Thermodynamic Activity and Fugacity Applied to Risk Assessment and Criteria Development of Petroleum Hydrocarbons

by

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Abstract

Thermodynamic activity and fugacity were used to describe the toxic effects concentrations of petroleum hydrocarbons (PHCs) across a range of PHCs and PHC mixtures in different media (water, sediment, soil, lipid), for a variety of effects (e.g., survival, growth) on a variety of species. There is a similar range in activity associated with PHC toxicity across chemicals, media, species, and effects, and PHC mixtures of varying composition. Therefore, the lower 5th percentile of all PHC toxicity data (activity = 0.003), or corresponding lipid-normalized concentration (4.36 mol/m^3) or volume fraction (0.0008 m^3/m^3) calculated from activity at equilibrium can be applied to integrate a broad range of effects data into risk assessments and criteria development. When expressed as activity, the current PHC mixture criteria for sediment, soil, and water that are applicable in British Columbia overlap or exceed the range of activities associated with toxic effects, and therefore, these criteria may be underestimating environmental risks from PHC exposure.

Keywords: petroleum hydrocarbons; thermodynamic activity; risk assessment

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List of Acronyms

a	Activity (unitless)
ANCOVA	Analysis of Co-Variance
BCF	Bioconcentration Factor
BCMoE	British Columbia Ministry of Environment
BSAF	Biota-Sediment Accumulation Factor
CCME	Canadian Council of Ministers of the Environment
C_L	Concentration in lipid phase (mol/m^3_{LIPID}) calculated at equilibrium
CSR	Contaminated Sites Regulation (1996)
EC	Effective Carbon number
EC25/50	Effect Concentration to 25%/50% of test organisms
EMA	Environmental Management Act (2003)
EPH	Extractable Petroleum Hydrocarbons
f	Fugacity (pascals)
FW	Freshwater
HEPH	Heavy Extractable Petroleum Hydrocarbons
HMW	High Molecular Weight
K_{OW}	octanol-water partition coefficient
LC50	Lethal Concentration to 50% of test organisms
LEPH	Light Extractable Petroleum Hydrocarbons
LMW	Low Molecular Weight
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
PHC(s)	Petroleum Hydrocarbon(s)
PAH(s)	Polycyclic Aromatic Hydrocarbon(s)
QSAR(s)	Quantitative Structure-Activity Relationship(s)
SW	Saltwater
TPHCWG	Total Petroleum Hydrocarbon Criteria Working Group
USEPA	United States Environmental Protection Agency
V_C/V_L	Volume fraction of chemical in lipid phase at equilbrium ($m^3_{CHEMICAL}/m^3_{LIPID}$)
VPH	Volatile Petroleum Hydrocarbons
Z	Fugacity Capacity ($mol/m^3/Pa$)

Glossary

activity (a)	a unitless thermodynamic description of how saturated a given medium is with a given chemical. Activity can be expressed as the ratio of the chemical's fugacity (f) to its reference fugacity (f^R) of the pure chemical at a defined standard state. It can also be defined as the product of a chemical's molar fraction (x, in $mol_{SOLUTE}/mol_{SOLVENT}$) and an activity coefficient (γ, unitless). Activity can also be approximated by a chemical's concentration (C, mol/m^3) divided by the chemical's solubility (S, mol/m^3) in the same medium.
aliphatic	petroleum hydrocarbon chemicals with molecular structure that does not include any benzene rings (e.g., molecular structure is straight or branched chain, or alicyclic)
aromatic	petroleum hydrocarbon chemicals with a molecular structure that includes one or more benzene rings
baseline toxicity	a synonym for "non-polar narcosis"
criteria	for the intent of this research, "criteria" and "guidelines" are used interchangeably to describe regulatory tools that specify the amount of chemical(s) below which ecosystems are expected to have an acceptable level of risk for adverse effects. In other contexts, these two terms may have more specific or legal definitions.
critical body burden	a synonym for "critical body residue"
critical body residue	the concentration of chemical in the whole-body of test organisms that is associated with a toxic effect. E.g., the critical body burden of organic chemicals associated with non-polar narcosis has been described between 0.2 and 8 mmol/kg (McCarty & Mackay, 1993). "Critical body residue" is synonymous with "critical body burden".
external	describes an abiotic medium (e.g., water, sediment, or soil) that is outside the body of an organism
fugacity (f)	a thermodynamic concept describing a chemical's escaping tendency, or tendency to move from one phase to another (e.g., from a water phase to a gas phase). Fugacity in pressure units of pascals (Pa) can be calculated as the chemical's concentration (C, mol/m^3) divided by the chemical's fugacity capacity (Z, $mol/m^3/Pa$).
fugacity capacity (Z)	in units of $mol/m^3/Pa$ is a chemical- and medium-specific parameter describing the number of moles of a chemical required to increase the chemical's partial pressure in one cubic meter of the medium by one pascal.

guideline	for the intent of this research, "criteria" and "guidelines" are used interchangeably to describe regulatory tools that specify the amount of chemical(s) below which ecosystems are expected to have an acceptable level of risk for adverse effects. In other contexts, these two terms may have more specific or legal definitions.
internal	describes a biotic medium (e.g., whole-body, or specific tissue) that is inside the body of an organism.
LC50	Lethal concentration to 50% of test organisms.
lipid-normalized concentration (C_L)	in units of mol/m^3_{LIPID}, an approximation of the amount of chemical inside an organism at equilibrium with external media, and normalized to the lipid content.
non-polar narcosis	non-specific mode of toxic action whereby organic chemicals interact with biological members and affect the general fluidity and/or functioning of membrane proteins (Escher et al., 2011). First described by Meyer (1899) and Overton (1899). "Baseline toxicity" is often used as a synonym for non-polar narcosis.
octanol-water partition coefficient (K_{OW})	the ratio of a chemical solute concentration in octanol (a surrogate lipid phase) saturated with water to the chemical solute concentration in water that is saturated with octanol (Mackay et al., 2006). Typically expressed on a log-transformed basis (i.e., log K_{OW}).
petroleum hydrocarbons (PHCs)	group of organic chemicals from petrogenic, pyrogenic, biogenic sources that all have a carbon-hydrogen structure, but vary widely in molecular structure by the number of carbon and hydrogen atoms, the types of chemical bonds (i.e., single, double, or triple), molecular shape, and presence of other elements like nitrogen.
polycyclic aromatic hydrocarbons (PAHs)	a group of aromatic petroleum hydrocarbons (PHCs) that have two or more benzene rings as part of their molecular structure.
risk assessment	a comparison between the levels of chemical exposure experienced by ecosystems in the environment and the level of chemicals associated with adverse effects to ecosystems.
tissue residue	a synonym for "critical body residue". Can also refer to concentration within a specific tissue type (e.g., liver or muscle) that is associated with a toxic effect.
volume fraction in lipid phase (V_C/V_L)	in units of m^3/m^3, an approximation of the fraction of the lipid volume inside an organism that is occupied by chemical(s), where the internal and external media are at equilibrium.

wildlands land use	"...use of land for the primary purpose of supporting natural ecosystems, including the use of land for ecological reserves, national or provincial parks, protected wetlands or woodlands, native forests, tundra and alpine meadows, but does not include uses defined as urban park land use." (CSR, 1996, Part 1)

1. Introduction

1.1. Petroleum Resource Industry in British Columbia

The oil and gas industry is active in the Canadian Province of British Columbia (BC) and is expected to continue its growth, particularly in remote north-eastern areas of the Province. There are presently about 20,400 active oil and gas well sites in BC (Office of the Auditor General of British Columbia, 2010). More are planned for the future, particularly in the north-eastern Peace region of the province (BC Oil & Gas Commission, 2012). In BC, 43,500 hectares of the 95 million hectares of crown land are tenured or protected for non-exclusive use by the petroleum and natural gas sector (Ministry of Forests, Lands & Natural Resource Operations (MFLNRO), 2011). Eighty percent of wells are on Crown land. Most oil processing and storage occurs at these Crown land well sites. Seventy percent of off-site processing and storage also takes place on Crown land (MFLNRO, 2011). The oil and gas industry operates within BC's ecosystems, and in many cases in close proximity to human populations. In the Peace Region, there are at least a dozen human communities within a 15 km radius of up to 600 well sites (Northern Health, 2006).

The oil and gas industry provides fossil fuel resources and various economical benefits. For example, the Peace region is the only region in the Province that entirely relies on the extraction of underground resources (including both the oil and gas sector and the mining sector) for its basic income source (MFLNRO, 2011). However, industrial activities have been associated with elevated environmental concentrations of many different chemicals including metals and organic compounds like petroleum hydrocarbons (Kelly et al., 2009; 2010). Sites that contain substances at concentrations exceeding established regulatory criteria are deemed unsuitable for various uses and are designated as contaminated sites (British Columbia Ministry of Environment (BCMoE), 2009). There are over 9000 current and historical contaminated sites listed in

the Provincial Contaminated Sites Registry for BC (BCMoE, 2009). Many of these sites are contaminated with petroleum hydrocarbons. A dense cluster of contaminated sites can be found in the northeast corner of the Province (Evans, 2008). Because of the prevalence of petroleum hydrocarbon contaminated sites and the expected growth in the number of such sites in the future, it is important to develop management strategies for the protection of ecosystems, and the animals and people who interact with and rely on these ecosystems.

1.2. Petroleum Hydrocarbons

Petroleum hydrocarbons (PHCs) are a diverse group of organic chemicals that have the potential to adversely affect exposed organisms and ecosystems. Hydrocarbons are produced through petrogenic processes (e.g., crude oil formed by geologic processes), through pyrogenic processes (i.e., burning or combustion of organic material), and through biogenic processes (biodegradation or biosynthesis by plants and bacteria), and all hydrocarbons can enter the environment from both anthropogenic and natural sources (Nagpal, 1993; Sikkema et al., 1995). The petroleum resource extraction industry is a major anthropogenic source of PHCs. There are thousands of individual chemicals that are classified as petroleum hydrocarbons. However, toxicity data are only available for 95 of these PHCs, and only approximately 25 of those PHCs have toxicity data sufficient for the methodology commonly used to manage pollution through criteria development (Edwards et al., 1997). Nevertheless, the environment is exposed to potentially 1000s of chemicals in the PHC category alone, which makes predicting or understanding the cumulative effects on organisms from PHC exposure a difficult task. The mandate of the British Columbia Ministry of Environment (BCMoE) includes “to encourage and maintain an optimum quality environment through specific objectives for the management and protection of land, water, air and living resources of British Columbia“ (Ministry of Environment Act, 1996). The Environmental Management Act (EMA; 2003) is one of the regulatory tools that the BCMoE can use to achieve this mandate. Under the EMA the BCMoE has the authority to specify environmental quality guidelines for evaluating risk and managing adverse effects on the environment resulting from PHC exposure.

1.3. Environmental Management of Petroleum Hydrocarbons

Management of chemicals in the environment is frequently based on an understanding of the relationship between environmental exposure and effects on ecological receptors and/or human health. Describing and quantifying relationships between exposure and effects for contaminants provides a basis for environmental management of contaminants. Environmental quality criteria and guidelines are intended to define the concentrations of contaminants that are protective of human health and the environment (i.e., effects thresholds), and thus are important management tools because they help to describe and quantify hazards to the environment associated with chemical contaminants.

The governments of Canada and British Columbia set environmental quality guidelines for individual media types (i.e., water, air, sediment, soil) with defined narrative intentions to protect specific organisms or specific land uses (e.g., aquatic life or agricultural land use). It is important to understand and respect the narrative intent when applying these to ensure that environmental receptors (e.g., aquatic invertebrates, wildlife, or humans) are afforded an appropriate level of protection.

1.4. Wildlands Ecological Context

The Contaminated Sites Regulation (CSR) in British Columbia was amended in 2008 to include “Wildlands” landuse (Stage 6 Amendments to the CSR; BCMoE, 2008). The “Wildlands Landuse” classification was added to the CSR to “accommodate wildlands standards for the oil and gas drilling sector, as well as wildlands in other parts of British Columbia” (BCMoE, 2008, para. 6). Wildlands are defined in the CSR as land used “for the primary purpose of supporting natural ecosystems, including the use of land for ecological reserves, national or provincial parks, protected wetlands or woodlands, native forest, tundra and alpine meadows, but does not include uses defined as urban park land use” (CSR, 1996, Part 1). After the operating lifespan of an oil or gas well site, certain numerical chemical criteria or guidelines must be met in order for the site to be considered not contaminated and also capable of supporting natural

ecosystems. These criteria and guidelines are ideologically intended to define the concentration of contaminants that would protect against adverse effects on wildlife, plant, and human use of the wildlands. This goal includes protecting all life-stages of all species from adverse effects associated with exposure to multiple different chemicals.

Table 1.1. Summary for guidelines for petroleum hydrocarbon mixtures in soil, sediment, and water that can be applied in wildlands.

Medium	Reference	Guideline	PHC Mixtures[1]	Narrative Intent
Soil	CCME (2008)	Canada-wide Standard	F1, F2, F3, F4	Agricultural, Residential Use[2]
Soil	CCME (2008)	Canada-wide Standard	F1, F2, F3, F4	Commercial, Industrial Use[3]
Soil	CSR (1996), Schedule 4	Generic Numerical Soil Standards	HEPH, LEPH, VPH	Agricultural, Urban Park, Residential, Commercial, or Industrial Use
Sediment	BCMoE (Nagpal et al., 2006)	Working Environmental Quality Guidelines	LMW PAH, HMW PAH	Marine: Protection of Aquatic Life: No or minor adverse effects on biota
Sediment	BCMoE (Nagpal et al., 2006)	Working Environmental Quality Guidelines	LMW PAH, HMW PAH	Freshwater: Protection of Aquatic Life - No effects threshold based on background approach
Sediment	BCMoE (Nagpal et al., 2006)	Working Environmental Quality Guidelines	Total PAH	Freshwater: Protection of Aquatic Life - severe effect level, effects range low or moderate
Sediment	CSR (1996), Schedule 9	Generic Numerical Sediment Standards	Total PAH	Freshwater & Marine: Protection of sensitive aquatic life, or typical aquatic life
Water	CSR (1996), Schedule 6	Generic Numerical Water Standards	VPH, LEPH	Freshwater: Protection of Aquatic Life
Water	CSR (1996), Schedule 6	Generic Numerical Water Standards	EPH	Freshwater: Protection of Aquatic Life, and use for irrigation, livestock, and drinking water (unfiltered at point of consumption)

BCMoE = British Columbia Ministry of the Environment; CCME = Canadian Council of Ministers of the Environment; CSR = Contaminated Sites Regulation; EPH = extractable petroleum hydrocarbons; F1, F2, F3, F4 = Fractions of PHCs based on boiling points; HEPH = heavy extractable petroleum hydrocarbons; HMW = high molecular weight; LEPH = light extractable petroleum hydrocarbons; LMW = low molecular weight; PAH = polycyclic aromatic hydrocarbons; VPH = volatile petroleum hydrocarbons.
1. PHC mixture compositions described in more detail in Table 3.12.
2. Derived from 25th%ile of toxicity data (LC/IC20(25) or EC/IC/LC50 for F4) to plants and invertebrates.
3. Derived from 50th%ile of toxicity data (LC/IC20(25) or EC/IC/LC50 for F4) to plants and invertebrates.

There are currently no guidelines for PHCs in British Columbia or Canada that are specifically designated to protect wildlands. Instead, the PHC guidelines (Table 1.1) designed to protect urban parks (CSR, 1996, Schedule 4), residential/parkland, or commercial land use (CCME, 2008, Table 1) are often applied to wildlands, even though environmental receptors in urban parks, residential or parkland environments are not necessarily representative of the environmental receptors or exposure pathways that exist in wildlands ecosystems. For example, the CCME Tier 1 guidelines for PHCs in soil identify the following exposure pathways (and environmental receptors) as pertinent for the protection of residential areas and parklands: direct contact (nutrient cycling, invertebrates, plants, and human toddler); soil ingestion (wildlife, and human toddler); groundwater and surface water (aquatic life, livestock, and human toddler); indoor vapour inhalation (human toddler); and, ingestion of produce grown on PHC-containing soil (human toddler) (Tables 5.2 and 5.3 in CCME - Supporting Technical Document, 2008). Some of these pathways in parklands and residential areas may not be relevant in a wildlands setting (e.g., exposure of human toddlers to surface or groundwater or soil). There are also potential exposure pathways that need to be considered in a wildlands setting but are not considered for parklands or residential areas: for example, ingestion of PHCs by wildlife through food-web transfer, preening, or dermal absorption by wildlife through direct contact with water, sediment, or soil (CCME - Supporting Technical Document, 2008; Haggarty et al., 2003). There may also be different exposure frequencies and durations in a wildlands environment compared to a residential/parklands setting (e.g., amount of plants or soil consumed by wildlife or people).

The effects data used to develop environmental quality guidelines need to support and reflect the narrative intent of the guideline. For example, the residential/parkland CCME Tier 1 guidelines for PHCs in soil are derived using data on PHCs' effects on plants and soil invertebrates through direct contact with soil, which were considered to be the most sensitive receptors in residential/parkland environments (CCME - Supporting Technical Document, 2008, Tables 5.2 and 5.3). Wildlands are designated to protect all life stages of all organisms in all food chains including plants and soil invertebrates, as well as vertebrate wildlife and humans that inhabit or utilize wildlands ecosystems. Therefore, numeric guidelines for PHCs need to be established

using appropriate data that will reflect the narrative intent behind wildlands ecosystems classification.

Unfortunately, the requisite data are often not always available when making management decisions. In reality, there are many data limitations when developing guidelines or criteria to protect ecosystems from PHC exposure. One of these limitations arises from an imperfect match between the actual exposure scenarios that occur in real environments and the laboratory-based toxicity tests from which numerical criteria and guidelines are often derived. Toxic effects data do not always provide a direct match between the species tested in the lab, and the species in the environment that are part of the protection goals. Toxicity data are available for only a subset of the chemicals that are anthropogenically released to the environment. Toxicity data for all the different unique mixtures of PHCs that may be released to the environment are also very limited (Landrum et al., 2012). Toxicity data typically expose test organisms to chemicals through one media at a time, whereas organisms in the environment may be exposed through multiple different media (e.g., soil, and water). In face of data limitations, the Canadian Environmental Protection Act recognizes the importance of applying a "weight of evidence approach and the precautionary principle" (CEPA, 1999, Section 76.1), to help make informed decisions for protecting the environment, incorporating the different types of data that are available.

1.5. Research Objectives

The overall goal of this research is to develop, evaluate, and apply an activity- and fugacity-based methodology for the purpose of conducting environmental risk assessments and developing environmental quality criteria for petroleum hydrocarbon contaminated sites. More specifically, this research addresses the following questions:

1. What are the activities and fugacities of individual and mixtures of PHCs in various environmental media that cause toxicological effects in biota?

2. How do the activities and fugacities causing toxicity of individual PHCs compare to those for PHC mixtures?

3. How does an activity- and fugacity-based risk assessment and guideline development approach compare to existing risk assessment and guideline development approaches?

4. How can the activity-based and fugacity-based approaches be used to inform risk assessments and environmental quality guideline development?

Although this research is focused on petroleum hydrocarbons in a wildlands context, the methodology explored here may also serve as a useful tool for addressing other contamination problems. Ultimately, this research strives to develop ecologically-relevant and empirically-supported assessment tools for chemicals in the environment. The methods explored through this research may ultimately be incorporated into future management of existing and potential anthropogenic chemical impacts.

2. Background: Chemistry and Toxicology of Petroleum Hydrocarbons

2.1. Chemistry and Toxicology of Individual Petroleum Hydrocarbons

All petroleum hydrocarbons have a carbon-hydrogen structure, but vary widely by the number of carbon and hydrogen atoms, the types of chemical bonds (i.e., single, double, or triple), the molecular shape (e.g., linear, or cyclic), and the presence of other elements like nitrogen (Figure 2.1). All PHCs can be classified as either aliphatic (i.e., molecular structure is a straight chain, branched chain, or alicyclic) or aromatic (i.e., molecular structure includes one or more benzene rings). These widely varying types of molecule structures and compositions result in a wide variety of chemical properties. For example, the water solubility of PHCs ranges from water soluble for smaller molecules to very insoluble for heavier substances (Verbruggen et al., 2008). Additionally, for a given number of carbons, aliphatics are typically less water-soluble than aromatics, and the difference in aqueous solubility between aliphatics and aromatics becomes increasingly larger with greater number of carbon atoms. The octanol-water partition coefficient (K_{OW}) also increases with number of carbons in the molecule, and spans several orders of magnitude for PHCs. These widely ranging chemical properties are environmentally relevant because they ultimately influence the fate and distribution of PHCs in the environment. Chemical properties can also be used to predict the environmental behaviour and effects of PHCs. For example, quantitative structure-activity relationships (QSARs) can be used to relate chemical properties to biological activity endpoints like survival which is helpful for managing environmental pollutants (e.g., Van Leeuwen et al., 1992).

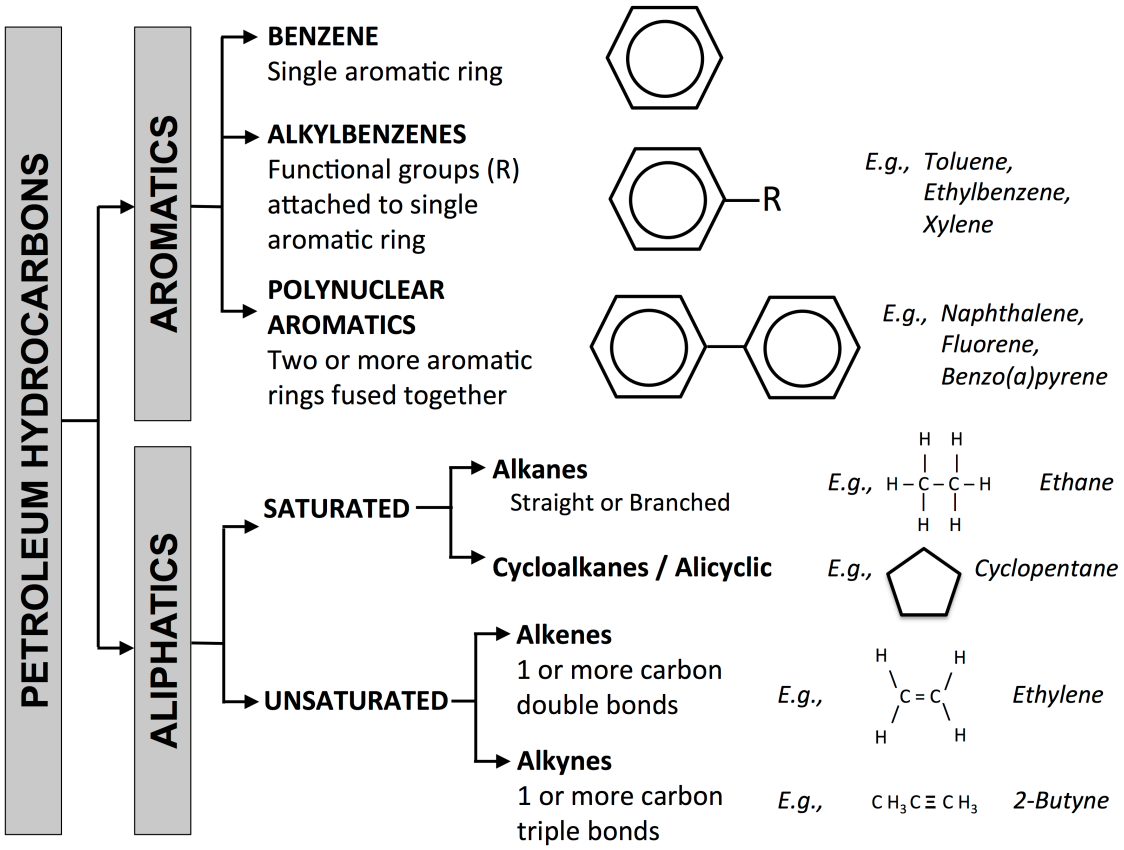


Figure 2.1. Petroleum hydrocarbon chemical structures. Adapted from Amherst Scientific (1998).

Despite the large diversity in structures and chemical properties of PHCs, all PHCs are thought to share non-polar narcosis (or baseline toxicity) as a common mode of toxic action (MacLeod et al., 2004; Muijs & Jonker, 2010; Verbruggen et al., 2008). Although the specific biochemical mechanics of non-polar narcosis remain unresolved, this nonspecific mode of action is understood to be a result of organic chemicals dissolving into the lipid phase of biological membranes and affecting the general fluidity of cell membrane lipids and/or functioning of membrane proteins (Escher et al., 2011). Hans Meyer (1899) and Charles Ernest Overton (1899) were the first scientists who each independently described nonpolar narcosis in studies of different gases used in anaesthesiology. They reported that the potency of different anaesthetic gases is constant relative to their solubility in oil (a surrogate lipid phase), even though potency appeared widely varying when exposure concentration was measured in the gas phase

(an abiotic phase outside of the organism). Subsequently, certain environmental pollutants were described as sharing non-polar narcosis as a common mode of action (Könemann, 1981). In fact, non-polar narcosis is believed to be a mode of toxicity shared by about 60% of all commercially used organic chemicals (van Wezel & Opperhuizen, 1995).

Some PHCs, depending on their chemical structure and properties, also have more specific mechanisms through which they interact with cells and cause effects on organisms. Some PHCs, many with log K_{OW} of 5 to 8 have demonstrated AhR receptor agonist activity (Vrabie et al., 2012). Indicators of exposure and effects can be found through genome expression profiles, protein expression patterns, and abnormalities in tissue morphology (Whitehead et al., 2011). Polycyclic aromatic hydrocarbons (PAHs), as well as benzene, toluene, ethylbenzene, and xylene (BTEX), are aromatic PHCs associated with carcinogenic effects (Melendez-Colon et al., 1999). Typically carcinogenicity occurs at much lower internal concentrations than non-polar narcosis (McCarty & Mackay 1993). In addition, there are also photo-induced modes of toxic action, such as photo-enhancement or photo-activation of PHC toxicity, particularly for microbial responses to PHC exposure (Barron et al., 2003). Various site-specific factors, such as organic carbon type and content in sediments and soils, can influence the bioavailability and, in turn, the apparent toxicity of PHCs (Hawthorne et al., 2006; Thorsen et al., 2004). In addition to internal cell-to-chemical-interaction mediated mechanisms of toxicity, PHCs can also cause direct physical impacts to organisms, particularly in the presence of pure-phase hydrocarbons. These physical effects include oiling of an animal's feathers or skin, and physical suffocating of an animal's respiratory surfaces (CCME - Supporting Technical Document, 2008; Haggarty et al., 2003).

2.2. Chemistry and Toxicology of Mixtures of Petroleum Hydrocarbons

In the real world, organisms are simultaneously exposed to many different PHCs and mixtures of PHCs varying in composition and chemistry depending on the source and degree of environmental weathering. For example, there are many different types of PHC mixtures (e.g., crude oil, fuel oil, lubricating oils) that vary in composition depending

on original geologic source, extent of weathering, and types of industrial processing. Furthermore, an organism may be exposed to PHC mixtures from multiple different sources. Chemical components of these environmentally relevant PHC mixtures range widely in terms of their chemical properties from volatile chemicals that more readily go into the air phase, to more hydrophobic chemicals with very low solubilities in water that more readily associate with organic material in sediments or resist environmental weathering. Individual components of PHC mixtures will therefore differentially partition between the environment and organisms, according to each component's individual properties.

Despite complex PHC exposure profiles, there are some commonalities in the behaviour of PHCs that may be helpful when assessing the unique toxicity of environmentally relevant mixtures of PHCs. Since all PHCs are thought to share non-polar narcosis as a common mode of action, organisms are generally thought to show an additive response to mixtures of PHCs (i.e., a half dose of two different chemicals is equal to full dose of either single chemical). Additive toxicity has been demonstrated for mixtures of organic chemicals sharing nonpolar narcosis as mode of toxic action (Hermens et al., 1984). Additive toxicity for mixtures of PAHs, thought to be the components of PHCs primarily responsible for toxicity, has also been demonstrated (di Toro & McGrath, 2000; Landrum et al., 2003, 2012). Furthermore, when aquatic PAH exposure is expressed in terms of internal lipid-based concentrations, an additive response has been observed (McGrath & Di Toro, 2009; Meador, 2006). There can also be deviations from this model of additivity, caused by components with additional more specific modes of toxic action, toxic metabolites, synergistic or antagonistic interactions between mixture components, and varying toxicokinetics between mixture components (Altenburger et al., 2003; Escher et al., 2011). Therefore, empirical cumulative toxicity effects data for PHC mixtures are informative and valuable when available, but unfortunately are just not practical for each possible unique environmentally-relevant mixture. Even when complex mixture toxicity data are available, there can be many different modifying factors (Landrum et al., 2012), which affect ability to make direct comparisons between different toxicity data, or between toxicity data and exposure data.

2.3. Brief History of Activity & Fugacity Applications

Activity and fugacity are two complementary thermodynamic concepts that have both been applied as valuable tools to understand behaviour of chemicals in various disciplines for over a century. Hans Meyer (1899) and Charles Ernest Overton (1899) worked in anesthesiology describing a common activity of nonpolar narcosis. Fugacity was first introduced and defined by Lewis (1900; 1901) as a chemical's "escaping tendency", or tendency to move from one phase to another (e.g., from a water phase to gas phase). To overcome the abstract nature of the fugacity concept, Lewis (1907) introduced thermodynamic activity, a ratio that describes how saturated a given medium is with a given chemical. John Ferguson (1939) proposed applying Gilbert Lewis's thermodynamic activity (Lewis 1907) to toxicology as a measure of chemical potential, assuming that at equilibrium, the activity of external phases can approximate the chemical potential at the cellular site of action. Chemical engineering (Prausnitz, 1969 as cited in Mackay & Arnot 2011) and, more recently environmental contaminant fate and transport modeling (e.g., Campfens & Mackay, 1997; Clark et al., 1990; Gobas, et al., 1999; Mackay, 1979; 2004; Mackay & Arnot, 2011; Parajulee & Wania, 2014) have also applied thermodynamic-based methodology. Passive samplers such as semi-permeable membrane devices (SPMDs) are one way that activity and fugacity can be measured in abiotic media to approximate exposure to PHCs at the cellular active site (e.g., Wilcockson & Gobas 2001). Recently, toxicity of organic chemicals has been studied in terms of activity (e.g., Engraff et al., 2011; Mayer & Holmstruf, 2008; Mayer & Reichenberg, 2006; Mackay et al., 2011). Activity and fugacity are also beginning to be used for guideline development and risk assessment of organic chemicals (Gobas & Otton, 2015; Mackay et al., 2011).

Some modes of toxic action, such as non-polar narcosis, can be identified by a specific thermodynamic activity. For example, chemical activities of non-polar organic chemicals between 0.01 and 0.09 (Mackay & Arnot, 2011; McCarty et al., 2013) tend to cause lethality through non-polar narcosis. Actual activities of PHCs in toxicity tests and environmental exposures can be compared to these values to assess whether non-polar narcosis can be expected to occur.

Activity (to a greater extent than fugacity) has also been suggested and explored as a method for evaluating exposure to mixtures of chemicals, particularly for mixtures of PAHs. For example, Engraff et al. (2011), and Witt et al. (2010) have both used activity to explore relationships between thermodynamic activity, chemical effects, and exposure to mixtures of PAHs, particularly where activity was empirically measured in the exposure media using passive sampling devices. Mayer and Reichenberg (2006) have used activity to further explore contributions of hydrophobic chemicals (i.e., log K_{OW} greater than around 6) to mixture toxicity. This present research focused on the use of activity and fugacity to explore the effects of environmentally relevant mixtures of PHCs. Often the assumption of thermodynamic equilibrium is made in environmental fate modeling. However, this research does not assume equilibrium, but rather expresses exposure to PHCs in terms of concentrations in multiple media on a common-unit basis to investigate the potential for thermodynamic activity and fugacity to inform PHC risk assessments and guideline development.

3. Methods

3.1. General Methodology

The description of the methodology of this research is divided into five sections. First, Section 3.2 describes how to calculate activity and fugacity. Then, each of the subsequent sections addresses one of the objectives listed in Section 1.5. Section 3.3 describes the methods used to determine activities and fugacities that cause toxicological effects in biota for individual and mixtures of PHCs in various environmental media. Section 3.4 describes the methods used to compare activities and fugacities associated with toxicological effects for individual PHCs with those for PHC mixtures. Finally, Section 3.5 describes the methods used to compare an activity- and fugacity-based approach with existing PHC risk assessment and guideline development approaches. All statistics were computed on logarithm (base 10)-transformed data using R open-source software version 2.14.2 (R Development Core Team 2012), and R Studio (2012). A p-value <0.05 was considered statistically significant. Upper and lower 95% confidence limits around estimates of mean log-transformed values were calculated as mean ± [2•standard deviation of log-transformed data ÷ (sample size)$^{0.5}$].

3.2. Activity & Fugacity

3.2.1. Thermodynamic Description of Activity & Fugacity

Activity and fugacity are two complimentary thermodynamic concepts that have both been applied for over a century to understand the behaviour of individual chemicals. Fugacity describes in pascals (Pa, a unit of pressure) the tendency for a chemical to move from one phase to another. Fugacity can there be measured directly in gaseous media like air. For example, the vapour pressure of a chemical is equal to the fugacity of pure chemical in equilibrium with air in a fixed volume. Fugacity (f ; in

units of pascals, Pa) is related to concentration (C ; in units of mol•m^{-3}) by the chemical's fugacity capacity, Z (in units of mol•m^{-3}•Pa^{-1}):

$$f = \frac{C}{Z} \qquad \text{…Equation 3.1}$$

Z is a chemical- and medium-specific parameter describing the number of moles of a chemical required to increase the chemical's partial pressure in one cubic meter of the medium by one pascal. Z can be calculated for any medium using chemical- and media-specific properties. Therefore, fugacity, or the "escaping tendency", can also be calculated for any chemical in any medium (Table 3.1).

Table 3.1. Methodology for calculation of activity and fugacity for PHCs in various biotic and abiotic media[1].

Medium	Activity "*a*" (unitless)	Fugacity "*f*" (Pa)
Air	$a = \frac{C_A}{S_G}$	$f = \frac{C_A}{Z_A}$
Water	$a = \frac{C_W}{S_W}$	$f = \frac{C_W}{Z_W}$
Soil or Sediment	$a = \frac{C_S}{S_S} = \frac{C_{OC}}{S_{OC}}$	$f = \frac{C_S}{Z_S} = \frac{C_{OC}}{Z_{OC}}$
Diet	$a = \frac{C_D}{S_D} = \frac{C_L}{S_L}$	$f = \frac{C_D}{Z_D} = \frac{C_L}{Z_L}$
Invertebrate, fish, or mammal tissues	$a = \frac{C_B}{S_B} = \frac{C_L}{S_L}$	$f = \frac{C_B}{Z_B} = \frac{C_L}{Z_L}$

[1] Specific equations for each term are in Table 3.4.
a = activity (unitless); C = Concentration (mol•m^{-3}); f = fugacity (Pa); S = solubility (mol•m^{-3}); Z = fugacity capacity (mol•m^{-3}•Pa^{-1}).
Subscripts: A = air; B = biota; D = diet; G = gas; L = lipid; OC = organic carbon; S = soil/sediment; W = water.

