhalation exposures, the following subchronic p-RfCs can be used as the denominator in the HQ equations: *n*-pentane (10 mg/m^3), *n*-hexane (2 mg/m^3), cyclohexane (18 mg/m^3), and *n*-heptane (4 mg/m^3). In this alternative approach, the subchronic p-RfC for *n*-hexane (2 mg/m^3) is recommended for use with the remainder of the fraction, including any other fraction members analyzed individually.

For chronic inhalation exposures, the following chronic p-RfCs can be used as the denominator in the HQ equations: *n*-pentane (1 mg/m^3), *n*-hexane (0.7 mg/m^3), cyclohexane (6 mg/m^3), cyclohexene (1 mg/m^3), and *n*-heptane (0.4 mg/m^3). In this alternative approach, the chronic p-RfC for *n*-heptane (0.4 mg/m^3) is recommended for use with the remainder of the fraction, including any other fraction members analyzed individually.

6.3. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

Table 8 summarizes the noncancer health references values for indicator chemicals used when available analytical data and exposure estimates are limited to either air concentrations of, or oral exposure rates associated with, the whole fraction. When analytical results, air concentrations, or exposure rate measures for individual compounds with reference values are available, then the hazards associated with these compounds can be assessed separately, using the HI approach and reference values reported in Tables 6 and 7.

Table 8. Summary of Noncancer Reference Estimates for Indicator Chemicals for Aliphatic Low Carbon Range (C5−C8 [EC5−EC8]) Fraction of Total Petroleum Hydrocarbons

Toxicity Type (units); Indicator Chemical	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED/HEC)	UF_C	Reference
Subchronic p-RfD (mg/kg-d); cyclohexene	Rat/M	Hepatotoxicity (increased total serum bilirubin)	5×10^{-2}	$BMDL_{1SD}$	4.81	100	MHLW (2001) as cited in U.S. EPA (2012b)
Chronic p-RfD (mg/kg-d); cyclohexene	Rat/M	Hepatotoxicity (increased total serum bilirubin)	5×10^{-3}	$BMDL_{1SD}$	4.81	1,000	MHLW (2001) as cited in U.S. EPA (2012b)
Subchronic p-RfC (mg/m^3); *n*-hexane	Rat/M	Neurotoxicity (peripheral neuropathy)	2×10^{0}	$BMCL_{1SD}$	215	100	Huang et al. (1989) as cited in U.S. EPA (2009a)
Chronic p-RfC (mg/m^3); *n*-heptane	Rat/M	Neurotoxicity (loss of hearing sensitivity)	4×10^{-1}	$BMCL_{1SD}$	1,170	3,000	Simonsen and Lund (1995) as cited in U.S. EPA (2016)

BMDL = benchmark dose lower confidence limit; C = carbon; EC = equivalent carbon; HEC = human equivalent concentration; HED = human equivalent dose; M = male; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; SD = standard deviation; UF_C = composite uncertainty factor.

6.4. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

The inhalation cancer assessment outcomes for mixtures and individual components of the aliphatic low carbon range fraction that have existing assessments are shown in Table 9. The only component of the fraction for which there is information available to adequately assess carcinogenic potential is commercial hexane. The PPRTV assessment for commercial hexane (U.S. EPA, 2009b) describes the WOE as follows:

> *Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005b), there is "Suggestive Evidence for [the] Carcinogenic Potential" of commercial hexane in humans. There are no data on carcinogenicity of commercial hexane in humans. A 2-year carcinogenicity bioassay in mice and rats exposed to commercial hexane showed an increased incidence of liver tumors (combined hepatocellular adenomas and carcinomas) in female mice (Daughtrey et al., 1999; Biodynamics, 1993a, b). No increase in liver tumor incidence was observed in treated male mice or in either sex of F344 rats exposed to commercial*

hexane under the same conditions. The study authors also identified a statistically significant increase in the incidence of pituitary tumors in female mice. Available data on the genotoxicity of commercial hexane are limited; no gene reversion or chromosomal aberrations in mammalian cells and no chromosomal aberrations in the bone marrow of rats exposed in vivo were observed in the only tests conducted.

Table 9. Available Cancer WOE Descriptors for Aliphatic Low Carbon Range Fraction (C5−C8 [EC5−EC8])

Compound or Mixture	Cancer WOE Descriptor	Source
n-Pentane (C5 [EC5])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2009e)
Commercial hexane (C6 [EC NA])	*"Suggestive Evidence of Carcinogenic Potential"*	U.S. EPA (2009b)
n-Hexane (C6 [EC6])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2005)
Methylcyclopentane (C6 [EC6.27])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2009d)
Cyclohexane (C6 [EC6.59])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2010)
Cyclohexene (C6 [EC6.74])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2012b)
2,2,4-Trimethylpentane (C8 [EC6.98])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2007)
n-Heptane (C7 [EC7])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2016)
Methylcyclohexane (C7 [EC7.22])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2013)
2,4,4-Trimethylpentene (C8 [EC6.8])	*"Inadequate Information to Assess Carcinogenic Potential"*	U.S. EPA (2015)

C = carbon; EC = equivalent carbon; NA = not applicable; WOE = weight of evidence.

While data on genotoxicity testing of compounds and mixtures in the aliphatic low carbon range fraction are limited, available information suggests little to no genotoxic potential (see Section 5).

6.5. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

None of the mixtures or constituents in this fraction had an oral slope factor (OSF) from IRIS, PPRTVs, HEAST, MassDEP, or TPHCWG. Thus, a provisional OSF (p-OSF) is not derived for the fraction. The only available inhalation unit risk (IUR) value for members of the aliphatic low carbon range fraction is a screening p-IUR for commercial hexane (U.S. EPA, 2009b). In the absence of data to support a clear 'best' surrogate for the mixture, the most health-protective value will be adopted to protect against the carcinogenicity of components of the mixture. The provisional IUR (p-IUR) of 2×10^{-4} (per mg/m^3) for combined pituitary adenomas and adenocarcinomas in female mice exposed to commercial hexane is selected to assess inhalation carcinogenicity for this fraction (see Table 10).

Table 10. Summary of Cancer Risk Estimates for Aliphatic Low Carbon Range (C5−C8 [EC5−EC8]) Fraction of Total Petroleum Hydrocarbons

Toxicity Type (units); Indicator Chemical	Species/ Sex	Tumor Type	Cancer Risk Estimate	Reference
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$; commercial hexane	Mouse/F	Pituitary adenomas or adenocarcinomas	2×10^{-4}	Daughtrey et al. (1989) and Biodynamics (1993), both as cited in U.S. EPA (2009b)

C = carbon; EC = equivalent carbon; F = female; NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. LITERATURE SEARCH AND SCREENING

Literature searches were conducted in February 2018 and updated in August 2021 for studies relevant to the derivation of provisional toxicity values the aliphatic low carbon range fraction of total petroleum hydrocarbons (TPHs). The following 27 constituents (CASRNs) were included for the aliphatic low carbon range fraction: cyclohexane (110-82-7), cyclohexene (110-83-8), cyclopentane (287-92-3), 2,2-dimethylbutane (75-83-2), 2,3-dimethylbutane (79-29-8), 2,3-dimethylpentane (565-59-3), 2,4-dimethylpentane (108-08-7), 3-ethylpentane (617-78-7), commercial hexane (no CASRN), *n*-hexane (110-54-3), 2-methyl-2-butene (513-35-9), 2-methyl-2-pentene (625-27-4), methylcyclohexane (108-87-2), methylcyclopentane (96-37-7), 2-methylheptane (592-27-8), 3-methylheptane (589-81-1), 2-methylhexane (591-76-4), 3-methylhexane (589-34-4), 2-methylpentane (107-83-5), 3-methylpentane (96-14-0), *n*-octane (111-65-9), *n*-pentane (109-66-0), 2,2,3,3-tetramethylbutane (594-82-1), 2,2,3-trimethylbutane (464-06-2), 2,2,4-trimethylpentane (540-84-1), 2,3,3-trimethylpentane (560-21-4), and 2,3,4-trimethylpentane (565-75-3). Initial searches were date limited from 2007 to 2018 and were conducted using the U.S. Environmental Protection Agency (U.S. EPA) Health and Environmental Research Online (HERO) database of scientific literature. The PubMed database was searched using the HERO interface. The updated search was conducted similarly using the same search strings in PubMed and Web of Science from February 2018 through August 2021. There was an additional search of Agency for Toxic Substances and Disease Registry (ATSDR) and U.S. EPA documents for health risk values for fraction members.

The results of the PubMed searches (title and abstract) were screened for relevance using the Population, Exposure, Comparison, and Outcome (PECO) criteria outline in Table A-1 below. Full-text screening for relevance to hazard identification was performed using the refined PECO criteria shown in Table A-2.

Table A-1. PECO Criteria for Title and Abstract Screening of Total Petroleum Hydrocarbon Constituent Literature Search Results

PECO Element	Inclusion Criteria
Population	Humans (any population) or laboratory mammals (any life stage).
Exposure	Human: Exposure to the subject material alone or as the primary component of a mixture, known or presumed to occur by oral, inhalation, and/or dermal routes. Animal: In vivo, exposure to the subject material alone, by oral or inhalation (including instillation) routes, for all durations of exposures (durations <28 d will be captured as supporting information), including any duration during gestation. Other routes of exposure will be captured as supporting information.
Comparison	Human: Includes any comparison/referent group (no exposure, lower exposure). Animal: Includes concurrent negative (untreated, sham-treated, or vehicle) control.
Outcomes	Assesses any cancer or noncancer endpoint in any tissue, organ, or physiological system.

PECO = Population, Exposure, Comparison, Outcomes.

Table A-2. PECO Criteria for Full Text Screening for Relevance to Hazard Identification

PECO Element	Inclusion Criteria
Population	Humans (any population) or laboratory mammals (any life stage).
Exposure	Human: Exposure to the subject material alone or as the primary component of a mixture, known or presumed to occur by oral or inhalation routes. Animal: In vivo, exposure to the subject material alone, by oral or inhalation routes, for durations ≥28 d or any duration during gestation.
Comparison	Human: Includes any comparison/referent group (no exposure, lower exposure). Animal: Includes concurrent negative (untreated, sham-treated, or vehicle) control.
Outcomes	Assesses any cancer or noncancer health outcome in any tissue, organ, or physiological system.

PECO = Population, Exposure, Comparison, Outcomes.

APPENDIX B. COMPOSITION OF MIXTURES RELEVANT TO THE ALIPHATIC LOW CARBON RANGE FRACTION

Information on the composition of the C5–7 alkene mixture used in the Springborn Labs study (Springborn Labs, 2003 as cited in OECD, 2004) is provided in Table B-1. Tables B-2, B-3, and B-4 list the compositions of practical-grade hexane, commercial hexane, and C6 mixture without *n*-hexane mixture in the Krasavage et al. (1980), U.S. EPA (2009b), and Egan et al. (1980) studies, respectively.

Table B-1. Composition of C5−C7 Alkene Mixture[a]

Category	C	Example Compounds	Percentage in Mix
C5 *n*-olefins	5	*n*-Pentene	0.5%
C5 *iso*-olefins	5	3-Methyl-1-butene/isopentene	1.3%
C5 *n*-paraffins	5	*n*-Pentane	3.3%
C5 *iso*-paraffins	5	2-Methylbutane/isopentane	9.3%
C6 *n*-olefins	6	*n*-Hexene	10.4%
C6 *iso*-olefins	6	4-Methyl-1-pentene/isohexene	55.6%
C6 *iso*-paraffins	6	2-Methylpentane/isohexane	17.8%
C7 *iso*-olefins	7	Isoheptene	1.0%
Total contribution from members of fraction			≥99.2%

[a]Springborn Labs (2003) as cited in OECD (2004).