Thermodynamic activity "a" can be expressed as the ratio of the chemical's fugacity f and the chemical's reference fugacity f^R of the pure chemical at a defined standard state. The reference fugacity, f^R, is generally the fugacity (or vapour pressure) of the pure chemical in a liquid state (for substances that are liquids at the temperature

and pressure of interest), or in a sub-cooled liquid state (for substances that are solids at the system's temperature and pressure):

$$a = \frac{f}{f^R} \qquad \text{…Equation 3.2}$$

Thermodynamic activity is also defined as the product of the chemical concentration "x" in units of (mol solute/mol solvent) and the activity coefficient γ (unitless):

$$a = \gamma \bullet x \qquad \text{…Equation 3.3}$$

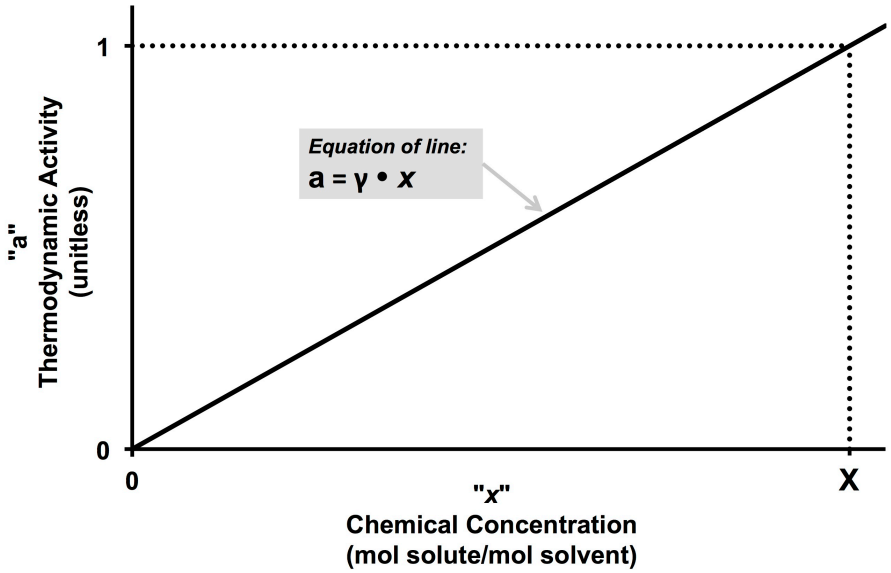


Figure 3.1. General relationship of the thermodynamic activity of a chemical substance in a solvent assuming activity coefficient γ (i.e., slope), is constant over range of thermodynamically possible concentrations ($0 \leq x \leq X$), where X is the chemical's solubility in the solvent.

Assuming that PHCs and water are immiscible, it can be shown that in typical saturated solution where the chemical concentration in solution is in a thermodynamic equilibrium with the pure chemical in either liquid or sub-cooled liquid form (for substances that are respectively liquids or solids at the system's temperature), the activity coefficient γ is equal to the reciprocal of X (moles chemical/moles solvent), the chemical's solubility:

$$\gamma = \frac{1}{X} \qquad \text{…Equation 3.4}$$

Assuming that the activity coefficient γ is constant over the range of thermodynamically possible concentrations (Figure 3.1), it follows that the activity of a chemical can be approximated by the ratio of the chemical's concentration x (mol•mol^{-1}) and its solubility X (mol•mol^{-1}) in the medium in which it occurs ($a = \frac{x}{X} = \frac{C}{S}$ …Equation 3.5). Dividing x and X by the molar volume of the solvent (e.g., water molar volume = 18•10^{-6} m^3•mol^{-1}) produces a method to approximate the activity in more conventional units of chemical concentration C (mol•m^{-3}) and solubility S (mol•m^{-3}):

$$a = \frac{x}{X} = \frac{C}{S} \qquad \text{…Equation 3.5}$$

The activity of chemicals can be calculated in any medium (Table 3.1).

For substances that are solids at the system's temperature, the activity coefficient γ is the ratio of the fugacity ratio F (i.e., the ratio of the fugacities of the solid and the sub-cooled liquid form of the substance) and the chemical's solubility X in the solvent in units of mol•mol^{-1}:

$$\gamma = \frac{F}{X} \qquad \text{…Equation 3.6}$$

Which gives the following equation for the calculation of the activity of chemicals in solid form:

$$a = x \bullet \frac{F}{X} = C \bullet \frac{F}{S} \qquad \text{…Equation 3.7}$$

The fugacity ratio F can be calculated following Walden's Rule (1908):

$$F = \exp\left\{-6.79 \bullet \left[\left(\frac{T_M}{T}\right) - 1\right]\right\} \qquad \text{[T in K]} \qquad \text{…Equation 3.8}$$

Where T_M is the melting point of the chemical (in Kelvin; K) and T is the temperature of the system (K).

Both activity and fugacity have established thermodynamic limits. The activity can range from 0 to a maximum value of 1 for liquids, and from 0 to a maximum value of F for solids. The fugacity can range from 0 to a maximum value equal to the chemical's vapour pressure (of either liquid or sub-cooled liquid). These maximum possible activity or fugacity value provides a means to identify whether reported chemical concentrations in the environment and in toxicological studies are thermodynamically possible in the environment (i.e., $a \leq 1.0$ for liquids or $a \leq F$ for solids) or whether they cannot occur in the environment (i.e., $a > 1.0$ for liquids or $a > F$ for solids). Activities greater than 1, or fugacities greater than the chemical's vapour pressure typically represent experimental artefacts and/or analytical error (e.g., exceedance of the chemical's solubility, unit errors, and others).

When comparing activities for solids and liquids, as I plan to do in this analysis, it is important to account for any differences in activities due to the formation of solids. Therefore, for substances that are solid at the system's temperature (i.e., T_M greater than 298 K), the solubility and vapour pressure of the sub-cooled liquid were used in this research. To account for formation of solids, sub-cooled liquid properties were calculated by dividing the solubility or vapour pressure measured for solids by F (where F was calculated with Equation 3.8).

Activity and fugacity are complementary quantities because they both describe the amount of a chemical in a medium, with respect the medium's capacity for the chemical. The difference between activity and fugacity is that fugacity is thermodynamically defined based on gas as the reference phase. Activity is usually defined based on water as the reference phase. Fugacity may be more appropriate for chemicals that readily go into the gas phase and therefore, can be measurable (i.e., above detection limits) in gas phase (e.g., air). Whereas activity based on water solubility may be more appropriate for chemicals that readily go into the water phase, and therefore can be measureable (i.e., above detection limits) in water.

Activity and fugacity were both calculated from concentration-based toxicity data in a variety of different media for individual PHCs as well as for PHC mixtures (Section 3.3). Activity and fugacity were also calculated for current guidelines regulating PHC mixture concentrations in water, sediment, and soil (Section 3.5).

3.2.2. Lipid-phase Concentration and Volume Fraction

Activity calculated for any medium can be used to estimate the chemical's lipid-normalized concentration (C_L) and lipid-normalized volume fraction (V_C/V_L) inside an organism at equilibrium with an external medium (Table 3.2).

Table 3.2. Equations for calculation of lipid phase concentration and volume fraction.

Media	Concentration in Lipid Phase: C_L (mol chemical / m^3 lipid)	Volume Fraction in Lipid Phase: V_C/V_L (m^3 chemical / m^3 lipid)
All media	activity • S_L	activity • S_L • V_M • (10^{-6} m^3 • cm^{-3})

S_L = Chemical Solubility in Lipid Phase (mol/m^3; see Table 3.4); V_M = Le Bas Molar Volume (cm^3/mol).

C_L and V_C/V_L may be easier to understand in terms of applications to risk assessment and guideline development of PHCs as they are in more familiar units (mol•m^{-3} and m^3•m^{-3}) than the more abstract, unitless activity. The lipid-normalized concentration (C_L) of a chemical approximates the body burden, or number of molecules of chemical interacting with the lipid phase of biological membranes inside the organism. The volume of space occupied by chemicals within lipid compartments of organisms (V_C/V_L), rather than the number of molecules has also been used to describe chemical exposure and toxicity, particularly for chemicals sharing non-polar narcosis as a shared underlying mode of toxic action (McCarty et al., 2013). Therefore these two lipid-normalized metrics were also investigated as potential methods in which activity could be applied for PHC risk assessment and guideline development.

In a system at equilibrium, the activity will be equal across all media. The activity calculated in any abiotic medium (e.g., water or sediment) will equal the activity in a biotic medium such as the lipids of organisms. For example:

$$a = \frac{C_{WATER}}{S_{WATER}} = \frac{C_{SEDIMENT}}{S_{SEDIMENT}} = \frac{C_{LIPID}}{S_{LIPID}}$$ …Equation 3.9

Equation 3.5 for activity can be rearranged to solve for concentration in any media:

$$C = a \bullet S$$ …Equation 3.10

Therefore, activity calculated in any medium (e.g., sediment, soil, water) multiplied by the chemical's lipid-phase solubility (S_L ; units=mol•m^{-3}_{LIPID}) estimates the lipid-normalized chemical concentration (C_L ; units=mol•m^{-3}_{LIPID}) for a system at equilibrium:

$$C_L = a_{\text{ANY MEDIUM}} \bullet S_L$$ …Equation 3.11

S_L was calculated using the octanol-water partition coefficient and (K_{OW}) and the liquid solubility (for chemicals that are liquid at system's temperature) or sub-cooled liquid (for chemicals that are solid at system's temperature) (Table 3.4):

$$S_L = K_{OW} \bullet S_W$$ …Equation 3.12

Activity was applied to calculate the volume-fraction based concentration of chemicals in the lipid-phase at equilibrium:

$$V_C/V_L = a_{\text{ANY MEDIUM}} \bullet S_L \bullet V_M \bullet (10^{-6})$$ …Equation 3.13

Where V_C/V_L is the volume of chemical per volume of lipid (m^3/m^3); V_M is the chemical's Le Bas molar volume (cm^3•mol^{-1}), and 10^{-6} is a unit conversion factor (m^3•cm^{-3}).

Activity, fugacity, C_L and V_C/V_L were calculated for PHC concentrations representing PHC toxicity (Section 3.3), as well as current PHC guidelines (Section 3.5). Activity, fugacity, C_L and V_C/V_L were calculated from concentrations of individual PHCs, as well as from concentrations of PHC mixtures.

3.2.3. PHC Mixture Calculations

Several hypotheses were explored to evaluate the combined toxicity of multiple chemicals in environmentally relevant mixtures of petroleum hydrocarbons. The total activity, fugacity, and lipid-phase concentration and volume fraction was calculated for PHC mixtures (Table 3.3).

Table 3.3. Methodology to calculate multiple chemical exposure and toxicity for chemical mixtures.

Method	Equation	Units
Activity	$a_{MIXTURE} = \sum_{i=1}^{n} a_i = \sum_{i=1}^{n} \left(\frac{C_i}{S_i} \right)$	unitless
Fugacity	$f_{MIXTURE} = \sum_{i=1}^{n} f_i = \sum_{i=1}^{n} \left(\frac{C_i}{Z_i} \right)$	Pa
Lipid Concentration	$C_{L,\,MIXTURE} = \sum_{i=1}^{n} C_{L,i} = \sum_{i=1}^{n} \left(a_i \bullet S_{L,i} \right)$	mol • (m^3 lipid)$^{-1}$
Volume Fraction in Lipid Phase	$\left(\frac{V_C}{V_L} \right)_{MIXTURE} = \sum_{i=1}^{n} \left(\frac{V_C}{V_L} \right)_i = \sum_{i=1}^{n} \left(a_i \bullet S_{L,i} \bullet V_{M,i} \bullet 10^{-6} \right)$	(m^3 chemical) • (m^3 lipid)$^{-1}$

Table Note: *i* = an individual chemical component of the PHC mixture; *n* = number of components measured in the PHC mixture.

The activity was calculated for each individual component (a_i) of PHC mixtures, using component-specific solubilities. Concentration of any PHC mixture component in any medium was limited to its solubility. Then, the total activity of the mixture ($\sum a_i$) was calculated as the sum of all the activities of all the individual mixture components:

$$a_{MIXTURE} = \sum_{i=1}^{n} a_i = \sum_{i=1}^{n} \left(\frac{C_i}{S_i} \right) \qquad \text{…Equation 3.14}$$

Similarly, the total fugacity of the PHC mixture was calculated as the sum of the fugacities calculated for each individual component:

$$f_{MIXTURE} = \sum_{i=1}^{n} f_i = \sum_{i=1}^{n} \left(\frac{C_i}{Z_i} \right) \qquad \text{…Equation 3.15}$$

The total lipid-normalized concentration of a mixture of PHCs was calculated as the sum of the lipid-normalized concentrations for each individual mixture component:

$$C_{L,MIXTURE} = \sum_{i=1}^{n} C_{L,i} = \sum_{i=1}^{n} \left(a_i \bullet S_{L,i} \right) \qquad \text{…Equation 3.16}$$

Activity of individual components (a_i) was limited to ≤ 1 in the calculation of $C_{L,MIXTURE}$.

The total volume fraction of the lipid phase occupied by PHCs was calculated as the sum of the volume fractions occupied by each individual component in the PHC mixture:

$$\left(\frac{V_C}{V_L} \right)_{MIXTURE} = \sum_{i=1}^{n} \left(\frac{V_C}{V_L} \right)_i = \sum_{i=1}^{n} \left(a_i \bullet S_{L,i} \bullet V_{M,i} \bullet 10^{-6} \right) \qquad \text{…Equation 3.17}$$

Activity of individual components (a_i) was limited to ≤ 1 in the calculation of $(V_C/V_L)_{MIXTURE}$.

3.2.4. Details of Activity, Fugacity, C_L, and V_C/V_L Calculations

A chemical's concentration in any media, whether it is a measured exposure concentration in the environment, a reported toxic concentration, or a guideline concentration, can be expressed as activity (unitless: activity = C/S; Equation 3.5) or fugacity (Pa; fugacity = f/Z; Equation 3.1; Table 3.1). Subsequently, an equilibrium-based lipid normalized concentration (C_L in mol/m^3) or volume fraction (V_C/V_L in m^3/m^3) can be calculated from activity in any medium (Table 3.2). Table 3.4 summarizes the chemical- and medium-specific properties used to calculate activity, fugacity, C_L, and V_C/V_L from reported toxicity, exposure, or guideline concentrations (mol/m^3) of single PHC chemicals or PHC mixture components in various media.

Table 3.4. Variables and constants used in calculations of activity, fugacity, and lipid-normalized concentration and volume fraction.

Symbol	Description	Constant Value or Equation	Units
Concentrations			
C_W	Concentration in water	measured	mol•m^{-3}
C_{OC}	Concentration in organic carbon	measured	mol•m^{-3}
C_S	Concentration in sediment or soil	measured	mol•m^{-3}
C_L	Concentration in lipid	measured	mol•m^{-3}
C_B	Concentration in biota	measured	mol•m^{-3}
C_D	Concentration in diet	measured	mol•m^{-3}
C_A	Concentration in air	measured concentration in air • (1 - ϕ_{AP})	mol•m^{-3}
C_{SALT}	Concentration of salt in saltwater	0.5	mol/L
$C_{P\text{-}SW}$	Concentration of particles in saltwater	0.66 (Mackintosh, 2002)	g•m^{-3}
$C_{TSP\text{-}A}$	Concentration of Total Suspended Particles in air	80	µg•m^{-3}
Solubilities			
S_G	Solubility in gas	$S_G = \frac{H \bullet S_W}{R_{GAS} \bullet T}$	mol•m^{-3}
S_W	Solubility in water	Freshwater: Appendix A Saltwater: $S_{W\text{-}Freshwater} \bullet R_{SALT}^{-1}$	mol•m^{-3}
S_{OC}	Solubility in organic carbon	$S_{OC} = K_{OC} \bullet S_W$ $= R_{OC} \bullet K_{OW} \bullet S_W$	mol•m^{-3}
S_S	Solubility in sediment or soil	$S_S = K_S \bullet S_W$ $= R_{OC} \bullet K_{OW} \bullet S_W \bullet \phi_{OC} \bullet \rho_S \bullet (0.001 L/m^3)$	mol•m^{-3}
S_L	Solubility in lipid	$K_{OW} \bullet S_W$	mol•m^{-3}

Symbol	Description	Constant Value or Equation	Units
S_B	Solubility in biota	$S_B = K_B \bullet S_W$ $= (\phi_L \bullet S_L) + (\phi_P \bullet 0.05 \bullet S_L) + (\phi_W \bullet S_W)$	mol•m^{-3}
Fugacity Capacities			
Z_A	Fugacity Capacity in air	$Z_A = \frac{1}{R_{GAS} \bullet T}$	mol•Pa^{-1}•m^{-3}
Z_W	Fugacity Capacity in water	$Z_W = \frac{S_W}{P} = \frac{1}{H}$	mol•Pa^{-1}•m^{-3}
Z_{OC}	Fugacity Capacity in organic carbon	$Z_{OC} = K_{OC} \bullet Z_W$ $= R_{OC} \bullet K_{OW} \bullet Z_W$	mol•Pa^{-1}•m^{-3}
Z_S	Fugacity Capacity in sediment or soil	$Z_S = K_S \bullet Z_W$ $= R_{OC} \bullet K_{OW} \bullet Z_W \bullet \phi_{OC} \bullet \rho_S \bullet (0.001 L/m^3)$	mol•Pa^{-1}•m^{-3}
Z_L	Fugacity Capacity in lipid	$Z_L = K_{OW} \bullet Z_W$	mol•Pa^{-1}•m^{-3}
Z_B	Fugacity Capacity in biota	$Z_B = K_B \bullet Z_W$ $= (\phi_L \bullet Z_L) + (\phi_P \bullet 0.05 \bullet Z_L) + (\phi_W \bullet Z_W)$	mol•Pa^{-1}•m^{-3}
Fractions			
ϕ_{OC}	Fraction of organic carbon in sediment/soil	0.01 unless otherwise measured	kg•kg^{-1}
$\phi_{OC\text{-}W}$	Fraction of organic carbon in particles suspended in water	0.2	kg•kg^{-1}
ϕ_D	Fraction of chemical in dissolved form in water	$\frac{1}{1 + \left[\phi_{OC-W} \bullet \left(\frac{V_P}{V_W}\right) \bullet R_{OC} \bullet K_{OW} \bullet \rho_{P-W} \bullet 10^{-3}\right]}$	unitless
ϕ_L	Fraction of lipid in biota or in diet	0.05 unless otherwise measured	kg•kg^{-1}
ϕ_P	Fraction of protein in biota	Invertebrates: 0.1 (Hendricks et al., 2005) Mammals: 0.21 (Hendricks et al., 2005) unless otherwise measured	kg•kg^{-1}
ϕ_W	Fraction of water in biota	0.8 unless otherwise measured	kg•kg^{-1}

Symbol	Description	Constant Value or Equation	Units
$\phi_{OC\text{-}A}$	Fraction of organic carbon in air particles	0.2	$kg \bullet kg^{-1}$
ϕ_{AP}	Fraction of chemical in air particles	$\frac{(K_{GP} \bullet C_{TSP-A})}{(1 + K_{GP} \bullet C_{TSP-A})}$	unitless
ϕ_G	Fraction of chemical in gas phase	$1 - \phi_{AP}$	unitless
Volume Fractions			
V_{AP}/V_A	Volume of air particles per Volume of air	$2 \bullet 10^{-11}$	$m^3 \bullet m^{-3}$
V_P/V_W	Volume of suspended particles per Volume of water	Freshwater: $5.0 \bullet 10^{-6}$ Saltwater: $4.4 \bullet 10^{-7}$ (Mackintosh, 2002)	$m^3 \bullet m^{-3}$
V_C/V_L	Volume of chemical per Volume of lipid	V_C/V_L = activity • S_L • V_M	$m^3 \bullet m^{-3}$
Densities			
ρ_A	Density of air (at 25°C)	1.1839	$kg \bullet m^{-3}$
ρ_{OC}	Density of organic carbon	1000	$kg \bullet m^{-3}$
ρ_S	Density of sediment or soil	1500	$kg \bullet m^{-3}$
ρ_L	Density of lipid	900	$kg \bullet m^{-3}$
$\rho_{P\text{-}W}$	Density of particles suspended in water	1500	$kg \bullet m^{-3}$
ρ_D	Density of diet/gavage fluid	900	$kg \bullet m^{-3}$
Constants & Ratios			
R_{OC}	Ratio of K_{OC} to K_{OW}	0.35 (Seth, Mackay, & Muncke 1999)	$L_{OCTANOL} \bullet (kg_{OC})^{-1}$
k_S	Setschenow or Salting-Out constant	0.0018 (Xie et al., 1997)	Unitless

Symbol	Description	Constant Value or Equation	Units
R_{SALT}	Ratio of freshwater to saltwater solubility	$10^{(k_S \bullet C_{SALT} \bullet V_M)}$ (Xie et al., 1997)	Unitless
F	Fugacity Ratio (Calculated with Walden's Rule)	$F = \exp\left\{-6.79 \bullet \left[\left(\frac{T_M}{T}\right) - 1\right]\right\}$	unitless (T_M , T in K)
R_{GAS}	Universal Gas Law Constant	8.314	$Pa \bullet m^3 \bullet mol^{-1} \bullet K^{-1}$
Partition Coefficients			
K_{OW}	Octanol / Water	Freshwater: Appendix A Saltwater: $K_{OW} \bullet R_{SALT}$	unitless
K_{OA}	Octanol / Air	Appendix A	unitless
K_{GP}	Gas / Particle	$(V_{AP}/V_A) \bullet (\phi_{OC\text{-}A}) \bullet (K_{OA})$	$m^3 \bullet \mu g^{-1}$
K_{OC}	Organic Carbon / Water	$R_{OC} \bullet K_{OW}$	$L_{WATER} \bullet kg_{OC}^{-1}$
Additional Chemical Properties			
P	Vapour Pressure	Appendix A	Pa
H	Henry's Law Constant	Freshwater: $P \bullet S_W^{-1}$ Saltwater: $P \bullet S_W^{-1} \bullet R_{SALT}$	$Pa \bullet m^3 \bullet mol^{-1}$
T	Temperature of System	298 (25)	K (°C)
T_M	Melting Temperature	Appendix A	Kelvin (K)
V_M	Le Bas Molar volume	Appendix A	$cm^3 \bullet mol^{-1}$

Values representing the fraction of organic carbon, fraction of lipid, fraction of water and fraction of protein in different media were obtained from the original study's data, where available. If information on these properties was not available, then some generalizations or standard values (listed in Table 3.4) were applied in the calculations.

Reported concentrations measured in water and air were considered to represent the chemical's total concentration. Total concentrations include both the fraction of dissolved chemical (in water) or fraction of chemical in pure gas phase (in air), as well as the fraction of chemical sorbed to dissolved or suspended organic particles. The fraction

of chemical in the dissolved phase (ϕ_D) was calculated to represent the fraction of chemical that is bioavailable in water:

$$\phi_D = \frac{1}{1+\left[\phi_{OC-W} \bullet \left(\frac{V_P}{V_W}\right) \bullet R_{OC} \bullet K_{OW} \bullet \rho_{P-W} \bullet 10^{-3}\right]} \qquad \text{…Equation 3.18}$$

Where ϕ_{OC-W} is the fraction organic carbon in particles suspended in water (0.2); V_P/V_W is the volume fraction of particles suspended in water (Freshwater: $5.0 \bullet 10^{-6}$; Saltwater: $4.4 \bullet 10^{-7}$ $m^3 \bullet m^{-3}$); R_{OC} ratio relating K_{OC} to K_{OW} (0.35), and ρ_{P-W} is the density of particles suspended in water (1500 $kg \bullet m^{-3}$).

The fraction of chemical in pure gas phase (ϕ_G) was calculated to represent the fraction of chemical that is bioavailable in air:

$$\phi_G = 1 - \phi_{AP} = 1 - \frac{\left(K_{GP} \bullet C_{TSP-A}\right)}{\left(1 + K_{GP} \bullet C_{TSP-A}\right)} \qquad \text{…Equation 3.19}$$

Where ϕ_{AP} is the fraction of chemical sorbed to air particles; K_{GP} is the gas-particle partition coefficient [$(V_{AP}/V_A) \bullet (\phi_{OM-A}) \bullet (K_{OA})$; $m^3 \bullet \mu g^{-1}$]; V_{AP}/V_A is the volume fraction of particles in air ($2 \bullet 10^{-11}$ $m^3 \bullet m^{-3}$), ϕ_{OM-A} is the fraction of organic carbon in air particles (0.2 $kg \bullet kg^{-1}$); K_{OA} is the octanol-air partition coefficient; and C_{TSP-A} is the concentration of particles in the air (80 $\mu g \bullet m^{-3}$).

For both single chemical concentrations, and chemical mixture component concentrations, the freely dissolved concentrations (i.e., the bioavailable concentrations) were calculated in water (C_W) or in air (C_A):

$$C_W = \phi_D \bullet C_{W\text{-reported}} \qquad \text{Equation 3.20}$$

$$C_A = \phi_G \bullet C_{A\text{-reported}} \qquad \text{Equation 3.21}$$

Chemical Property Data of PHCs

The following chemical property data were required for activity, fugacity, C_L, and V_C/V_L calculations: molar mass ($g \bullet mol^{-1}$); vapour pressure (P ; Pa), solubility in water (S_w ; $mol \bullet m^{-3}$), octanol-water partitioning coefficient (K_{OW} ; unitless), Le Bas molar volume (V_M ; $cm^3 \bullet mol^{-1}$), and melting temperature (T_M ; K). All chemical property data for individual PHCs were obtained from Mackay et al. (2006), or, if not available in Mackay et al., were obtained from EpiSuite (USEPA, 2014). Values identified as "recommended" in Mackay et al. (2006) were preferred, but if no recommended values were identified, other available data from Mackay et al. (2006) were selected. Where available, empirical values, and values measured at 298 K were preferred over modelled or QSAR-predicted values. Finally, QSAR-predicted values were applied for some chemicals, particularly some mixture components when no recommended or empirical values were available (Table 3.5). Appendix A presents all PHC chemical property data for individual PHCs (including alkylated PAHs) and mixture components that were applied in calculations.

For thermodynamic consistency and comparability between chemicals (Section 3.2.1), all calculations used the aqueous solubility and vapour pressure of liquids (for chemicals with $T_M \leq 298K$), or the solubility and vapour pressure of sub-cooled liquids (for chemicals with $T_M > 298K$). If only data for vapour pressure and/or solubility of solids were available, then the fugacity ratio (F) calculated using Walden's Rule (Equation 3.8) was applied to convert solid-based properties, to a sub-cooled liquid-based properties:

$$S_{SUB\text{-}COOLED\ LIQUID} = S_{SOLID} \bullet F^{-1} \qquad \text{…Equation 3.22}$$

$$P_{SUB\text{-}COOLED\ LIQUID} = P_{SOLID} \bullet F^{-1} \qquad \text{…Equation 3.23}$$

Organic chemicals have been shown to have a lower aqueous solubility in salt solutions than in freshwater (Xie et al., 1997). For toxicity or guideline concentrations of chemicals in saltwater, chemicals' aqueous solubility, Henry's Law Constant, and K_{OW}, were corrected to account for altered behaviour in presence of ions. The unitless ratio of freshwater aqueous solubility to saltwater solubility was calculated using the following equation (Xie et al., 1997):

$$R_{SALT} = 10^{(k_S \bullet C_{SALT} \bullet V_M)} \quad \text{…Equation 3.24}$$

Where k_s is the Setschenow or "salting-out" constant (with value = 0.0018; Xie et al., 1997), C_{SALT} is the concentration of salt (0.5 mol/L), and V_M is the molar volume (cm^3/mol) of the chemical. The following saltwater-corrected properties were applied in activity, fugacity, C_L, and V_C/V_L calculations involving saltwater-based media:

$$S_{W\ in\ SALTWATER} = R_{SALT} \bullet (S_{W\ in\ FRESHWATER})^{-1} \quad \text{…Equation 3.25}$$

$$H_{\ in\ SALTWATER} = R_{SALT} \bullet (H_{\ in\ FRESHWATER}) \quad \text{…Equation 3.26}$$

$$K_{OW\ in\ SALTWATER} = R_{SALT} \bullet (K_{OW\ in\ FRESHWATER}) \quad \text{…Equation 3.27}$$

Le Bas molar volume was calculated for individual PHCs if it was not available in either Mackay et al. (2006) or in EpiSuite (USEPA, 2014). Molar volume was calculated for each element in the molecule as the number of atoms per element multiplied by the elemental molar volume. The molar volumes (in $cm^3 \bullet mol^{-1}$) for carbon (14.8), hydrogen (3.7), sulfur (25.6), 5-carbon aromatic rings (-11.5), and 6-carbon aromatic rings (-15) were obtained from Mackay et al. (1993). The chemical's molar volume was then calculated as the sum of the total molar volumes for each element in the compound.

Henry's Law Constant (H ; units of $Pa \bullet m^3 \bullet mol^{-1}$) was calculated for all chemicals as the ratio of the liquid vapour pressure to aqueous solubility of liquid (for chemicals with $T_M \leq 298K$), or as the ratio of the sub-cooled liquid vapour pressure to the aqueous solubility of the sub-cooled liquid (for chemicals with $T_M > 298K$).

Chemical Properties of PHC Mixture Components

Many mixture components were identified as specific individual PHCs, in which case chemical-specific properties were applied. But, for those PHC mixture components that were characterized by fractions based on the number of carbon atoms in PHC molecules, established QSARs (Table 3.5) were applied to calculate chemical properties for individual fractions. The median effective carbon (EC) number in a carbon fraction's range was used to calculate physical-chemical properties using QSAR relationships

(e.g., An effective carbon number of 10.5 was used to represent the C10 to C11 fraction).

Table 3.5. Methods to calculate chemical properties for PHC mixture components described by carbon fractions.

Property	Equation	Units	Reference
Molecular Weight (MW)	$MW = 14.07 \bullet EC + 3.51$ …for aliphatic hydrocarbons $MW = 6.36 \bullet EC + 60.86$ …for aromatic hydrocarbons	$g \bullet mol^{-1}$	Verbruggen et al., 2008
Log K_{OW}	$\text{Log } K_{OW} = 0.53 \bullet EC + 0.55$ …for aliphatic hydrocarbons $\text{Log } K_{OW} = 0.15 \bullet EC + 1.76$ …for aromatic hydrocarbons	unitless	Verbruggen et al., 2008
Aqueous Solubility (S_W) *(Liquid or sub-cooled)*	$\text{Log}K_{OW} \leq 10$: $S_W = 1000 \bullet (10\text{^}(-1.175 \bullet \text{Log } K_{OW} + 0.658))$ $\text{Log}K_{OW} > 10$: $S_W = 1000 \bullet (10\text{^}(-1.175 \bullet 10 + 0.658))$	$mol \bullet m^{-3}$	Verbruggen et al., 2008
Vapour Pressure (P)	$EC \leq 12$: $P = 101325 \bullet (10\text{^}(-0.5 \bullet EC + 2.3))$ $EC > 12$: $P = 101325 \bullet (10\text{^}(-0.36 \bullet EC + 0.72))$	Pa	Gustafson et al., 1997
Le Bas Molar Volume (V_M)	Number of Carbons = EC Number of Hydrogen = $(EC \bullet 2 + 2)$ …for aliphatic hydrocarbons Number of Carbons = EC Number of Hydrogen = $(EC \bullet 2)$ …for aromatic hydrocarbons V_M = (Number of Carbons • 14.8 $m^3 \bullet mol^{-1}$) + (Number of Carbons • 3.7 $m^3 \bullet mol^{-1}$) …for all hydrocarbons	$cm^3 \bullet mol^{-1}$	MacKay et al., 2003

EC= effective carbon number
These equations were applied to aliphatic components of soil-based mixture data (Cermak et al., 2008) and sediment- and lipid-based mixture toxicity data (Verbruggen et al., 2008).

Some mixture components were reported as alkylated PAHs (e.g., C1 fluorenes). These alkylated PAH components include all chemicals that share the same PAH base and the same number of carbons attached in any configuration to the PAH base. For example, C1-fluorenes includes both 1-methylfluorene and 9-methylflourene because they both share a fluorene base, and both have one carbon group attached but in different molecular positions. The physical-chemical properties of either a representative

chemical, or the average of multiple representative chemicals were assigned to alkylated PAHs (Table 3.6; Appendix A).

Table 3.6. Representative chemicals used to assign physical-chemical properties to alkylated polycyclic aromatic hydrocarbons (PAHs).

Alkylated PAH	Representative Chemical(s)[1]
C1 chrysenes	1-methylchrysene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene
C1 dibenzothiophenes	1-methyldibenzothiophene
C1 fluorenes	1-methylfluorene, 9-methylfluorene
C1 naphthalenes	1-methylnaphthalene, 2-methylnaphthalene
C1 phenanthrenes	1-methylphenanthrene, 2- methylphenanthrene, 3-methylphenanthrene, 4- methylphenanthrene
C2 dibenzothiophenes	dimethyldibenzothiophene, ethyldibenzothiophene
C2 chrysenes	1,2-dimethylchrysene,1,6-dimethylchrysene, 5,6-dimethylchrysene, 6-ethylchrysene
C2 fluorenes	2-ethylfluorene
C2 naphthalenes	1,2-dimethylnaphthalene, 1,3-dimethylnaphthalene, 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, 2,3-dimethylnaphthalene, 2,6-dimethylnaphthalene
C2 phenanthrenes	1,2-dimethylphenanthrene, 1,3-dimethylphenanthrene, 3,6-dimethylphenanthrene
C3 chrysenes	7,8,12-trimethylbenz(a)anthracene
C3 dibenzothiophenes	trimethyldibenzothiophene
C3 fluorenes	2,3,9-trimethylfluorene, 9-isopropylfluorene
C3 naphthalenes	1,4,5-trimethylnaphthalene, 1,6,7-trimethylnaphthalene
C3 phenanthrenes	1,2,3-trimethylphenanthrene, 9-isopropylphenanthrene
C4 naphthalenes	1,2,3,4-tetramethylnaphthalene
C4 phenanthrenes	1-methyl-7-(1-methylethyl)phenanthrene, 1,2,3,4-tetramethylphenanthrene, 9,10-diethylphenanthrene

[1] If more than one chemical represents the alkylated PAH group, then the average property value across all chemicals in the group was applied.

3.3. Toxicological Effects Data for Individual and Mixtures of Petroleum Hydrocarbons Expressed as Activity, Fugacity, and Lipid-phase Concentration and Volume Fraction

3.3.1. Compilation of Toxicological Effects Data for Individual PHCs

A variety of laboratory-based toxicological effects data on freshwater, saltwater, and terrestrial species for different individual PHCs measured in water, porewater, sediment, air, diet, or internally (i.e., whole-body invertebrate and fish tissue) were compiled from the literature. The objective of the compilation was to consider a variety of PHC toxicity data for different ecological receptors and effects. Toxicity data consist of a concentration of a PHC in a specific medium in units of moles per cubic meter associated with a specific effect to a specific species.

A dataset including 1169 toxicity data points was compiled from a number of different sources (Table 3.7). In summary, toxicity data for 59 different petroleum hydrocarbon chemicals (42 aromatic and 17 aliphatic PHCs) measured in seven different media (water, porewater, sediment, soil, body tissue, diet, and air), seven different species categories, seven different types of toxicological effects and eight different types of endpoints were compiled (Table 3.8; Appendix B).

Table 3.7. Toxicological Data Sources for Individual Petroleum Hydrocarbons

Reference	n Toxicity Data-points	Number of Different Chemicals	Media (n)	Species Categories	Effects	Reported Endpoints
ECOTOX Database (USEPA, 2012a)	40	8 aromatics	Water (28); Soil (12)	Soil Invertebrates; FW/SW Fish; FW/SW Invertebrates;	Growth, Reproduction, Survival	NOAEL, MATC, LOAEL
Edwards et al. (1997)	44	4 aliphatics; 12 aromatics	Air (17); Diet (29)	Mammals	Survival, Growth, Hemo/histo-pathology, Detectable by smell/onset of headache	NOAEL, LOAEL
IRIS Database (USEPA, 2012b)	14	3 aliphatics; 4 aromatics	Air (9); Diet (5)	Mammals	Growth; Reproduction; Development; Hemo/histopathology	NOAEL, LOAEL
Hansen et al. (2003)	272	13 aromatics	Water (225); Porewater (21); Sediment (26)	FW Amphibians; FW/SW Fish; FW/SW Invertebrates	Survival; Growth; Reproduction; Behavioural (sediment avoidance)	NOAEL, EC25, EC50, LC100, LC50, chronic
Di Toro et al. (2000)	27	1 aliphatic; 13 aromatics	Body (33)	FW Fish; SW Invertebrates	Survival	LC50
Batelle (2007)	772	12 aliphatics; 27 aromatics	Water (782)	FW Amphibians; FW/SW Fish; FW/SW Invertebrates	Survival, Growth, Reproduction	LC50, LOAEL, NOAEL

USEPA = United States Environmental Protection Agency; FW = freshwater; SW = saltwater; EC = Effect Concentration; LC = Lethal Concentration; LOAEL = Lowest Observed Adverse Effect Level; MATC = Maximum Acceptable Toxicant Concentration; NOAEL = No Observed Adverse Effect Level; Chronic = 21-90 day duration toxicity test with varying levels of effects on survival, growth, and/or reproduction.

Table 3.8. Toxicological Data Description for Individual Petroleum Hydrocarbons (number of data-points in brackets)

Chemicals	17 different aliphatic PHCs (71); 42 different aromatic PHCs (1098)
Media	Air (26); Diet (32); Body (27); Porewater (21); Sediment (26); Soil (12); Water (1025)
Species Categories	FW Amphibians (5); FW Fish (471); FW Invertebrates (257); Mammals (58); Saltwater Fish (76); SW Invertebrates (290); Soil Invertebrates (12)
Effects	Development (2), Growth (87); Reproduction (42); Survival (1001); Other (2); Behavior – sediment avoidance (1); Hemapathology or Histopathology (34)
Endpoints	EC25 (2); EC50 (1); LC100 (1); LC50 (953); LOAEL (65); MATC (4); NOAEL (78); Chronic (65)

FW = freshwater; SW = saltwater; EC = Effect Concentration; LC = Lethal Concentration; LOAEL = Lowest Observed Adverse Effect Level; MATC = Maximum Acceptable Toxicant Concentration; NOAEL = No Observed Adverse Effect Level; Chronic = 21-90 day duration toxicity test with varying levels of effects on survival, growth, and/or reproduction.

The data sources incorporated into this research represent data reported by government and international working groups. These sources were chosen in part because they are already familiar data sources to regulatory agencies, which may aid in future communication, development, and applications of the activity- and fugacity-based approach to risk assessment and guideline development. In addition, the toxicity data that have already been identified by other expert working groups, and have formed the basis for existing environmental quality criteria, have already undergone some degree of quality screening, and therefore, are expected to be of sufficient quality to be used in this analysis. For example, acute toxicity data from Hansen et al. (2003) have been deemed appropriate for deriving the USEPA National Water Quality Criteria.

Water, sediment, and diet-based toxicity data for individual PHCs, as compiled by Mandeep Purewal (2012) provided the starting point for this data compilation. Toxicity data in Purewal's thesis were obtained through a search of the United States Environmental Protection Agency (USEPA) ECOTOX database (USEPA, 2012a) for aquatic and terrestrial toxicity data on target chemicals likely to occur in a typical crude oil hydrocarbon mixture. Terrestrial toxicity data, primarily for mammals, were identified by Purewal from a review by the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG; Edwards et al., 1997), and from the USEPA Integrated Risk Information System (IRIS) database (USEPA, 2012b). All data provided in Purewal's thesis were

reviewed against original references to check for error and also to identify additional study-specific information required for subsequent calculations and analyses (e.g., specific medium in which chemical concentration was measured, duration of toxicity test). The full original reference papers were not located for some PHC toxicity data cited in Edwards et al., or USEPA (2012a,b). These data were not included in this project's dataset, unless sufficient supplementary information required to calculate activity and fugacity (e.g., chemical dose, exposure medium) were available from other sources. Through this quality assurance process, additional PHC toxicity data (n = 57) were identified from Edwards et al. (1997), and the USEPA IRIS and ECOTOX databases (USEPA 2012a,b), and added to the dataset used in this research. All studies in Appendix B of Edwards et al. (1997) were reviewed against the following criteria. An effect must have been reported. Only toxicity datapoints that were significantly different from control were used. If the study indicated that there were no controls, then reported data were not included in the dataset. If an effect was reported as not having a dose-response relationship, then the reported data were not included in the dataset. If the effect was observed to be reversible in a recovery-phase following exposure, then the datapoint was not included in the dataset (even though effects from many PHCs operating through non-specific modes of action are reversible). Finally, cancer or tumor-based endpoints were not included in the database.

The USEPA document on "Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures" (Hansen et al. 2003) provided toxicity data for freshwater amphibians, and fresh- and salt-water invertebrate and fish species, where concentrations were measured in water (n=225), porewater (n=21), and sediment (26). This dataset included the lethal concentrations (LC50s) that were used to calculate the final acute and chronic values used by USEPA in developing their PAH sediment quality guidelines. Di Toro et al. (2000) reported 33 body burden data points that had been cited by the USEPA (Hansen et al. 2003). These data describe the quantity of individual PAHs per gram of lipid in invertebrates and in fish that was associated with 50% mortality (LC50) in a laboratory toxicity test.

The data from Di Toro et al. (2000) and Hansen et al. (2003) were checked against the original data citations for their accuracy and applicability to this research. If the original citation for the data as referenced in Di Toro et al. (2000) or in Hansen et al. (2003) could not be located, or data were cited as unpublished data, then these data were not included in the dataset. For example, Di Toro et al. (2000) contained a large data set of acute toxicity values, including toxicity data for at least fifteen species that are not represented in this project's dataset. These data, however, were not included in this project dataset because the original acute toxicity data were not included in Di Toro et al. (2000) and, therefore, unavailable.

Internal concentrations of dichlorobenzene from Table 3 of Di Toro et al. (2000) and originally determined by Van Wezel et al. (1995) were not included in study because it is not a single chemical, but rather a mixture of two isomers [1,2-dichlorobenzene and 1,4-dichlorobenzene with different melting points (-17°C and 53°C respectively), and different aqueous solubilities (at 25°C, liquid solubility of 1,2-dichlorobenzene = 0.952 mol/m^3 and sub-cooled liquid solubility of 1,4-dichlorobenzene = 0.544 mol/m^3).

Battelle (2007) contains a compilation of aquatic toxicity data (n=782) on both aromatic (n=724) and aliphatic (n=58) hydrocarbons that had been prepared for the Massachusets Department of Environmental Protection, in support of guideline development for petroleum hydrocarbon fractions. These data were obtained by Batelle through a search of the USEPA ECOTOX database for laboratory-based aquatic toxicity data on 35 different aromatic hydrocarbons, and 43 different aliphatic hydrocarbons. The data included results for both freshwater and saltwater invertebrate and fish species, on survival, growth, and reproduction, and a variety of effects levels (i.e., LC50s, LOECs, and NOECs). Original studies from the ECOTOX database were not checked because the data described by Batelle was considered to be consistent with the data screening process used in the present study.

Additional datasets that compile a large amount of PHC toxicity data include the Petrotox database (Redman et al., 2012), and the USEPA Toxicity Residue database (Jarvinen & Ankley, 1999; compiled from over 500 literature references; toxicity data for 190 species, 200 organic and inorganic chemicals, survival, growth and reproduction

effects). These datasets have not been included in this analysis at this time due to likely overlap with some of the data already included in the project database. However, these data may be useful in future research.

The majority of the toxicity data for individual PHCs were LC50s (i.e., concentrations of PHCs that were lethal to 50% of test organisms; Table 3.8). Therefore, the majority of the analyses in this research focused on LC50 subset of the toxicity dataset. Table 3.9 summarizes the composition of this LC50 subset of the toxicity dataset for individual PHCs.

Table 3.9. LC50s for Individual Petroleum Hydrocarbons.

PHC Category	Datapoints	Number of Unique Chemicals	Media (number of datapoints)	Species Categories	Number of Different Species
Aliphatic	49	12	Water (48); Body (1)	FW Fish (15); SW Fish (14); SW Invertebrate (20)	6
Aromatic	904	40	Water (834); Porewater (21); Sediment (23); Body (26)	FW Amphibian (5); FW Fish (395); FW Invertebrate (209); SW Fish (56); SW Invertebrate (239)	111

PHC = Petroleum Hydrocarbon; FW = freshwater; SW = Saltwater

3.3.2. Compilation of Toxicological Effects Data for PHC Mixtures

Water-, sediment-, lipid-, and soil-based toxicity test data for mixtures of PHCs were compiled from the primary literature (Table 3.10). Studies with detailed mixture composition characterization were selected to test the additivity of activity, fugacity, and lipid-phase concentration and volume fraction in environmentally-relevant complex mixtures of PHCs.

Table 3.10. Toxicological Data Sources for Mixtures of Petroleum Hydrocarbons

Reference	Media	Chemical Mixtures	Test Species (Species Categories)	Number of Datapoints
Barron, Podrabsky, & Ricker (1999)	Water	Weathered underwater plume (3 different plume sources)	SW Invertebrate: • *Mysidopsis bahia*	3
Cermak, Stephenson, Birkholz, Want, & Dixon (2010)	Soil	Four different crude oil distillates (F2, F3, F3a, and F3b)	Soil Invertebrates: • *Orthonychirus folsomi* • *Eisenia andrei*	9
Verbruggen et al. (2008)	Sediment	One light fuel oil mixture, and one heavy lubricant oil mixture	FW Invertebrates: • *Chironomus riparius* • *Ephoron virgo* • *Hyalella azteca* • *Plectus acuminatus* SW Invertebrates: • *Corophium volutator* • *Echinocardium cordatum*	12
Verbruggen et al. (2008)	Lipid	*Same toxicity test as sediment-based PHC mixture toxicity test*	*Same toxicity test as sediment-based PHC mixture toxicity test*	12

Table Note: FW = freshwater; SW = saltwater.

All selected PHC mixture-based toxicity data-points were LC50s, to be comparable with the LC50 subset of toxicity data compiled for individual PHCs.

Water-based PHC Mixture Toxicity Data

Water-based PHC mixture toxicity data were obtained from Barron et al. (1999). Barron et al. tested the toxicity of the saltwater-accommodated fraction of PHC mixtures prepared from three different plumes of oil spilled from an underwater oil field. The composition of the mixture in the water was characterized using 34 named non-substituted and alkylated PAHs, and 26 alkanes. The experimental organisms, *Mysidopsis bahia* (Mysid shrimp), were exposed to PHC mixtures prepared in water for six to seven days. Only the LC50 endpoints were included in this research, but Barron et al. also reported NOEC, LOEC, LC20/EC20, and EC50 for survival and growth endpoints. Individual component concentrations in pure product were interpolated from Figures 2 and 3 in Barron et al. Mixture components with concentrations reported as less than the lower calibration limit or less than the method detection limit were not

included in the summation calculations for activity, fugacity, lipid-normalized concentration, or volume fraction concentration. The concentrations for each individual component in the mixture associated with toxic endpoints (i.e., LC50) were calculated:

$$\left(\frac{\text{Concentration of component in 100\% product}}{\text{Concentration of TPH in 100\% product}}\right) \bullet \text{TPH concentration for toxic endpoint}$$

Where TPH = total petroleum hydrocarbons.

Soil-based PHC Mixture Toxicity Data

Soil-based PHC mixture toxicity test data were from Cermak et al. (2010). Crude oil was distilled into four different fractions based on boiling points, labelled F2, F3, F3a, and F3b, and then mixed with soil for toxicity exposure experiments. The composition of each distillate was characterized based on concentrations of n-alkanes per carbon number (ranging from 9 to 38 carbons), and concentrations of 29 non-substituted and alkylated PAHs. LC50 toxic endpoints were reported for two different soil invertebrate species: *Orthonychirus folsomi* (Collembola; 7 and 35 day exposures) and *Eisenia andrei* (earthworms; 14 day exposure for F2 mixture, and 28 day exposure to the F3, F3a, and F3b mixtures).

Sediment-based PHC Mixture Toxicity Data

Sediment-based exposure to two different PHC mixtures was tested for toxicity by Verbruggen et al. (2008). One mixture was a lighter fuel oil, and the other mixture was a heavier lubricant oil mixture. Both mixtures were characterized using carbon fractions (as opposed to specific individual PHC compounds): composition was described using the concentration of PHCs in 31 aromatic carbon fractions and in 31 aliphatic carbon fractions. For example, the ">C23-C24" aromatic fraction includes all aromatic PHC mixture components with greater than 23 carbons, and less than or equal to 24 carbons in their chemical structure. Toxicity of the PHC mixtures was reported as LC50s for four freshwater invertebrate species [*Chironomus riparius* (midge), *Ephoron virgo* (mayfly), and *Hyalella azteca* (amphipod), and *Plectus acuminatus* (nematode); all 10-day exposures], and two saltwater invertebrate species [*Corophium volutator* (mud shrimp; 10 day exposure), and *Echinocardium cordatum* (urchin; 14 day exposure).

Verbruggen et al. (2008) also reported NOAECs for survival, and NOAECs and EC50s for growth from these toxicity tests. However, only the LC50s were included in this present analysis.

Lipid-based PHC Mixture Toxicity Data

Concentrations of PHC mixtures in the lipid phase of test organisms were also obtained from Verbruggen et al. (2008) – the same study from which sediment-based PHC mixture toxicity data were obtained. The PHC mixture composition and toxicity test species and durations for these lipid-based PHC mixture concentrations were the same as described above for sediment. Verbruggen et al. calculated the internal lipid-phase composition of PHC mixture components (in mmol/L_{LIPID}) by applying an equilibrium partitioning model in 2 phases. The first phase calculated porewater PHC concentration in equilibrium with the organic carbon in sediment and a pure oil phase (e.g., oil droplets or layer coating sediment particles). The second phase calculated the lipid-normalized body burden by applying an equilibrium model between porewater and cell membranes (i.e., the lipid-phase of test organisms).

3.3.3. Categorization of Toxicological Effects Data

All toxicity data were categorized by chemical, media, effect, effect level, and species category. Chemicals were broadly designated as either aliphatic or aromatic. Furthermore, the individual chemicals included in this toxicity dataset are thought to be representative of chemicals often found in environmentally relevant mixtures of PHCs. For example, the chemicals in this dataset are also listed in the PETROTOX database (Redman et al., 2012) as typical oil mixture constituents.

Toxicity data were categorized into the different media in which the chemical measurements were made, including air, water, sediment, porewater, soil, diet, and internal (i.e., in the organism, such as critical body burden for example). Toxicity tests involving administration of chemical via gavage were considered in the diet category. Media types were considered as either external media (those that were measured in an abiotic medium; i.e., sediment, soil, water, porewater, diet, air), or as internal media (those that were measured in a biotic medium; i.e., in tissue or whole body).

Toxic effects categories included survival, growth, reproduction, development, sediment avoidance, internal (e.g., liver weight, or altered hematology), and other effects. Effect levels for toxicity data were reported at various effect observation frequencies (e.g., EC10, the effect concentration at which 10% of test organisms are expected to show an effect). Lower effect concentrations (e.g., EC5, EC10) at which a low percentage of organisms are adversely affected are appropriate for this research, because they align with the goal of protecting whole wildlands ecosystems. However, EC50s like the lethal concentrations to 50% of organisms (i.e., LC50) are commonly reported, and therefore were also included in the dataset for this research. There is greater statistical certainty within a given study when determining an LC50 at the middle of a dose-response curve, compared to a LOAEL or EC5 which typically fall at the tails of dose-response curves. Ultimately, toxicity data belonged to one of the following effect level categories: EC25, EC50, LC100, LC50, LOAEL, MATC, NOAEL, or chronic.

Species were categorized into one of the following seven taxonomic and habitat-based categories: freshwater invertebrate, freshwater fish, saltwater invertebrate, saltwater fish, amphibians, terrestrial soil invertebrates, and air-breathing mammals.

3.3.4. Activities and Fugacities Describing the Toxicity of Individual PHCs

The toxicity of individual PHCs was compared across different chemicals, media, species, effects and endpoints. For each of these comparisons, individual PHC toxicity data (e.g., LC50s) were expressed in four different ways: as activity, fugacity, and lipid-phase concentration (C_L) and volume fraction (V_C/V_L). All statistical tests describing individual PHC toxicity (t-tests, ANVOAs, ANCOVAs, linear regressions) were run using log-transformed toxicity data.

Across-Chemical Comparison of Individual PHC Toxicity

To test for differences in the toxicity of individual PHCs (represented by log-transformed LC50s) between aliphatic petroleum hydrocarbons (i.e., saturated or unsaturated straight-chained, branch-chained, and cyclic hydrocarbons) and aromatic hydrocarbons (i.e., containing one or more benzene ring; Figure 2.1), Welch's t-test for

difference of means assuming unequal variances was used. Toxicity of individual PHCs (represented by log-transformed LC50s) was also compared by linear regression across a range of chemical hydrophobicity as described by the logarithm of chemical's octanol-water partition coefficient, log K_{OW}. Differences in the linear regression of log LC50 against log K_{OW} between aliphatic and aromatic hydrocarbons were tested using Analysis of Co-variance (ANCOVA).

Across-Media Comparison of Individual PHC Toxicity

Internal and External Media

To test for differences in the toxicity of individual PHCs (represented by log-transformed LC50s) between internal-based media (i.e., biotic media representing measurements made inside an organism: lipid media) and external media (i.e., abiotic media including water, porewater, and sediment), Welch's t-tests for difference of means assuming unequal variances were used.

Water, Porewater, Sediment, and Lipid Media

Analysis of variance (ANOVA) was used to test for differences in the mean toxicity of individual PHCs (represented by log-transformed LC50s) between any individual media (water, porewater, sediment, and lipid). Tukey's post-hoc honest-significant difference (HSD) multiple comparison was also used to test for differences in mean log LC50s between all possible pair-wise combinations of media.

Across-Species Comparison of Individual PHC Toxicity

Species Categories

Analysis of variance (ANOVA) was used to test for differences in the mean toxicity of individual PHCs (represented by log-transformed LC50s) between five different categories of species (freshwater amphibians, invertebrates, fish, or saltwater invertebrates or fish). Tukey's post-hoc honest-significant difference (HSD) multiple comparison was also used to test for differences in mean log LC50s between all possible pair-wise combinations of species categories.

Individual Species

PHC toxicity (represented by log-transformed LC50s) was compared across individual species by developing species-sensitivity distribution curves, where the order of species was presented in order from lowest to highest mean toxicity (where LC50s were expressed as log-transformed activity).

Across-Effects and Across-Endpoints Comparison of Individual PHC Toxicity

The mean and range between the 5th and 95th percentiles of activities associated with seven different types of effects [survival, growth, reproduction, development, behaviour, hemapathology or histopathology, or other (detectable by human smell and onset of headache in humans)] were graphically compared. These effects were identified by one or more of the following eight different endpoints: LC50, EC50, EC25, LC100, LOAEL, NOAEL, MATCH, or chronic (i.e., 21-90 day duration toxicity test with varying levels of affected survival, growth, and/or reproduction). All toxicity data described in Table 3.7, for all effects and endpoints were first log-transformed and then included in this comparison between effects (not just LC50 data).

3.3.5. Activities and Fugacities Describing the Toxicity of PHC Mixtures

The toxicity (i.e., LC50s) of mixtures of PHCs was expressed as activity, fugacity, and lipid-phase concentration and volume fraction, using equations in Table 3.3. PHC mixture toxicity was summarized across all media (n=36), as well as by each individual medium: lipid (n=12), sediment (n=12), water (n=3), and soil (n = 9).

3.4. Toxicity of Petroleum Hydrocarbon Mixtures Compared to Toxicity of Individual Petroleum Hydrocarbons

Toxicity data for PHC mixtures (LC50s; Section 3.3.2) were compared to toxicity data for individual PHCs (LC50s only; Section 3.3.1). The range between the 5th and 95th percentile of individual PHC toxicity across all media, species, and individual

chemicals was used to evaluate the contributions to overall PHC mixture toxicity from each individual PHC mixture component (a_i, f_i, $C_{L,i}$, $(V_C/V_L)_i$), as well as from sub-totals of only aromatic components in PHC mixtures ($\sum a_{i,aromatic}$, $\sum f_{i,aromatic}$, $\sum C_{L,i,aromatic}$, $\sum (V_C/V_L)_{i,aromatic}$), sub-totals of only aliphatic components in PHC mixtures ($\sum a_{i\text{-},aliphatic}$, $\sum f_{i,aliphatic}$, $\sum C_{L,i,aliphatic}$, $\sum (V_C/V_L)_{i,aliphatic}$), and sum total of all chemicals components in PHC mixtures ($\sum a_i$, $\sum f_i$, $\sum C_{L,i}$, $\sum (V_C/V_L)_i$). These comparisons between individual PHC toxicity and PHC mixture toxicity were made on an activity, fugacity, lipid concentration, and volume fraction basis.

3.5. Comparison of Activity- and Fugacity-based Approach with Current Approaches to PHC Risk Assessment and Guideline Development

How does an activity- or fugacity-based risk assessment and guideline development approach compare to existing risk assessment and guideline development approaches? Typically, risk assessments and guideline development are confined to evaluations of a single media at a time, and require a set of exposure concentrations, toxic effects concentrations and guideline concentrations all from the same single medium being evaluated. Current media-specific concentration-based guidelines (which essentially also represent threshold effects concentrations) for PHC mixtures are compared against the broad set of toxicity data considered in this research for PHCs across media, species, and chemicals (Section 3.4). This comparison provides a basis for evaluating current regulatory tools for managing of chemical mixtures relative to the level of protection that they provide for plants and animals in BC wildlands ecosystems.

3.5.1. Current PHC Mixture Guidelines

Existing guidelines for PHC mixtures in water, sediment, and soil that were developed for the Canadian Council of Ministers of the Environment (CCME), the British Columbia Ministry of Environment (BCMoE), and consensus-based guidelines (Long et al., 1995; MacDonald et al., 1996, 2000) were converted to activity, fugacity, concentration in lipid-phase, and volume fraction in lipid-phase, in order to compare existing PHC mixture guidelines with the toxicity data compiled in this research.

Guidelines for individual PHCs (e.g., the BC Contaminated Sites Regulation standards for individual PAHs) are also relevant to BC wildlands, but were not included in this analysis in order to keep the focus on PHC mixtures. Table 3.11 lists the media-specific guidelines for PHC mixtures that are presently applied in wildlands settings.

Table 3.11. Numerical petroleum hydrocarbon mixture guidelines for soil, sediment, and water that can be applied in wildlands.

Medium	Reference	PHC Mixture Description[1]	Value	Units	Narrative Intent
SOIL					
Soil (coarse or fine)	CCME (2008)	F1 (EC# 6-10)	210	µg/g	Agricultural/ Residentia Use
Soil (coarse or fine)	CCME (2008)	F1 (EC# 6-10)	320	µg/g	Commercial/ Industrial Use
Soil (coarse or fine)	CCME (2008)	F2 (EC# 10-16)	150	µg/g	Agricultural/ Residential
Soil (coarse or fine)	CCME (2008)	F2 (EC# 10-16)	260	µg/g	Commercial/ Industrial
Soil (fine)	CCME (2008)	F3 (EC# 16-34)	1300	µg/g	Agricultural/ Residential
Soil (fine)	CCME (2008)	F3 (EC# 16-34)	2500	µg/g	Commercial/ Industrial
Soil (coarse)	CCME (2008)	F3 (EC# 16-34)	300	µg/g	Agricultural/ Residential
Soil (coarse)	CCME (2008)	F3 (EC# 16-34)	1700	µg/g	Commercial/ Industrial
Soil (fine)	CCME (2008)	F4 (EC# >34)	5600	µg/g	Agricultural/ Residential
Soil (fine)	CCME (2008)	F4 (EC# >34)	6600	µg/g	Commercial/ Industrial
Soil (coarse)	CCME (2008)	F4 (EC# >34)	2800	µg/g	Agricultural/ Residential
Soil (coarse)	CCME (2008)	F4 (EC# >34)	3300	µg/g	Commercial/ Industrial
Soil	CSR (1996), Schedule 4	HEPH (EC# 19-32)	1000	µg/g	Agricultural, Urban Park, Residential
Soil	CSR (1996), Schedule 4	HEPH (EC# 19-32)	5000	µg/g	Commercial, Industrial

Table continued next page…

Medium	Reference	PHC Mixture Description[1]	Value	Units	Narrative Intent
Soil	CSR (1996), Schedule 4	LEPH (EC# 10-19)	1000	µg/g	Agricultural, Urban Park, Residential
Soil	CSR (1996), Schedule 4	LEPH (EC# 10-19)	2000	µg/g	Commercial, Industrial
Soil	CSR (1996), Schedule 4	VPH (EC# 5-10)	200	µg/g	Agricultural, Urban Park, Residential, Commercial, Industrial
SEDIMENT					
Sediment (marine)	BCMoE (Nagpal et al., 2006)	LMW PAH (∑6 PAHs)	3.7	µg/g	Protection of Aquatic Life – no adverse effects on biota
Sediment (marine)	BCMoE (Nagpal et al., 2006)	LMW PAH (∑6 PAHs)	7.8	µg/g	Protection of Aquatic Life – minor adverse effects on biota
Sediment (freshwater)	BCMoE (Nagpal et al., 2006)	LMW PAH (∑PAHs with molecular weight < 200g/mol)	0.1	µg/g	Protection of Aquatic Life – No effects threshold based on background approach
Sediment (marine)	BCMoE (Nagpal et al., 2006)	HMW PAH (∑9 PAHs)	9.6	µg/g	Protection of Aquatic Life – no adverse effects on biota
Sediment (marine)	BCMoE (Nagpal et al., 2006)	HMW PAH (∑9 PAHs)	53	µg/g	Protection of Aquatic Life – minor adverse effects on biota
Sediment (freshwater)	BCMoE (Nagpal et al., 2006)	HMW PAH (∑PAHs with molecular weight > 200g/mol)	1	µg/g	Protection of Aquatic Life – No effects threshold based on background approach
Sediment (freshwater)	BCMoE (Nagpal et al., 2006)	Total PAHs (∑16 PAHs)	100	µg/g	Protection of Aquatic Life – severe effect level
Sediment (freshwater)	BCMoE (Nagpal et al., 2006)	Total PAHs (anywhere between 4 and 21 not-specified individual PAHs)	4	µg/g	Protection of Aquatic Life – Effects Range Low
Sediment (freshwater)	BCMoE (Nagpal et al., 2006)	Total PAHs (anywhere between 4 and 21 not-specified individual PAHs)	35	µg/g	Protection of Aquatic Life – Effects Range Moderate
Sediment (freshwater or marine)	CSR (1996), Schedule 9	Total PAHs (∑13 PAHs)	10	µg/g dryweight	Protection of sensitive aquatic life

Table continued next page…

Medium	Reference	PHC Mixture Description[1]	Value	Units	Narrative Intent
Sediment (freshwater or marine)	CSR (1996), Schedule 9	Total PAHs (∑13 PAHs)	20	µg/g dryweight	Protection of typical aquatic life
Sediment (freshwater)	MacDonald et al. (2000)	Total PAHs (∑13 PAHs)	1.61	µg/g dryweight	Protection of Aquatic Life – Threshold Effect Concentration, below which harmful effects are unlikely to be observed
Sediment (freshwater)	MacDonald et al. (2000)	Total PAHs (∑13 PAHs)	22.8	µg/g dryweight	Protection of Aquatic Life – Probable Effect Concentration, above which harmful effects are likely to be observed
Sediment (marine)	MacDonald et al. (1996)	Total PAHs (∑13 PAHs)	1.68	µg/g	Protection of Aquatic Life – Threshold Effect Level, below which harmful effects are unlikely to be observed
Sediment (marine)	MacDonald et al. (1996)	Total PAHs (∑13 PAHs)	16.8	µg/g	Protection of Aquatic Life – Probable Effect Level, above which harmful effects are likely to be observed
Sediment (marine)	MacDonald et al. (1996)	LMW PAHs (∑7 PAHs)	0.312	µg/g	Protection of Aquatic Life – Threshold Effect Level, below which harmful effects are unlikely to be observed
Sediment (marine)	MacDonald et al. (1996)	LMW PAHs (∑7 PAHs)	1.44	µg/g	Protection of Aquatic Life – Probable Effect Level, above which harmful effects are likely to be observed
Sediment (marine)	MacDonald et al. (1996)	HMW PAHs (∑6 PAHs)	0.655	µg/g	Protection of Aquatic Life – Threshold Effect Level, below which harmful effects are unlikely to be observed
Sediment (marine)	MacDonald et al. (1996)	HMW PAHs (∑6 PAHs)	6.68	µg/g	Protection of Aquatic Life – Probable Effect Level, above which harmful effects are likely to be observed
Sediment (marine)	Long et al. (1995)	Total PAHs (∑13 PAHs)	4.02	µg/g dryweight	Protection of Aquatic Life – Effects Range Low
Sediment (marine)	Long et al. (1995)	Total PAHs (∑13 PAHs)	44.8	µg/g dryweight	Protection of Aquatic Life – Effects Range Moderate
Sediment (marine)	Long et al. (1995)	LMW PAHs (∑7 PAHs)	0.552	µg/g dryweight	Protection of Aquatic Life – Effects Range Low

Table continued next page…

Medium	Reference	PHC Mixture Description[1]	Value	Units	Narrative Intent
Sediment (marine)	Long et al. (1995)	LMW PAHs (∑7 PAHs)	3.16	µg/g dryweight	Protection of Aquatic Life – Effects Range Moderate
Sediment (marine)	Long et al. (1995)	HMW PAHs (∑6 PAHs)	1.70	µg/g dryweight	Protection of Aquatic Life – Effects Range Low
Sediment (marine)	Long et al. (1995)	HMW PAHs (∑6 PAHs)	9.60	µg/g dryweight	Protection of Aquatic Life – Effects Range Moderate
WATER					
Water (freshwater)	CSR (1996), Schedule 6	VPH (EC# 5-10)	1500	µg/L	Protection of Aquatic Life
Water (freshwater)	CSR (1996), Schedule 6	LEPH (EC# 10-19)	500	µg/L	Protection of Aquatic Life
Water (freshwater)	CSR (1996), Schedule 6	EPH (EC# 10-19)	5000	µg/L	Protection of Aquatic Life, and use for irrigation, livestock, and drinking water (unfiltered at point of consumption).

1. See Table 3.12 for detailed description of PHC mixture guidelines' designated composition.

Sediment quality guidelines for PHC mixtures were grouped into four different categories (no effect, low effect, moderate, and severe effect levels) based on similar narrative intents. Sediment quality guidelines with narrative intents falling into the "No Effect" category included "no adverse effects on biota" and "no effects threshold based on background approach" from Nagpal et al. (2006); and "protection of sensitive aquatic life" from CSR (1996). Sediment quality guidelines with "Low Effect" narrative intents included "minor adverse effects on biota" and "effects range – low" from Nagpal et al. (2006); "protection of typical aquatic life" from CSR (1996); threshold effect concentrations or levels from MacDonald et al. (1996, 2000), and "effects range low" from Long et al. (1995). Sediment quality guidelines with "Moderate Effect" intents included "effects range moderate" from Nagpal et al. (2006); probable effect concentration or levels from MacDonald et al. (1996, 2000); and "effects range moderate" from Long et al. (1995). Finally, sediment quality guidelines with "Severe Effect" intent included the "severe effect level" from Nagpal et al. (2006). All sediment guidelines are stipulated for protection of aquatic and/or marine life.

All water quality guidelines for PHC mixtures were from Schedule 6 of the Contaminated Sites Regulation (1996) and shared the same narrative intent, protection

of aquatic life. Soil quality guidelines were either from CCME (2008), or from Schedule 4 of the CSR (1996), and were intended to protect either agricultural, residential and urban park land uses, or commercial and industrial land uses, or all five land uses.

Current guidelines for PHC mixtures stipulate the mixture composition using a variety of methods, including both media-specific sum concentrations of specified suites of individual polycyclic aromatic hydrocarbons (PAHs; e.g., sum of 13 individual PAHs), as well as media-specific concentrations by carbon-fractions (designated by boiling point ranges). Table 3.12 lists the compositions of PHC mixtures associated with each of the current PHC mixture guidelines.

Table 3.12. Composition of petroleum hydrocarbon mixture guidelines.

Medium	**Sediment**											**Soil**							**Water**		
Freshwater (F) or Saltwater (S)	F	S	F	F	F	S	S	F	S	S	F	na	na	na	na	na	na	na	F	F	F
PHC Mixture	**Total PAHs**					**LMW PAHs**			**HMW PAHs**			**F1**	**F2**	**F3**	**F4**	**VPH**	**LEPH**	**HEPH**	**VPH**	**LEPH**	**EPH**
Reference	1	2,3	4	4	5	2,3	4	4	2,3	4	4	6	6	6	6	7	7	7	8	8	8
Number of Components in Mixture	13	13	16	4 to 21	13	7	6	na	6	9	na	na	na	na	na	na	na	na	na	na	na
Component																					
naphthalene	✔	✔	✔		✔	✔	✔										(-)	(-)		(-)	
acenaphthylene	✔	✔	✔			✔	✔														
acenaphthene	✔	✔	✔		✔	✔	✔													(-)	
fluorene	✔	✔	✔		✔	✔	✔													(-)	
phenanthrene	✔	✔	✔		✔	✔	✔										(-)	(-)		(-)	
anthracene	✔	✔	✔		✔	✔	✔													(-)	
2-methylnaphthalene		✔			✔	✔															
fluoranthene	✔	✔	✔		✔				✔	✔											
pyrene	✔	✔	✔		✔				✔	✔							(-)	(-)			
benzo(a)anthracene	✔	✔	✔		✔				✔	✔							(-)	(-)			
chrysene	✔	✔	✔		✔				✔	✔											
dibenz(a,h)anthracene		✔	✔		✔				✔	✔							(-)	(-)			
benzo(b)fluoranthene	✔																(-)	(-)			
benzo(k)fluoranthene	✔		✔							✔											
benzo(b+k+j)fluoranthene																					
benzo(a)pyrene	✔	✔	✔		✔				✔	✔							(-)	(-)			

…Table continued next page

Medium	Sediment											Soil							Water		
Freshwater (F) or Saltwater (S)	F	S	F	F	F	S	S	F	S	S	F	na	na	na	na	na	na	na	F	F	F
PHC Mixture	**Total PAHs**					**LMW PAHs**			**HMW PAHs**			**F1**	**F2**	**F3**	**F4**	**VPH**	**LEPH**	**HEPH**	**VPH**	**LEPH**	**EPH**
Reference	1	2,3	4	4	5	2,3	4	4	2,3	4	4	6	6	6	6	7	7	7	8	8	8
Number of Components in Mixture	13	13	16	4 to 21	13	7	6	na	6	9	na	na	na	na	na	na	na	na	na	na	na
Component																					
acenapthalene					✔																
benzo(b)fluorene			✔																		
benzo(g,h,i)perylene			✔							✔											
indeno(1,2,3-c,d)pyrene			✔							✔							**(-)**	**(-)**			
benzene																**(-)**					
toluene																**(-)**					
ethylbenzene																**(-)**					
xylenes																**(-)**					
∑PAHs with MW < 200g/mol								✔													
∑PAHs with MW > 200g/mol											✔										
EC 6-10												✔									
EC 10-16													✔								
EC 16-34														✔							
EC >34															✔						
EC 5-10																✔			✔		
EC 10-19								✔									✔			✔	✔
EC 19-32											✔							✔			

na = not applicable; F = freshwater; S = saltwater; PAHs = polycyclic aromatic hydrocarbons; LMW = low molecular weight; HMW = high molecular weight; VPH = volatile petroleum hydrocarbons; LEPH = light extractable petroleum hydrocarbons; HEPH = heavy extractable petroleum hydrocarbons; EPH = extractable petroleum hydrocarbons; EC = effective carbon; MW = molecular weight;
✔ = included in mixture composition; **(-)** = excluded from mixture composition.
1. MacDonald et al. (2000); **2.** Long et al. (1995); **3.** MacDonald et al. (1995); **4.** Nagpal et al. (2006); **5.** CSR (1996), Schedule 9; **6.** CCME (2008); **7.** CSR (1996), Schedule 4; **8.** CSR (1996) Schedule 6.

Media-specific guidelines for PHC mixtures were converted to activity, fugacity, and lipid-concentration and lipid-volume fraction by applying the same methodology described in Section 3.2. Activities of sediment and soil quality guidelines were calculated using 1% organic carbon content. Soils and sediments with organic carbon content greater than 1% will result in increased solubility of chemicals, thereby lowering the activity associated with these sediment and soil quality guidelines from what is

presented in this research. For example, doubling the organic carbon content to 2% would halve the activity associated with the guideline. Conversely, sediment with an organic carbon content less than 1% would have lower sorptive capacity for chemicals, and activity would be higher than presented. The calculations of activity, fugacity, C_L, and V_C/V_L associated with water quality guidelines assumed that the guidelines represented dissolved fraction of chemicals, and therefore, no correction was made to water guideline concentrations to account for the amount of chemical sorbed to suspended particles. This approach represents a conservative assumption. If these water quality guidelines instead represent total concentrations (i.e., dissolved plus fraction of chemical bound to organic carbon), then their associated activities would decrease with increasing dissolved organic carbon concentrations. Activity was limited to less than or equal to 1 for PHC mixture components when calculating lipid concentration and volume fraction associated with existing PHC mixture guidelines in all media.

The current guidelines can be expressed as a range of molar concentrations, depending on the distribution of the total PHC mixture composition between the various chemical components. Therefore, a range in activity, fugacity, C_L, and V_C/V_L associated with each guideline was calculated using a range in possible mixture compositions (Figure 3.2).