C = carbon.

Table B-2. Composition of Practical-Grade Hexane[a]

CASRN	Name	EC	C	Percentage in Mix
287-92-3	Cyclopentane	5.66	5	9%
79-29-8	2,3-Dimethylbutane	5.68	6	24%
107-83-5	2-Methylpentane/isohexane	5.72	6	1.8%
96-14-0	3-Methylpentane	5.85	6	24%
110-54-3	*n*-Hexane	6	6	40%
110-82-7	Cyclohexane	6.59	6	2.5%
Total contribution from members of fraction				100%

[a]Krasavage et al. (1980).

C = carbon; EC = equivalent carbon.

Table B-3. Composition of Commercial Hexane[a]				
CASRN	**Name**	**EC**	**C**	**Percentage in Mix**
107-83-5	2-Methylpentane/isohexane	5.72	6	13%
96-14-0	3-Methylpentane	5.85	6	16%
110-54-3	*n*-Hexane	6	6	52%
96-37-7	Methylcyclopentane	6.27	6	16%
110-82-7	Cyclohexane	6.59	6	<3%
108-08-7	2,4-Dimethylpentane	6.31	7	<3%
Total contribution from members of fraction				100%

[a]U.S. EPA (2009b).

C = carbon; EC = equivalent carbon.

Table B-4. Composition of C6 Mixture without *n*-Hexane[a]				
CASRN	**Name**	**EC**	**C**	**Percentage in Mix**
79-29-8	2,3-Dimethylbutane	5.68	6	3.4%
107-83-5	2-Methylpentane/isohexane	5.72	6	35.3%
96-14-0	3-Methylpentane	5.85	6	30.0%
110-54-3	*n*-Hexane	6	6	0.3%
96-37-7	Methylcyclopentane	6.27	6	24.6%
110-82-7	Cyclohexane	6.59	6	6%
Total contribution from members of fraction				≥99.6%

[a]Egan et al. (1980).

C = carbon; EC = equivalent carbon.

APPENDIX C. POTENTIALLY RELEVANT NONCANCER EVIDENCE

DEVELOPMENT OF EXPOSURE-RESPONSE ARRAYS

As described in the main document, dose-response data were presented in exposure-response arrays by health outcome and exposure route. The following sections summarize the evidence provided by human and experimental animal studies of noncancer health outcomes. In order to assess consistency in effects and potency across the components of the fraction, experimental data from compound-specific Integrated Risk Information System (IRIS) and Provisional Peer-Reviewed Toxicity Value (PPRTV) documents and primary data sources (identified from literature searches) were used to create exposure-response arrays. Exposure-response arrays present dose-response data by health outcome and exposure route. From left to right, compounds exhibiting an effect are shown before those not exhibiting an effect, to enable identification of patterns. Within the group exhibiting an effect, compounds are ordered from lowest lowest-observed-adverse-effect levels (LOAELs) to highest. For compounds that do not exhibit an effect, no-observed-adverse-effect levels (NOAELs) in the arrays are ordered by equivalent carbon (EC) number index (low to high from left to right), with mixtures shown last. Both administered doses and exposure concentrations reported in the arrays and in text reflect time-weighted average (TWA) exposures, to facilitate comparisons across studies and compounds. Consistency across the fraction was evaluated by assessing if comparable outcomes were observed for members of the fraction, and if these effects were observed at similar dose levels. Unless otherwise specified, the term "significant," used throughout this appendix, refers to statistical significance at a p-value < 0.05.

NEUROLOGICAL EFFECTS

Peripheral nervous system effects are the critical effect for the subchronic and chronic reference concentrations (RfCs) and subchronic provisional reference dose (p-RfD) for *n*-hexane (U.S. EPA, 2009a), and a cocritical effect (with decreased body weight) for the subchronic provisional RfC (p-RfC) for commercial hexane (U.S. EPA, 2009b). A neurological endpoint (decreased hearing sensitivity) is also the critical effect for the subchronic and chronic p-RfCs for *n*-heptane (U.S. EPA, 2016). Neurological effects in humans have been studied for several additional fraction members, but the majority of the data pertain to peripheral neuropathy associated with *n*-hexane. Animal studies examining neurological effects are available for about half of the compounds or mixtures with toxicity data; however, the studies varied widely with respect to the spectrum of the neurological effects evaluated.

Human Studies

Neurotoxicity has been observed in humans exposed to aliphatic compounds in the low carbon range fraction. *n*-Hexane is the most intensely-studied compound in this fraction, with studies of occupational exposure resulting in peripheral neuropathy characterized by loss of distal motor and sensory function (Wang et al., 2014; Kutlu et al., 2009; Puri et al., 2007; U.S. EPA, 2005). Clinical symptoms of neurotoxicity include weakness, motor impairment, paresthesia (burning or tingling sensation in limbs), hypoesthesia (partial loss of sensation and/or diminished sensibility), and changes in tendon reflexes and muscle tone. These symptoms were usually confined to distal portions of the limbs, and the degree of intensity depended on the extent of exposure (Wang et al., 2014; Kutlu et al., 2009; Puri et al., 2007; U.S. EPA, 2005). Electrophysiology measurements in exposed workers revealed decreased maximum conduction velocity (MCV) and reduced amplitude of the sensory nerve action potential (SNAP) (Wang et

al., 2014; Kutlu et al., 2009; Puri et al., 2007; U.S. EPA, 2005). Reduced SNAP amplitude was also observed in asymptomatic workers exposed to *n*-hexane, and the magnitude of the effect was correlated with urinary concentrations of 2,5-hexanedione (Neghab et al., 2012). Examination of sural nerve biopsy samples showed axonal swelling, demyelination, and a selective decrease in long myelinated neurons in workers exposed to *n*-hexane (Puri et al., 2007; U.S. EPA, 2005).

Some human studies have suggested central nervous system (CNS) toxicity resulting from *n*-hexane exposure, including clinical signs of Parkinsonism (i.e., tremor, bradykinesia, and rigidity), memory loss, and impaired visual motor response to neurological assessment (U.S. EPA, 2005). Pathology and magnetic image resonance findings in these patients indicated loss of dopaminergic neurons, gliosis in the substantia nigra, and cerebral cortical atrophy. *n*-Hexane also affects vision in exposed workers, demonstrated by decreased visual evoked potentials, color discrimination deficits, and maculopathy, characterized by damage to blood vessels, fluid leakage into the retina, and pigment dispersion (Beckman et al., 2016; Kutlu et al., 2009; U.S. EPA, 2005).

No clinical signs of peripheral neuropathy were reported in 18 workers exposed to a solvent containing >90% *n*-heptane for 1−9 years (U.S. EPA, 2016). However, electrophysiology testing of 12 workers revealed a decrease in motor nerve conduction velocity (NCV) correlated with increased exposure duration and an increase in amplitude desynchronization of the evoked muscle action potential (U.S. EPA, 2016).

Neurological symptoms (i.e., fatigue, headache, dizziness) were reported in workers exposed to glue containing at least 75% cyclohexane (U.S. EPA, 2010). Electrophysiological abnormalities were also noted (i.e., shorter motor distal latency); however, workers were previously exposed to *n*-hexane. Other study limitations included small group sizes (n = 15−18) and poorly matched controls. No neurological symptoms were reported in a different study of workers exposed to glue containing at least 75% cyclohexane; however, the findings of this study were limited by small cohort size, discrepancies in reporting of analytical air concentrations, and absence of details related to the measured health outcomes (U.S. EPA, 2010). Print shop workers exposed to methylcyclohexane and other solvents for an average of 15 years experienced sleep apnea, mood disturbances, and decreased hand-eye coordination (U.S. EPA, 2013). Volunteers exposed to 4,000, 8,000, 14,000, or 20,000 mg/m^3 *n*-heptane for up to 15 minutes reported vertigo, with severity increasing with exposure concentration (U.S. EPA, 2016). Additional effects observed at the highest concentration included hilarity, incoordination, and inability to walk straight.

Neurological effects were not observed in volunteers exposed to 15,000 mg/m^3 *n*-pentane for 10 minutes (Mckee et al., 2015), or cyclohexane at 86 or 860 mg/m^3 for 4 hours in two test sessions (U.S. EPA, 2010).

Animal Studies

Animals exposed orally to alkane compounds containing six carbons have exhibited peripheral nervous system effects; few data on the neurotoxicity of other members of the fraction were located. Studies for which neurotoxicity effect levels could be determined are shown in an exposure-response array (see Figure C-1). Decreases in NCV occurred after oral exposure to several C6 alkanes at doses between 785 and 1,168 mg/kg-day in a comparative toxicity study by Ono et al. (1981). The relative potency of effects on NCV, based on severity of changes, was *n*-hexane > methylcyclopentane >2-methylpentane > 3-methylpentane. In a 24-week neurotoxicity study of *n*-hexane that was published after development of the PPRTV and IRIS documents for that compound (Yin et al., 2014), a LOAEL of 500 mg/kg-day was identified for gait abnormalities; this value is comparable to the LOAEL of 785 mg/kg-day identified by Ono et al. (1981) and was used as the basis for the subchronic oral p-RfD for that compound. An 8-week study focused on evaluating whether diallyl sulfide mitigates neurotoxic effects of *n*-hexane reported gait abnormalities and decreased grip strength in rats exposed to 3,000 mg/kg-day *n*-hexane (the only dose tested) (Wang et al., 2017). Krasavage et al. (1980) reported no hindlimb paralysis in a group of five male rats exposed to 4,000 mg/kg-day (5 days/week for 13 weeks) practical-grade hexane containing 40% *n*-hexane, but one of the five rats exhibited histologic evidence of neuropathy (giant axonal neuropathy) while no control rats exhibited this effect; the small number of animals tested and the lack of statistically significant change preclude determination of effect levels for this mixture.

Limited data in rats exposed orally to alkenes do not show evidence of neurotoxicity. Exposure to 1-hexene did not induce sciatic nerve histopathology at doses up to 1,000 mg/kg-day for 6−7 weeks (Gingell et al., 2000 as cited in OECD, 2004) and there was no change in rotarod performance at doses up to 3,365 mg/kg-day for 4 weeks (Dotti et al., 1994 as cited in OECD, 2004). Exposure of rats to 2,4,4-trimethylpentene (up to 1,000 mg/kg-day for 4 weeks) did not result in treatment-related effects on functional observational battery (FOB), sensory reactivity, grip strength, motor activity, or histopathology in the brain, spinal cord, or sciatic nerve (U.S. EPA, 2015). Similarly, oral exposure of rats to the C5−C7 alkene mixture at doses up to 1,000 mg/kg-day for 4 weeks did not alter FOB or histopathology of brain, spinal cord, or optic or peripheral nerve (Springborn Laboratories, 2003 as cited in OECD, 2004).

Neurological effects seen after inhalation exposure to aliphatic low carbon range compounds include peripheral neuropathy and related signs (abnormal gait and peripheral nerve atrophy), decreased hearing sensitivity, and mild narcosis or sedation (see Figure C-2). Studies examining CNS effects, including hearing sensitivity, are displayed in Figure C-3.