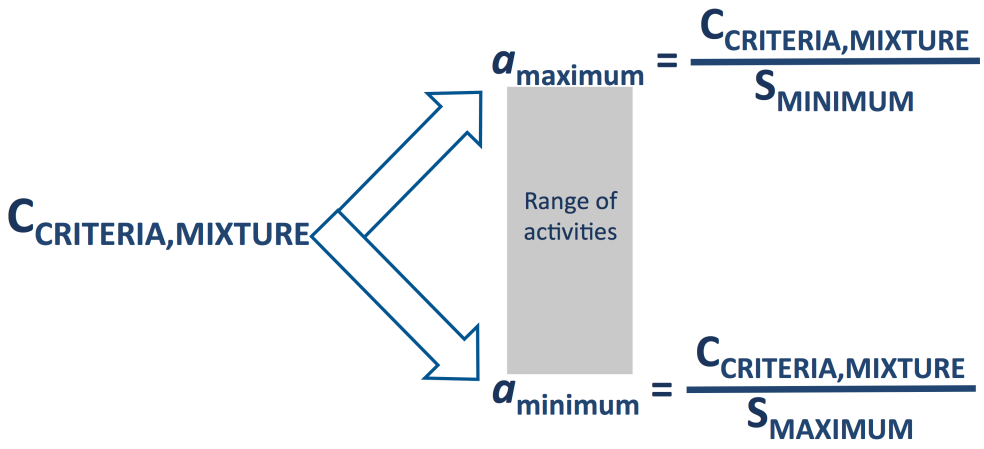


Figure 3.2. Illustration of method used to calculate a range of activities (or fugacity, C_L, and V_C/V_L) associated with each single concentration-based PHC mixture criteria.

Three different activity, fugacity, C_L, and V_C/V_L values were calculated for each PHC mixture guideline that designated its composition with specific chemicals (e.g., the composition of the sediment quality guideline for total PAHs from the BC Contaminated Sites Regulation was designated as ∑16 PAHs; Table 3.12). A maximum value associated with the PHC mixture was calculated by applying a composition comprised of 100% of the lightest mixture component by molecular weight (e.g., 100% naphthalene in the MacDonald et al., 2000 sediment quality guideline for total PAHs). Conversely, a minimum value associated with the PHC mixture guidelines was calculated by applying a composition equal to 100% of the heaviest component (by molecular weight). A third value representing a median activity, fugacity, C_L, or V_C/V_L was calculated by applying a composition that was equi-molar between all stipulated components.

For the PHC mixture guidelines that designated mixture composition by fractions based on number of carbons (e.g., CCME soil quality guidelines, Table 3.12), a total of six different values were calculated to represent a range in activity, fugacity, C_L, and V_C/V_L associated with each guideline. Values were calculated using a composition with i) 100% of components at the bottom-limit of the prescribed fraction; ii) 100% of components at the median of the prescribed fraction; and iii) 100% of components at the upper-limit of the prescribed fraction. Furthermore, for each of those three variants in composition, two variants in the PHC mixture composition were calculated: i) 100% of mixture was comprised of aromatic PHCs (a conservative estimate since aromatic PHCs are more potent than aliphatic PHCs), and ii) 80% of mixture was comprised of aliphatic PHCs and 20% was comprised of aromatic components (an approximation of a typical environmentally relevant PHC mixture; CCME, 2008). The British Columbia guidelines for freshwater sediment quality (Nagpal et al., 2006; Table 3.12) described two PHC mixture fractions designated by molecular weight. For these two guidelines, an equivalent carbon was used to calculate a range of activity, fugacity, C_L, and V_C/V_L values: heavy PAHs with molecular weight greater than 200 g/mol were represented by a carbon range of 19 to 32, and light PAHs with molecular weight less than 200 g/mol were represented by a carbon range of 10 to 19. Chemical properties for the PHC mixture guidelines designated by carbon fractions were calculated from the carbon number using equations in Table 3.5.

A comparison between current guidelines and the distributions of toxicity data developed in this research, expressed as either activity, lipid concentration and volume fraction, will help evaluate the potential applicability of activity-based approaches to guideline development for PHC mixtures, as well as help evaluate current guidelines used to manage PHC mixtures in wildlands.

4. Results and Discussion

4.1. Toxicity of Petroleum Hydrocarbons using Activity- and Fugacity-based Approaches

4.1.1. Toxicity of Individual Petroleum Hydrocarbons

The compiled toxicity dataset for individual petroleum hydrocarbons is presented in Appendix B. In summary, at least one LC50 toxicity datapoint was collected for 55 different chemicals (13 aliphatic and 42 aromatic). At least one toxicity datapoint was collected for 67 different chemicals (18 aliphatics and 49 aromatic PHCs) describing at least one type of effect level (e.g., LC50 or NOAEL) for at least one type of endpoint (e.g., survival or reproduction). These chemicals covered a broad range of molecular weights (aliphatics: 70.1 to 167.9 g/mol; aromatics: 78.1 to 284.8 g/mol), water solubilities (aliphatics: $1.72 \cdot 10^{-3}$ to 18.1 mol/m^3; aromatics: $3.96 \cdot 10^{-6}$ to 22.7 mol/m^3), vapour pressures (aliphatics: 1.43 to $4.2 \cdot 10^4$ Pa; aromatics: $6.1 \cdot 10^{-7}$ to $1.3 \cdot 10^4$ Pa), Henry's Law Constants (H, calculated as vapour pressure divided by water solubility; aliphatics: 48 to $3.3 \cdot 10^5$ Pa•m^3•mol^{-1}; aromatics: 0.035 to 8125 Pa•m^3•mol^{-1}), and log K_{OW}s (aliphatics: 2.31 to 5.65; aromatics: 2.11 to 5.73) (Appendix A).

The geometric mean activity of individual PHCs in LC50s across all single petroleum hydrocarbons in the dataset (n=953) is 0.046 (95%CI: 0.042 to 0.052); the geometric mean fugacity is 1.21 Pa (95%CI: 0.83 to 1.77 Pa); the geometric mean lipid concentration is 81.6 mol/m^3 (95%CI: 73.0 to 91.3 mol/m^3); the geometric mean volume fraction is 0.012 m^3/m^3 (95%CI: 0.011 to 0.14 m^3/m^3) (Table 4.1). Because of the large sample size (n=969), the standard errors (SE) for these estimates of the mean were small (standard errors were less than 2% of means). However, the variation of the data spanned several orders of magnitude; the 5th and 95th percentiles for activity were 0.003 to 0.62, for fugacity were 0.0001 to 2660 Pa, for lipid concentration were 4.35 to

1146 mol/m^3, and for volume fraction were 0.0008 to 0.157 m^3/m^3 (Table 4.1; Figure 4.1). These broad distributions and cumulative frequencies of all LC50 data for single petroleum hydrocarbons expressed as activity, fugacity, lipid concentration, and volume fraction are illustrated in Figure 4.2.

Table 4.1. Summary description of LC50s for single petroleum hydrocarbons expressed as activity, fugacity, and lipid–phase concentration and volume fraction.

Summary Statistic	Log10 Activity (unitless)	Log10 Fugacity (Pa)	Log10 Lipid Concentration (mol/m^3)	Log10 Volume Fraction in Lipid Phase (m^3 chemical/m^3 lipid)
n	953	953	953	953
Arithmetic Mean	-1.33	0.083	1.91	-1.91
95% CI	*-1.38 to -1.29*	*-0.081 to 0.248*	*1.86 to 1.96*	*-1.95 to -1.86*
Median	-1.29	0.266	1.96	-1.85
5th to 95th %iles	-2.51 to -0.208	-3.97 to 3.42	0.636 to 3.06	-3.12 to -0.804
Minimum	-7.47	-8.44	-4.60	-8.30
Maximum	0.585	4.43	3.69	0.002
Standard Deviation	0.712	2.53	0.748	0.721

Summary Statistic	Activity (unitless)	Fugacity (Pa)	Lipid Concentration (mol/m^3)	Volume Fraction in Lipid Phase (m^3 chemical/m^3 lipid)
n	953	953	953	953
Arithmetic Mean	0.135	480	245	0.035
95% CI	*0.116 to 0.154*	*377 to 584*	*216 to 274*	*0.03 to 0.039*
Geometric Mean	0.046	1.21	81.6	0.012
95% CI	*0.042 to 0.052*	*0.83 to 1.77*	*73 to 91.3*	*0.011 to 0.014*
Median	0.052	1.84	91.3	0.014
5th to 95th %iles	0.003 to 0.62	0.0001 to 2660	4.35 to 1146	0.0008 to 0.157
Minimum	3.36×10^{-8}	3.60×10^{-9}	0.00003	5.06×10^{-9}
Maximum	3.84	27028	4928	1.00
Standard Deviation	0.288	1601	446	0.068

CI = Confidence Interval on estimate of the mean.

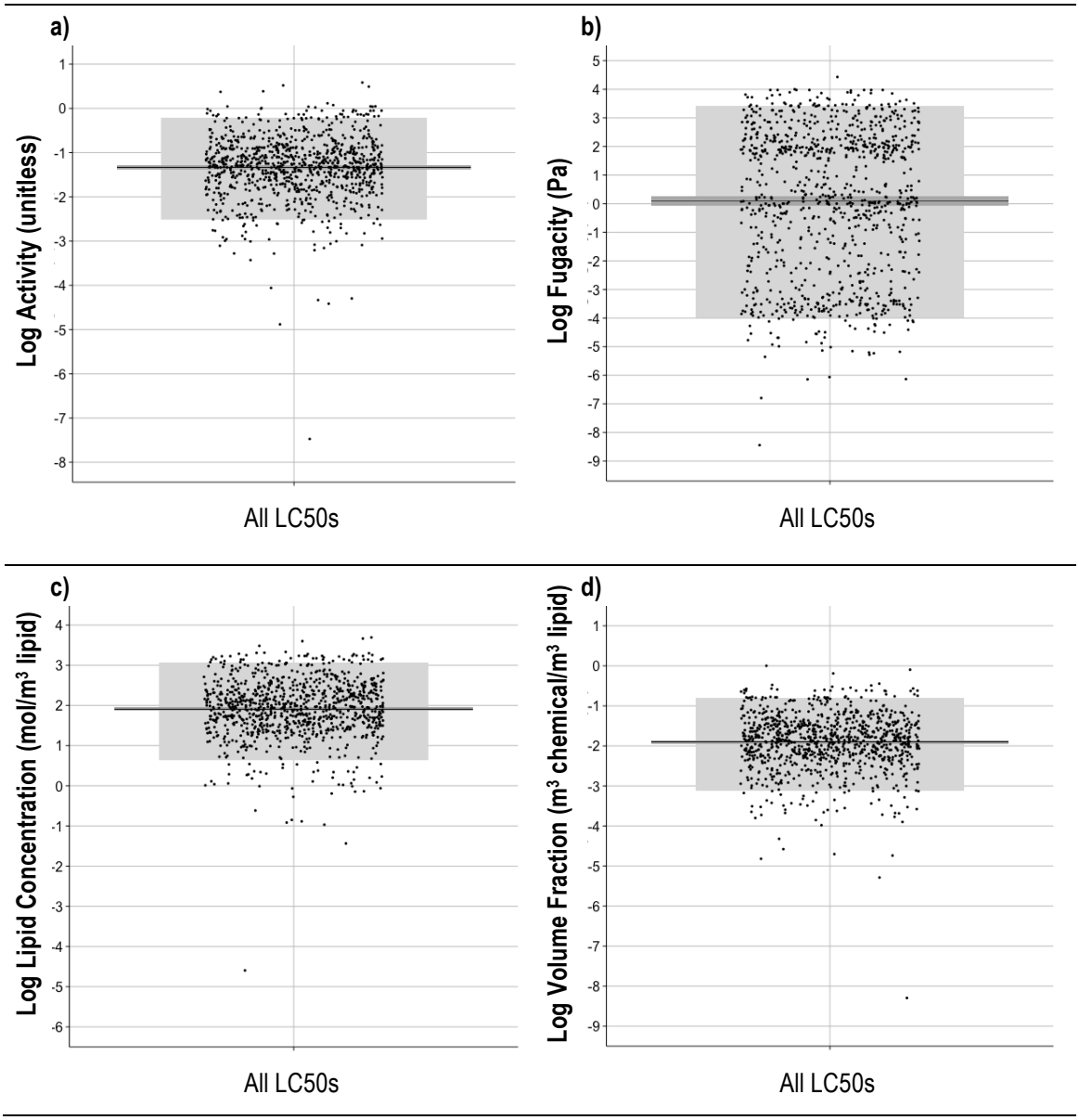
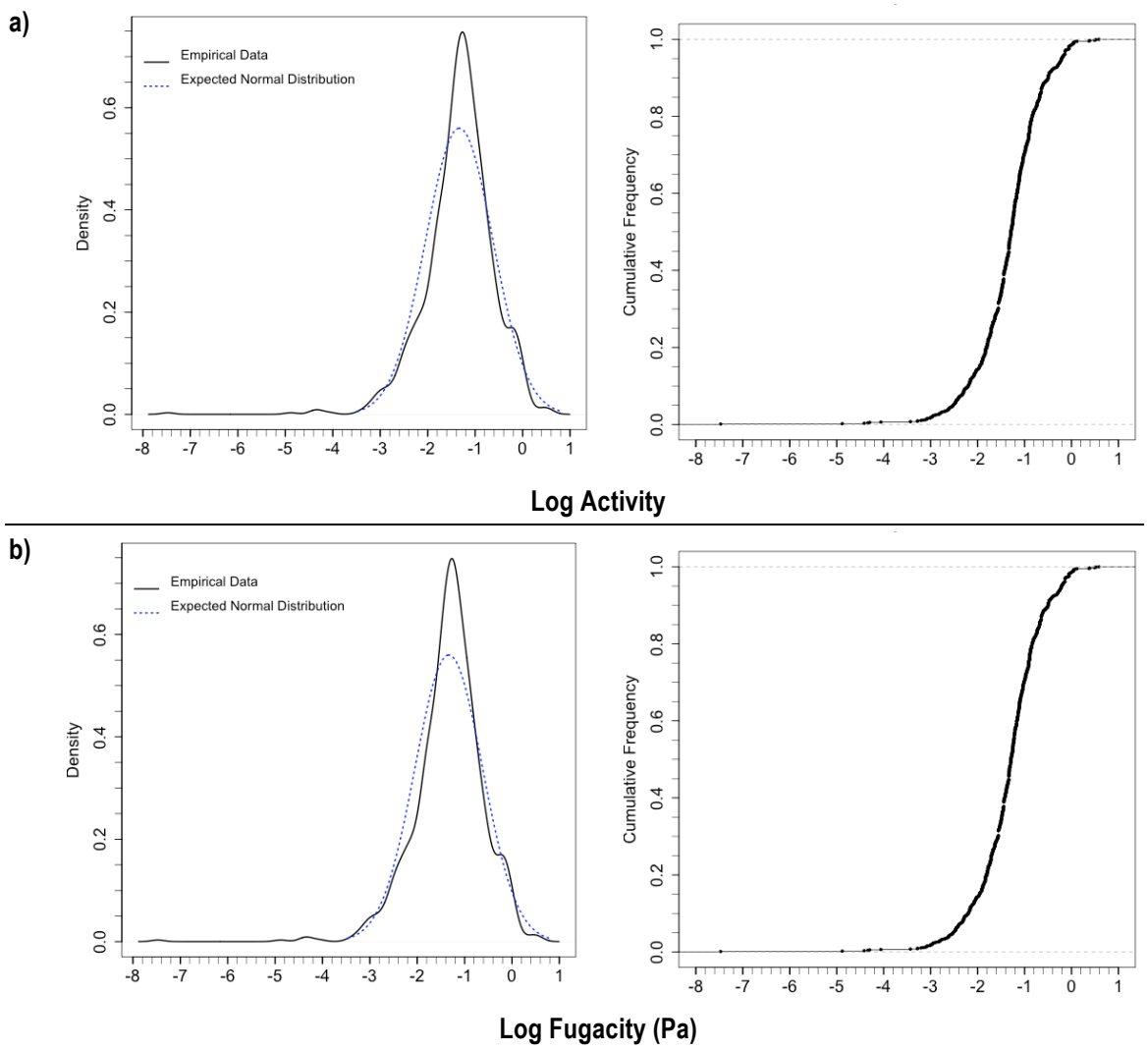


Figure 4.1. LC50s of petroleum hydrocarbons expressed as a) activity; b) fugacity; c) lipid concentration; and d) volume fraction.

• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility;
Solid black horizontal line represents the mean;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.



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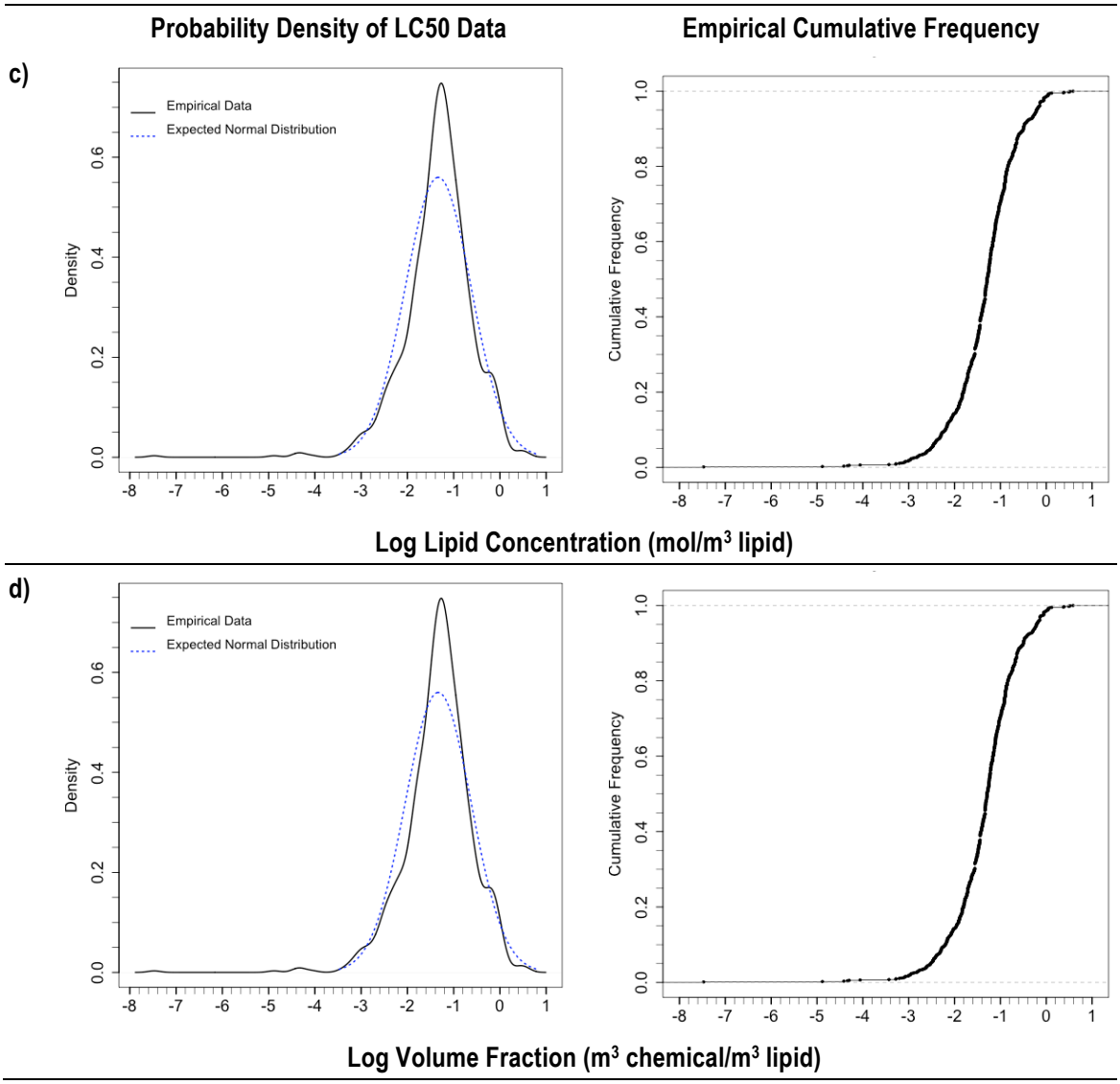


Figure 4.2. Probability density distribution and empirical cumulative frequency of LC50s for individual petroleum hydrocarbons expressed as a) activity; b) fugacity; c) lipid concentration; and d) volume fraction in lipid phase.

Data points with Activity Greater than 1

There were 14 LC50 data points with activity ≥ 1, and they were all from water-based measurements. An activity value ≥ 1 means that toxicity was observed only when the test medium was at ≥100% of its capacity for that chemical. These datapoints with activity greater than or equal to one were measured for seven different chemicals with log K_{OW} ranging from 2.6 to 5.2. Activity of a chemical in a given media theoretically can

not exceed its solubility at an environmentally relevant pressure and temperature. Therefore, these datapoints with activity calculated to be beyond their thermodynamic limit may have arisen from experimental factors or assumptions incorporated into activity calculations (e.g., aqueous solubility of chemical is accurately measured). These datapoints were included throughout the analyses, but were not thought to have a large effect on the overall results since they comprised only 1% of the total data. In other applications however, where datapoints with activity ≥1 comprise a larger percent of the database, it will be important to carefully review their utility and appropriate treatment.

Consistency with other reported values

This study found 90% of LC50s expressed as activity fell between .003 and 0.62 (Table 4.1), which is consistent with the 0.001 to 1.0 range of activities calculated from critical body residues associated with non-polar narcosis by organic chemicals (McCarty et al., 2013). In addition the mean log-transformed activity from this current research (-1.33; sd = 0.712; Table 4.1) is almost identical to the -1.33 (sd = 0.508) log-activity value from McCarty et al. (2013).

The mean log-transformed lipid concentration calculated in this study (1.91 mol/m^3 lipid; sd = 0.748; Table 4.1) was very similar to the mean log-transformed critical body residue (1.73 mol/m^3 lipid; sd = 0.458; n = 161) reported by McCarty et al. (2013). Comparing the standard deviations, there was more variation associated with the data used in this study than in McCarty et al.'s research. In part, this difference in variation can be attributed to the larger sample size (n=953) used in this research compared to n=161 in McCarty et al. These differences are likely due to the data screening criteria used by McCarty et al. to isolate critical body residues specifically associated with the non-polar narcosis mechanism. This current research included a broader range of toxicity data, which may be more likely to include mechanisms other than non-polar narcosis. Incorporating a broad range of toxicity data is relevant to risk assessment applications because ultimately the goal is to protect organisms from chemicals' impacts, regardless of the specific underlying mechanism causing the effect.

The geometric mean volume fraction in this study (0.012 m^3/m^3 ; 95% CI: 0.011 to 0.014 ; Table 4.1) was within the confidence interval for the geometric mean volume

fraction associated with non-polar narcosis (0.007 m^3/m^3 ; 95% CI: 0.001 to 0.06) reported by McCarty (2013). The smaller confidence interval around the geometric means of the volume fraction in this research can be attributed to the larger sample size (n=953) and subsequent smaller standard error in this research compared to McCarty et al. (n=161).

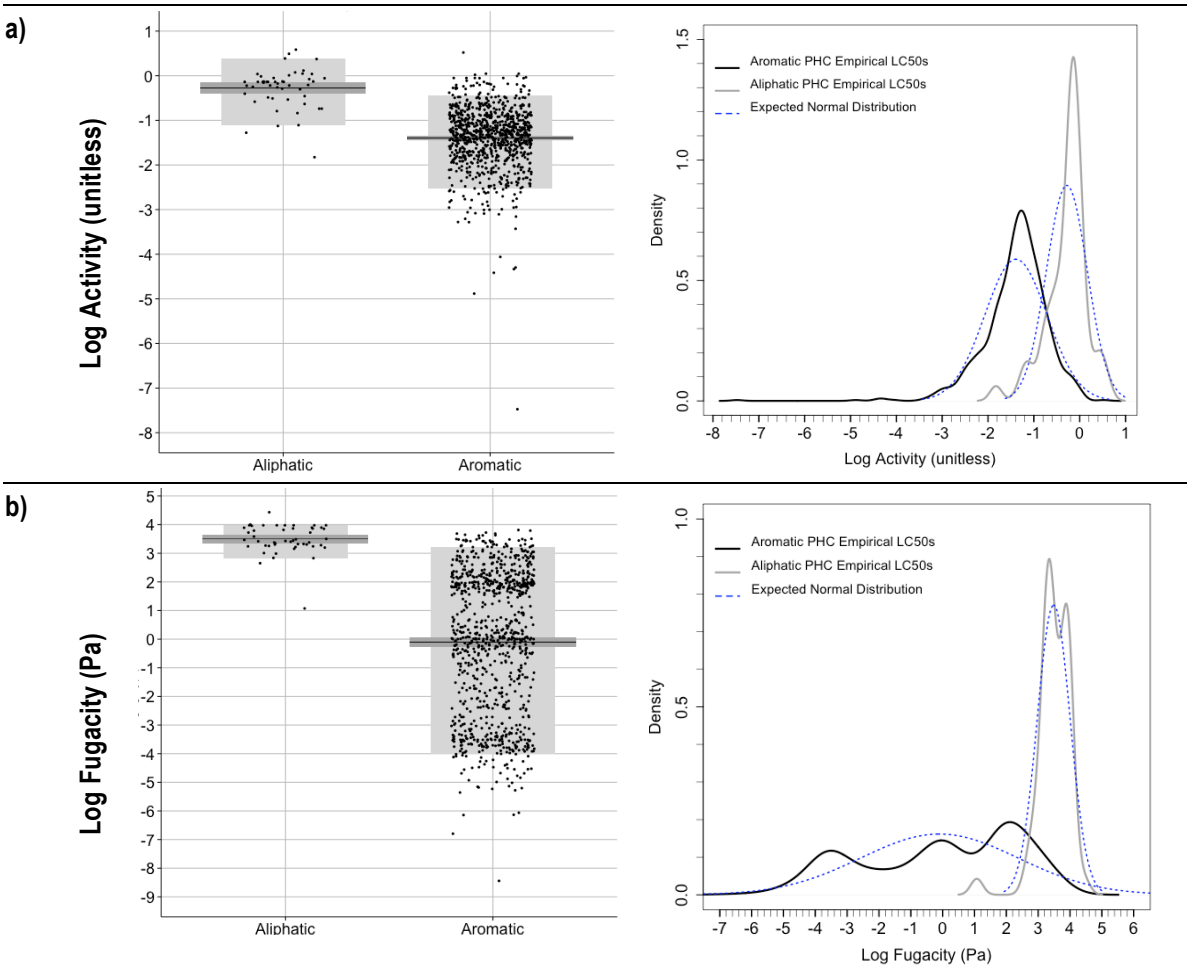
These activity, fugacity, lipid concentration, and volume fraction approaches allow integration of a broad variety of toxicity data for a wide variety of different PHC chemicals, media, and species onto a single plot. This single plot can then be used to select a value at the lower range of the distribution across all available data describing toxicity (Figure 4.2). The selection of probabilistic value which can then be applied for the purposes of managing petroleum hydrocarbons in wildlands. For example, the 5^{th} percentile of all activity values (activity = 0.003; Table 4.1) integrates data describing toxicity from a wide variety of different PHC chemicals, media, and species. Similarly, the 5^{th} percentile of fugacity (0.0001 Pa), lipid concentration (4.35 mol/m^3) or volume fraction (0.0008 m^3/m^3) also incorporate a wide variety of toxicity data, which can be used to inform risk assessment and guideline development.

Across-Chemical Comparison of Individual PHC Toxicity

Aliphatic and Aromatic PHC Toxicity Comparison

When expressed as activity, fugacity, lipid concentration or volume fraction, the toxicity for aliphatic PHCs, as measured by LC50s, was, on average, greater than that for aromatic PHCs (t-test, all $p<0.001$; Figure 4.3; Table 4.3). More specifically, the median activity corresponding to LC50s of aliphatic hydrocarbons is 12.9 times greater than that of aromatics (95% CI: 9.46 to 17.6 times). The median fugacity corresponding to aliphatic LC50s is 3881 times greater than that of aromatics (95% CI: 2328 to 6470). The median lipid concentration corresponding to LC50s for aliphatics is 10.6 times that of aromatics (95% CI: 8.06 to 14.0). The volume fraction in the lipid phase for aliphatics' LC50s is 9.68 times greater than that for aromatics (95% CI: 7.25 to 12.9). This observed difference between the two categories of PHCs is consistent with concentration-based observations that aromatics are generally considered more toxic than aliphatic PHCs (Barron et al., 1999). Aliphatic chemicals are thought to cause

effects primarily by non-polar narcosis and physical interference (e.g., suffocation from clogged fish gills). Physical effects can occur at concentrations greater than the solubility when there is a pure chemical phase present, like oil droplets suspended in water or coating sediment particles. Aromatic chemicals are thought to share non-polar narcosis as a toxic mechanism, but in addition, also often have more specific mechanisms of toxic action (e.g., aryl hydrocarbon receptor mediated toxicity). Metabolism of aromatic PHCs can form metabolites that are also toxic. The presence of these toxic metabolites results in an effect to organisms at lower chemical exposures to parent compounds than expected if the parent compound was the only toxic compound present.



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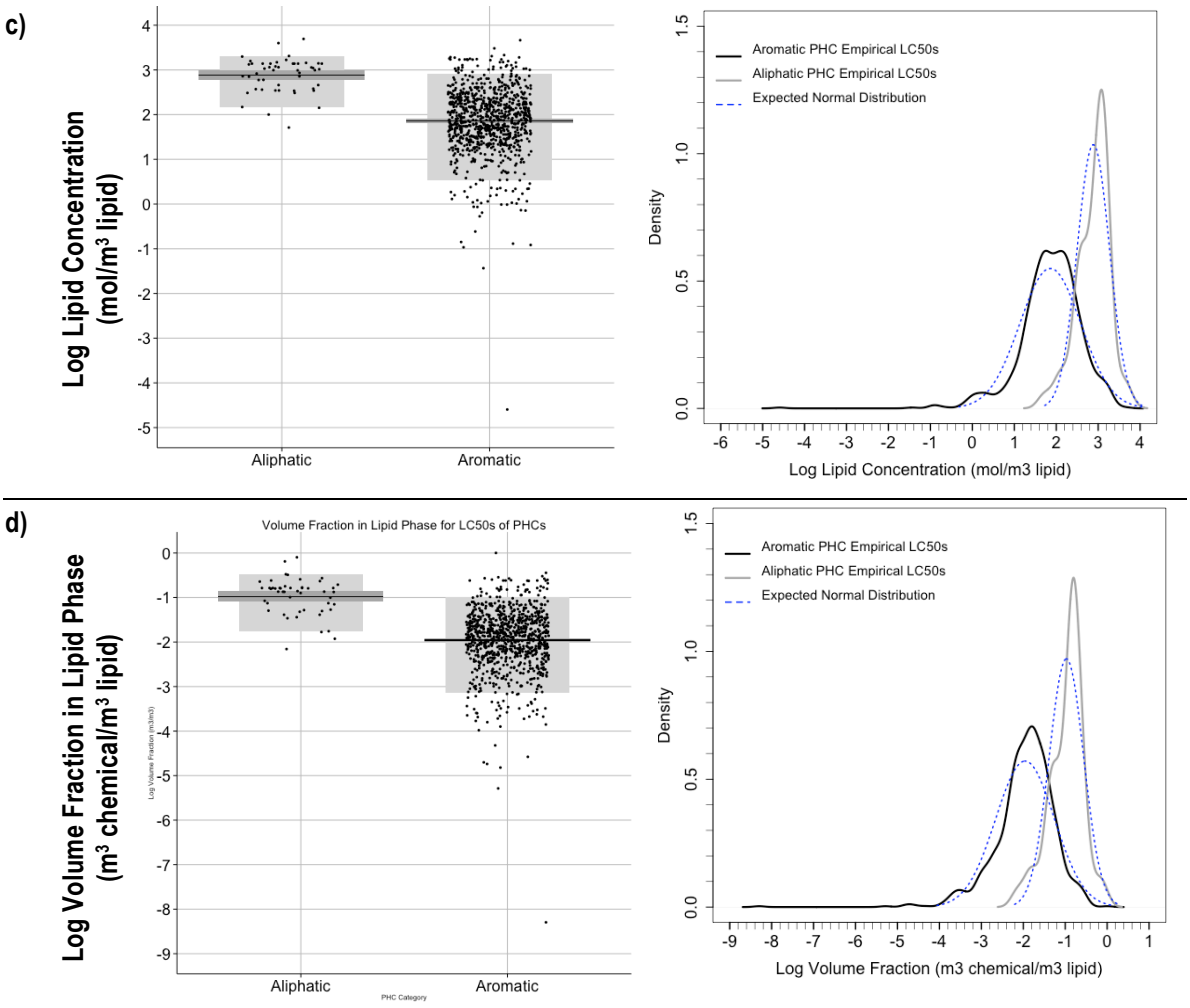


Figure 4.3. Comparison of aliphatic and aromatic LC50s expressed as a) activity; b) fugacity; c) lipid concentration; and d) volume fraction in lipid phase.

• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility;
Solid black horizontal line represents the mean;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.

Table 4.2. Summary description of LC50s for single aliphatic and aromatic petroleum hydrocarbons expressed as (A) the logarithm-transformed and (B) the non-transformed activity, fugacity, and lipid–phase concentration and volume fraction.

A	Log Activity (unitless)		Log Fugacity (Pa)		Log Lipid Concentration (mol/m^3)		Log Volume Fraction (m^3/m^3)	
Summary Statistic	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**
n	49	904	49	904	49	904	49	904
Mean$_A$	-0.280	-1.39	3.49	-0.101	2.89	1.86	-0.971	-1.96
95% CI	-0.408 to -0.153	-1.44 to -1.35	3.34 to 3.64	-0.265 to 0.063	2.78 to 3.00	1.81 to 1.91	-1.09 to -0.854	-2.00 to -1.91
Median	-0.156	-1.32	3.44	0.129	2.99	1.92	-0.868	-1.87
5th to 95th %iles	-1.12 to 0.382	-2.53 to -0.449	2.83 to 3.98	-4.00 to 3.21	2.16 to 3.31	0.541 to 2.91	-1.77 to -0.482	-3.13 to -0.988
Minimum	-1.83	-7.47	1.07	-8.44	1.71	-4.60	-2.158	-8.296
Maximum	0.585	0.520	4.43	3.81	3.69	3.66	-0.096	0.002
Standard Deviation	0.446	0.679	0.52	2.47	0.39	0.73	0.411	0.699

B	Activity (unitless)		Fugacity (Pa)		Lipid Concentration (mol/m^3 lipid)		Volume Fraction (m^3/m^3)	
Summary Statistic	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**	**Aliphatic**	**Aromatic**
n	49	904	49	904	49	904	49	904
Mean$_A$	0.785	0.100	4849	244	1057	201	0.154	0.028
95% CI	0.572 to 0.997	0.088 to 0.112	3544 to 6155	197 to 291	806 to 1308	177 to 225	0.113 to 0.195	0.025 to 0.032
Mean$_G$	0.524	0.041	3074	0.792	768	72.3	0.107	0.011
95% CI	0.391 to 0.703	0.037 to 0.045	2187 to 4322	0.543 to 1.16	596 to 990	64.7 to 80.8	0.082 to 0.14	0.01 to 0.012
Median	0.699	0.048	2790	1.35	989	82.9	0.136	0.013
5th to 95th %iles	0.076 to 2.41	0.003 to 0.356	680 to 9484	0.0001 to 1609	144 to 2025	3.47 to 814	0.017 to 0.329	0.001 to 0.103
Minimum	0.015	3.36×10^{-8}	11.8	3.60×10^{-9}	51	0.00003	0.007	5.06×10^{-9}
Maximum	3.84	3.31	27028	6493	4928	4619	0.801	1.00
Std.Dev.	0.743	0.184	4571	707	879	362	0.144	0.054

Mean$_A$ = arithmetic mean; Mean$_G$ = geometric mean; CI = confidence interval around the mean; Std.Dev. = standard deviation.

Table 4.3. Welch's t-test comparing aromatic and aliphatic toxicity expressed as the logarithm of the activity, fugacity, lipid concentration, and volume fraction.