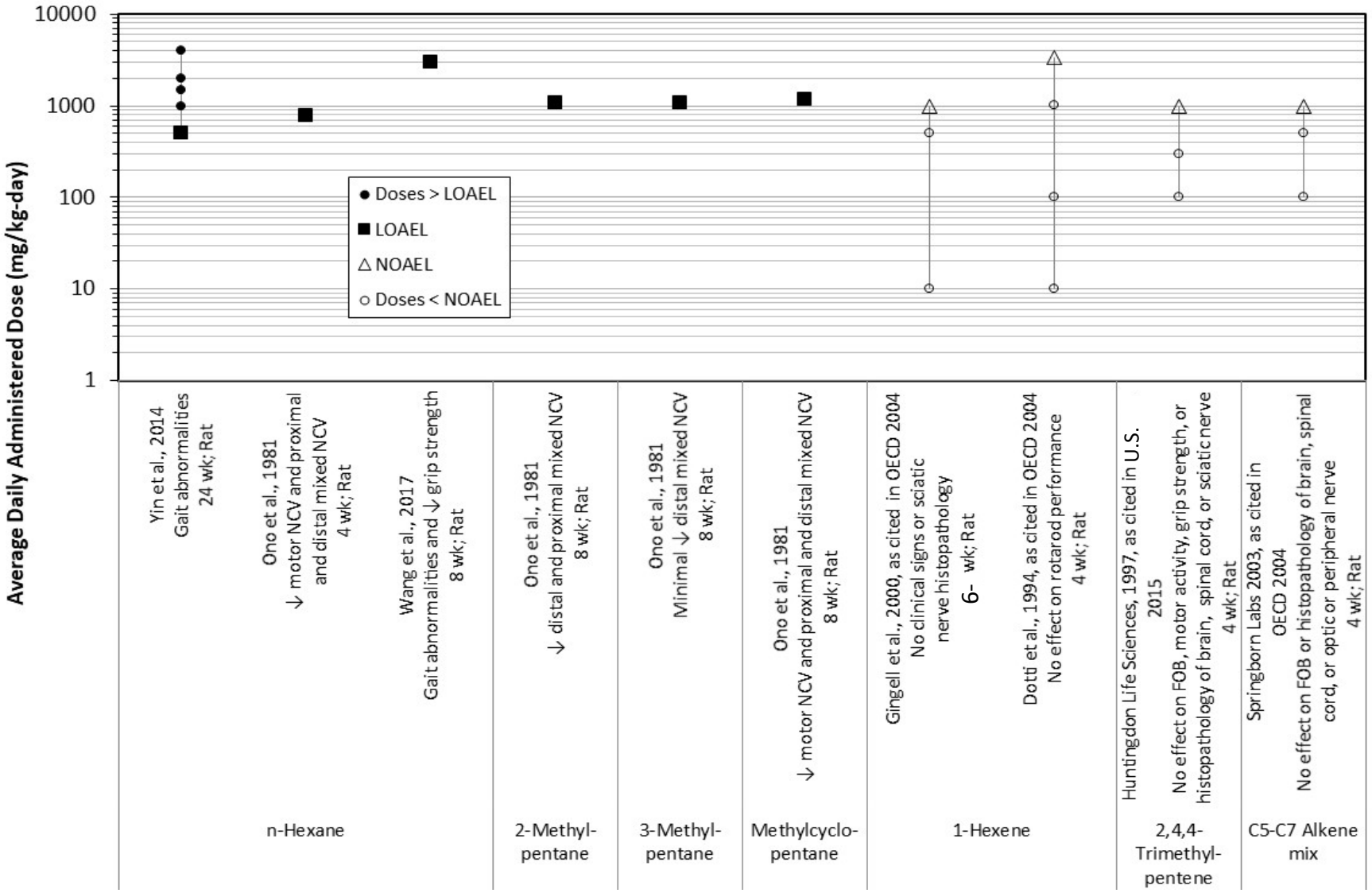

Figure C-1. Neurological Effects in Animals after Oral Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

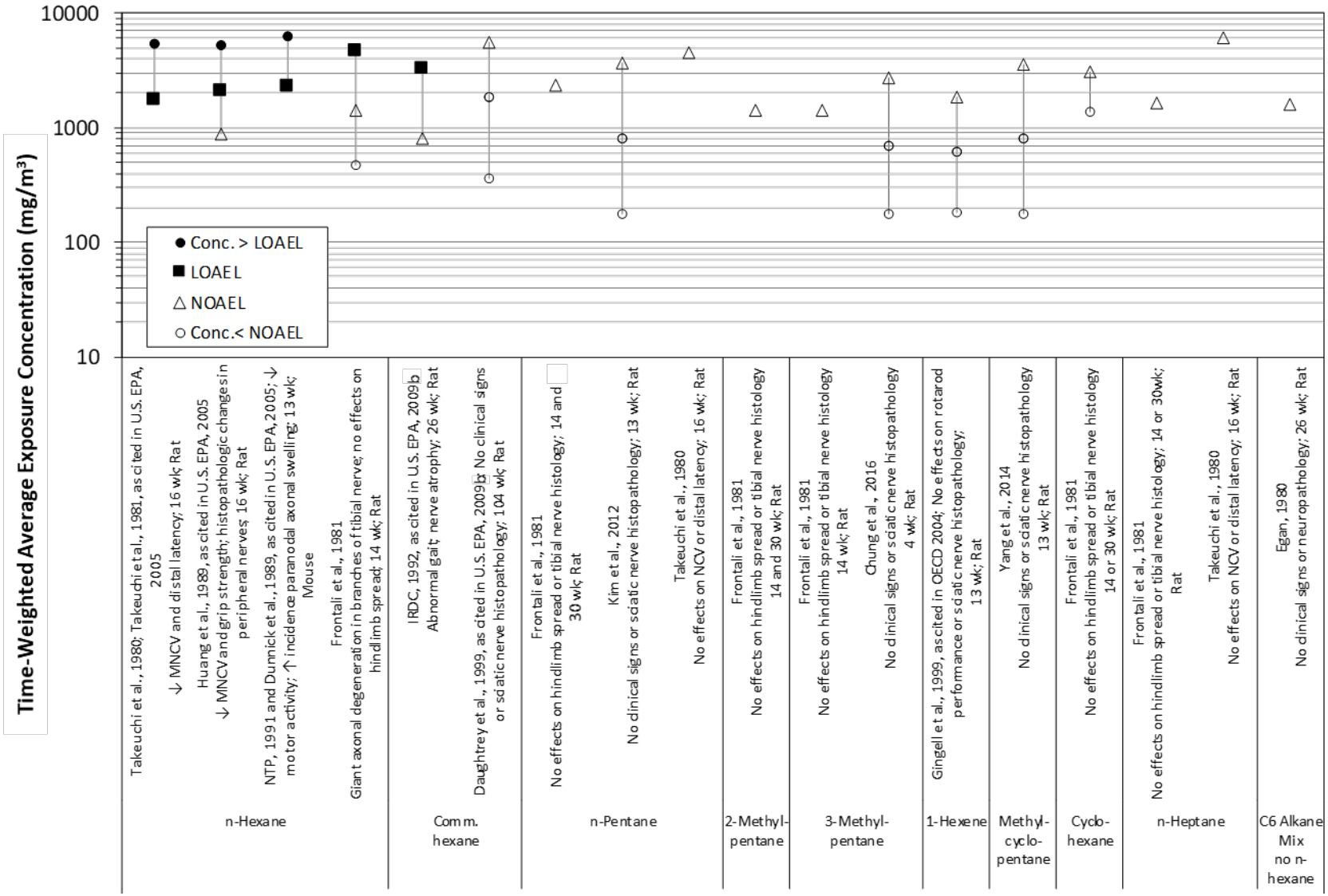

Figure C-2. Peripheral Nervous System Effects in Animals after Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

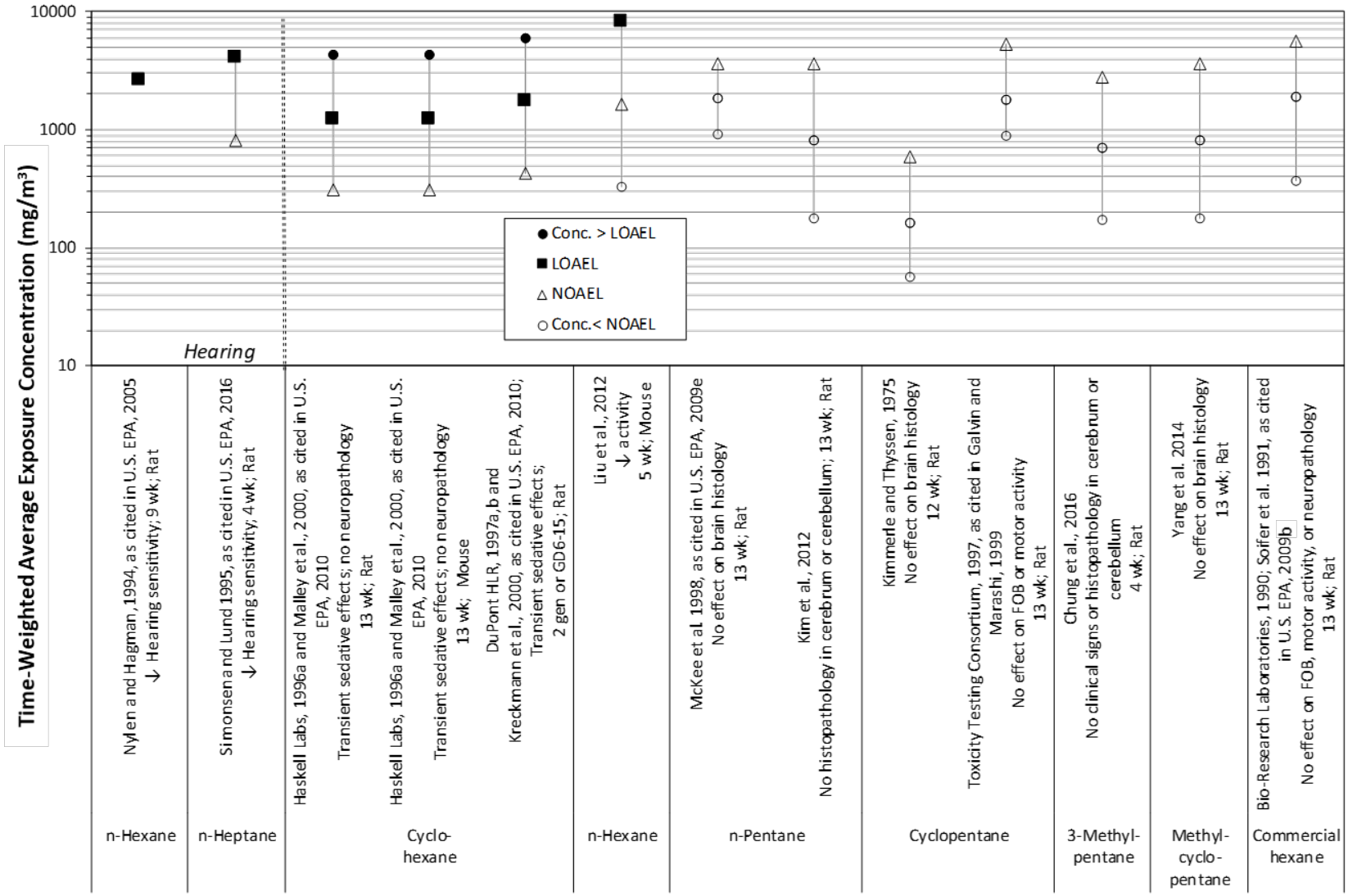

Figure C-3. Hearing Sensitivity and Other Central Nervous System Effects in Animals after Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

As Figure C-2 indicates, eight compounds, commercial hexane, and the C6 alkane mixture without *n*-hexane have been tested for different measures of peripheral neuropathy (e.g., NCV, hindlimb spread, rotarod performance, and tibial or sciatic nerve histopathology) in studies of at least 13 weeks in duration. Of the compounds tested for any peripheral nervous system effect, only *n*-hexane and commercial hexane exhibited evidence of peripheral neuropathy. Of note, exposure to 2- and 3-methylpentane and methylcyclopentane by inhalation did not result in significant effects on hindlimb spread or tibial or sciatic nerve histology, despite the fact that these compounds induced effects on NCV after oral exposure (Ono et al., 1981). The study authors of the inhalation study for methylcyclopentane (Yang et al., 2014) noted that their study was likely not adequate to evaluate potential neurological effects, as specialized histopathology preparations (teased nerve fiber preparations or Epon-embedded specimens) may be necessary to detect axonal changes. Many of the other available studies suffer from similar limitations; thus, the data from these studies should not be interpreted as providing incontrovertible evidence for a lack of peripheral nerve damage.