	Activity (unitless)	Fugacity (Pa)	Lipid Concentration (mol/m^3)	Volume Fraction (m^3/m^3)
T-test on log-transformed data				
Difference in $Mean_A$	1.111	3.59	1.026	0.986
SE	0.068	0.111	0.060	0.063
95% CI	0.976 to 1.25	3.37 to 3.81	0.906 to 1.15	0.860 to 1.11
T-statistic	16.4	32.5	17.1	15.6
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
T-test interpreted on non-transformed data[1]				
Ratio Aliphatic:Aromatic[1]	12.91	3881	10.6	9.68
95% CI on Ratio[1]	9.46 to 17.6	2328 to 6470	8.06 to 14.0	7.25 to 12.9

$Mean_A$ = arithmetic mean; SE = standard error; CI = confidence interval
[1] Ratio = 1 indicates no difference between toxicity of aliphatic and aromatic PHCs (non-transformed data).

The geometric mean lipid-phase concentrations for aromatic hydrocarbons (72.3 mol/m^3 lipid; 95% CI: 64.7 to 80.8; Table 4.2) was almost double the critical body residue of PAHs reported in McCarty et al. (2013; 38.9 mol/m^3 lipid, assuming lipid density is 1000 kg/m^3; 95% CI: 30.2 to 50.1). The difference in confidence intervals on the estimate of the mean between the two studies is, in part, attributed to larger sample size (n=904) and subsequent smaller standard error in this study compared to McCarty et al. (n=66). However, the geometric mean lipid concentration from McCarty et al. (2013) was still within the range between the 5^{th} and 95^{th} percentiles for aromatic PHCs from this research (3.47 to 814 mol/m^3 lipid ; Figure 4.3c ; Table 4.2).

The aliphatic hydrocarbons' LC50s (n=49) had activities nearing 50% saturation (geometric mean = 0.52; 95% CI: 0.39 to 0.70), and volume fractions over 10% (geometric mean = 0.11 m^3/m^3; 95% CI: 0.08 to 0.14; Table 4.2). The geometric mean of lipid-phase concentrations for aliphatic hydrocarbons (768 mol/m^3 lipid; 95% CI: 596 to 990; Table 4.2) was more than an order of magnitude greater than the geometric mean lipid-phase concentration associated with baseline neutral narcosis reported in McCarty et al. (2013; 53 mol/m^3 lipid, assuming lipid density is 1000kg/m^3; 95% CI =

45.5 to 63.4). These values for activity, lipid-phase concentration and volume fraction are all high, drawing attention to test conditions that may have contained pure chemical phase (in addition to any dissolved chemical). Presence of oil droplets, particularly for those aliphatic chemicals with low aqueous solubility (the lowest solubility of aliphatic hydrocarbons in the LC50 dataset = 0.0017 mol/m^3) and high log K_{OW} (maximum log K_{OW} for aliphatic hydrocarbons in the LC50 dataset = 5.65), may be indicated by activities nearing one. More than half of the LC50s for aliphatic PHCs were at an activity greater than 50% saturation, indicating that oil droplets or other pure chemical phase may have been present and contributed to the observed impacts on test organisms' survival.

The geometric mean activity, fugacity, lipid concentration, and volume fraction were all different between aromatic and aliphatic chemical groups (Table 4.3). However, the 5th percentile of the distribution of the aromatic LC50s (activity = 0.003; Table 4.2) equals the 5th percentile of the distribution of LC50s for all PHCs including both aliphatic and aromatic PHCs (activity = 0.003; Table 4.1). Managing PHCs by applying an activity at the lower limit of the distribution of activities across all chemicals (e.g., the 5th percentile = 0.003; Table 4.1) would be a conservative approach to protecting wildlands from effects on survival from both aromatic and aliphatic PHCs. Similarly, the 5th percentile of the distribution of all toxicity data expressed as lipid concentration (4.35 mol/m^3; Table 4.1) or as volume fraction (0.0008 m^3/m^3; Table 4.1) applies to the management of both aliphatic and aromatic PHCs.

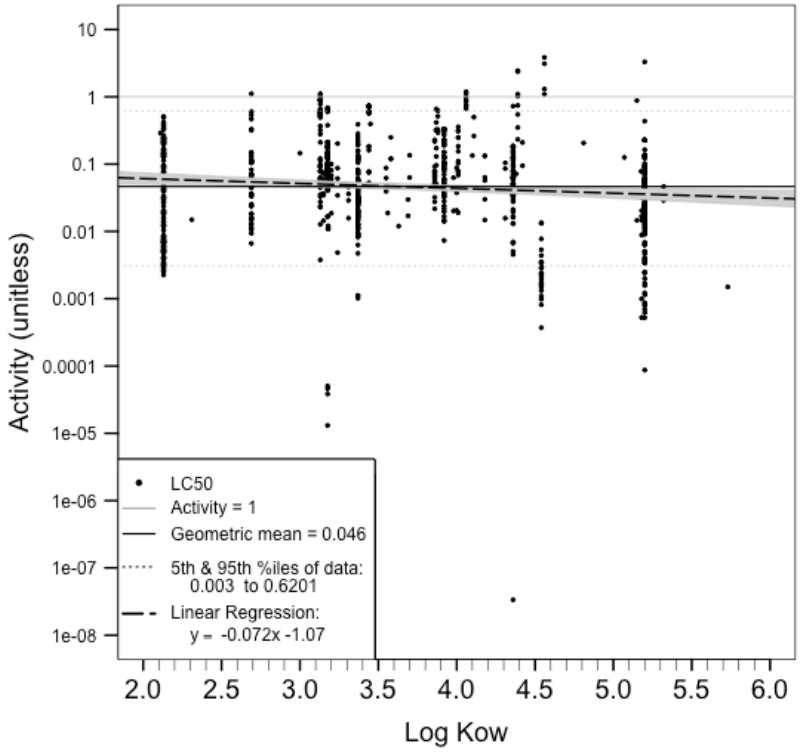
Log K_{OW} as Descriptor of PHC Toxicity

The toxicity (LC50s) of different PHC chemicals, expressed as either activity, fugacity, lipid concentration, or volume fraction was evaluated across a range of chemicals in terms of their hydrophobicity (log K_{OW}) (Figure 4.4).

Log-transformed activity associated with LC50s for PHCs decreased with log octanol-water partitioning coefficient (K_{OW}) of the PHCs (Figure 4.4a; slope [95% CI] = -0.072 [-0.118 to -0.026]; p-value of slope=0.002). For each one-unit increase in log K_{OW}, activity decreased by a factor of 0.85 (95% CI: 0.76 to 0.94; Table 4.4). This factor, although statistically significant, would result in a small change in activity associated with LC50s of PHCs from 0.06 for PHCs with log K_{OW} of 2, to activity of 0.03 for PHCs with

log K_{OW} of 6. Therefore, these data indicate that the activity associated with LC50s for PHCs does not vary considerably across a wide range of different PHCs.

a)



b)

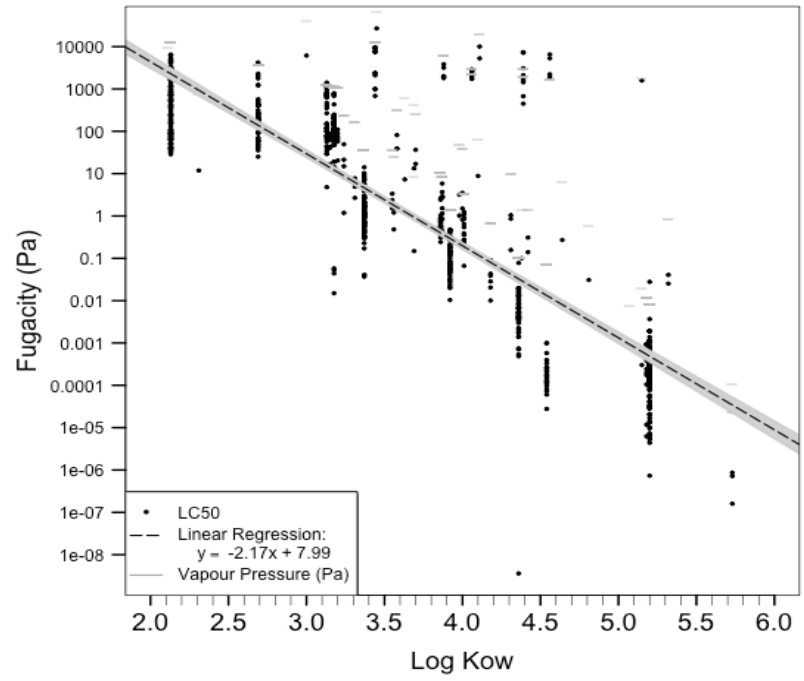
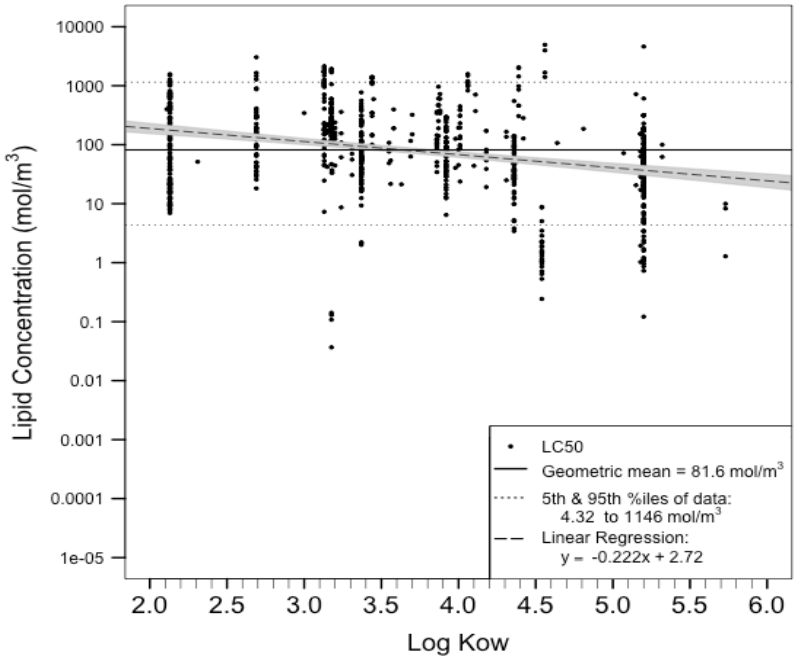


Figure 4.4 continued on next page…

c)



d)

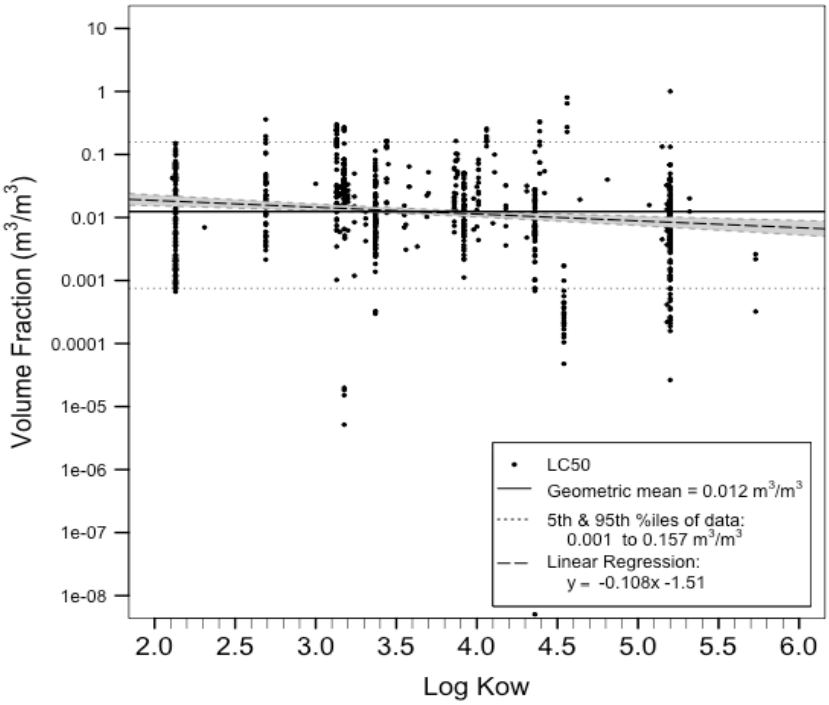


Figure 4.4. Linear regression of LC50s (n=953) for PHCs expressed as the logarithm of a) activity, b) fugacity, c) lipid-phase concentration, and d) volume fraction in lipid-phase, as a function of log K_{OW}. p-value for all slopes ≤0.002.

Log-transformed lipid concentration (Figure 4.4c) and volume fraction (Figure 4.4d) associated with LC50s for PHCs also showed similar statistically significant decreasing relationship with log K_{OW} (log lipid concentration slope [95% CI] = -0.22 [-0.27 to -0.18]; log volume fraction slope [95% CI] = -0.11 [-0.15 to -0.06]; Table 4.4). The lipid concentration associated with LC50s decreases by 40% per one unit change in log K_{OW} (95%CI=33 to 46%; Table 4.4). This rate of change corresponds to a range from 190 to 25 mol/m^3 over the range of log K_{OW} from 2 to 6. This range is well between the 5^{th} and 95^{th} percentiles for LC50s across all PHCs (4.35 to 1146 mol/m^3; Table 4.1). The volume fraction associated with LC50s decreases by 22% per one unit change in log K_{OW} (95%CI=13 to 30%; Table 4.4). This rate of change corresponds to a range in volume fraction associated with LC50s for PHCs between 0.02 and 0.007 m^3/m^3 over a log K_{OW} range from 2 to 6. This range in volume fractions is within the range between the 5^{th} and 95^{th} percentiles of observed volume fractions associated with LC50s across all PHCs (0.0008 to 0.157 m^3/m^3; Table 4.1).

Table 4.4. Linear regression of petroleum hydrocarbon toxicity (LC50s) against log K_{OW}.

Model	Model Parameter	Estimate	SE	LCI	UCI	p-value
log (Activity) = -0.073 • logK_{OW} - 1.07						**0.002**
	Intercept	-1.07	*0.087*	*-1.24*	*-0.900*	**<0.001**
	Slope	-0.072	*0.023*	*-0.118*	*-0.026*	**0.002**
log (Fugacity) = -2.17 • logK_{OW} +7.99						**<0.001**
	Intercept	7.99	*0.611*	*7.67*	*8.31*	**<0.001**
	Slope	-2.17	*0.043*	*-2.26*	*-2.08*	**<0.001**
log (C_L) = -0.221 • logK_{OW} + 2.72						**<0.001**
	Intercept	2.72	*0.088*	*2.55*	*2.90*	**<0.001**
	Slope	-0.221	*0.023*	*-0.270*	*-0.18*	**<0.001**
log (V_C/V_L) = -0.108 • logK_{OW} - 1.51						**<0.001**
	Intercept	-1.51	*0.087*	*-1.68*	*-1.34*	**<0.001**
	Slope	-0.108	*0.023*	*-0.150*	*-0.06*	**<0.001**
Estimated multiplicative-change in y-value per 1-unit change in log K_{OW}[1,2]:						
Activity at (LogK_{OW} + 1) : Activity at LogK_{OW}		0.847		0.762	0.942	
Fugacity at (LogK_{OW} + 1) : Fugacity at LogK_{OW}		0.007		0.006	0.008	
C_L at (LogK_{OW} + 1) : C_L at LogK_{OW}		0.601		0.541	0.668	
V_C/V_L at (LogK_{OW} + 1) : V_C/V_L at LogK_{OW}		0.780		0.701	0.868	

K_{OW} = octanol-water partition co-efficient; SE = standard error; LCI = lower 95% confidence interval; UCI = upper 95% confidence interval; C_L = concentration in lipid phase (mol•m^{-3}); V_C/V_L = volume chemical per volume lipid (m^3•m^{-3}).

[1] Ratio = 1 indicates no difference.

[2] Estimated multiplicative-change in y-value per 1-unit change in logK_{OW} = 10^{Slope}

Significant terms determined as $p<0.05$ are highlighted in **bold**.

The change in activity, lipid concentration, and volume fraction across PHCs of varying log K_{OW} is statistically significant, but small. For the purposes of managing PHCs, a common value (of either activity, lipid concentration, or volume fraction) can be applied to wildlands that will limit effects to survival from a wide range of PHCs.

The log-transformed fugacity that was associated with LC50s across all PHCs, in contrast to activity, lipid concentration, and volume fraction, had a steeply decreasing relationship with log K_{OW} (slope [95% CI]: -2.17 [-2.26 to -2.08; Figure 4.4b). This result was unexpected since the pressure exerted by a chemical in an organism resulting in a given effect (e.g., an LC50) was expected to be the same across different chemicals.

However, based on this result, the fugacity associated with LC50s for heavier, more hydrophobic PHCs is much lower than that for lighter, more hydrophilic PHCs. The fugacity capacity of various media (water, sediment, lipid) is higher for hydrophobic chemicals than it is for hydrophilic PHCs. In other words, it takes more moles of hydrophobic PHCs to increase the chemical's partial pressure by one Pa in one cubic meter of a medium, compared to hydrophilic PHCs.

Because there is a different fugacity associated with toxic effects (in this case, LC50s) across different PHC chemicals (Figure 4.4b), the fugacity associated with effects will also be different between PHC mixtures of different chemical compositions. There is no single fugacity value that can be applied for PHC management on wildlands across different PHCs or different PHC mixtures of varying compositions.

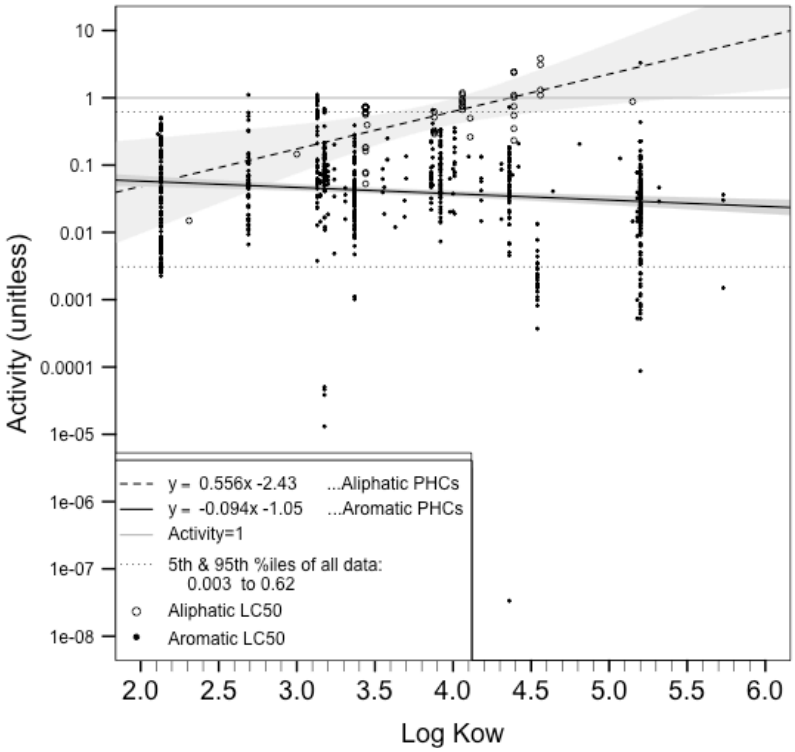
Aliphatic and Aromatic PHC Toxicity described by Log K_{OW}

The PHC toxicity to log K_{OW} relationships were further refined by taking into account the aromatic or aliphatic classification of the various chemicals. The toxicity:log K_{OW} models for aromatic PHCs (Figure 4.5) was similar to the toxicity:log K_{OW} models that considered all aromatic and aliphatic PHCs together (Figure 4.4). The toxicity:log K_{OW} models for only aliphatic PHC however, were significantly different from the toxicity:log K_{OW} models for only aromatic PHCs (ANCOVA; all $p \leq 0.002$; Table 4.6), regardless of whether toxicity (LC50s) was expressed as activity, fugacity, lipid concentration or volume fraction. These ANCOVA results are consistent with the previous t-test results (Figure 4.3) that also showed differences in toxicity between aromatic and aliphatic PHCs, regardless of how toxicity (LC50s) was expressed (either activity, fugacity, lipid concentration, or volume fraction).

The LC50s for aliphatic PHCs expressed as log activity have an increasing relationship with log K_{OW} (slope = 0.556 [95%CI: 0.187 to 0.925]; Figure 4.5a; Table 4.5). However, because of the small sample size for aliphatic PHC toxicity (n=49), it is possible that the single aliphatic datapoint at log K_{OW} = 2.5 may be skewing this linear regression, and weighting the slope more positive than the true toxicity:log K_{OW} relationship. In addition, the activity values greater than 1 for aliphatic PHCs with log K_{OW} above 4 are likely a result of experimental error, since activities greater than 1 are

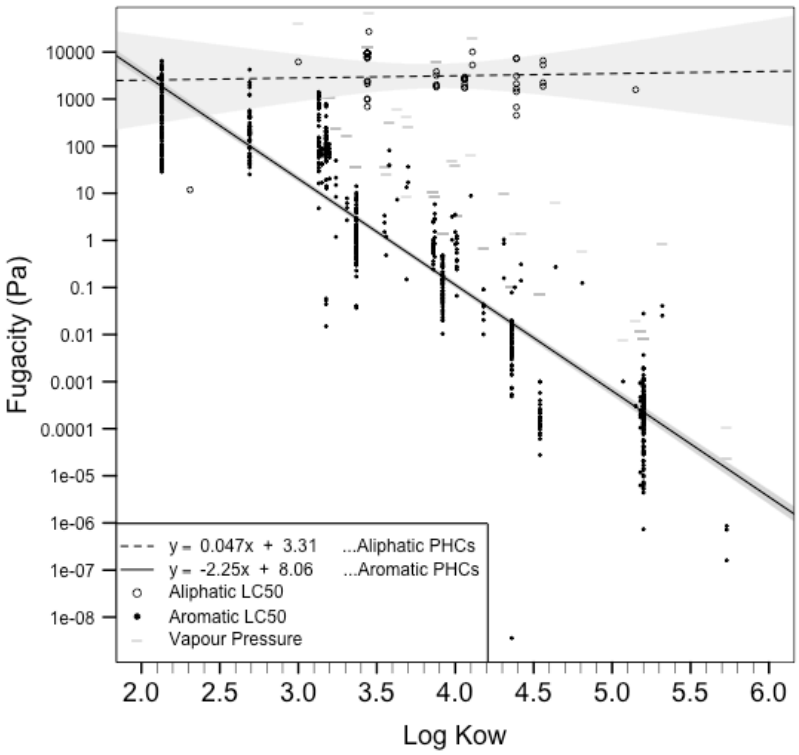
thermodynamically not possible. These values greater than one may also be providing leverage to the linear relationships for aliphatic PHCs. These same observations about the toxicity:log K_{OW} relationship for aliphatic PHCs can be made when toxicity is expressed as log lipid concentration (slope = 0.336 [95%CI: -0.040 to 0.712]; Figure 4.5c; Table 4.5) or as log volume fraction (slope = 0.449 [95%CI: 0.0.073 to 0.826]; Figure 4.5d; Table 4.5).

a)



p-value of slope
Aliphatic PHCs:
<0.001
Aromatic PHCs:
<0.001

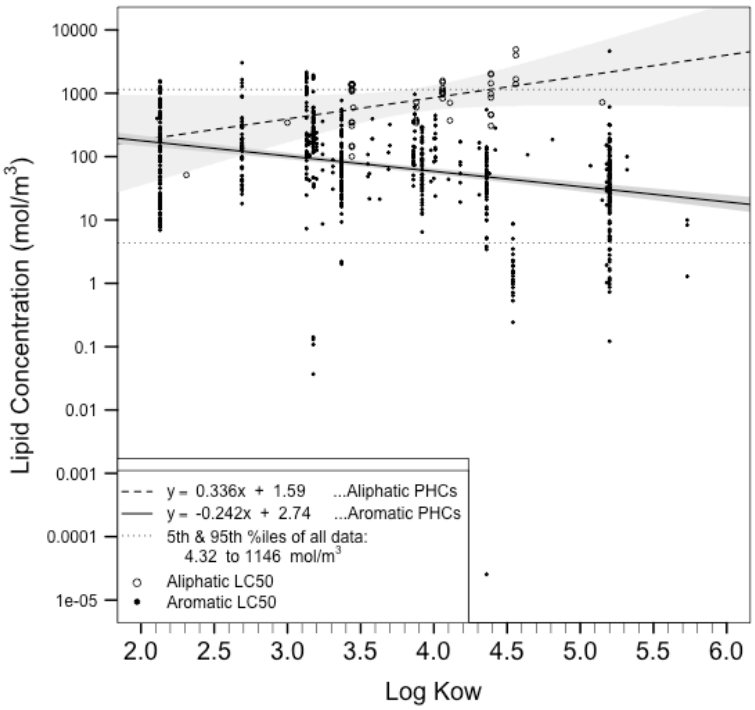
b)



p-value of slope
Aliphatic PHCs:
0.855
Aromatic PHCs:
<0.001

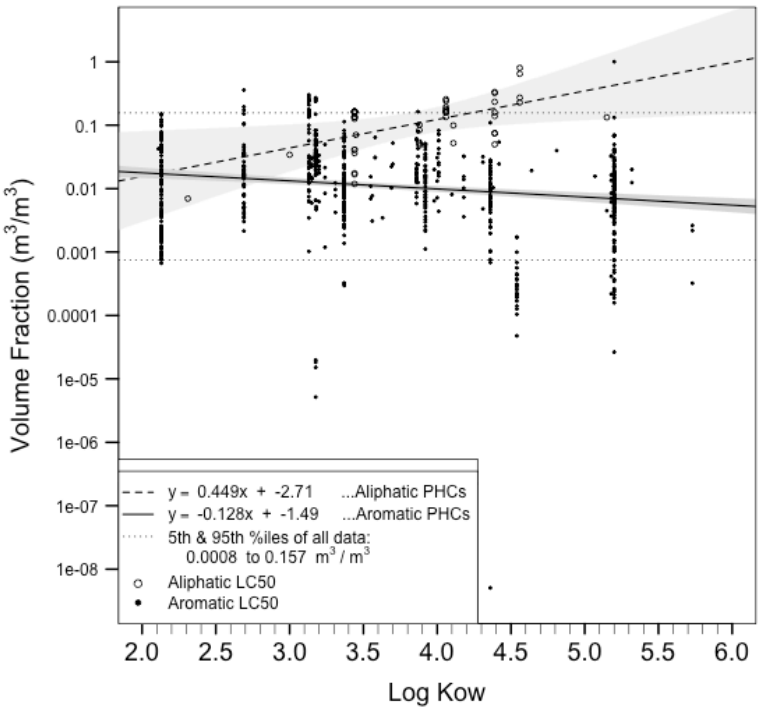
…Figure 4.5 *continued on next page*

c)



p-value of slope
Aliphatic PHCs:
0.074
Aromatic PHCs:
0.002

d)



p-value of slope
Aliphatic PHCs:
0.017
Aromatic PHCs:
0.002

Figure 4.5. Linear regression of aliphatic (n=49) and aromatic (n=904) PHC toxicity (LC50s) against log K_{OW}, where toxicity is expressed as a) activity, b) fugacity, c) lipid-phase concentration, and d) volume fraction in lipid-phase.

Grey-shaded areas represent 95% confidence intervals around the linear regression.

Table 4.5. Linear regression of aliphatic and aromatic PHC toxicity (LC50s) against log K_{OW} (aliphatic PHC n=49; aromatic PHC n=904).

Model	Model Parameter	Estimate	SE	LCI	UCI	p-value
Activity ~ log K_{OW} with LogK_{OW}:PHC interaction term						**<0.001**
Aliphatic PHCs: log (Activity) = 0.556•logK_{OW} - 2.43						
	Intercept	-2.43	*0.719*	*-3.88*	*-1.00*	**<0.001**
	Slope	0.556	*0.185*	*0.187*	*0.925*	**<0.001**
	Estimated multiplicative change in Activity per 1-unit change in log K_{OW}[1,2]	3.60		*1.54*	*8.42*	
Aromatic PHCs: log (Activity) = -0.094•logK_{OW} – 1.06						
	Intercept	-1.06	*0.724*	*-2.50*	*0.40*	0.057
	Slope	-0.094	*0.186*	*-0.466*	*0.278*	**<0.001**
	Estimated multiplicative change in Activity per 1-unit change in log K_{OW}[1,2]	0.805		*0.342*	*1.89*	
Fugacity ~ log K_{OW} with LogK_{OW}:PHC interaction term						**<0.001**
Aliphatic PHCs: log (Fugacity) = 0.047•logK_{OW} + 3.31						
	Intercept	3.31	*0.992*	*1.33*	*5.29*	**<0.001**
	Slope	0.047	*0.255*	*-0.460*	*0.557*	0.855
	Estimated multiplicative change in Fugacity per 1-unit change in log K_{OW}[1,2]	1.11		*0.344*	*3.61*	
Aromatic PHCs: log (Fugacity) = -2.25•logK_{OW} + 8.07						
	Intercept	8.07	*0.998*	*6.07*	*10.1*	**<0.001**
	Slope	-2.25	*0.256*	*-2.77*	*-1.74*	**<0.001**
	Estimated multiplicative change in Fugacity per 1-unit change in log K_{OW}[1,2]	0.006		*0.002*	*0.018*	
C_L ~ log K_{OW} with LogK_{OW}:PHC interaction term						**<0.001**
Aliphatic PHCs: log (C_L) = 0.336•logK_{OW} + 1.59						
	Intercept	1.59	*0.731*	*0.128*	*3.052*	**0.03**
	Slope	0.336	*0.188*	*-0.040*	*0.712*	0.074
	Estimated multiplicative change in C_L per 1-unit change in log K_{OW}[1,2]	2.17		*0.912*	*5.15*	
Aromatic PHCs: log (C_L) = -0.242•logK_{OW} + 2.74						
	Intercept	2.74	*0.736*	*1.27*	*4.21*	0.119
	Slope	-0.242	*0.189*	*-0.620*	*0.136*	**0.002**
	Estimated multiplicative change in C_L per 1-unit change in log K_{OW}[1,2]	0.573		*0.240*	*1.37*	

…Table 4.5 continued on next page

Model	Model Parameter	Estimate	SE	LCI	UCI	p-value
V_C/V_L ~ log K_{OW} with LogK_{OW}:PHC interaction term						**<0.001**
Aliphatic PHCs: log (V_C/V_L) = 0.449•logK_{OW} - 2.71						
	Intercept	-2.71	*0.734*	*-4.18*	*-1.24*	**<0.001**
	Slope	0.449	*0.188*	*0.073*	*0.826*	**0.017**
	Estimated multiplicative change in V_C/V_L per 1-unit change in log K_{OW}[1,2]	2.81		*1.18*	*6.70*	
Aromatic PHCs: log (V_C/V_L) = -0.128•logK_{OW} - 1.49						
	Intercept	-1.49	*0.739*	*-2.97*	*-0.017*	0.101
	Slope	-0.128	*0.190*	*-0.507*	*0.252*	**0.002**
	Estimated multiplicative change in V_C/V_L per 1-unit change in log K_{OW}[1,2]	0.745		*0.311*	*1.79*	

K_{OW} = octanol-water partition co-efficient; Aro = aromatic petroleum hydrocarbon; Ali = aliphatic petroleum hydrocarbon; SE = standard error; LCI = lower 95% confidence interval; UCI = upper 95% confidence interval; C_L = concentration in lipid phase (mol•m^{-3}); V_C/V_L = volume chemical per volume lipid (m^3•m^{-3}).
[1] A Ratio = 1 indicates no difference.
[2] Estimated multiplicative-change in y-value per 1-unit change in logK_{OW} = 10^{Slope}
Significant terms ($p<0.05$) are highlighted in **bold**.

Table 4.6. Comparison of the toxicity to log K_{OW} linear relationships between aliphatic and aromatic PHCs using ANCOVA.

Model	Model Parameter	df	F	p-value
Activity ~ log K_{OW} with LogK_{OW}:PHC interaction term				
	Log K_{OW}	1	11.3	**<0.001**
	PHC-type	1	135	**<0.001**
	LogK_{OW}:PHC Interaction term	1	12.2	**<0.001**
	Residuals	949		
Fugacity ~ log K_{OW} with LogK_{OW}:PHC interaction term				
	Log K_{OW}	1	5390	**<0.001**
	PHC-type	1	947	**<0.001**
	LogK_{OW}:PHC Interaction term	1	80.4	**<0.001**
	Residuals	949		
C_L ~ log K_{OW} with LogK_{OW}:PHC interaction term				
	Log K_{OW}	1	103	**<0.001**
	PHC-type	1	120	**<0.001**
	LogK_{OW}:PHC Interaction term	1	9.36	**0.002**
	Residuals	949		
V_C/V_L ~ log K_{OW} with LogK_{OW}:PHC interaction term				
	Log K_{OW}	1	24.4	**<0.001**
	PHC-type	1	105	**<0.001**
	LogK_{OW}:PHC Interaction term	1	9.26	**0.002**
	Residuals	949		

Table Note: Significant terms ($p<0.05$) are highlighted in bold.

When expressed as log fugacity, the toxicity (LC50s) of aliphatic PHCs was constant across the range of log K_{OW} (slope [95% CI] = 0.047 [-0.46 to 0.557], p-value = 0.855; Figure 4.5b; Table 4.5). A constant fugacity around 3000 Pa is associated with LC50s of aliphatic chemicals regardless of log K_{OW}. However, this constant fugacity associated with aliphatic LC50s would not be protective of effects from aromatic PHCs which have LC50s at fugacities as low as $3.6 \bullet 10^{-9}$ Pa. Therefore, fugacity does not normalize toxicity across different chemicals, and the fugacity associated with effects can not be compared between different PHCs of varying log K_{OW}, or between PHC mixtures of varying compositions.

Across-Media Comparison of Individual PHC Toxicity

Internal and External Media-based Toxicity Measurements

There was no statistically significant difference in mean LC50s for PHCs expressed as either activity, fugacity, lipid concentration or volume fraction between concentration measurements taken in external media (i.e., sediment, porewater, or water) and concentration measurements taken internal to the organism (i.e., lipid-based measurements that more directly measure the amount of chemical at the cellular-chemical interaction) (Welch's t-test: all p-values > 0.05; Table 4.7). In this research, all internal media-based measurements were based on whole-body tissue of either fish or invertebrates, and were reported as a total body burden on a lipid-normalized basis (available data for lipid content of test organisms ranged between 0.5 and 8.5%). Therefore, all internal-media based measurements are considered equivalent.

Table 4.7. Welch's t-test comparing external-media and internal-media based measurements of toxicity (LC50s) expressed as activity, fugacity, lipid concentration, and volume fraction.

	log10(Activity)	log10(Fugacity)	log10(Lipid Concentration)	log10(Volume Fraction)
T-test for difference between log-transformed LC50s measured in internal and in external media				
Difference in $Mean_A$	0.079	0.05	0.073	0.074
SE	0.076	0.298	0.076	0.073
95% CI	-0.073 to 0.231	-0.546 to 0.645	-0.079 to 0.225	-0.072 to 0.220
T-statistic	1.03	-0.167	0.964	1.01
p-value	0.309	0.868	0.342	0.318
T-test interpreted on non-transformed data[1]:				
Ratio Internal:External[1]	1.20	1.12	1.18	1.19
95% CI on Ratio[1]	0.845 to 1.70	0.285 to 4.42	0.83 to 1.68	0.85 to 1.66

$Mean_A$ = arithmetic mean; SE = standard error; CI = confidence interval
[1] Ratio = 1 indicates no difference between toxicity of internal and external-media based non-transformed LC50 measurements.

There were fewer datapoints for internal-based measurements of toxicity (n=27) than for external-media-based measurements (n=926; Table 4.8), but the overall ranges between the 5th and 95th percentiles of the distributions for internal-based

measurements fell within the ranges of the external-based measurements (light-grey shaded regions in Figure 4.6). These results suggest that on average externally-based measurements can represent the amount of chemical inside a test organism.

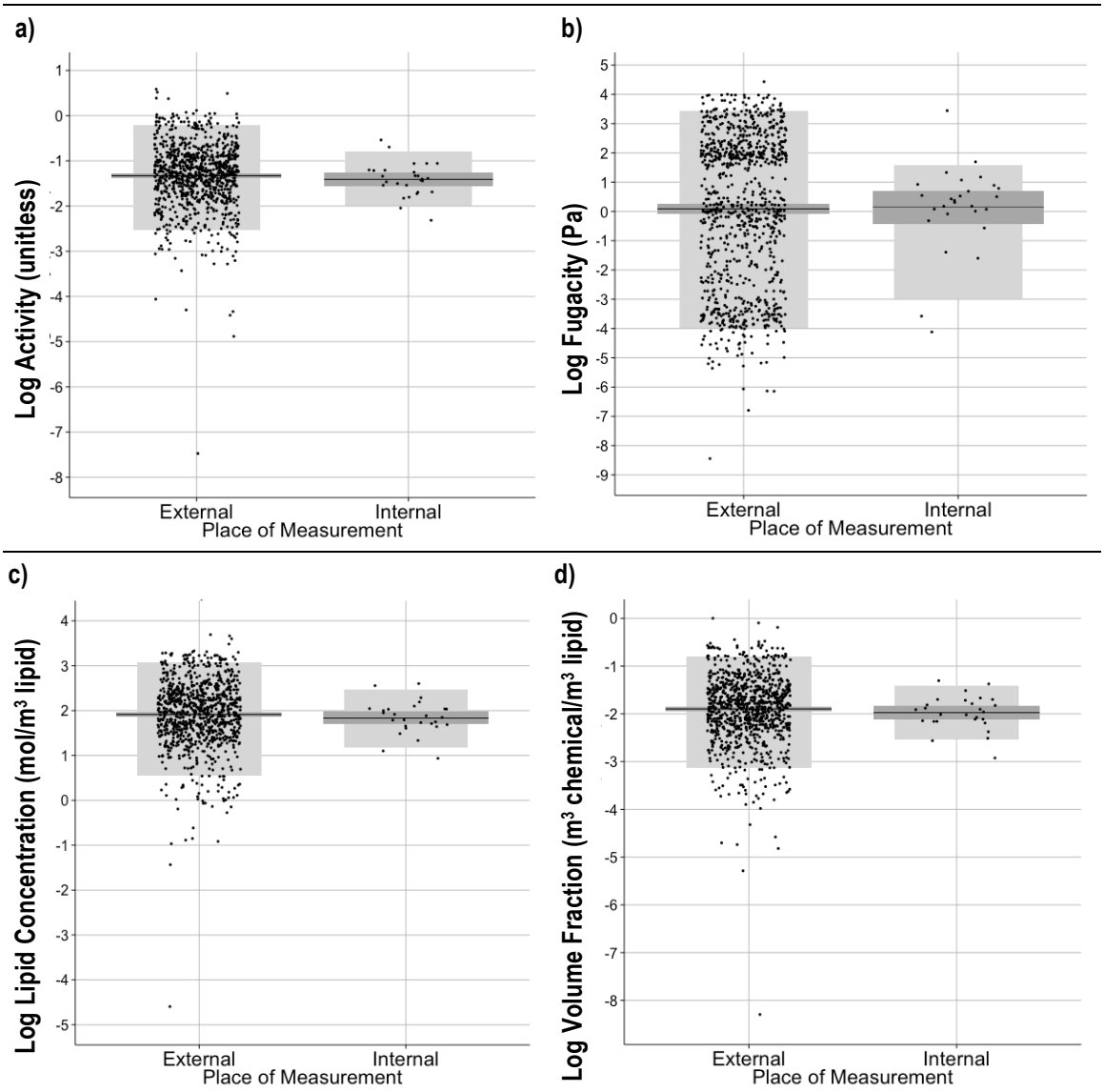


Figure 4.6. Comparison of external media-based measurements (i.e., water, pore-water, and sediment) and internal (i.e., lipid)-based measurements of toxicity (LC50s) expressed as a) activity (p=0.309); b) fugacity (p=0.868); c) lipid-phase concentration (p=0.342), and d) volume fraction in lipid-phase (p=0.318).

• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility;
Solid black horizontal line represents the mean;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.
p-values refer to t-test between mean log external and mean log internal toxicity.

Table 4.8. Summary of internally and externally-measured concentrations associated with 50% mortality, expressed as activity, fugacity, and lipid-phase concentration and volume fraction.

	Log Activity		Log Fugacity		Log Lipid Concentration		Log Volume Fraction	
Summary Statistic	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**	External
n	27	926	27	926	27	926	27	926
$Mean_A$	-1.411	-1.33	0.13	0.082	1.84	1.91	-1.978	-1.90
95% CI	-1.55 to -1.26	-1.38 to -1.28	-0.440 to 0.703	-0.086 to 0.250	1.70 to 1.98	1.86 to 1.96	-2.12 to -1.84	-1.95 to -1.86
5th to 95th %iles	-1.98 to -0.804	-2.52 to -0.207	-2.98 to 1.58	-3.98 to 3.43	1.17 to 2.48	0.552 to 3.07	-2.55 to -1.415	-3.13 to -0.800
Minimum	-2.32	-7.47	-4.12	-8.44	0.94	-4.60	-2.92	-8.30
Maximum	-0.538	0.585	3.44	4.43	2.60	3.69	-1.30	0.002
Standard Deviation	0.375	0.720	1.48	2.56	0.373	0.756	0.358	0.728

	Activity (unitless)		Fugacity (Pa)		Lipid Concentration (mol/m^3 lipid)		Volume Fraction (m^3/m^3)	
Summary Statistic	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**
n	27	926	27	926	27	926	27	926
$Mean_A$	0.056	0.137	108	491	97.2	249	0.014	0.035
95% CI	0.033 to 0.079	0.118 to 0.156	-97 to 313	385 to 598	62 to 133	219 to 279	0.01 to 0.018	0.031 to 0.04
$Mean_G$	0.039	0.047	1.35	1.21	69	82.0	0.011	0.012
95% CI	0.028 to 0.054	0.042 to 0.052	0.363 to 5.05	0.82 to 1.78	50 to 96	73.2 to 92	0.008 to 0.014	0.011 to 0.014
5th to 95th %iles	0.011 to 0.17	0.003 to 0.621	0.008 to 41	0.0001 to 2677	15 to 310	3.57 to 1170	0.003 to 0.039	0.001 to 0.159
Minimum	0.005	3.36x10^-8	0.0001	3.60x10^-9	9	0.00003	0.001	5.06x10^-9
Maximum	0.290	3.84	2774	27028	400	4928	0.050	1.00
Standard Deviation	0.060	0.291	533	1621	92	451	0.011	0.069

$Mean_A$ = arithmetic mean; $Mean_G$ = geometric mean; CI = confidence interval.

Toxicity data where the chemical measurement was made directly within an organisms' tissue were limited in terms of PHC diversity. Only 1 aliphatic chemical was represented (1,1,2,2-tetrachloroethane, log K_{OW} = 2.31) and there were 26 datapoints for 11 different aromatic PHCs (log K_{OW} from 2.11 to 5.32). Whereas, external-media based

chemical measurements included eight different aliphatic PHCs and 25 different aromatic PHCs. Even if only aromatic PHCs are considered, there is still no difference in activity, fugacity, lipid concentration or volume fraction between external- and internal-media based measurements of toxicity (LC50s) (Table 4.9).

Table 4.9. Welch's t-test comparing aromatic PHC toxicity (LC50s) between internal and external media based measurements.

	Activity (unitless)	**Fugacity (Pa)**	**Lipid Concentration (mol/m^3)**	**Volume Fraction (m^3/m^3)**
Difference between log-external and log-internal LC50s (95% CI)	0.003 (-0.15 to 0.16)	0.3 (-0.41 to 0.82)	0.015 (-0.136 to 0.165)	0.015 (-0.136 to 0.165)
Ratio External to Internal LC50s (95% CI)	1.01 (0.71 to 1.44)	1.59 (0.389 to 6.53)	1.03 (0.72 to 1.48)	1.03 (0.73 to 1.46)
p-value	0.965	0.514	0.861	0.847

There appears to be lower variation in the activity, lipid concentration, and volume fraction for PHC toxicity (LC50s) when based on internal-media measurements, compared to external-media based measurements, evident by the narrower range between the 5^{th} and 95^{th} percentiles (lighter-grey shaded regions in Figure 4.6). This lower variation can in part be attributed to the smaller sample size, fewer chemicals (only PAHs measured internally), and fewer species in the internal-media based measurements compared to the external-media based toxicity data. However, this narrower variation is also consistent with the merits of established tissue residue and critical body burden approaches to chemical risk assessment and guideline development (Meador et al., 2006). Both established tissue residue/critical body burden concepts and activity and lipid-phase concentrations and volume fractions in this research are attempting to quantify, and incorporate bioavailability into exposure measurements (exposure can either be environmental or exposure during a laboratory-based toxicity test). There is reduced toxicokinetic variability when looking at internal-media based concentrations or activities, compared to external-based measurements. Variability in the rates of uptake and elimination, and in bioavailability, which ultimately determine an organism's exposure, are excluded in measurements of internal-based measurements.

The internal-media based measurements are not paired with the external-media based measurements, and they all come from many different independent studies (Figure 4.7). Therefore, the wider range in values associated with external media compared to internal media (Figure 4.6) may in part reflect variation between different laboratory test conditions in terms of the kinetics of partitioning between the abiotic environment and inside the organism. The duration of toxicity tests can be too short to reach equilibrium between the exposure media (e.g., water), and inside the test organism, particularly for higher log K_{OW} substances. For PHCs with higher K_{OW}, it is possible that some toxicity data describe test systems that did not reach an equilibrium between external exposure media and internal organism tissue. In case of equilibrium not being reached, shorter toxicity test durations may result in inflated concentrations at which toxicity is observed, particularly for higher K_{OW} chemicals which typically move from external media (i.e., water or sediment) into organisms at a much slower rate than lower K_{OW} chemicals. For example, the datapoint with the lowest activity in this study was from a 56-day long toxicity test, which was also the longest duration test in the LC50 dataset (the chemical used in this long-term toxicity test was phenanthrene, log K_{OW} = 4.36, test species was a salmonid, *Oncorhynchus mykiss*, activity = $3.4 \bullet 10^{-8}$, fugacity = $3.6 \bullet 10^{-9}$ Pa; $C_L = 2.5 \bullet 10^{-5}$ mol/m^3; $V_C/V_L = 5.1 \bullet 10^{-9}$ m^3/m^3; Appendix D). Toxicity tests longer than 40 days had generally lower activity and fugacity than tests of shorter duration (Appendix D), suggesting that a chemical's kinetic rate of uptake in toxicity tests is an important consideration when evaluating chemical's true potency. Further exploration of those studies with the lowest activity values (bottom of Figure 4.7) will be important for future development and applications of this activity-based approach for wildlands settings, to ensure that wildlands receptors are adequately protected.

Within a single experiment, kinetics are an important consideration in evaluating toxicity, and normalizing for a chemical's bioavailability. But, when toxicity data are considered across wide variety of different studies as well as across a wide variety of PHC chemicals, as is the case inFigure 4.6, the external-media based measurements of toxicity (as activity, fugacity, lipid concentration, or volume fraction) are good representations of the amount of chemical required inside an organisms to cause the same effect (an LC50). On average, these data suggest that an equilibrium has been achieved between internal and external media. Therefore, in a management context, for

the range of PHC chemicals considered (log K_{OW} 2 to 6), the 5th percentile of all data (e.g., activity = 0.003) would be representative of adverse effects measured in both external and internal media.

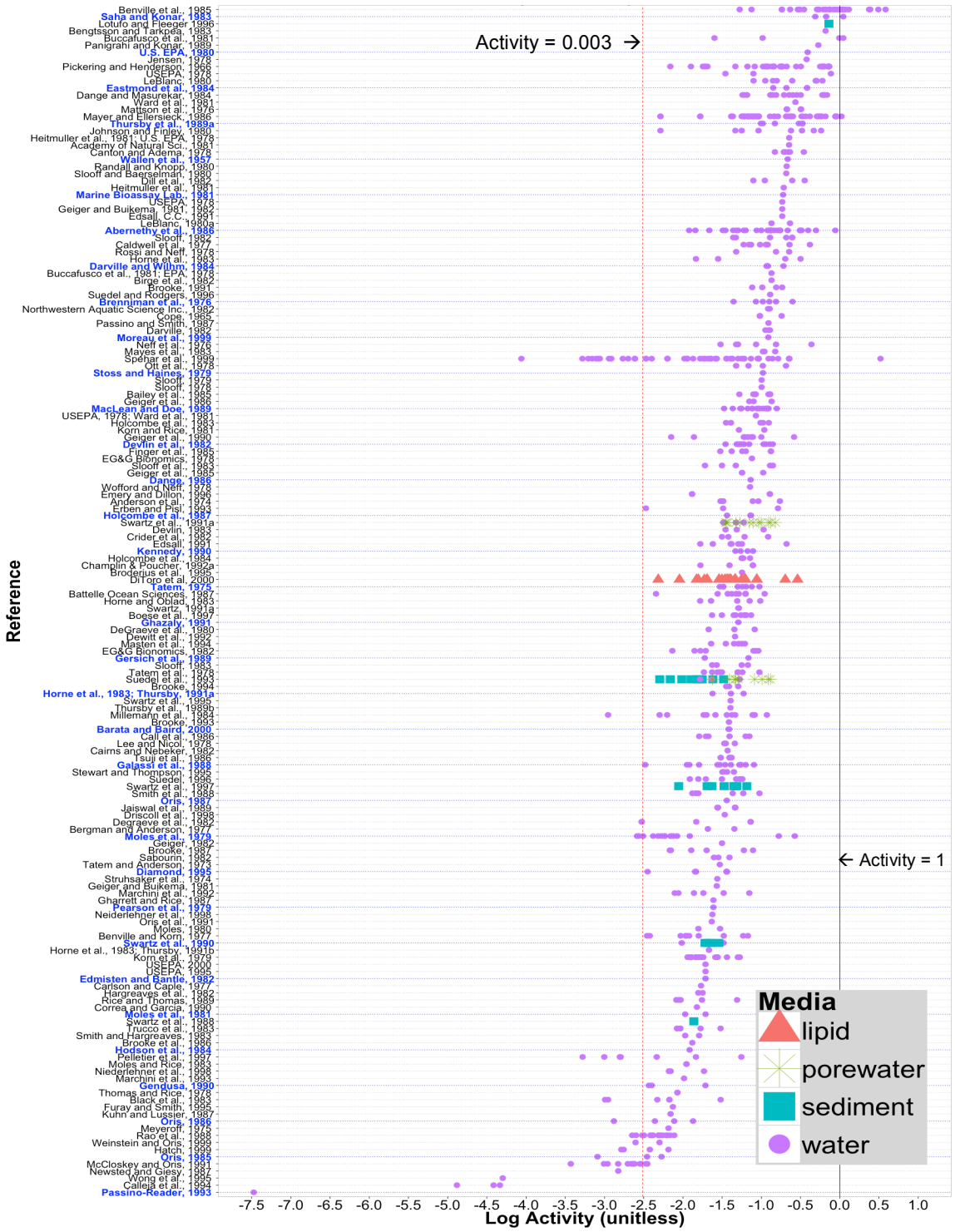


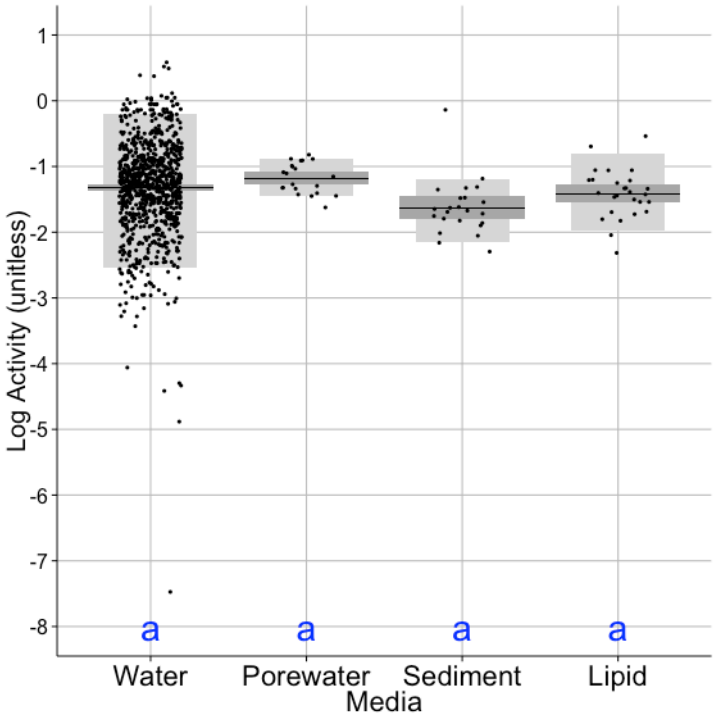
Figure 4.7. Distribution of PHC toxicity (LC50s) across original reference data sources and media, ordered by toxicity expressed as activity.

Water, Porewater, Sediment, and Lipid Media

There were no statistically significant differences in mean log-activity associated with LC50s for PHCs between any of the four different media types, water, porewater, sediment, and lipid (ANOVA: F=1.78, p=0.179; Figure 4.8a). In addition, there was no statistically significant difference in mean log-volume fraction associated with LC50s between the four media (ANOVA: F = 1.43, p = 0.233; Figure 4.8d). There was a statistical difference between media when PHC LC50s were expressed as lipid concentration (ANOVA: F=2.89, p=0.035; Figure 4.8c), however. The mean log-lipid concentration associated with LC50s of PHCs was significantly higher in water (mean = 1.93; 95%CI 1.88 to 1.98) than in sediment (mean = 1.48; 95%CI = 1.33 to 1.62), but all other possible two-way media comparisons were not statistically different (Tukey's HSD multiple comparison of means; Appendix E).

The methodology applied in this research uses a generalized relationship across all chemicals for the organic carbon:water partition coefficient (log K_{OC}) equal to 0.35 • log K_{OW} (Seth & Mackay, 1999). However, variation between chemical classes for the co-efficient in this K_{OC} : K_{OW} relationship has been reported (from 0.14 to 0.89; Seth & Mackay, 1999). If this methodology used 0.2 instead of 0.35 to calculate K_{OC} from K_{OW} the geometric mean activity for LC50s measured in sediment would double from 0.05 to 0.1. Subsequently, the lipid concentration and volume fraction calculated for sediment-based LC50s would also double (since lipid concentration = activity • solubility in lipid ; and volume fraction = activity • solubility in lipid • molar volume; Table 3.2). Calculations of activity, and lipid concentration and volume fraction for water-based LC50s do not include this 0.35 coefficient. Therefore, it is possible that the statistical differences in the mean calculated lipid concentration between water- and sediment-based LC50s are sensitive to the choice in the K_{OC} : K_{OW} co-efficient.

a)



b)

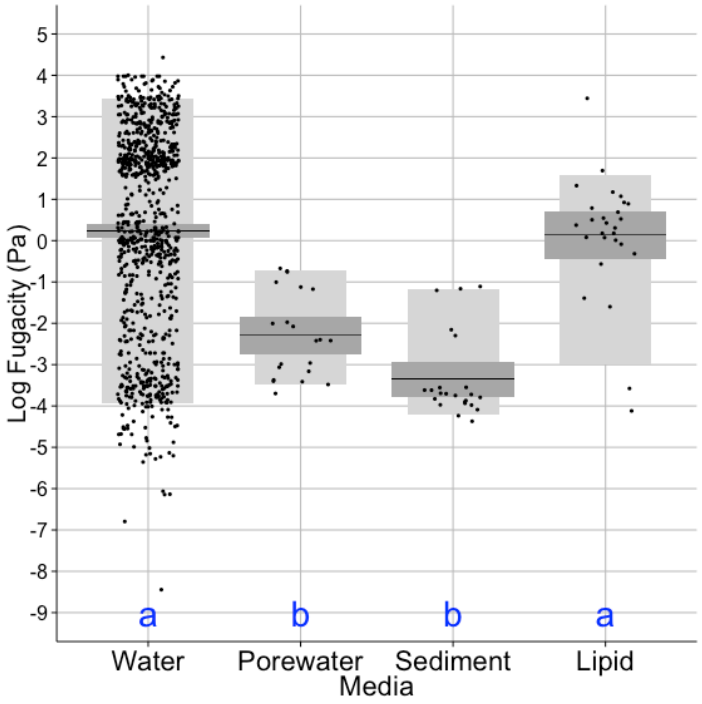
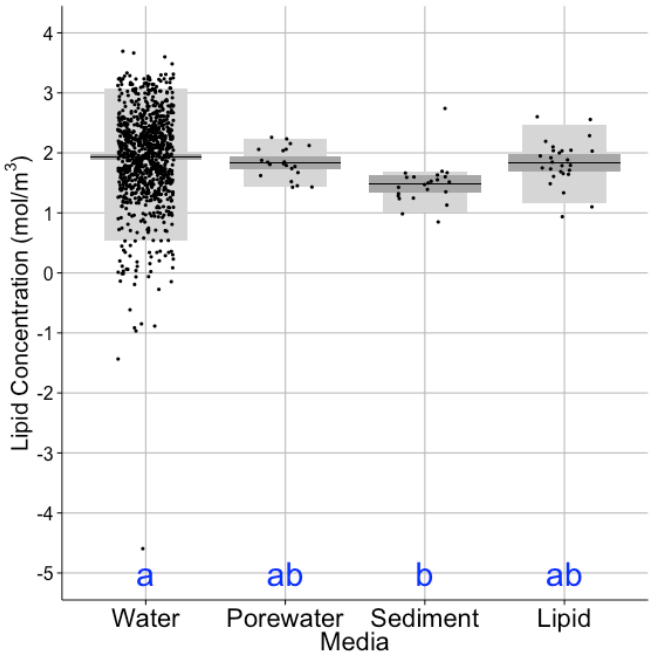


Figure 4.8 continued on next page…

c)



d)

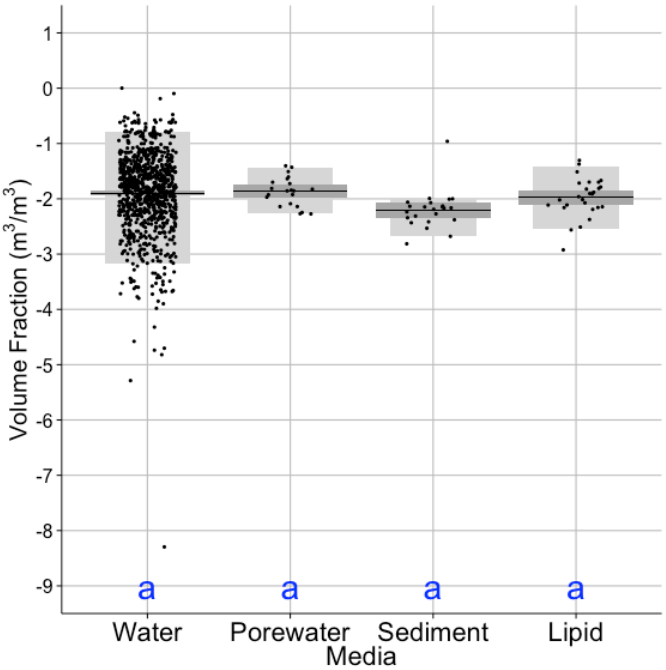


Figure 4.8. Comparison of LC50s expressed as a) activity, b) fugacity, and c) internal concentrations, between different media types.

Letters indicate similarities using ANOVA and Tukey's Honest-Significant-Difference tests;
• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility;
Solid black horizontal line represents the mean;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.

PHC LC50s expressed as fugacity were significantly lower in porewater and sediment media than in water and lipid media (Figure 4.8b; ANOVA: $F=22.7$, $p<0.001$; Appendix E). Not all chemicals are equally represented in each media. Porewater-based toxicity data were available for three different aromatic PHCs (acenaphthene, phenanthrene, and fluoranthene, with log K_{OW}s of 3.92, 4.36, and 5.2 respectively). Sediment-based toxicity data were available for the same three chemicals as porewater, plus pyrene (log K_{OW} = 5.18). Water-based toxicity data and lipid-based toxicity data were available for a greater number of different chemicals (42 PHCs, and 12 PHCs respectively), spanning a broader range of log K_{OW} (2.13 to 5.73 for water, and 2.11 to 5.32 for lipid). Because toxicity data expressed as fugacity varied significantly across chemicals of different K_{OW} (Figure 4.4b), these statistical differences between media likely reflect the fact that not all chemicals are represented evenly across the four different media.

In addition to the chemical-dependence of fugacities associated with toxicity, the majority of the porewater and sediment-based toxicity are from the same original study (Figure 4.7). Therefore, the toxicity data are more closely related between sediment and porewater than toxicity data for sediment and for water for example. The toxicity data for water come from 163 different original references (Figure 4.7), and therefore incorporate a lot more inter-laboratory and other sources of variation than the toxicity data for sediment and porewater which come from only six different original references.

Despite the sources of variation in toxicity data within media and between media, there was good overlap in the range between the 5^{th} and 95^{th} percentiles of the distributions of log-activity (Figure 4.8a), log-lipid concentration (Figure 4.8c), and lipid volume fraction (Figure 4.8d) between all four media. Because of this overlap, the combined distribution of all toxicity data across all media is inclusive of effects to organisms exposed in any medium, and can then be applied to risk assessments and guideline development for PHCs.

Across-Species Comparison of Individual PHC Toxicity

Species Categories

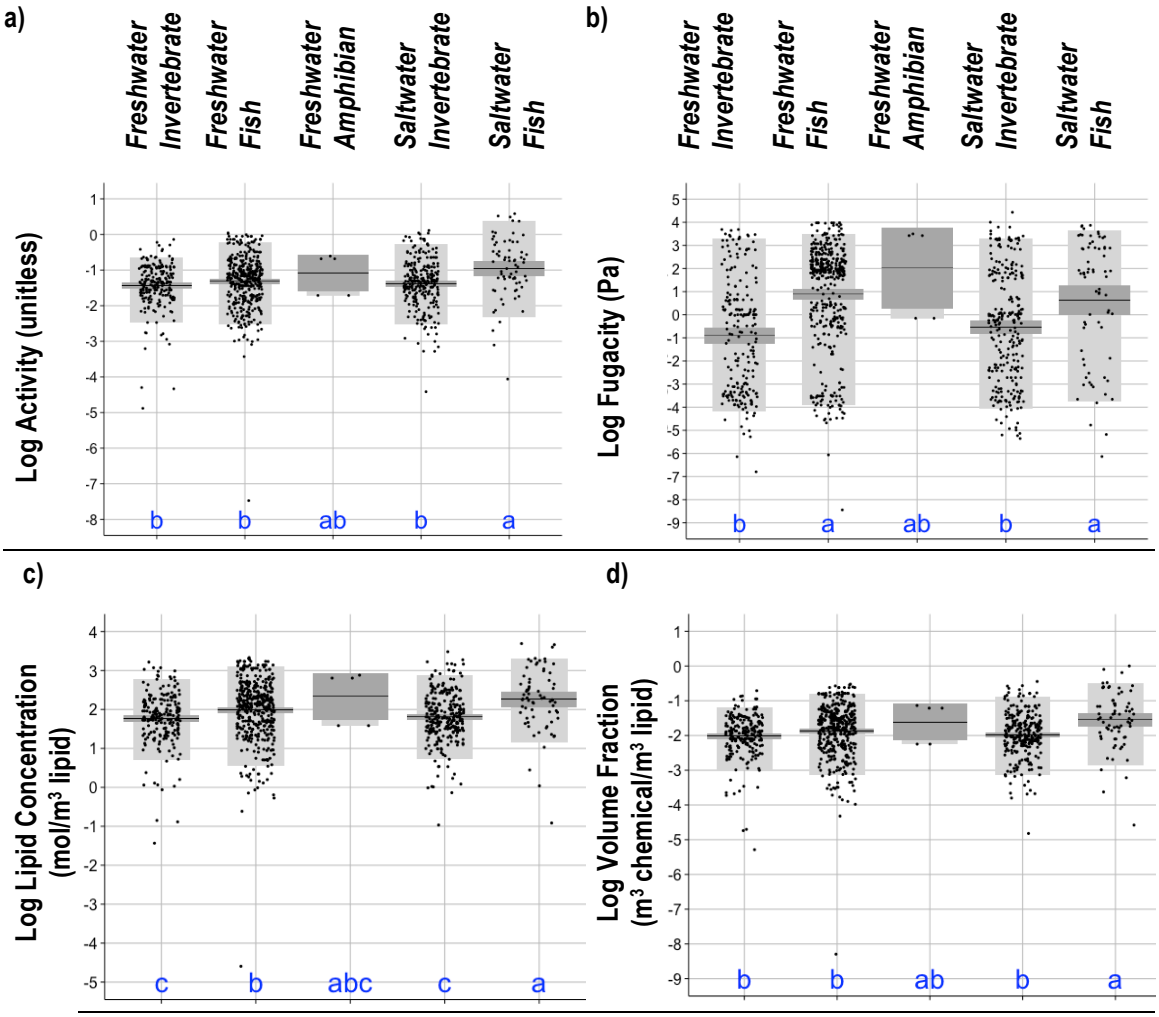


Figure 4.9. Comparison between freshwater invertebrates, fish, amphibians, and saltwater invertebrates and fish species categories in PHC toxicity (LC50s) expressed as a) activity, b) fugacity, c) lipid concentrations, and d) lipid-phase volume fraction.

Letters indicate similarities using ANOVA and Tukey's Honest-Significant-Difference tests;
• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility;
Solid black horizontal line represents the mean;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.

The dataset of LC50s for single PHCs was grouped into five different categories of test species: freshwater amphibians (2 species), invertebrates (33 species), and fish (23 species), and saltwater invertebrates (43 species) and fish (10 species) (111 species in total; Appendix C). There was broad overlap in the ranges between the 5^{th} and 95^{th} percentiles of PHC toxicity across all five species categories, when toxicity was expressed as activity, fugacity, lipid concentration, and volume fraction (Figure 4.9). Therefore, the lower 5^{th} percentile of all PHC toxicity data associated with 50% mortality, expressed as either activity (0.003), lipid concentration (4.35 mol/3), or volume fraction (0.0008 m3/m3) would be representative of a broad range of species.

There were statistical differences in the mean log-toxicity (LC50s) between the different species groups (ANOVA, all p–values <0.001; Appendix F). For example, the geometric mean activity for saltwater fish (0.11; 95%CI: 0.07 to 0.18; n=70) was slightly higher than the geometric mean activity for saltwater invertebrates (0.04; 95%CI: 0.035 to 0.05; n=259), freshwater invertebrates (0.04; 95%CI: 0.03 to 0.05; n=209), and freshwater fish (0.05; 95%CI: 0.04 to 0.06; n=410); (Figure 4.9, Appendix F). These statistical differences may be due to variations in sampling design and analytical techniques between different laboratories completing the original experiments. Additionally, the estimates of the geometric means in most species group (all except amphibians) have narrow confidence intervals because the large variation and large sample sizes per group cancel out when calculating the standard error per group (standard error = standard deviation ÷ sample size$^{0.5}$). The majority of individual data points fall outside the confidence interval around the mean. Therefore, regardless of any statistical differences, the overall broad range of activities associated adverse effects is more relevant to management of PHCs in wildlands than the mean. The ranges of LC50s within each of the five species categories all fell within the range of activities associated with critical body residues for neutral narcosis (0.001 to 1.0; McCarty et al., 2013).

Individual Species

Figure 4.10 presents all individual species ordered by their mean LC50 to PHCs expressed as activity. This species distribution curve presents another way of looking at toxicity data for a range of PHCs measured in a variety of different media across a broad

range of species. Figure 4.10 corroborates Figure 4.9 by showing that even within a single species, there can be a wide range in activities (over two orders of magnitude in some species) associated with LC50s. Therefore, there are likely other factors contributing to variation in toxicity observed even with a single species.

There has been some evidence indicating different toxicity of PHCs between invertebrates and fish, resulting from different cellular and biochemical mechanisms of toxicity and metabolism. For example, fish sometimes have more specific pathways than invertebrates for metabolism of chemicals. Toxicity data for soil or other terrestrial organisms are more limited compared to aquatic species, and therefore, soil-dwelling organisms may also have different toxic responses to contaminants. Toxicity of PHCs to plants is also an area of current research (e.g., Princz et al., 2012). Much important research continues to address these questions regarding different toxicity between different types of species. The data explored in this research do not suggest strong differences in LC50s between different species categories, when toxicity is expressed as activity, lipid concentration or volume fraction (Figure 4.9). This lack of difference is likely because membrane intercalation (i.e., non-polar narcosis) is shared between all species categories as underlying mechanism for effects observed in this dataset. The structure of cellular membranes is highly conserved, evolutionarily speaking, across many species. Stronger differences in general toxicity (or LC50s specifically) may be observed between organism categories for more specific biochemical mechanisms of toxic action. These differences may be more readily apparent by looking at the toxicity of individual PHCs (particularly aromatic PHCs) that are known to produce toxic metabolites.

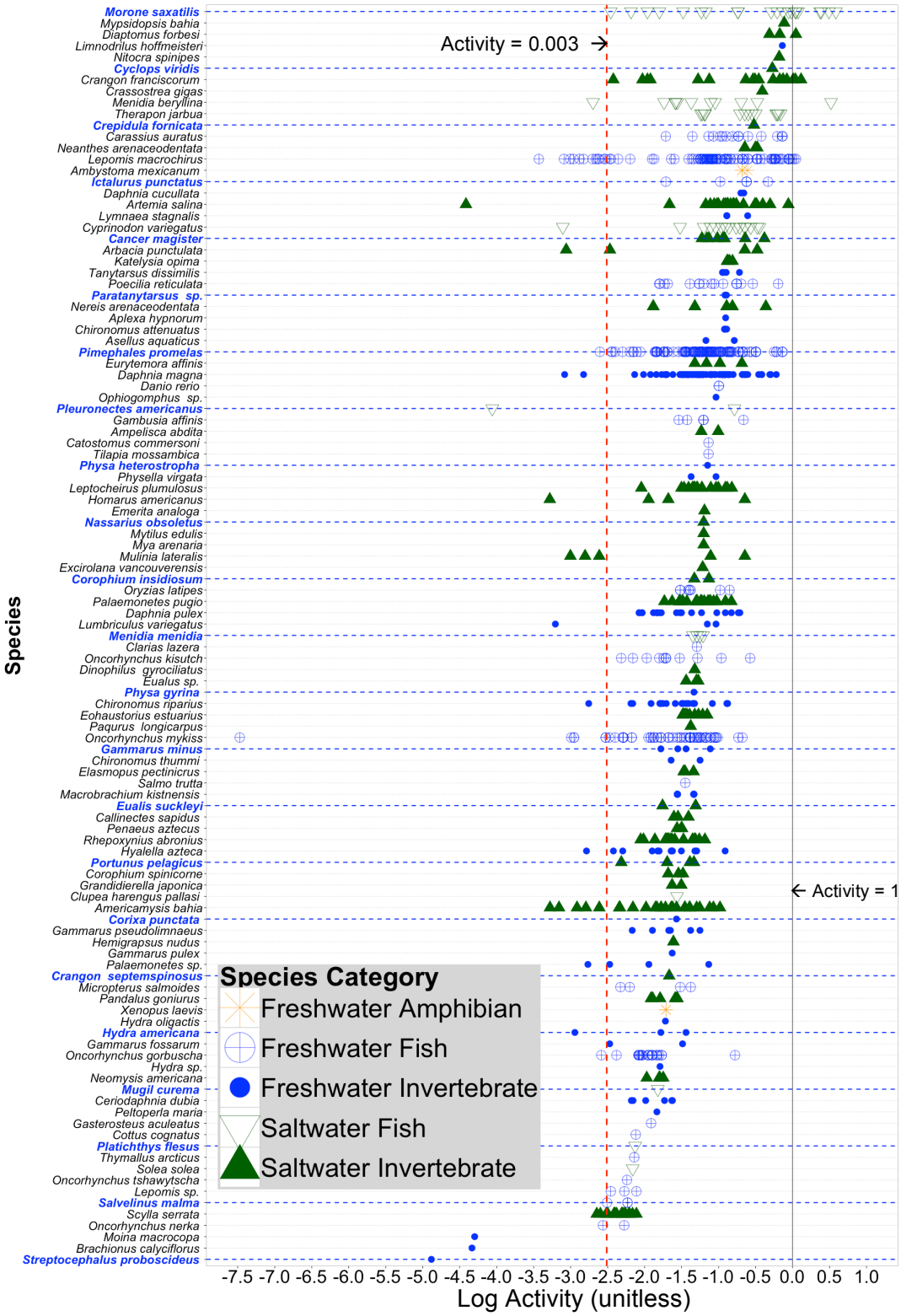


Figure 4.10. Species distribution curves of single chemical petroleum hydrocarbon LC50s expressed as activity.