Decreased hearing sensitivity was observed in rats following inhalation exposure to *n*-heptane and *n*-hexane (see Figure C-3), but little information is available for this endpoint. A single study of brainstem evoked potentials in rats exposed to 1,000 ppm *n*-hexane for 18 hours/day for 9 weeks suggested slight loss of auditory sensitivity; no effect on auditory sensitivity was seen after only 4 weeks of exposure to *n*-hexane (U.S. EPA, 2005). For *n*-heptane, decreased hearing sensitivity was the critical effect in the 4-week study used to derive the p-RfC (U.S. EPA, 2016). No other fraction members were specifically tested for auditory sensitivity. In mice and rats exposed to cyclohexane, transient decreases in the sensitivity of the animals to auditory stimuli were reported, but these effects were attributed to sedation (U.S. EPA, 2010).

Volatile hydrocarbons are well-known to induce narcotic effects after acute exposure to high concentrations (Mckee et al., 2015). In longer-term studies of cyclohexane and *n*-hexane at lower exposure levels, some evidence of narcosis was observed. Transient sedative effects in the absence of histopathology changes were observed in 13-week studies of rats and mice exposed to 6,886 mg/m^3 cyclohexane (U.S. EPA, 2010); the effects were transient and generally occurred during the exposure period (U.S. EPA, 2010). Decreased activity was reported in female mice exposed to *n*-hexane at a concentration of 8,340 mg/m^3; one mouse died at this exposure level (Liu et al., 2012). Narcotic effects were not reported in other studies reviewed.

In studies examining primarily other CNS endpoints (including FOB, motor activity, and histopathology of brain), no effects were seen in rats exposed by inhalation to *n*-pentane (U.S. EPA, 2009e), cyclopentane (Toxicity Testing Consortium, 1997 as cited in Galvin and Marashi, 1999; Kimmerle and Thyssen, 1975), 3-methylpentane (Chung et al., 2016), or commercial hexane (U.S. EPA, 2009b).

Summary of Potentially Relevant Neurological Evidence

Available data indicate that neurological effects associated with oral or inhalation exposure to saturated members of the aliphatic low carbon range fraction include peripheral neuropathy, decreased hearing sensitivity, visual deficits, and CNS effects. The lowest LOAELs (by compound or mixture) for neurological endpoints (excluding transient effects for cyclohexane) ranged from 1,230 to 8,340 mg/m^3 in inhalation studies in rats and mice (see Figures C-2 and C-3) and from 500 to 1,168 mg/kg-day in subchronic oral studies in rats

(see Figure C-1). In contrast, the limited available data on unsaturated fraction members and mixtures (1-hexene, 2,4,4-trimethylpentene, and the C5−C7 alkene mixture) do not indicate neurological effects. There are data demonstrating a causal relationship between *n*-hexane exposure and peripheral neuropathy in both humans and animals. Available oral and inhalation studies of other fraction members suggest that other six carbon alkanes (including 2- and 3-methylpentane and methylcyclopentane) and commercial hexane (a mixture of primarily C6 alkanes) may also induce peripheral neuropathy. While other studies may be limited by lack of specialized histopathological evaluation for peripheral nerve damage, the remaining studies do show that compounds other than *n*-hexane do not induce severe peripheral neuropathy that would be observed as clinical signs (e.g., gait abnormalities). Exposure to *n*-hexane and *n*-heptane via inhalation have been shown to reduce auditory sensitivity in rats. Supporting data in humans are lacking, and no other studies evaluating this endpoint in animals exposed to other compounds in the fraction were located in the sources reviewed. Humans exposed to *n*-hexane have shown visual deficits, but data in animals, or in humans after exposure to other members of the fraction, were not identified. Other CNS effects have been reported to occur in humans (dizziness, headache, signs of Parkinsonism, memory loss) and animals (sedation) exposed by inhalation to several aliphatic low carbon range fraction members (*n*-hexane, cyclohexane, methylcyclohexane, and *n*-heptane).

Taken together, the available data indicate that C6 alkanes and *n*-heptane can induce neurological effects. However, because most of the other compounds in the fraction have not been explicitly tested for sensitive measures of peripheral neuropathy or hearing, it is not possible to evaluate the consistency in these endpoints and their potencies across members of the fraction.

HEPATIC EFFECTS

Hepatic effects are the critical effects for the subchronic and chronic p-RfDs and chronic p-RfC for cyclohexene (U.S. EPA, 2012b), and for the subchronic and chronic p-RfD for 2,4,4-trimethylpentene (U.S. EPA, 2015). Critical hepatic effects of cyclohexene exposure included increased serum bilirubin and spongiosis hepatis, while the critical effect of 2,4,4-trimethylpentene was increased liver weight. Few human data pertaining to the hepatotoxicity of aliphatic low carbon range fraction members are available, and those data are limited to clinical chemistry measurements in workers exposed to mixtures. As shown in Table 3, data on hepatic effects in animals were located for 14 members of the fraction. In general, the hepatic endpoints evaluated in the animal studies were liver weight and histology, with a few studies measuring clinical chemistry.

Human Studies

Few data are available to evaluate potential hepatic effects of aliphatic low carbon range fraction exposures in humans. Workers exposed to methylcyclohexane and *n*-heptane (in addition to toluene and xylene) exhibited statistically significant elevations of urinary bile acids, urinary 6β-hydroxycortisol, and ratio of 6β-hydroxycortisol to urinary free cortisol (considered by the study authors to be sensitive measures of hepatotoxicity) compared with the control workers with normal liver function (U.S. EPA, 2013). No differences were seen between these two groups in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyl transferase (GGT), bilirubin, or urinary D-glucaric acid levels. No changes to clinical chemistry parameters were reported in a study of workers exposed to glue containing at least 75% cyclohexane; however, the findings of this study were limited by the

small cohort size (n = 38), discrepancies in reporting of analytical air concentrations, and absence of details related to the clinical chemistry parameters that were evaluated (U.S. EPA, 2013).

Animal Studies

As shown in Figure C-4, data on hepatic effects of oral exposure to aliphatic low carbon range compounds are limited to 4−8-week rat studies. Exposure to either >300 mg/kg-day 2,4,4-trimethylpentene (U.S. EPA, 2015) or 1,000 mg/kg-day C5−C7 alkene mixture (Springborn Laboratories, 2003 as cited in OECD, 2004) induced increases in absolute and/or relative liver weight, without concomitant histopathology changes. Rats exposed to cyclohexene for 7 weeks exhibited increased serum total bilirubin and bile acids at doses ≥50 mg/kg-day (U.S. EPA, 2012b). No changes in liver weight or histology were observed in rats exposed to 1-hexene (up to 1,000 mg/kg-day) for 6−8 weeks (Gingell et al., 2000 as cited in Carreón and Herrick, 2012; OECD, 2004).

Figure C-5 displays the exposure-response array for hepatic effects of inhalation exposures up to 26 weeks in duration. Only two fraction members were tested in longer-term (1−2-year) studies (cyclohexene and commercial hexane); these data were not arrayed as they were not considered to be comparable to the shorter-duration studies. Hepatic effects, primarily consisting of liver weight changes without effects on hepatic histopathology, were reported in rats exposed for up to 26 weeks to 3-methylpentane, commercial hexane, methylcyclopentane, cyclohexane, and n-hexane. Histologic changes in the liver were seen only with chronic exposure to cyclohexene and subchronic exposure to commercial hexane and cyclohexane. Chronic (2-year) exposure to cyclohexene resulted in an increased incidence of spongiosis hepatis at concentrations ≥720 mg/m^3 (U.S. EPA, 2012b). Slight hemorrhage and inflammation in the livers were noted in a few male rats exposed to 5,639 mg/m^3 commercial hexane for 13 weeks (U.S. EPA, 2009b). However, chronic (2-year) exposure to commercial hexane at concentrations up to 5,639 mg/m^3 did not result in any histopathology changes in the livers of rats or mice (U.S. EPA, 2009b). Increased liver weights and an increase in the incidence of centrilobular hepatocellular hypertrophy were observed in rats after exposure to 24,101 mg/m^3 cyclohexane for 13 weeks (U.S. EPA, 2010). In a companion experiment in mice, liver weights were increased without clinical chemistry or histology changes (U.S. EPA, 2010).

Exposure of rats to 2,648 mg/m^3 3-methylpentane for 4 weeks (Chung et al., 2016) or 3,608 mg/m^3 methylcyclopentane for 13 weeks (Yang et al., 2014) resulted in increased relative liver weights (in the absence of body-weight changes), but no effects on histopathology. Increased relative liver weights without histopathology changes were observed in mice exposed to 6,294 mg/m^3 n-hexane for 13 weeks, but body-weight decreases also occurred in this group (U.S. EPA, 2005). Increases in total serum cholesterol and serum albumin were observed in male, but not female, rats exposed for 13 weeks to 167 mg/m^3 n-octane, but there were no other clinical chemistry changes or effects on liver weight or histopathology; these effects were not considered to be adverse (Sung et al., 2010). No hepatic effects were noted in rats after subchronic exposure to n-pentane (Kim et al., 2012), cyclopentane (Toxicity Testing Consortium, 1997 as cited in Galvin and Marashi, 1999; Kimmerle and Thyssen, 1975), 1-hexene (Gingell, 1999 as cited in OECD, 2004), or 2,2,4-trimethylpentane (IUCLID, 2000 as cited in Johnson et al., 2012).