Across-Effects and Across- Endpoints Comparison of Individual PHC Toxicity

In addition to the 953 LC50 data-points, there were also 216 additional data-points for other effects and endpoints available in this study's dataset (Appendix B). These additional data points included information on effects other than survival, including effects on an organism's growth (n=87), reproduction (n=42), development (n=2), hemo/histopathology (n=34), behaviour (sediment avoidance by invertebrates; n=1), and other (n=2; level at which cyclopentene was detectable by human smell, and induced headache) (Figure 4.11). These data included endpoints describing the amount of chemical affecting 50% of the test organisms (LC50 or EC50), as well as additional endpoints measuring different effect concentrations including no observed adverse effects levels (NOAELs), low observed adverse effects levels (LOAELs), effect observed by 25 or 100% of test species (EC25 and LC100, respectively), and chronic effects (test ranged from 41 to 56 days, at least some effect on survival, reproduction, and/or growth was reported in original reference). There were also an additional 48 non-LC50 survival effect data-points. In total, this expanded dataset included data for five additional aliphatic chemicals and seven additional aromatic chemicals that weren't represented in the refined LC50 dataset (Appendix B).

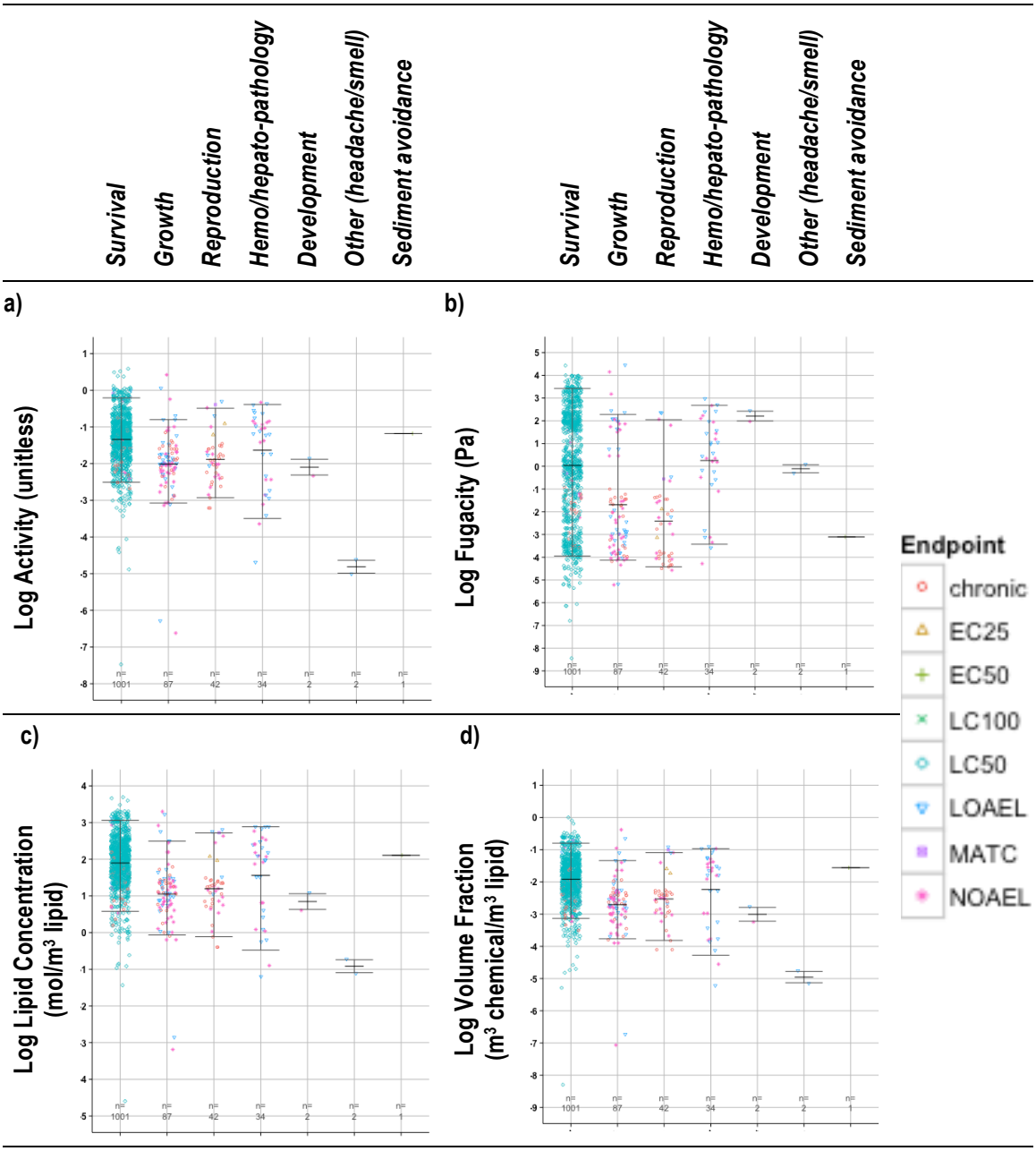


Figure 4.11. Comparison of a) activity, b) fugacity, c) lipid concentration, and d) volume fraction between different types of effects. Effects were quantified using one or more of 8 different endpoint types.

Long horizontal lines represent 5th and 95th percentiles of data per effect category; short horizontal lines represent the mean of log-transformed data (or geometric mean of non-transformed data).

• = one single LC50 datapoint; random jittering of datapoints about the x-axis for visibility; LOAEL = lowest observed adverse effects level; MATC = maximum acceptable toxicant concentration (typically MATC = geometric mean of NOAEL and LOAEL in same study); NOAEL = no observed adverse effect level.

There was great overlap in the distributions of toxicity datapoints across the different effects categories when toxicity was expressed as activity, fugacity, internal concentration, or volume fraction (Figure 4.11). This overlap suggests that the various different observable effects are a result of non-polar narcosis as a shared underlying mode of toxic action. Even within survival-based endpoints from many different studies (n=1001), the NOAELs and LOAELs are not consistently lower than the LC50s, as would be expected if comparing a NOAEL, LOAEL, and LC50 from within the same study. This observation suggests that other sources of variation, including random biological variation, are greater than the variation across different chemicals, species, effects, or endpoints.

Although overlapping, the more sensitive endpoints (e.g., development;

Figure 4.11) appear to have data distributions shifted slightly lower than the survival-based datapoints. However, the datapoints for development are almost entirely NOAELs and LOAELs. The survival-based datapoints are dominated by 50% effect level measurements (953 LC50s, and only 48 non-LC50 survival based datapoints). Hypothetically, if only the NOAELs and LOAELs for survival were compared to the NOAELs and LOAELs for development, the distributions of these different types of endpoints may be more aligned. Consideration of these additional 216 datapoints for effects and endpoints other than LC50s perhaps provides a quantitative way to derive criteria in place of arbitrary safety factors that are typically factors of 10.

Variation in individual PHC toxicity data

There is wide variation in the dataset, regardless of how toxicity data are expressed (Figure 4.2), including expression in original medium-specific concentrations (e.g., mol/kg_{OC}). Although this research specifically explored different chemicals, media, species, and effects as factors contributing to the variation in the toxicity data, these factors did not explain the orders of magnitude-scale variation seen in these data. There is still a large variation in these data when expressed in the original media-specific measured concentrations as well. When all LC50 data were considered together, the 5th and 95th percentiles of the distribution of activity, lipid concentration and lipid-phase volume fraction spanned 3 orders of magnitude, and fugacity spanned seven orders of magnitude.

The extreme lower tails of the distribution of these data were several orders of magnitude below the 5th percentile (Figures 4.1, 4.2) The minimum activity ($10^{-7.5}$), lipid-phase concentration ($10^{-4.6}$ mol/m^3 lipid), and lipid-phase volume fraction ($10^{-8.3}$ m^3/m^3) were roughly five orders of magnitude less than the respective 5th percentiles. Those datapoints below the 5th percentile potentially represent chemicals that either exert effects on organisms through more specific modes of action (like AhR-receptor mediated toxicity), or chemicals that produce toxic metabolites. The PHCs with the lowest toxicity included phenanthrene, and fluoranthene which are both chemicals that have been observed to be metabolised into toxic metabolites (Landrum et al., 2011). The bottom outliers may also be attributed to other causes such as analytical techniques or experimental methodology of the individual studies.

4.1.2. Toxicity of Environmentally Relevant PHC Mixtures Using Activity- and Fugacity-based Approaches

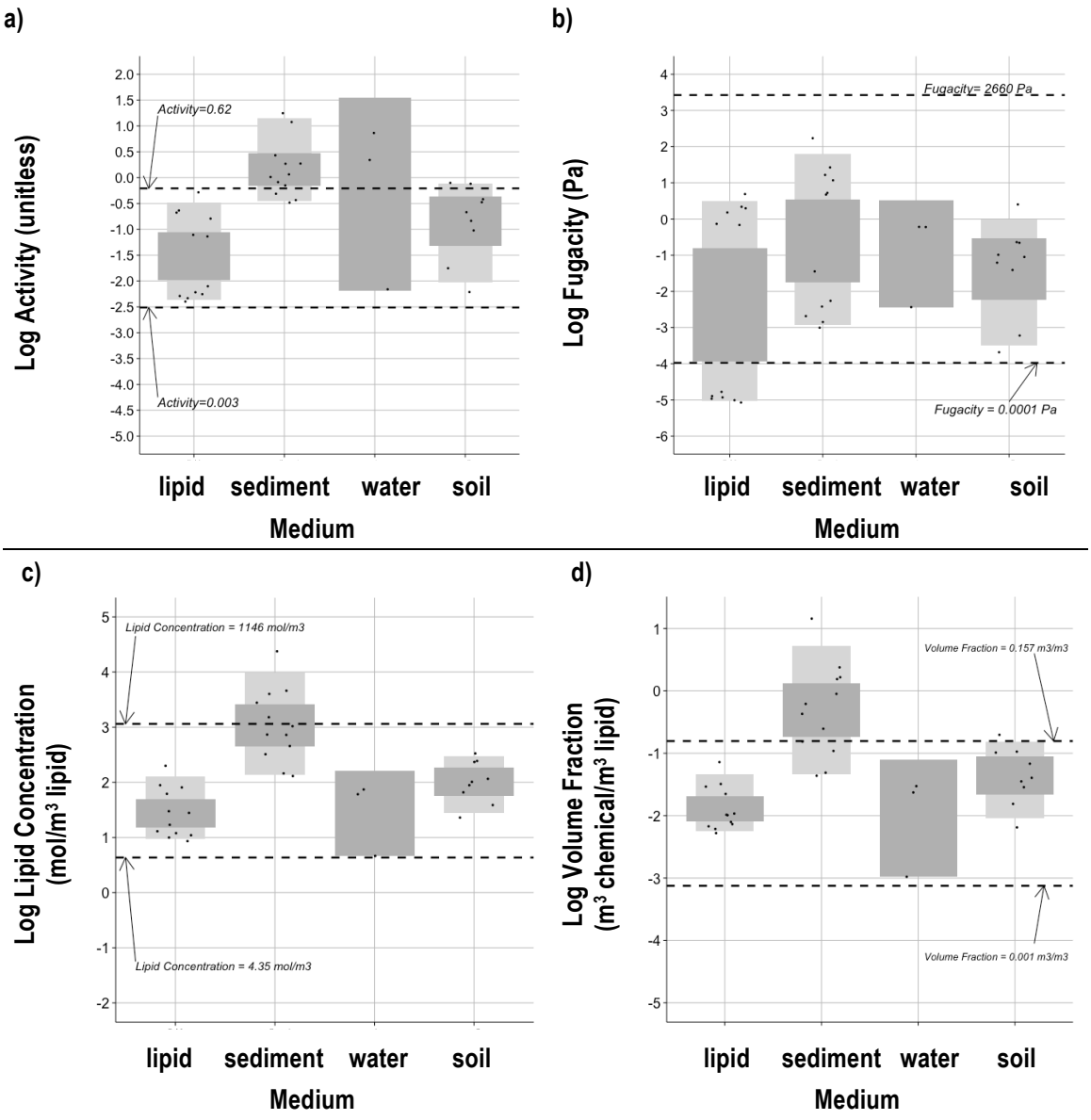


Figure 4.12. Toxicity of PHC mixtures (LC50s) measured in lipid, sediment, water, and soil, expressed as a) activity, b) fugacity, c) lipid concentrations, and d) lipid-phase volume fraction.

Dashed horizontal lines indicate 5th and 95th percentiles of toxicity data (LC50s) for single petroleum hydrocarbon chemicals.
• = ∑activity, ∑fugacity, ∑lipid concentration, or ∑volume fraction for all individual components indentified in the mixture at a mixture concentration associated with 50% mortality;
Random jittering of datapoints about the x-axis for visibility;
Dark-grey shaded area represents the 95% confidence interval around the estimate of the mean;
Light-grey shaded area represents the range between the 5th and 95th percentiles of the data.

This methodology applies an additive approach to describe toxicological effects associated with PHC mixtures, by adding the amount of each individual component present in the mixture. Each datapoint in Figure 4.12 represents the ∑ activity, ∑ fugacity, ∑ lipid-phase concentration, or ∑ lipid-phase volume fraction of all components in a mixture, where the total mixture concentration is associated with 50% mortality in test species. Twenty-three of the 36 PHC mixture datapoints expressed as activity fall within the 95th percentiles of the activities of single PHC LC50s (Figure 4.12a). Similarly, 31 of 36 PHC mixture datapoints expressed as lipid-phase concentration (Figure 4.12c) and 29 of 36 PHC mixture datapoints expressed as lipid-phase volume fraction (Figure 4.12d) fell within the respective 95th percentiles of CL and VC/VL for LC50s of single PHC chemicals. This observation of overlap in activities, C_L, and V_C/V_L between individual chemicals and PHC mixtures supports non-polar narcosis as an underlying shared mode of toxic action between mixture components, and that the response is additive.

Although 30 of 36 datapoints for ∑ fugacity of PHC mixtures fell within the 95th percentile of fugacity for single chemical LC50s (Figure 4.12b), the ∑ fugacity of mixtures' composition since fugacity associated with LC50s varies widely across different individual chemicals (Section 4.1.1). For example, a fugacity of 0.001 Pa may be sufficient for mixture components with log K_{OW} of 5 to elicit 50% mortality. However, that same 0.001 Pa fugacity is only 1% of the fugacity required to elicit the same response from exposure to mixture components with log K_{OW} of 4. Therefore, there is no common fugacity that consistently describes toxicity (LC50s) across different types of PHC mixtures (Figure 4.12b). Fugacity is not additive when describing the toxicity of mixtures containing chemical components with a variety of physical-chemical properties. For example, the fugacity-based mixture toxicity data based in sediment or lipid (Appendix G) highlight the dependence of the mixtures' combined ∑ fugacity on their chemical composition. There were two different types of mixtures tested in sediment and lipid media. All of the ∑ fugacity datapoints for the heavier mixture (comprised of PHCs with higher molecular weights) were clumped together at a lower fugacity (<0.003 Pa) than the datapoints for the lighter mixture (comprised of PHCs with lower molecular weights) which all had fugacity greater than 1 Pa (Figure 4.12b). Fugacity may be a useful tool for comparing toxicity of mixtures

across different species or media, when the mixtures' compositions are the same. But, fugacity is not a useful tool for comparing toxic effects between different mixtures with varying chemical compositions.

Table 4.10. Summary of activity, fugacity, and lipid-phase concentration (C_L) and volume fraction (V_C/V_L) associated with toxicity (LC50s) of mixtures of petroleum hydrocarbons in lipid (n=12), sediment (n=12), water (n=3), and soil (n=9).

Metric	Media	ANOVA (F, p) and Tukey HSD[1,2]	Mean $\sum^3$	Lower 95% CI on Mean	Upper 95% CI on Mean	Lower 5%ile	Upper 5%ile
Log(Activity)	ALL	F=9.37; p<0.001	-0.691	-1.04	-0.346	-2.30	0.917
	Lipid	b[2]	-1.52	-1.99	-1.05	-2.36	-0.477
	Sediment	a[2]	0.159	-0.157	0.475	-0.456	1.153
	Water	ab[2]	-0.318	-2.19	1.55	-1.91	0.812
	Soil	b[2]	-0.845	-1.33	-0.364	-2.03	-0.107
Activity	ALL	na	0.204	0.092	0.451	0.005	8.25
	Lipid	na	0.030	0.010	0.089	0.004	0.333
	Sediment	na	1.441	0.696	2.98	0.350	14.21
	Water	na	0.481	0.007	35.4	0.012	6.48
	Soil	na	0.143	0.047	0.433	0.009	0.781
Log(Fugacity)	ALL	F=1.48; p=0.240	-1.42	-2.13	-0.708	-4.98	1.27
	Lipid	a[2]	-2.37	-3.93	-0.81	-5.04	0.495
	Sediment	a[2]	-0.61	-1.76	0.539	-2.92	1.79
	Water	a[2]	-0.959	-2.44	0.519	-2.22	-0.219
	Soil	a[2]	-1.38	-2.24	-0.526	-3.50	-0.015
Fugacity	ALL	na	0.038	0.007	0.196	0.00001	18.624
	Lipid	na	0.004	0.0001	0.153	0.000009	3.13
	Sediment	na	0.245	0.017	3.46	0.0012	61.5
	Water	na	0.110	0.004	3.30	0.006	0.603
	Soil	na	0.041	0.006	0.298	0.0003	0.97

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Table 4.10 *continued…*

Metric	Media	ANOVA and Tukey HSD[1,2]	Mean ∑ [3]	Lower 95% CI on Mean	Upper 95% CI on Mean	Lower 5%ile	Upper 5%ile
Log(C_L)	ALL	F=19.7; p<0.001	2.114	1.825	2.403	0.983	3.617
	Lipid	b[2]	1.44	1.18	1.70	0.970	2.11
	Sediment	a[2]	3.04	2.66	3.42	2.14	3.98
	Water	b[2]	1.44	0.663	2.22	0.777	1.86
	Soil	b[2]	2.01	1.75	2.26	1.45	2.47
C_L	ALL	na	130	66.8	253	9.61	4142
	Lipid	na	27.4	15.1	50.0	9.33	128
	Sediment	na	1090	454	2622	138.1	9611
	Water	na	27.5	4.60	164	5.98	72.7
	Soil	na	102	56.4	183	28.3	294
Log(V_C/V_L)	ALL	F=17.4; p<0.001	-1.24	-1.54	-0.946	-2.23	0.255
	Lipid	a[2]	-1.89	-2.09	-1.68	-2.24	-1.33
	Sediment	b[2]	-0.313	-0.74	0.117	-1.33	0.726
	Water	a[2]	-2.04	-2.98	-1.11	-2.84	-1.54
	Soil	a[2]	-1.36	-1.66	-1.05	-2.04	-0.813
V_C/V_L	ALL	na	0.057	0.029	0.113	0.006	1.80
	Lipid	na	0.013	0.008	0.021	0.006	0.046
	Sediment	na	0.487	0.181	1.309	0.046	5.32
	Water	na	0.009	0.001	0.078	0.001	0.029
	Soil	na	0.044	0.022	0.089	0.009	0.154

na = not applicable
1. Difference in mean log-transformed data between media was tested using ANOVA (F-, and p-values), and Tukey HSD (to identify where differences are if ANOVA indicated a difference between means exists). ANOVA and Tukey HSD were only run on log-transformed data, not on untransformed data.
2. Same letters indicate no significant difference in means between pair-wise comparisons of media using Tukey's Honest-Significant-Difference test
3. Mean ∑ for log-transformed data. Mean ∑ represents geometric mean for non-transformed values.

All external (sediment, water, soil) media-based PHC mixture toxicity datasets included data points with ∑ activity greater than 1 (Figure 4.12a). Unlike the activity for

individual chemicals which is thermodynamically limited to ≤ 1 (i.e., concentration ≤ solubility), the ∑ activity for chemical mixtures is not necessarily thermodynamically constrained to ≤ 1. PHC mixtures considered in this research are comprised of chemical components with water solubilities ranging from $8 \bullet 10^{-9}$ mol/m^3 to 1.2 mol/m^3. Hypothetically, a mixture comprised of two chemicals, both with an activity = 0.75, but with solubilities differing by several orders of magnitude, would have a ∑ activity = 1.5, but neither individual component would be in excess of their respective solubilities.

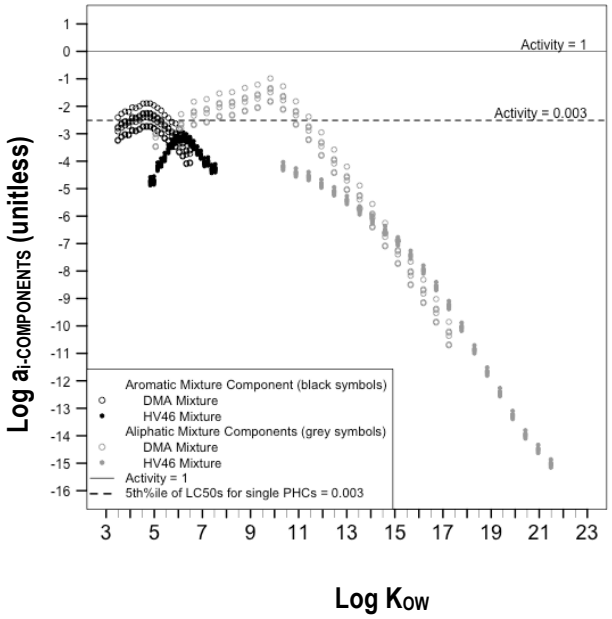
The ∑ activity of PHC mixtures in lipid, water, and soil agrees with previous studies of the activity of less complex mixtures. Mayer and Holmstrup (2008) reported an activity in lipid and water of 0.058 (95%CI = 0.057 to 0.059; or mean log activity = -1.24) that was associated with 50% mortality to *Folsomia candida*, a soil springtail invertebrate, after 7-day exposure to 10 different PAHs or a binary pyrene/anthracene mix. Schmidt et al. (2013) reported a ∑ activity in water and lipid of 0.027 (log activity = -1.57), and a lipid-based concentration based on SPME concentrations of 133 mol/m^3_{LIPID} (log lipid concentration = 2.1) that were associated with toxicity (LC50s) of mixtures of three PAHs (phenanthrene, pyrene, and naphthalene) to springtail soil invertebrates.

4.2. PHC Mixture Toxicity Compared to Individual PHC Toxicity Using Activity-based Approaches

The ∑ activity associated with LC0s of PHC mixtures in lipid, sediment, water, and soil (Figure 4.12a) was comprised of the individual activities of each separate component (ai) present in PHC mixtures. These activities for each of the individual mixture components in lipid, sediment, water, and soil are presented in Figures 4.13a, 4.14a, 4.15a, and 4.16a. The ∑ activity for all components (aromatic plus aliphatic), ∑ activity for all aromatic components, and ∑ activity for all aliphatic components of PHC mixtures are presented in Figures 4.13b, 4.14b, 4.15b, and 4.16b. The individual components and sub-totals of aromatic and aliphatic components of PHC mixtures expressed as fugacity, lipid concentration, and lipid-phase volume fraction are also included in Appendices G, H, and I, respectively. The focus of this discussion will be on activity. However, the same explanations for differences between individual PHCs and

PHC mixtures also apply to lipid concentration and volume fraction. Fugacity was not considered an appropriate tool for looking at mixtures of chemicals with widely varying chemical properties, and therefore was not further considered in this discussion.

a) Activity of Individual PHC Mixture Components (a_i) in Lipid



b) ∑Activity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures in Lipid

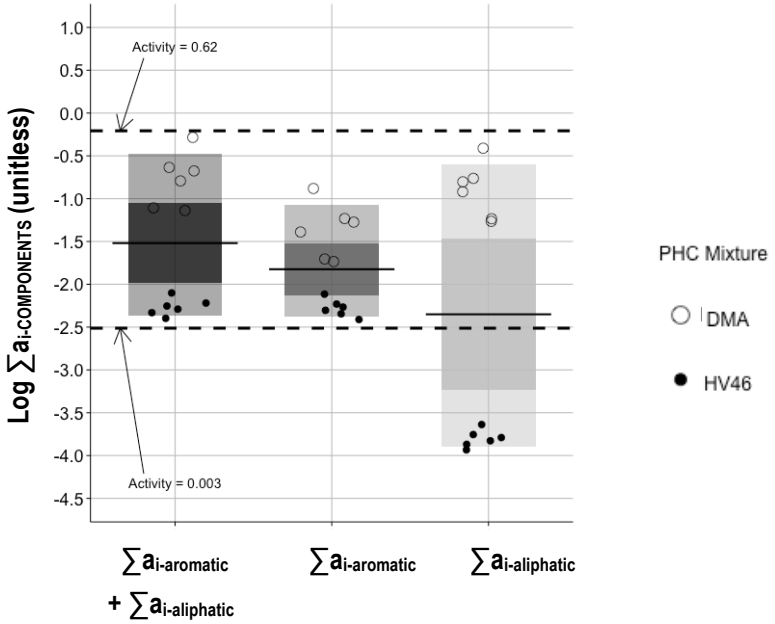
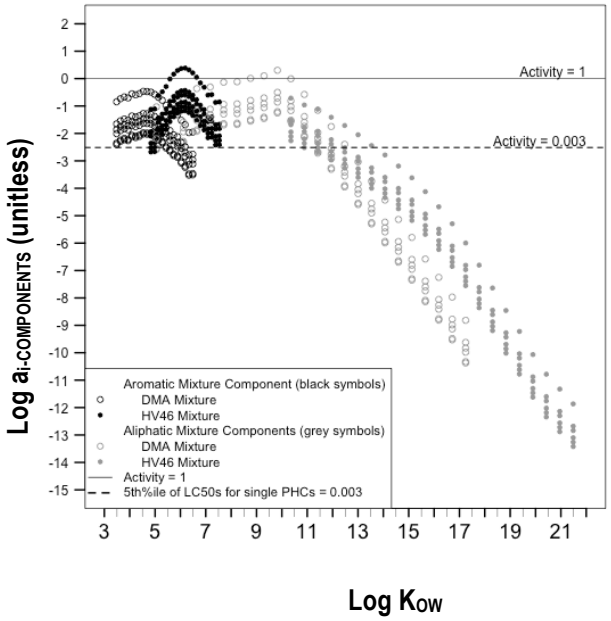


Figure 4.13. Activity in lipid of a) individual components (a_i) and b) ∑activity of all components, ∑activity of only aromatic components, and ∑activity of only aliphatic components in PHC mixtures. Total PHC concentrations in lipid were reported as toxic (LC50s).

Random jittering of datapoints about the x-axis for visibility;
Dark shaded areas represents the 95% confidence interval around the estimate of the mean;
Light shaded areas represents the range between the 5th and 95th percentiles of the data.

a) Activity of Individual PHC Mixture Components (a_i) in Sediment



b) ∑Activity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures in Sediment

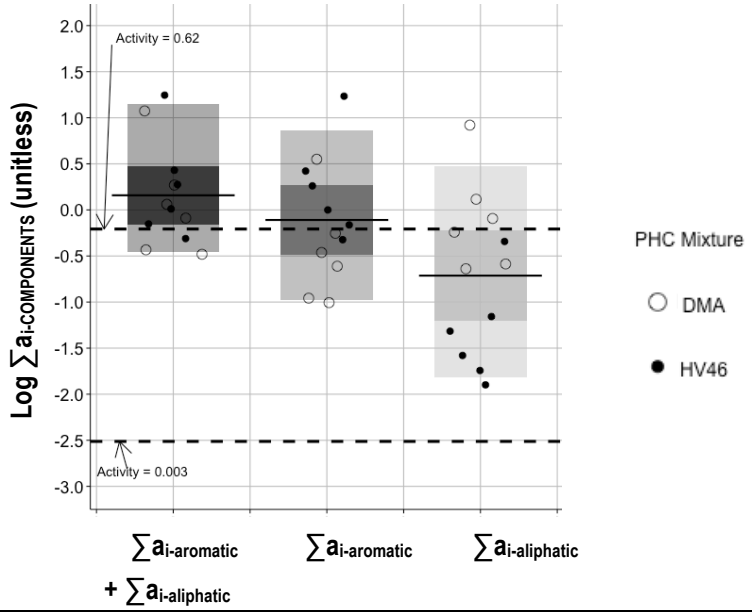
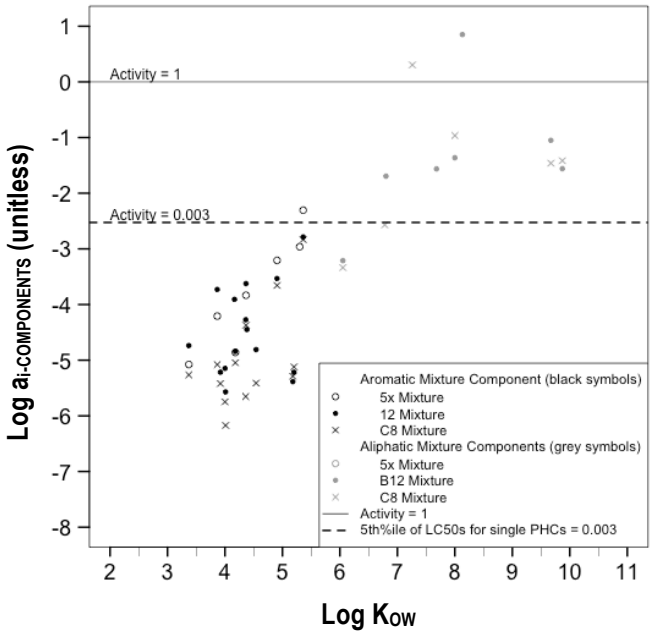


Figure 4.14. Activity in sediment of a) individual components (a_i) and b) ∑activity of all components, ∑activity of only aromatic components, and ∑activity of only aliphatic components in PHC mixtures. Total PHC concentrations in sediment were reported as toxic (LC50s).

Random jittering of datapoints about the x-axis for visibility;
Dark shaded areas represents the 95% confidence interval around the estimate of the mean;
Light shaded areas represents the range between the 5th and 95th percentiles of the data.

a) Activity of Individual PHC Mixture Components (a_i) in Water



b) ∑Activity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures in Water

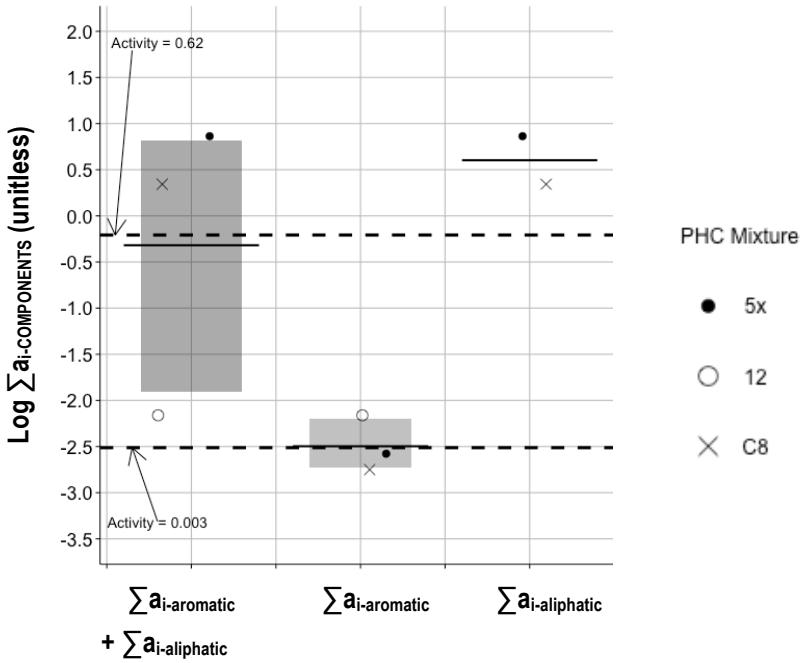
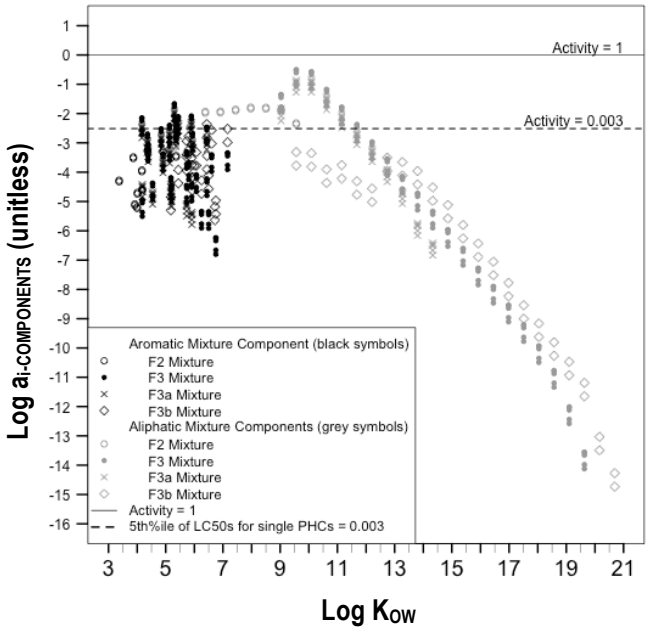


Figure 4.15. Activity in water of a) individual components (a_i) and b) ∑activity of all components, ∑activity of only aromatic components, and ∑activity of only aliphatic components in PHC mixtures. Total PHC concentrations in water were reported as toxic (LC50s).

Random jittering of datapoints about the x-axis for visibility;
Dark shaded areas represents the 95% confidence interval around the estimate of the mean;
Light shaded areas represents the range between the 5th and 95th percentiles of the data.

a) Activity of Individual PHC Mixture Components (a_i) in Soil



b) ∑Activity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures in Soil

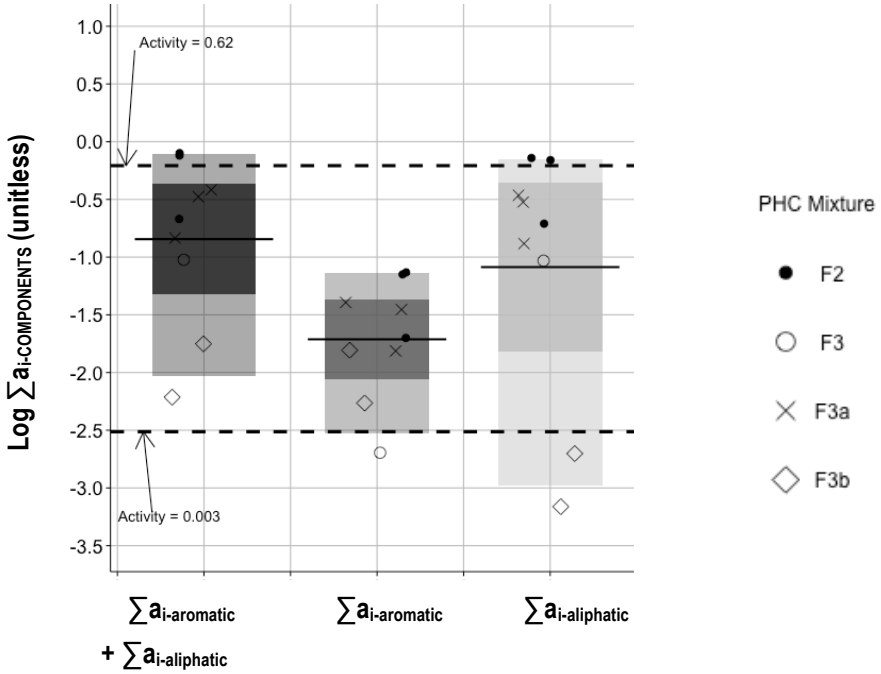


Figure 4.16. Activity in soil of a) individual components (a_i) and b) ∑activity of all components, ∑activity of only aromatic components, and ∑activity of only aliphatic components in PHC mixtures. Total PHC concentrations in soil were reported as toxic (LC50s).

Random jittering of datapoints about the x-axis for visibility;
Dark shaded areas represents the 95% confidence interval around the estimate of the mean;
Light shaded areas represents the range between the 5th and 95th percentiles of the data.

Individual components in mixture with activity greater than one

Sediment- and water-based PHC mixture toxicity included one or more components in the mixture with activity greater than one (Figure 4.14a, and Figure 4.15a). There were no individual components in lipid- or soil-based mixtures (Figure 4.13a, and Figure 4.16a) with activity greater than one. Mixture components in sediment and water with an activity (a_i) greater than one indicate that there may be oil droplets or a pure chemical phase present, since the external media are saturated with respect to those components' solubility. These pure-chemical phases may contribute to toxicity through an external physical mode of toxic action such as suffocating respiratory surfaces rather than non-polar narcosis, an internal cellular-level mode of toxic action. Therefore, toxicity observed in the sediment- and water-based PHC mixture tests may have been a result of these physical effects, rather than solely non-polar narcosis.