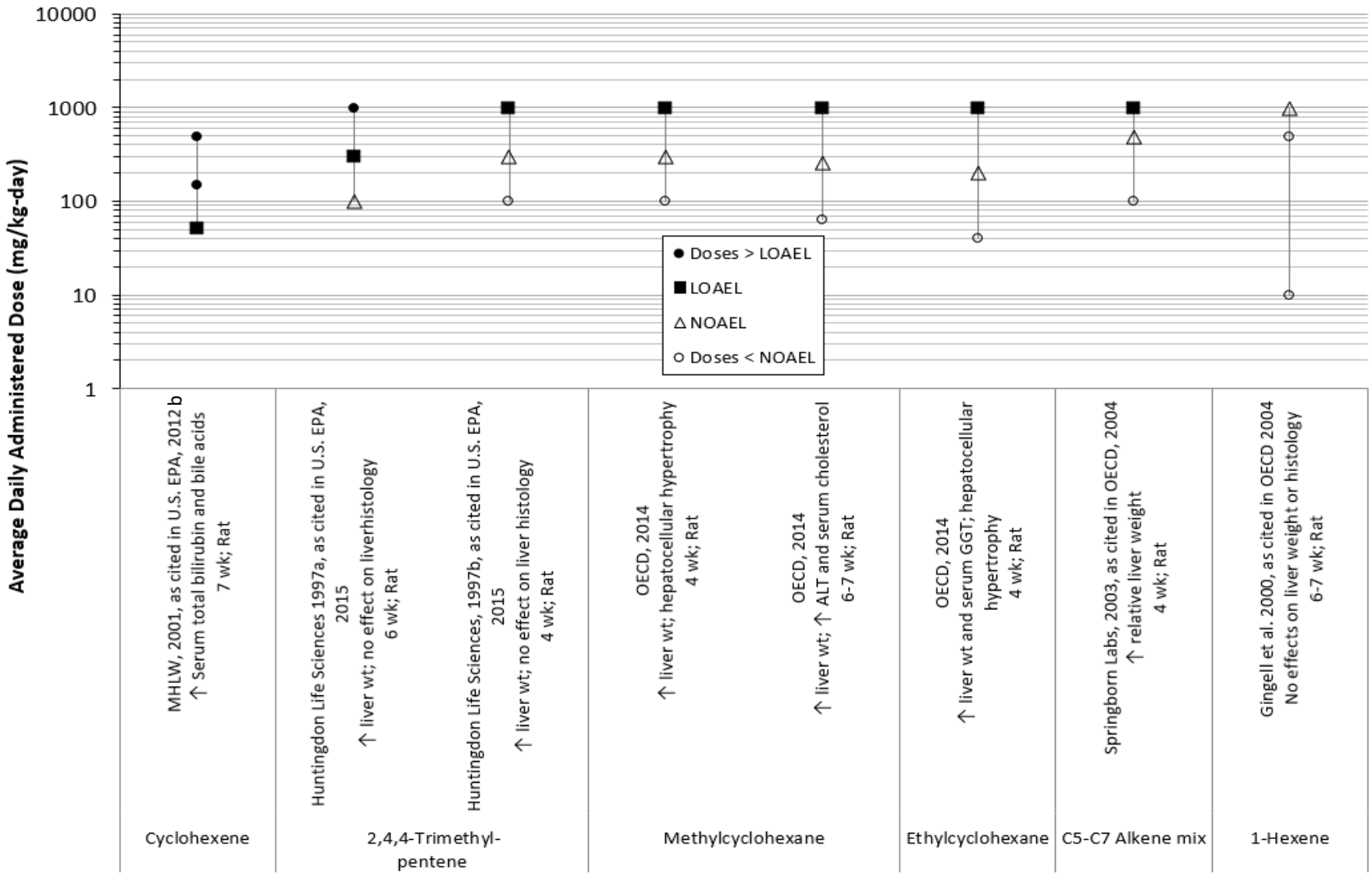

Figure C-4. Hepatic Effects in Animals after Oral Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

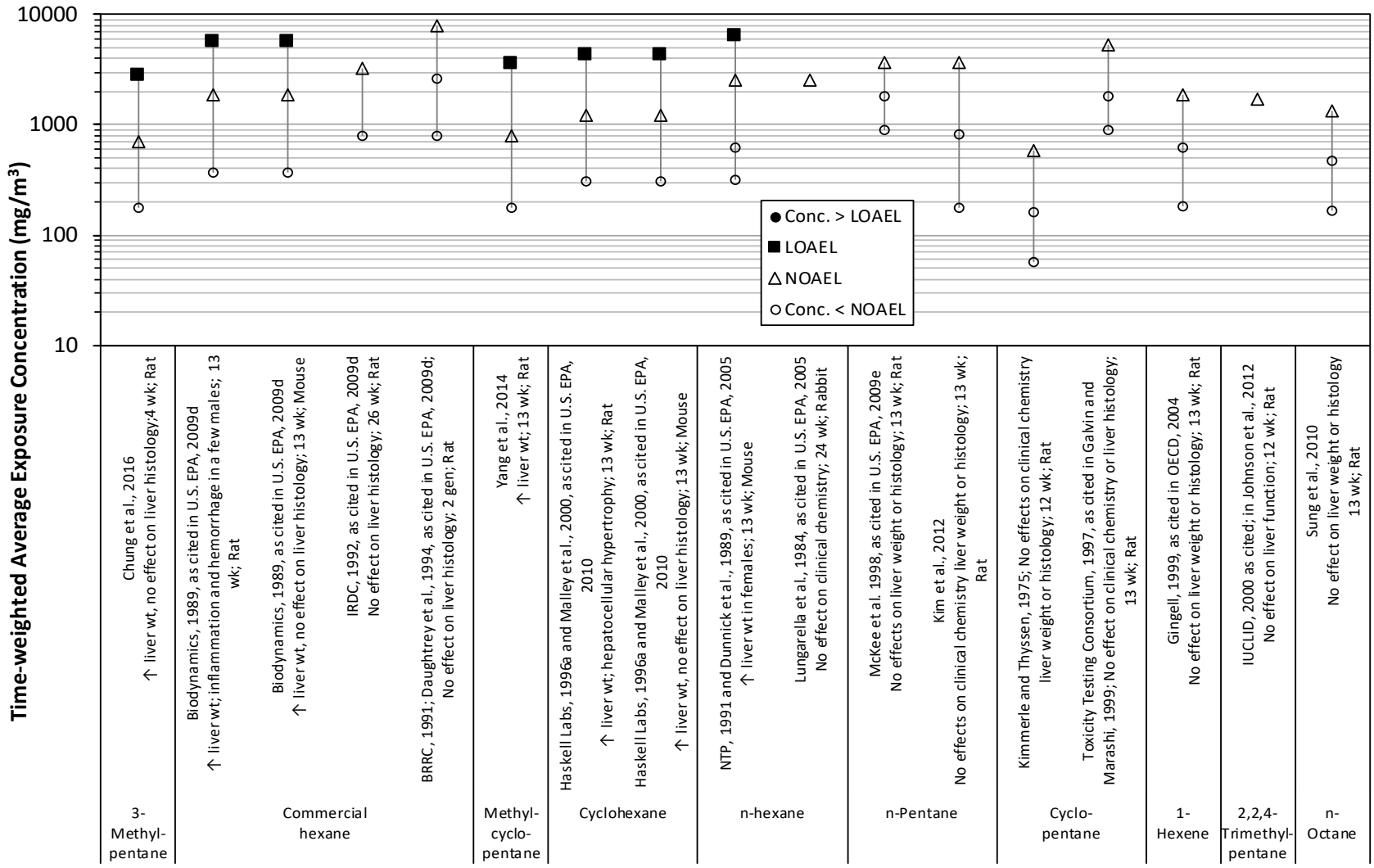

Figure C-5. Hepatic Effects in Animals after Subchronic Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

Summary of Potentially Relevant Hepatic Evidence

Oral studies examining liver effects were limited to five compounds and one mixture (C5−C7 alkenes) in studies of 4−7 weeks in duration, and most showed increases in liver weight. Hepatic effects, primarily consisting of increased relative liver weights in the absence of body-weight changes, were also seen in inhalation studies in laboratory animals exposed to at least one compound with six, seven, and eight carbons, and with linear, branched, cyclic, and unsaturated structures. Histopathological changes in the livers of animals exposed to aliphatic low carbon range fraction members varied, consisting of hepatocellular hypertrophy in subchronic oral and inhalation studies and spongiosis hepatis in a chronic inhalation study. Lowest LOAELs (by compound or mixture) for hepatic endpoints ranged between 2,763.3 and 6,294 mg/m^3 in subchronic inhalation studies in rats and mice (see Figure C-5) and varied from 50 to 1,000 mg/kg-day in subchronic oral studies in rats (see Figure C-4). Too few chronic studies were available to compare effects and potencies after longer exposures. In aggregate, the data suggest that many aliphatic low carbon range fraction compounds and mixtures can produce increases in rodent liver weight, occasionally in tandem with histological (hepatocellular hypertrophy) or serum chemistry (increases in bilirubin, ALT, or GGT) changes, and that potencies are generally comparable in inhalation studies, but more variable in oral studies.

BODY-WEIGHT EFFECTS

Decreased body weight was a cocritical effect in the study used to derive the subchronic p-RfC for commercial hexane (U.S. EPA, 2009b). No human studies examining body-weight effects of aliphatic low carbon range compounds were identified in the sources reviewed.

As Table 3 shows, animal studies that examined body weight as an endpoint are available for nearly all of the compounds and mixtures with toxicity data; exceptions are ethylcyclohexane and practical-grade hexane. In this section, body-weight changes of at least 10% relative to controls in adult animals are considered LOAELs, and smaller changes are not. For studies that reported body-weight gain but did not report absolute body weights, and for studies of maternal weight gain during gestation, statistically significant changes from control are described.

Animal Studies

Figure C-6 shows the effects of orally-administered aliphatic low carbon range compounds and mixtures on body weight; data are available for 14 compounds and one mixture, including compounds with carbon numbers across the entire range (C5−C8). Body-weight decreases were seen with several C5−C6 compounds: *n*-pentane, 2,3-dimethylbutane, *n*-hexane, 2-methyl-2-pentene, and methylcyclopentane. No effects on body weight were seen in studies of compounds of higher (EC ≥ 6.68) equivalent carbon number (U.S. EPA, 2015, 2012b; Til et al., 1986 as cited in OECD, 2004; Halder et al., 1985).

Body-weight effects in animals exposed by inhalation for subchronic (up to 16 weeks) or chronic durations (26 weeks to 2 years) are shown in Figures C-7 and C-8. In inhalation studies, reductions in body weight were reported to occur in rats and/or mice exposed for up to 16 weeks to *n*-hexane, 2-methylpentane, 1-hexene, and 2,2,4-trimethylpentene at concentrations >1,000 mg/m^3; and in rats, mice, or hamsters exposed for ≥26 weeks to *n*-pentane, *n*-hexane, methylcyclohexane, and commercial hexane at concentrations ranging from 268 to 5,639 mg/m^3.

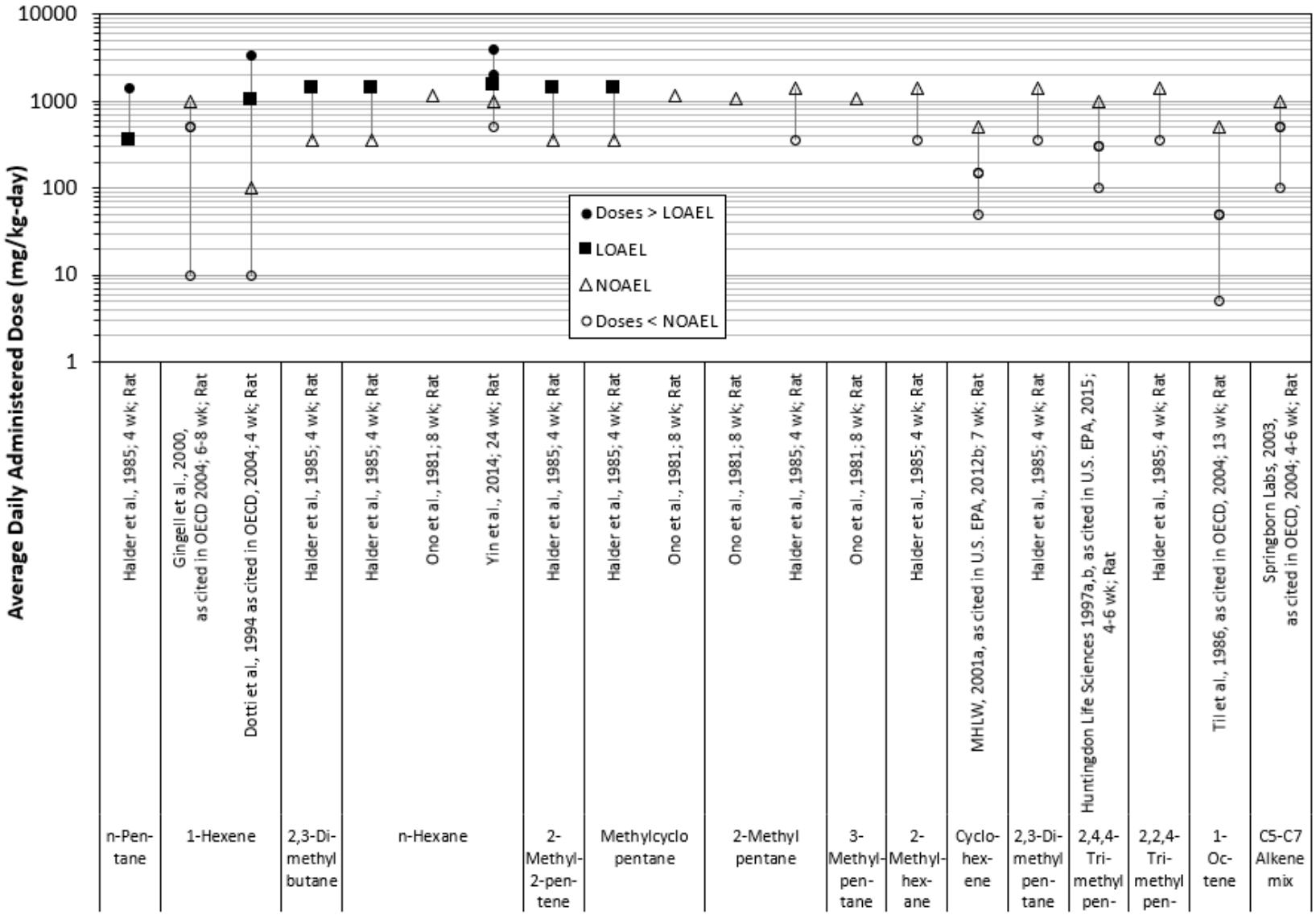

Figure C-6. Decreases in Body Weight in Animals after Oral Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

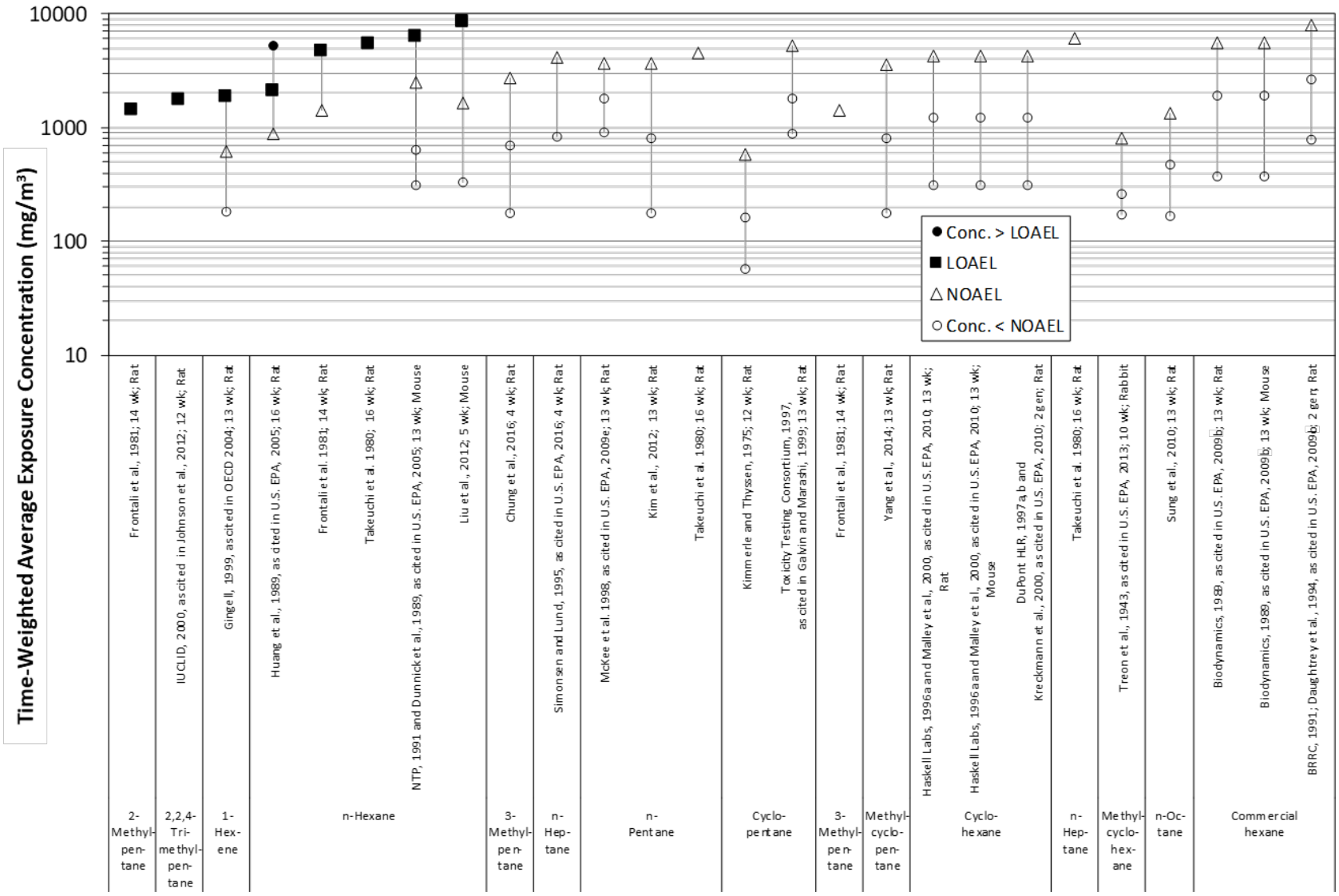

Figure C-7. Decreases in Body Weight in Animals after Subchronic (4−16 weeks) Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

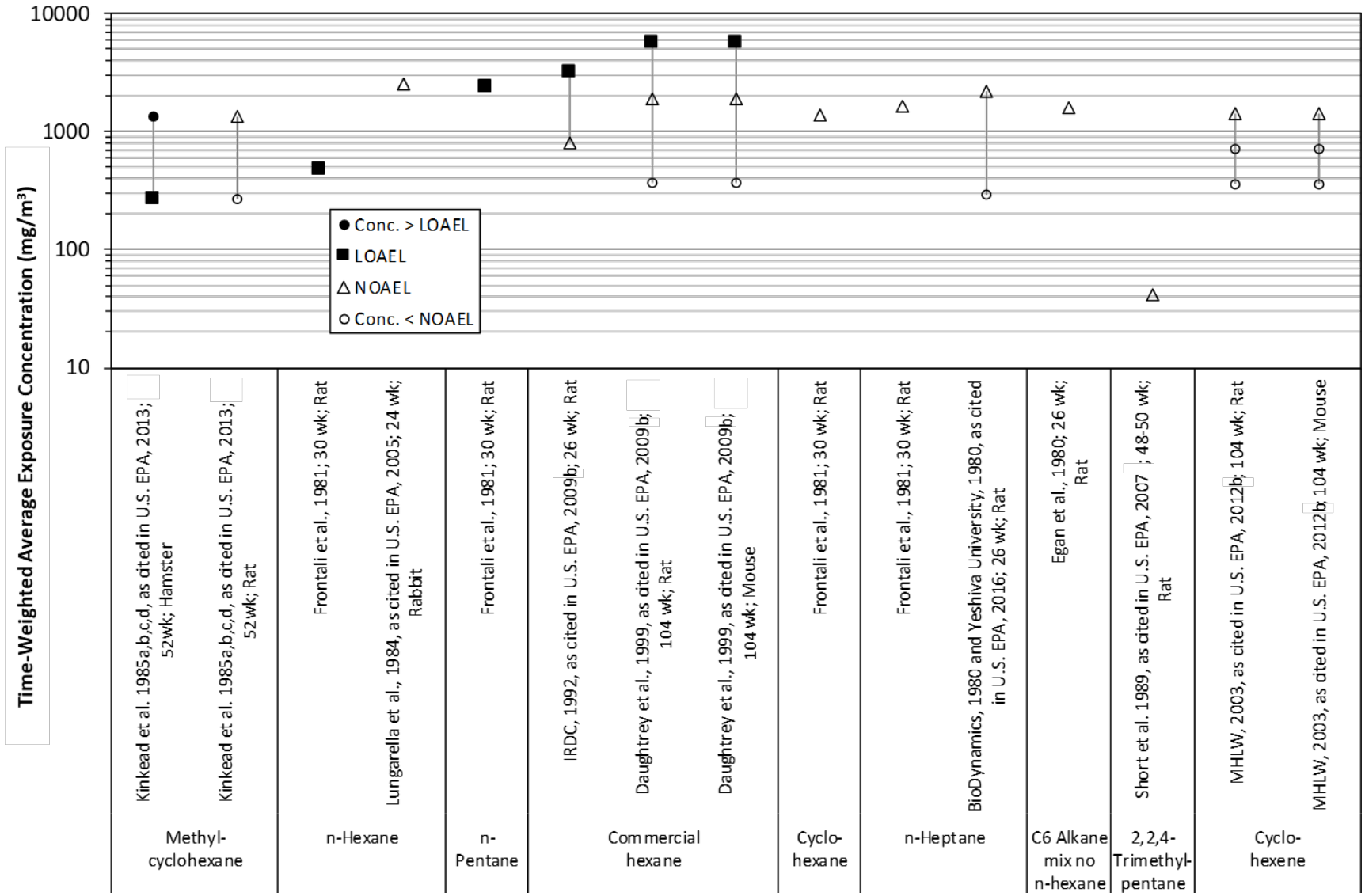

Figure C-8. Decreases in Body Weight in Animals after Chronic (24−104 weeks) Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

Body-weight changes associated with gestational exposure are not shown in the figures. Maternal body-weight reductions were reported in pregnant rats and mice exposed during gestation to *n*-hexane at a concentration of 14,686 mg/m^3 (U.S. EPA, 2005) and in pregnant rats exposed to commercial hexane (≥2,632 mg/m^3) (U.S. EPA, 2009b) or cyclohexane (≥1,722 mg/m^3) (U.S. EPA, 2010). No effect on maternal body weight was noted in pregnant rats exposed to *n*-pentane concentrations up to 7,377 mg/m^3 on gestation days (GDs) 6−15 (U.S. EPA, 2009e).

No body-weight changes were observed in studies of adult rats, mice, or rabbits exposed by inhalation or oral administration for at least 4 weeks to cyclopentane, 3-methylpentane, *n*-heptane, *n*-octane, or the C6 alkane mixture without *n*-hexane.

Summary of Potentially Relevant Body Weight Evidence

Compounds and mixtures in the aliphatic low carbon range fraction have been shown to reduce body weights of rats, mice, and hamsters after oral and inhalation exposure. Individual compounds that induced body-weight changes after inhalation exposure include compounds across the entire carbon range (C5−C8) and compounds representing linear, branched, cyclic, and unsaturated structures. Lowest LOAELs ranged between 1,414 and 5,357 mg/m^3 in rats and between 6,294 and 8,340 mg/m^3 in mice in subchronic inhalation studies (see Figure C-7). In chronic inhalation studies, a LOAEL of 268 mg/m^3 was identified in hamsters; LOAELs ranged between 472 and 5,639 mg/m^3 in rats and mice (see Figure C-8). In oral studies, body-weight decreases were seen with several C5−C6 compounds, but compounds with higher equivalent carbon numbers (EC ≥ 6.68) did not induce body-weight changes. Lowest LOAELs (by compound or mixture) for body-weight endpoints ranged between 357 and 1,500 mg/kg-day in subchronic oral studies in rats (see Figure C-6). Taken together, the inhalation and oral animal data indicate that compounds in the aliphatic low carbon range fraction can be expected to induce body-weight reductions at sufficiently high doses (generally ≥1,000 mg/kg-day for most compounds or duration-adjusted concentrations ≥1,000 mg/m^3 after less-than-chronic exposures).

GASTROINTESTINAL EFFECTS

The *n*-heptane screening subchronic and chronic p-RfDs are based on analogue read-across analysis using *n*-nonane as the analogue; forestomach lesions were the critical effect in the study of *n*-nonane (U.S. EPA, 2016). No human studies examining gastrointestinal (GI) effects of aliphatic low carbon range compounds were identified in the sources reviewed. Data on GI effects of aliphatic low carbon range compounds in animals exposed by oral and inhalation routes were limited, so exposure-response arrays are not developed for this endpoint.

Animal Studies

The subchronic and chronic oral p-RfDs for *n*-heptane are based on an analogue, *n*-nonane (C9 [EC9]), and forestomach histopathology (hyperplasia and hyperkeratosis at doses ≥100 mg/kg-day administered by gavage as neat compound 7 days/week) (U.S. EPA, 2016). Irritation of the gastric mucosa was noted at both gross and microscopic examination of rats exposed by gavage to 1-hexene (as neat compound) at doses ≥1,010 mg/kg-day for 4 weeks (Dotti et al., 1994 as cited in OECD, 2004). No histopathology findings were observed in the stomach or large or small intestines of rats exposed to 2,4,4-trimethylpentene in maize oil at doses up to 1,000 mg/kg-day for 4 weeks (U.S. EPA, 2015) or the C5−C7 alkene mixture in corn oil at doses up to 1,000 mg/kg-day for 4−6 weeks (Springborn Laboratories, Inc., 2003 as cited

in OECD, 2004). None of the remaining oral studies of compounds within the C5−C8 range evaluated GI tract histopathology, and the only related data available were gross necropsy findings in the stomach. In the unpublished version of the Halder et al. (1985) gavage study, API (1985) reported grossly observed stomach changes including ulcers, edema, and reddened areas; these effects were seen in 80−100% of the animals treated with each of the tested compounds in the C5−C8 range (affected dose groups were not reported; duration-adjusted doses tested in the study were 357 and 1,429 mg/kg-day). All of the compounds (including *n*-pentane, 2,3-dimethylbutane, 2-methylpentane, *n*-hexane, 2-methyl-2-pentene, methylcyclopentane, 2-methylhexane, 2,3-dimethylpentane, and 2,2,4-trimethylpentane) were administered neat (without solvent) in that study.

No inhalation studies of aliphatic low carbon range compounds or mixtures have identified GI effects. Studies that examined the GI tract for histopathology changes reported no effects in rats after exposure for 4−13 weeks to *n*-pentane (U.S. EPA, 2009e), cyclopentane (Kimmerle and Thyssen, 1975), 3-methylpentane (Chung et al., 2016), methylcyclopentane (Yang et al., 2014), or *n*-octane (Sung et al., 2010), or in mice exposed to *n*-hexane for 13 weeks (U.S. EPA, 2005). Chronic (2-year) studies of commercial hexane in rats and mice exposed by inhalation to duration-adjusted concentrations up to 5,639 mg/m^3 also showed no treatment-related histopathology in the GI tract (U.S. EPA, 2009b).

Summary of Potentially Relevant Gastrointestinal Evidence

Irritant responses in the GI tract were observed macroscopically in rats exposed by gavage to neat alkanes in the C5−C8 range (Halder et al., 1985), and forestomach histopathology was seen in rats exposed by gavage to neat *n*-nonane (the analogue for *n*-heptane). API (1985) and Halder et al. (1985) reported gross changes in the stomach collectively for the tested C5−C8 compounds as a group; thus, effect levels could not be determined. Histopathology changes were not seen after inhalation exposure to compounds in the fraction, and histopathology evaluations of the GI tract were lacking for most of the available oral studies. It appears from these observations that oral exposure to undiluted members of the fraction may result in direct effects on the GI tract. However, available data are not considered sufficient to evaluate the consistency in GI effects and potencies across fraction members.

RESPIRATORY EFFECTS

Nasal and laryngeal lesions represent the critical effect for the chronic RfC for commercial hexane (U.S. EPA, 2009b). No information on respiratory effects in humans exposed to aliphatic low carbon range compounds or mixtures was identified in the sources reviewed. Animal studies examining respiratory tract endpoints are available for nine compounds and two mixtures (see Table 3); the preponderance of the animal data is from subchronic inhalation studies.

Animal Studies

Only two of the available oral studies of compounds or mixtures relevant to the aliphatic low carbon range fraction examined respiratory tract effects in animals, and no oral studies examined nasal pathology. No histopathology changes were observed in the lungs of rats given 2,4,4-trimethylpentene at doses up to 1,000 mg/kg-day for 4 weeks (U.S. EPA, 2015) or in the lungs or tracheas of rats given the C5−C7 alkene mixture at doses up to 1,000 mg/kg-day for 4−6 weeks (Springborn Laboratories, Inc., 2003 as cited in OECD, 2004). Due to the limited data

and lack of effects, an exposure-response array is not presented for respiratory effects after oral exposure.

Figure C-9 shows the exposure-response data for respiratory effects from studies of animals exposed by inhalation. Animal studies in which the nasal cavity, nasal turbinates, and/or larynx were examined after inhalation exposure include a 4-week rat study of 3-methylpentane; subchronic rat and mouse studies of *n*-pentane, 1-hexene, *n*-hexane, methylcyclopentane, and *n*-octane; and chronic studies of commercial hexane in rats and mice. In mice exposed to ≥629 mg/m^3 *n*-hexane for 13 weeks, nasal histopathology changes included inflammation, erosion, regeneration, and metaplasia in the olfactory and/or respiratory epithelium (U.S. EPA, 2005). Nasal and laryngeal histopathology changes (hyperplasia of epithelial and goblet cells, chronic inflammation, and increased incidence of intracytoplasmic eosinophilic material in nasal turbinates; squamous metaplasia/hyperplasia of the columnar epithelium in the larynges) were observed in rats after 2 years of exposure to commercial hexane concentrations ≥564 mg/m^3 (U.S. EPA, 2009b). No histopathology changes in the nasal cavity, nasal turbinates, and/or larynx were observed in rats exposed to *n*-pentane by inhalation for 13 weeks (Kim et al., 2012), 3-methylpentane for 4 weeks (Chung et al., 2016), 1-hexene for 13 weeks (Gingell, 1999 as cited in OECD, 2004), methylcyclopentane for 13 weeks (Yang et al., 2014), or *n*-octane for 13 weeks (Sung et al., 2010), generally at concentrations exceeding 1,000 mg/m^3.

Few reports of lower respiratory tract effects were located in the sources reviewed. Enlargement of the air spaces in respiratory bronchioles and alveolar ducts and pulmonary fibrosis, along with papillary tumors of nonciliated bronchial epithelial cells were observed in rabbits exposed to *n*-hexane for 24 weeks at a concentration of 2,610 mg/m^3 (U.S. EPA, 2005). Gestational exposure studies of commercial hexane in rats and mice reported gross observations of pulmonary color change in dams at 7,894 mg/m^3 (U.S. EPA, 2009b). Other studies in rats or mice reported no treatment-related effects on the lung or lower respiratory tract histopathology after exposure to *n*-pentane, cyclopentane, 3-methylpentane, 1-hexene, methylcyclopentane, cyclohexane, or *n*-octane for 4−13 weeks (see Figure C-9).

Summary of Potentially Relevant Respiratory Evidence

Respiratory effects consisting of nasal and/or laryngeal lesions were reported in animals exposed to *n*-hexane and commercial hexane by inhalation, and limited evidence for bronchiolar and pulmonary changes after exposure to these materials has been reported. LOAELs ranged from 629 mg/m^3 in mice to 2,517 mg/m^3 in rabbits (see Figure C-9). Studies of other compounds did not show effects on the upper and/or lower respiratory tract. Thus, respiratory effects have not been consistently shown to be associated with oral or inhalation exposure to members of the aliphatic low carbon range fraction.

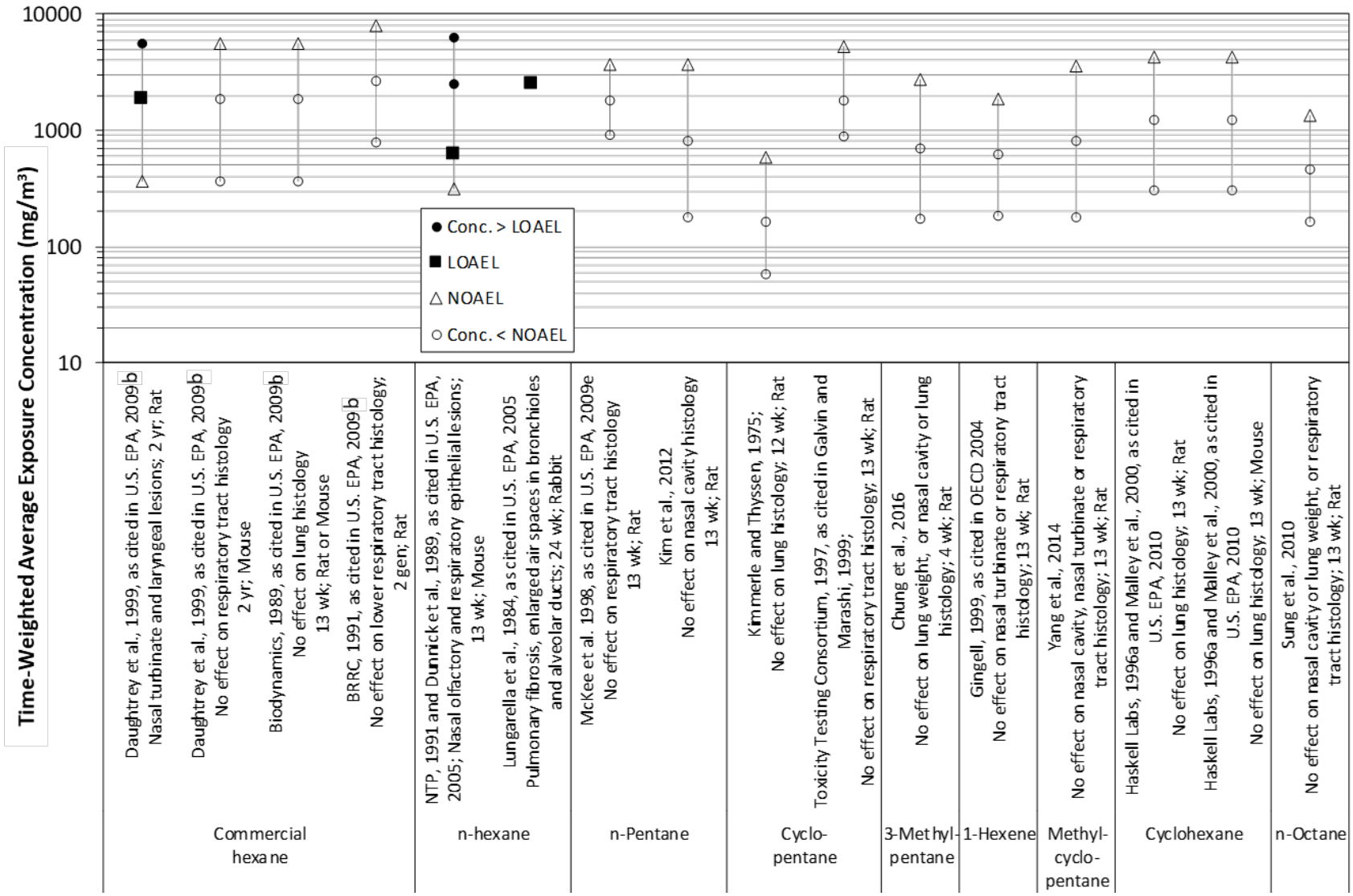

Figure C-9. Respiratory Tract Effects in Animals after Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

DEVELOPMENTAL EFFECTS

Developmental toxicity, manifested as reduced offspring weights, is the critical effect for the subchronic and chronic RfCs for cyclohexane (U.S. EPA, 2010, 2003). No human studies were available to address the potential for developmental toxicity of the aliphatic low carbon range total petroleum hydrocarbon (TPH) fraction. Animal studies of developmental toxicity are available for seven compounds and two mixtures; most of the data are from inhalation studies.

Animal Studies

Developmental studies of aliphatic low carbon range compounds and mixtures in animals exposed orally include teratogenicity studies of *n*-pentane and *n*-hexane, as well as combined repeated-dose and reproductive/developmental screening studies in rats exposed to 1-hexene, cyclohexene, 2,4,4-trimethylpentene, methylcyclohexane, ethylcyclohexane, or the C5−C7 alkene mixture. In mice exposed to *n*-hexane on GDs 6−15, fetal birth weights were decreased at doses ≥7,920 mg/kg-day, but maternal mortalities also occurred at these doses (U.S. EPA, 2005). No developmental effects were seen in rats exposed to *n*-pentane at doses up to 1,000 mg/kg-day during gestation (U.S. EPA, 2009e). The screening reproductive and developmental toxicity studies showed no developmental effects at doses up to 500 mg/kg-day (cyclohexene) (U.S. EPA, 2012b) or 1,000 mg/kg-day (1-hexene, 2,4,4-trimethylpentene, methylcyclohexane, ethylcyclohexane, and the C5−C7 alkene mixture) (U.S. EPA, 2015; OECD, 2014; Gingell et al., 2000 and Springborn Laboratories, Inc., 2003 as cited in OECD, 2004); however, these studies included only limited developmental toxicity evaluations (some were limited to pup weight and viability) and did not assess teratogenicity. Due to the limited data and absence of effects, an exposure-response array is not presented for developmental effects after oral exposure.

Data on developmental toxicity in animals exposed by inhalation are available for *n*-pentane, *n*-hexane, cyclohexane, and commercial hexane. *n*-Pentane has been studied only in a screening-level developmental toxicity assay, while more complete developmental toxicity data in two species are available for the remaining compounds, and two-generation reproductive toxicity studies are available for cyclohexane and commercial hexane. Figure C-10 displays the exposure-response information from these studies. In the screening-level study of *n*-pentane, no effects on number of implantations, viable fetuses, or incidences of external malformations were observed in rats exposed to concentrations up to 7,380 mg/m^3 on GDs 6−15 (U.S. EPA, 2009e). Decreased pup growth was observed in rats exposed to *n*-hexane during gestation to concentrations ≥881 mg/m^3 (duration-adjusted) and in mice exposed to 14,686 mg/m^3 (U.S. EPA, 2005). Increased incidences of skeletal variations were also reported in rats exposed to 14,686 mg/m^3 *n*-hexane (U.S. EPA, 2005); this finding may have been influenced by decreased fetal body weights at this exposure level. In mice exposed to *n*-hexane during gestation, decreases in the number of live fetuses per litter were reported at concentrations ≥7,500 mg/m^3 (Li et al., 2015; Li et al., 2014; U.S. EPA, 2005); a decrease in percent live implants and an increase in the incidence of late resorptions were also seen at 14,686 mg/m^3 (U.S. EPA, 2005).

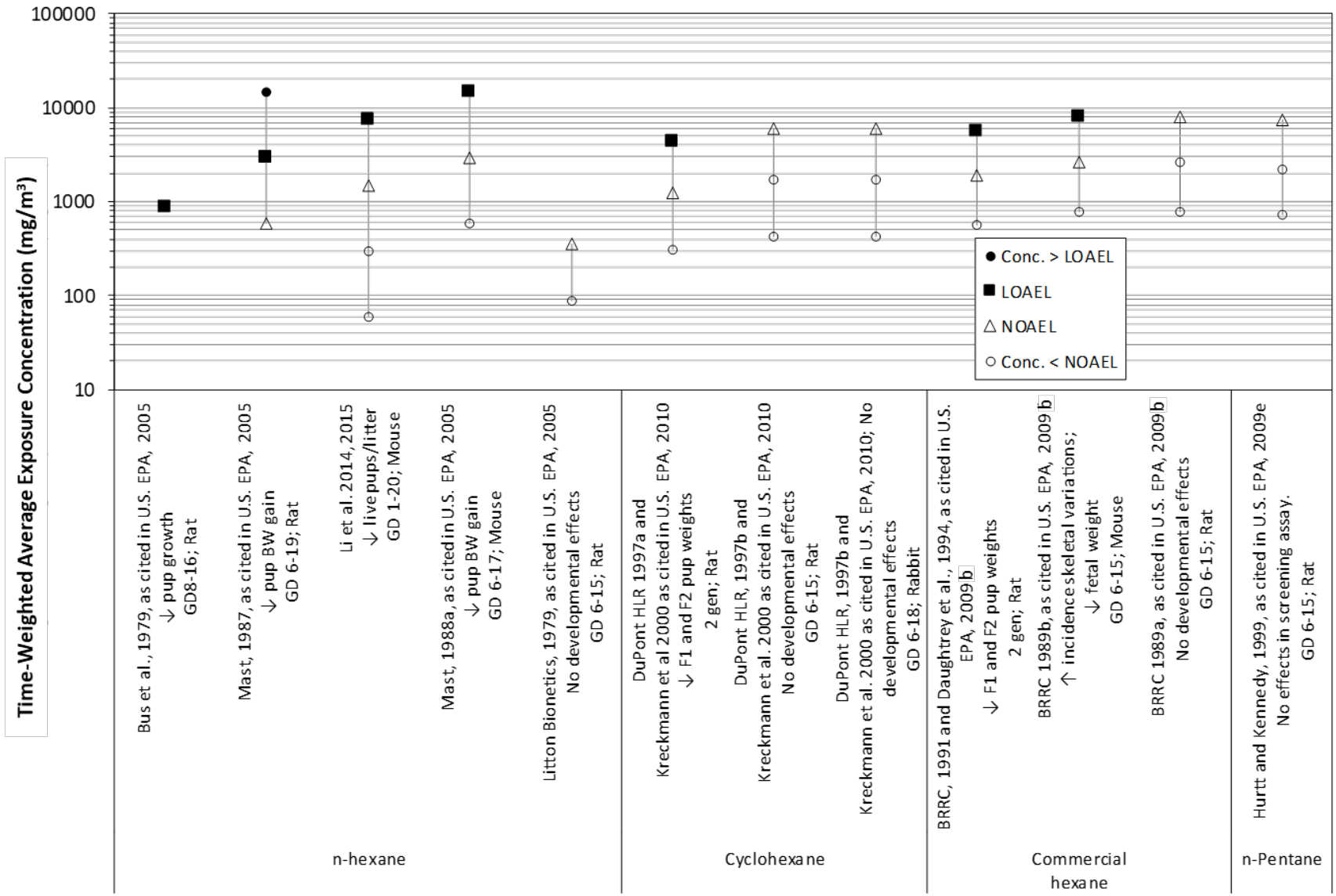

Figure C-10. Developmental Effects in Animals after Inhalation Exposure to Aliphatic Low Carbon Range Compounds and Mixtures

Cyclohexane induced decreases in F_1 and F_2 pup weights (during lactation) at a duration-adjusted concentration of 4,304 mg/m^3 in a two-generation rat reproductive toxicity study, while no effects on fetal weights or other measures of developmental toxicity were seen in rats and rabbits exposed to cyclohexane at 6,025 mg/m^3 during gestation [GDs 6−15 in rats or GDs 6−18 in rabbits (U.S. EPA, 2010)]. A two-generation reproductive toxicity study of commercial hexane also reported decreased F_1 and F_2 offspring weights (postnatal days [PNDs] 14 and 7, respectively) in rats at a duration-adjusted concentration of 5,639 mg/m^3 (U.S. EPA, 2009b). Exposure to 7,985 mg/m^3 commercial hexane had no effect on GD 21 fetal weights or developmental toxicity endpoints in rats when exposure was limited to GDs 6−15 (U.S. EPA, 2009b). In mice exposed to commercial hexane at 7,895 mg/m^3 during gestation, an increase in the incidence of skeletal variations was seen in the absence of pup weight changes (U.S. EPA, 2009b).

Summary of Potentially Relevant Developmental Evidence

Limited developmental toxicity data, which lack teratogenicity assessments, are available for *n*-pentane, 1-hexene, cyclohexene, 2,2,4-trimethylpentene, and the C5−C7 alkene mixture. More robust developmental toxicity data are available for *n*-hexane, cyclohexane, and commercial hexane. The available oral and inhalation data suggest that *n*-hexane, cyclohexane, and commercial hexane reduced body weights in rat offspring, while 1-hexene, cyclohexene, 2,4,4-trimethylpentene, methylcyclohexane, ethylcyclohexane, and the C5−C7 alkene mixture did not. Exposure to *n*-hexane and commercial hexane via inhalation increased the incidences of skeletal variations in rats and mice, respectively, when exposed during gestation, but cyclohexane and *n*-pentane did not; data on skeletal variations and malformations were not available for the remaining compounds. Only *n*-hexane exposure (by inhalation) has been shown to affect embryonic or fetal viability. In summary, too few compounds have received rigorous testing for developmental effects, so the available developmental toxicity data are not adequate to assess consistency in effects or potencies of the compounds and mixtures in the fraction.

OTHER EFFECTS

New studies identified in the PubMed searches for *n*-hexane identified effects on ovarian function in female mice exposed by inhalation. Liu et al. (2012) reported reduced egg production and serum progesterone levels at duration-adjusted *n*-hexane exposure concentrations ≥330 mg/m^3 and decreases in diestrus duration and number of ovarian follicles after 5 weeks of exposure (4 hours/day, 7 days/week). Alterations in the proportions of secondary and atretic ovarian follicles, estrous cycle disruptions, and changes in the secretion of progesterone and estradiol by cultured ovarian granulosa cells from exposed offspring were also reported in female offspring of mice exposed to *n*-hexane during gestation (Li et al., 2015; Li et al., 2014). No other studies of ovarian function in humans or animals exposed to aliphatic low carbon range compounds or mixtures were located in the sources reviewed. Takeuchi et al. (1980)

APPENDIX D. REFERENCES

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