Individual components in mixture with activity less than 0.003

In lipid-, sediment-, water- and soil- based PHC mixture toxicity tests, many of the mixtures' components (Figures 4.13a, 4.14a, 4.15a, 4.16a) have an activity that is lower than 0.003, the 5th percentile of single-chemical-based LC50s expressed as activity (Table 4.1; Figure 4.1). This observation is consistent with the additivity hypothesis of toxicity for chemicals sharing the same mode of toxic action (Könemann 1981; Hermens 1989). Under this hypothesis, a given effect (in this case, an LC50) will be observed at one activity, regardless of whether the activity of PHCs in a medium comes from one single chemical, or from a mixture of multiple chemicals. The individual mixture components (Figures 4.13a, 4.14a, 4.15a, 4.16a) should all have an activity (a_i) that is lower than the activity of LC50s for single chemicals, in order for the $\sum a_i$ of the mixture to be equal to the activity of LC50s for single chemicals.

Cumulative activity of mixture components greater than 0.003

In lipid, sediment, water, and soil, the $\sum a_i$ of all mixture components (both aromatic and aliphatic components) in PHC mixtures associated with 50% mortality is greater than the activity = 0.003 (the lower 5^{th} percentile of LC50s for single PHCs expressed as activity) (Figures 4.13b, 4.14b, 4.15b, 4.16b). This observation suggests

that guidelines and risk assessments developed from distribution of single PHC chemical toxicity will also be protective of complex, environmentally relevant mixtures of PHCs.

Furthermore, the $\sum a_i$ of only the aromatic mixture components is also greater than 0.003 for the majority of the PHC mixture toxicity datapoints (Figures 4.13b, 4.14b, 4.15b, 4.16b), suggesting that in these cases, the aromatic components alone are present in sufficient quantities to cause 50% mortality. However, in the water-based and soil-based PHC mixture toxicity tests, there are some data where the $\sum a_i$ of only the aromatic mixtures is less than 0.003, suggesting that aromatic components alone are not present in sufficient amounts to cause 50% mortality (Figure 4.15b and Figure 4.16b). In these cases, the $\sum a_i$ of the mixture's aliphatic components make an important contribution to the $\sum a_i$ of all mixture components towards reaching activity values of PHC mixtures sufficient to cause an effect. Individual aliphatic components in mixtures are not typically present in sufficient quantities to cause an effect (Figures 4.13a, 4.14a, 4.15a, 4.16a: most $a_{i\text{-aliphatic}}$ less than 0.076, the lower 5th percentile of LC50s for individual aliphatic PHCs). But the cumulative effect of the aliphatic components ($\sum a_{i\text{-aliphatic}}$) may still be making an important contribution to overall PHC mixture toxicity when aromatic components of PHC mixtures are not present at activities associated with effects (i.e., $\sum a_{i\text{-aromatic}}$ less than 0.003).

Individual components in mixture with activity greater than 0.003

In all media, the toxicity tests on PHC mixtures had at least one mixture component (Figures 4.13a, 4.14a, 4.15a, 4.16a) with activity greater than 0.003, the 5th percentile of single-chemical-based LC50s expressed as activity (Figure 4.1). Based on these observations and calculations, the presence of only one or a few of the mixtures' components is sufficient to cause an LC50 effect. However, in the test conditions there were many more chemicals present in the PHC mixtures. There may be some behaviours of chemical mixtures that are not sufficiently captured in the calculations of activity for mixtures. The presence of co-solvents in PHC mixtures can enhance the solubility of individual components through the formation of micelles or micro-emulsions. The activity of mixture components (and therefore also the $\sum$ activity for mixtures) would be lower than presented in this research if chemicals' solubility is actually enhanced in mixtures. There may also be effects taking place in which the toxicity of mixtures or

exposure to multiple chemicals is reduced by the presence of other components in the mixture (e.g., competitive inhibition between chemicals).

Equilibrium considerations with PHC mixtures

The K_{OW} of mixtures' components (Figures 4.13a, 4.14a, 4.15a, 4.16a) suggest that not all mixture components measured in external media (sediment, water, or soil) are reaching steady-state with the tissue of the test organisms. Components with a log K_{OW} greater than approximately six may not be significantly contributing to the total contaminant loading inside organisms. Biota-sediment-accumulations factors (BSAFs) for example have been demonstrated to decrease with increasing log K_{OW} for organic chemicals with log K_{OW} above approximately 6 or 7 (Parkerton et al., 1993). In the PHC mixture toxicity datasets there are components with log K_{OW} values greater than six. These mixture components with high log K_{OW} that were measured in external media (i.e., water, sediment, or soil) are unlikely to reach steady state concentrations between outside and inside the organism within the duration of the toxicity test, and therefore unlikely contribute significantly to test organisms' total internal PHC exposure. Molecular volume is thought to be correlated with log K_{OW}, and therefore, it may be their physically large size that limits movement of highly hydrophobic chemicals across cellular membranes from external to internal media. By contrast, the highest log K_{OW} in the dataset for toxicity of single PHCs (Section 4.1.1) was 5.73 (less than 6), and there was evidence that steady state was reached since there were no significant differences in activities associated with LC50s of single chemicals between abiotic and biotic media (Figure 4.6a).

A comparison between the activity of sediment-based mixtures' components and activity of lipid-based mixtures' components (Figures 4.13, 4.14) provides evidence that high K_{OW} substances are not entering test organisms, since both of these datasets are from the same study (Verbruggen et al., 2008). Verbruggen et al. (2008) included a pure oil phase when calculating chemical partitioning between organic carbon in the sediment, aqueous porewater, and organisms' membrane lipid phase. In turn, the activity calculated in the lipid medium for PHC mixtures' components (Figure 4.13) are subsequently lower than the activity calculated in the sediment medium for PHC mixtures' components (Figure 4.14). This comparison suggests that equilibrium

between abiotic and biotic media was not reached for all PHC mixture components in these laboratory-based results.

Environmental exposures are typically longer than laboratory-based exposures. Longer exposure durations in the field allow more time for chemicals to reach the site of toxic action inside an organism. Heavy, hydrophobic chemicals can take long times (up to years long) to reach steady state between external, abiotic media and an internal biotic medium. Laboratory-based PHC mixture toxicity tests were on the order of days in duration. Water-based PHC mixture toxicity tests on invertebrates were 6 or 7 days long. Sediment- and lipid-based PHC mixture toxicity tests on invertebrates were 10 or 14 days long. Soil-based PHC mixture toxicity tests on invertebrates were 7, 14, 28, or 35 days long. Therefore, it is possible that not all mixture components, particularly the heavy hydrophobic mixture components measured in abiotic media were at equilibrium between the biotic and abiotic media. Lab-based tests of shorter duration are more susceptible to underestimates of PHC mixture toxicity than longer field-based studies.

Field-based measurements of PHC uptake from soil by biota provides evidence that equilibrium between abiotic and biotic media is also not reached in environmental settings. For example, Kreitinger et al. (2007) reported field-measured biota-sediment accumulation factors (BSAF)s for 16 PAHs in terrestrial soil earthworms that were much lower than BSAFs predicted by an equilibrium-partitioning model. Kreitinger et al. (2007) suggested that PAHs's affinity for organic carbon varies depending on the source of the organic carbon. Anthropogenically derived carbon (e.g., from combustion sources) has a higher affinity for PAHs than other more natural forms of organic carbon. The time to reach steady state between the external exposure media (e.g., soil) and the internal tissues of the organism is on the order of magnitude of years, and possibly greater than the lifespan of some organisms such as invertebrates. Therefore, these short-lived organisms never reach steady state, particularly for the heavy PHC components, and ultimately are not exposed. Therefore, internal-concentrations calculated using assumptions of equilibrium conditions can overestimate the activity and internal lipid concentration (and volume fraction) that an organism is ultimately exposed to.

The kinetic rates of uptake and elimination by organisms likely vary between different mixture components and different media. For heavier, hydrophobic mixture components (i.e., higher log K_{OW}), the rate of chemical elimination from organisms is very slow. The rate of metabolism then drives overall rates of accumulation (or dilution) in organisms' tissue. For lighter, lipophobic mixture components, rates of elimination drive overall rates of accumulation (or dilution) in the organisms' tissue since rates of elimination are much higher than rates of metabolism. The rate-limiting steps also differ between different media. In water, the rate limiting step is more likely to occur inside the organism: the uptake by the organism from external exposure media, and the rate of metabolism inside the organism. In contrast, the rate-limiting step in sediment more likely occurs in the abiotic medium: the desorption rate of the chemical from the organic carbon component of the soil or sediment into aqueous phase.

Future incorporation of uptake rates, kinetic limitations, and non-equilibrium conditions would potentially improve this method of adding activity to predict toxicity of PHC mixtures. A log K_{OW} cut-off, above which components are not included in the sum calculation would be one way of adjusting the method. Alternatively, correction factors derived from a bioaccumulation model could be applied to individual mixture components to account for the fraction of chemical in external media (i.e., sediment, water, or soil) that is taken up by organisms. For example, bioconcentration factors (BCF = concentration in organism ÷ concentration in water) or biota-sediment accumulation factors (BSAF = concentration in organism ÷ concentration in sediment) can be modelled using log K_{OW} (Arnot & Gobas 2004). Empirical BCF and BSAFs can also be calculated with chemicals' activities in the biotic and abiotic media. Modelling the kinetic rate of different PHC components, and scaling components' activity according to duration of the toxicity test, and the relative lifespan of the organism could influence the activity model (and subsequent lipid concentration and volume fraction models) for PHC mixture toxicity.

Despite their low uptake by organisms, these higher log K_{OW} components may still contribute to effects on organisms through physical effects if they are present in quantities greater than their solubilities (i.e., activity greater than 1). Additionally, an organism is exposed to more than just one type of external medium in a real

environmental setting; total exposure includes multiple pathways from multiple abiotic media (e.g., air, water, sediment) and diet.

Chemical thermodynamic considerations in PHC mixtures

Interactions between the chemical components in mixtures may be a contributing factor to differences in toxicity between PHC mixtures and individual PHCs. The solubility of PHCs can potentially be enhanced in the presence of other chemicals. Hydrocarbons with very high K_{OW} and low aqueous solubility can form micelles (an aggregate of PHC molecules), allowing more chemical to effectively dissolve. Therefore, the solubility of a sole chemical may be lower than its solubility if it is present in a complex mixture. If the solubility of a mixture component increases, then a_i would decrease. Therefore, solubility may be underestimated, and subsequently the activity may be over-estimated for mixture components that may have enhanced solubility in the presence of other chemicals in PHC mixtures.

The equation that calculates activity as concentration divided by solubility (Equation 3.5) assumes that the chemical is in an infinitely dilute solution (i.e., very low mole fraction in solvent) and its physical-chemical properties (particularly water solubility) are not influenced by the presence of other chemicals. Furthermore, the calculations in this research assume that the activity coefficient (γ, unitless) in Equation 3.3 (activity = $\gamma \bullet x$; x = molar fraction of chemical in solvent; Figure 3.1) is the same for all chemicals at all concentrations. However, it is possible that these assumptions do not hold in all environmentally relevant conditions. It is possible that the activity coefficient (γ) varies non-linearly with x, depending on the medium and chemical.

For chemicals in water the assumption of a linear relationship between activity coefficient (γ) and molar concentration in water is likely applicable. Chemical concentration in water is constrained by PHCs' low aqueous solubilities. Subsequently, water-based mixture components are also limited to a low range of mole fractions (x [in $mol_{chemical}/mol_{solvent}$] = molar concentration [in $mol_{chemical}/m^3_{solvent}$] • molar volume of solute [in $m^3_{solvent}/mol_{solvent}$]). The chemical components of the water-based PHC mixture all had low aqueous solubilities (less than 0.84 mol/m^3 for all components in the water-based PHC mixtures) and low values of x (between 10^{-12} and 10^{-9} $mol_{chemical}/mol_{solvent}$),

such that the activity coefficient, γ, can be assumed constant over that low range of values of x.

Conversely, PHCs with low solubility in water typically have high solubility in organic phases like organic carbon or in organisms' lipid membranes. High solubility in organic phases means that chemicals also have potential to reach high molar fractions (x) in organic phases. Chemicals close to their solubility in organic phases may reach mole fractions in organic phases sufficiently high to interact with other mixture components. Chemical components of PHC mixtures in organic phases may also be present over a broad range of molar fractions (x), such that the activity coefficient (γ) may change non-linearly over the environmentally relevant range of x. The γ of a hydrophobic mixture component that is present close to its saturation may not equal the γ of the same hydrophobic component present in very dilute quantities in an organic phase. The question remains for future research: how does the activity coefficient vary in different phases, at different concentrations. What implications does the nature of this activity-to-concentration relationship have for activity when calculating the ∑ activity of a PHC mixture?

To account for the interactions of chemicals when they exist in quantities close to their thermodynamic maximum (i.e., close to solubility), and in the presence of other mixture components, a corrected solubility can be calculated using equations from Gobas and Russel (1991):

$$\text{Log } S = \log S_W + f \bullet \log K_{OW} \qquad \text{Equation 4.1}$$

Where S = solubility of component of interest in water (mol/L), corrected for presence of other mixture components; S_W = solubility of single component in water (mol/L); f = volume fraction of co-solvent (i.e., volume of all components in the mixture, except for the chemical component of interest divided by total volume of all mixture components; L/L); K_{OW} = octanol-water partition coefficient of single component.

Gobas and Russell (1991) report that dissolved organic carbon acting as a single co-solvent at range in f of 10^{-5} to 10^{-7} reduces the bioavailable solute fraction (i.e., bioavailability) of a chemical with log K_{OW} = 6 by about 0.001%, or increases its solubility

in water by a negligible factor of 1.00001. However, in mixtures involving multiple chemicals acting as co-solvents, such as is the case for complex mixtures of petroleum hydrocarbons, the concentration of multiple chemicals may be sufficiently high (much greater than the 10^{-5} volume fraction in the Gobas & Russell example, 1991) and may significantly affect the solubility of individual mixture components. For example in sediment, a mixture component with a log K_{OW} = 6, and f = 0.01 (i.e., 1% of sediment volume is co-solvent), the bioavailable solute fraction is reduced by 13%, and the solubility subsequently increases by a factor of 1.15. Adjusting the solubility of multiple components in a mixture may also have an additive effect, and the resulting ∑ activity of mixtures may be lower than presented in this research. Future research on activity-based PHC mixture models could incorporate a correction to media-specific solubility values that accounts for presence of multiple mixture components. This type of correction would likely have the greatest impact in organic media for mixture components with high log K_{OW} (e.g., log K_{OW} > 5), and may improve the ability of activity (and subsequently C_L and V_C/V_L) to describe and predict mixture toxicity that is more in line with toxicity of single PHC chemicals and the additive hypothesis for PHC mixtures.

4.2.1. Limitations & Uncertainties of Activity-based Approaches to Evaluation of Toxicological Effects Data for PHCs

The chemical properties (e.g., log K_{OW}, aqueous solubility) used for mixture components can be difficult to empirically measure, particularly for heavier, lipophilic chemicals. The analytical detection limits may not be adequate to quantify chemical properties for some chemicals. For example, the aqueous solubility measured for PHCs with more than 10 carbons may be overestimated by some analytical methodologies due to the formation and measurement of colloids (i.e., insoluble, suspended PHC particles), rather than true aqueous solubility (Verbruggen et al., 2000). Environmental pH can affect solute structure, thereby affecting water solubility of the chemical. Additionally, temperature also affects solubility; generally, higher environmental temperatures result in higher solubilities compared to solubility at lower temperatures. All of these different sources of uncertainty and variability around chemical properties will contribute to uncertainty around the activity calculated for PHCs. For example, overestimated solubility will result in under-estimated activity, and vice versa.

Even though there is considerable inherent uncertainty in chemical properties due to precision and accuracy of available analytical techniques (Verbruggen et al., 2000), since there are currently not a lot of experimental data available, particularly for the higher molecular weight PHCs, calculated values are often relied upon. There is also uncertainty associated with the chemical property data that were calculated using various relationships (Table 3.5; e.g., aqueous solubility as a function of the log K_{OW}, or log K_{OW} as a function of the number of carbons). Calculated properties are particularly uncertain when they are extrapolated from beyond the domain of applicability for the established relationships. Often, the relationships used to calculate chemical properties were assumed to be linear, however, given lack of experimental data for higher molecular weight components, it is possible that these relationships could deviate from linearity. Heavier chemicals can form colloids, their molecular shape becomes more folded, and chemical property relationships based on molecular structures may no longer apply. Subsequently log K_{OW} may be overestimated, and water solubility and vapour pressures may be underestimated for higher molecular weight compounds.

Environmentally relevant mixtures of petroleum hydrocarbons include chemical components with a broader range of properties than the set of single chemicals for which toxicity data are available. Single chemical toxicity data were available for chemicals up to molecular weight of around 250 g/mol. Whereas, mixture-based toxicity data included PHC mixtures with components that had molecular weights predicted to be as high as 560 g/mol. Log K_{OW} and aqueous solubility for a range of mixture components were found to be beyond the range of chemical properties represented in the single chemical LC50 dataset (Section 4.2; Appendix A). This discrepancy in chemical properties between the single chemical toxicity dataset and the mixture toxicity dataset is a consideration when making comparisons between toxicity of PHC mixtures and toxicity of single chemicals.

4.3. Comparison of Activity-based Approaches to Current Approaches to PHC Mixture Risk Assessment and Guideline Development

Sediment quality guidelines for PHC mixtures were available for sum of high molecular weight (HMW) PAHs, sum of low molecular weight (LMW) PAHs, and total PAHs. Each of these PHC mixture guidelines includes only aromatic components (i.e., none of the sediment quality guidelines included any aliphatic components). Figure 4.17 illustrates that if these sediment quality guidelines are expressed as activities, they represent activities of PAHs mixtures between 0.0002 and 0.272 (log activity between -3.7 and -0.56). This range overlaps with the 95th percentile range of activity associated with LC50s for single PHCs which was between 0.003 and 0.62 (log activity -2.51 to -0.208; dotted lines in Figure 4.17). This overlap suggests that these sediment quality guidelines are protective of organisms from adverse non-polar narcosis-type effects.

The range in activities associated with these sediment quality guidelines in part comes from the variety of different narrative intents behind the guidelines. For example, the Contaminated Sites Regulation lists two guidelines for total PAHs (CSR, 1996), one for the protection of “sensitive aquatic life”, which is lower than the guideline for the protection of “typical aquatic life”. Also, the LMW and HMW PAH guidelines from MacDonald et al. (1996) included both probable effect levels (PEL, above which harmful effects are likely to be observed), and threshold effect levels (TEL, below which harmful effects are unlikely to be observed). Therefore, the range of activities calculated for each PHC mixture and reference (Figure 4.17) in part arises from these different narrative intents.

The range in activities associated with the sediment quality guidelines also comes in part from differences between the different guidelines in terms of how the PHC mixture compositions were prescribed (Table 3.12). For example, the total PAHs guideline from the BC Contaminated Sites Regulation (CSR 1996) is composed of 13 specific individual PAHs. Whereas the British Columbia Environmental Quality Criteria for total PAHs (Nagpal et al., 2006) includes two different guidelines: one where the mixture composition is prescribe as sum of 16 different and specific PAHs, and a second

guideline, where the mixture composition can include anywhere between 4 and 16 individual PAHs.

Regardless of the PHC mixture (either HMW, LMW, or total PAHs), the guideline's prescribed mixture compositions (e.g., ∑ of 13 or ∑ of 16 PAHs), and the narrative intents of the various sediment quality guidelines, they all overlap or fall below the 5th and 95th percentile of LC50s for single PHCs.

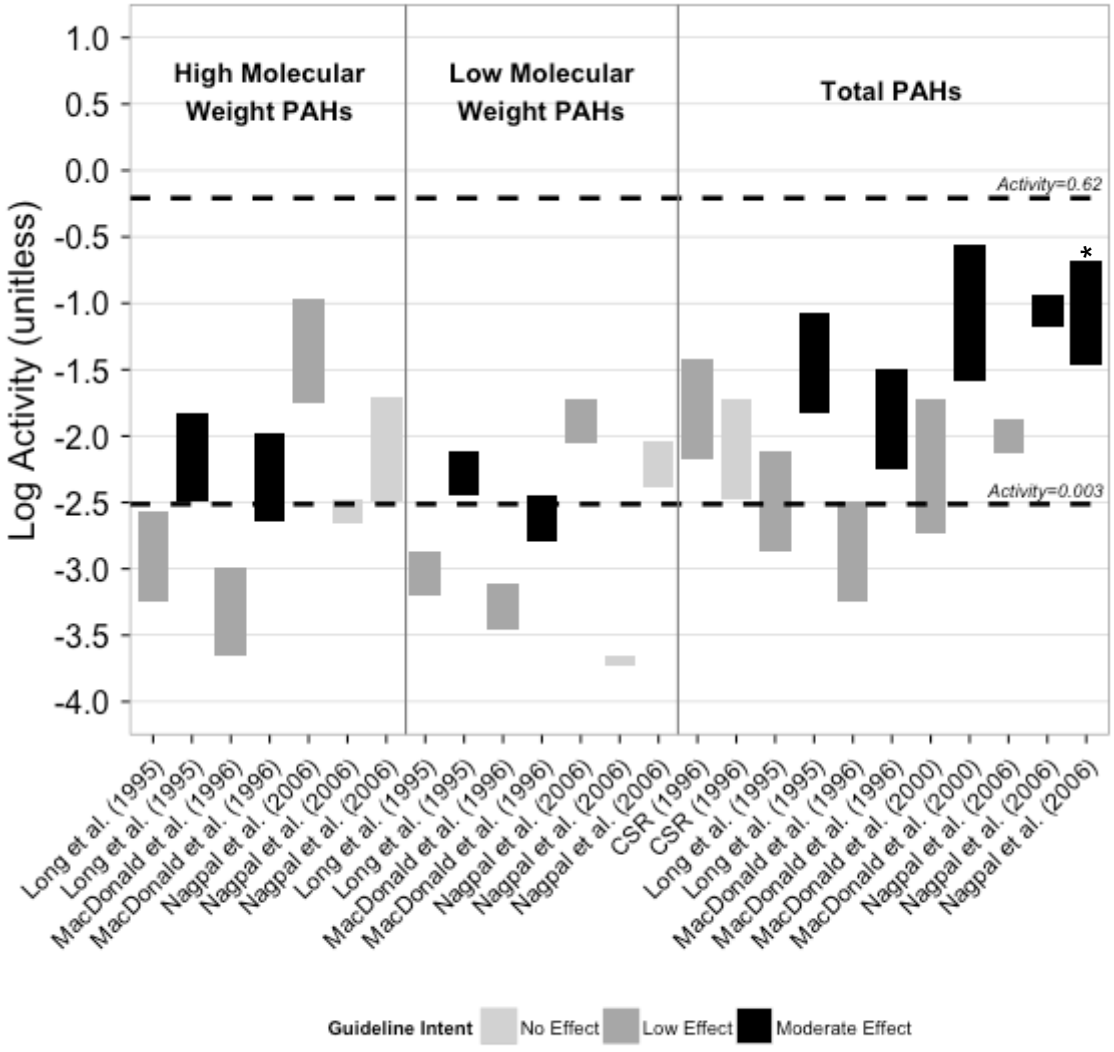


Figure 4.17. Sediment quality guidelines for PHC mixtures from British Columbia environmental quality guidelines (Nagpal et al., 2006), British Columbia Contaminated Sites Regulation (CSR, 1996), Long et al. (1995), and MacDonald et al. (1996, 2000) expressed as activity.

* This guideline represents a "severe effect level to aquatic life".

Soil quality guidelines for PHC mixtures that may be applied to terrestrial components of wildlands ecosystems are also expressed as activities in Figure 4.18. These guidelines are for PHC mixtures that include both aromatic and aliphatic components. The activities representing the various soil quality guidelines for PHC mixtures ranges from 0.158 to 49.8 (or log activity ranges from -0.8 to 1.7). The range of activities represented by the various guidelines include values greater than one. For mixtures, the ∑ai can be greater than one without individual mixture components exceeding their individual solubilities. This range in activities for soil quality guidelines is greater than the 5th and 95th percentiles of activities associated with LC50s for single chemicals (activity: 0.003 and 0.62; log activity -2.51 to -0.208; dotted lines in Figure 4.18). The majority of activities calculated for soil quality guidelines were greater than 0.62, the 95th percentile of activity associated with 50% mortality for single PHCs (log activity = -0.21). These soil quality guidelines are also higher than the mean $\sum a_i$ of PHC mixtures causing LC50s for soil organisms (geometric mean ∑ai for LC50s of PHC mixtures measured in soil = 0.14 [log $\sum a_i$ = -0.84]; minimum to maximum $\sum a_i$ = 0.006 to 0.79 [log = -2.2 to -0.10]; Figure 4.12a). This activity-based comparison between soil quality guidelines and toxicity data for PHCs suggests that there is a risk of false negatives (or Type II errors) associated with current soil quality guidelines. Soil samples evaluated against CCME (2008) and CSR (1996) guidelines and determined to be protective of ecological receptors may still have PHC mixtures present in sufficient quantities to adversely affect soil-based organisms. For example, activities associated with these soil quality guidelines were greater than the activities of crude oil PHC mixtures in soil causing 50% mortality to collembolan and earthworms. These soil guidelines applicable in BC wildlands may be inadequate for protecting all wildlands species (e.g., earthworms) from toxic effects associated with PHC exposure, including biological and ecological effects resulting from not only physical effects but also non-polar narcosis, or more specific modes of toxic action.

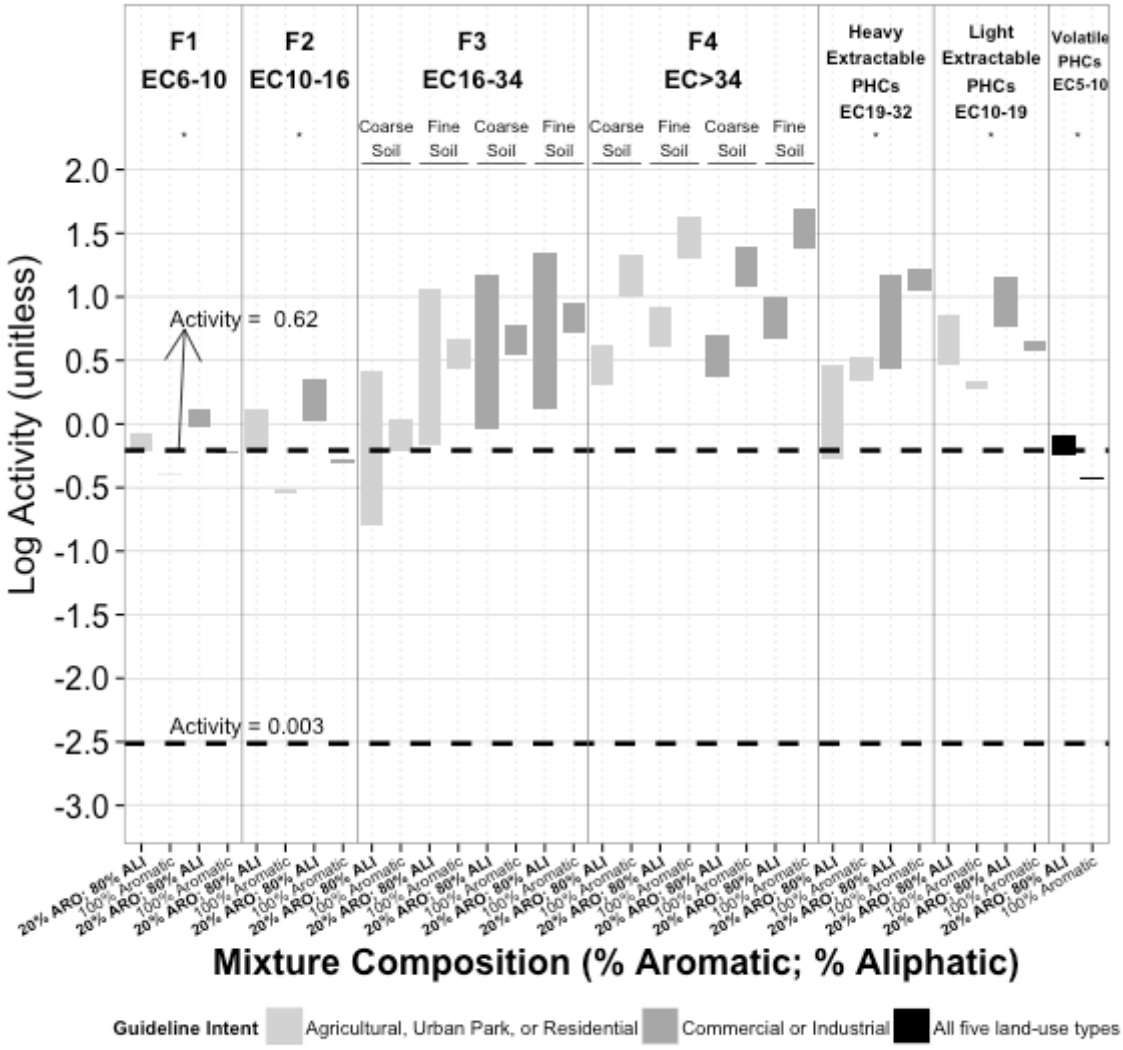


Figure 4.18. Soil quality guidelines for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996; heavy and light extractable PHCs, and volatile PHCs) and Canadian Council of Ministers of the Environment (CCME, 2008; F1, F2, F3, F4) expressed as activity.

* Soil quality guideline for F1, F2 from CCME (2008), and all CSR (1996) guidelines apply to both coarse and fine soil.
ARO = aromatic; ALI = aliphatic.

There is at least agreement in soil quality guidelines for different PHC mixtures expressed as activities between the two different guideline sources, CCME (2008) and the CSR (1996). The activities of the CCME guideline for F1 (PHCs with between 6 and 10 carbons) and the CSR guideline for volatile PHCs (PHCs with between 5 and 10 carbons) are almost identical (Figure 4.18). The CCME guidelines for F3 (between 16-

34 carbons) and for F4 (>34 carbons) also overlap with the CSR guideline for heavy extractable PHCs (between 19 and 32 carbons) (Figure 4.18).

Figure 4.19 illustrates the range of activities associated with the British Columbia Contaminated Sites Regulation (CSR, 1996) water quality guidelines for extractable petroleum hydrocarbons (EPH; mixture includes PHCs with between 10 and 19 carbons), light extractable petroleum hydrocarbons (LEPH; mixture of PHCs with between 10 and 19 carbons, excluding six specific PAHs: acenapthene, acridine, anthracene, fluorine, naphthalene, and phenanthrene); and volatile PHCs (mixture of PHCs with between 5 and 10 carbons, excluding benzene, toluene, ethylbenzene, and xylenes).

When the activity of the water quality guidelines for PHC mixtures is calculated using a mixture composition that is comprised of solely aromatic PHCs, these water quality guidelines fall within the 95th percentile range of activities associated with LC50s for single PHCs (Figure 4.19). Therefore, in this case, these water quality guidelines are likely to not offer sufficient protection of wildlands receptors from adverse effects of PHC mixture exposure. The incidence of false negative (or type II errors) associated with these criteria may not support the narrative intention of wildlands-specific criteria. When activity associated with the water quality guidelines is calculated using a 20% aromatic PHC, 80% aliphatic PHCs mixture composition representing a more environmentally relevant exposure composition, then the activity associated with these water quality guidelines increases (Figure 4.19). This increase is because PHCs are hydrophobic, and increasingly so for both heavier PHC mixtures, and for aliphatic PHCs. Therefore, aliphatic components of heavier PHC mixtures are close to there solubility limits, and their summed activities are close to or greater than one.

When using activity to compare environmental exposure data to either toxic effects data (as in risk assessments), or to environmental quality guidelines, the result is not so dependant on determining solubility in the media, so long as the calculation of the activities for guidelines or effects uses the same solubilities as the calculation of the activities for environmental exposures.

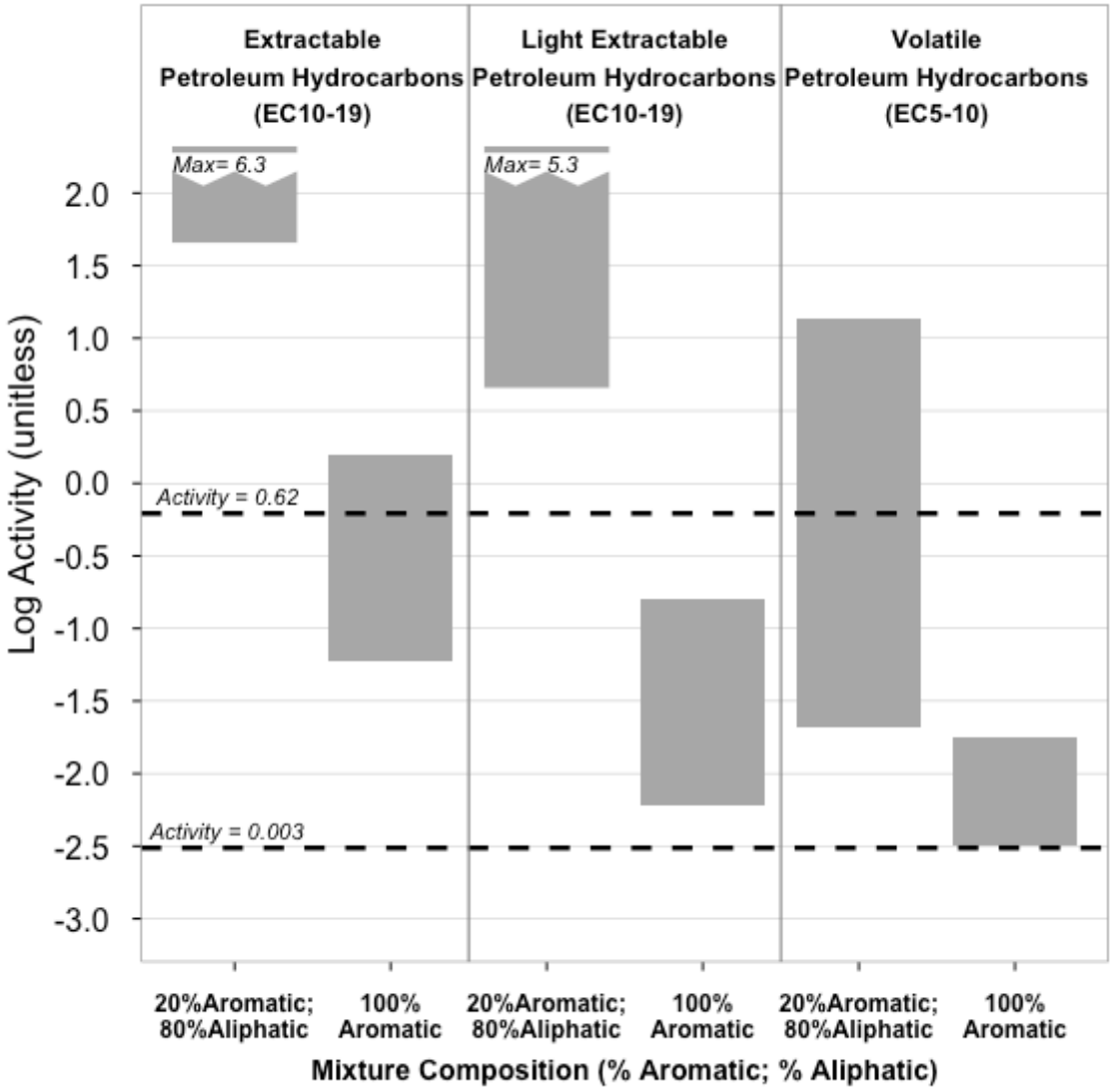


Figure 4.19. Water quality standards for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996) expressed as activity.

Activity can be used to calculate a lipid-normalized concentration associated with each of the guidelines (Figure 4.20 to Figure 4.22), and also the fraction of lipid volume occupied by PHCs (Figure 4.23 to Figure 4.25). By limiting activity to less than 1, the calculation of these two lipid-basis metrics includes only the amount of chemical that is below its saturation limit in the abiotic media (sediment, soil, or water). This method incorporates the maximum possible amount of chemical that can enter the lipid of an organism at equilibrium with abiotic media because chemicals in excess of their solubilities are not thought to reach the lipid of an organism, and therefore not contribute to non-polar narcosis.

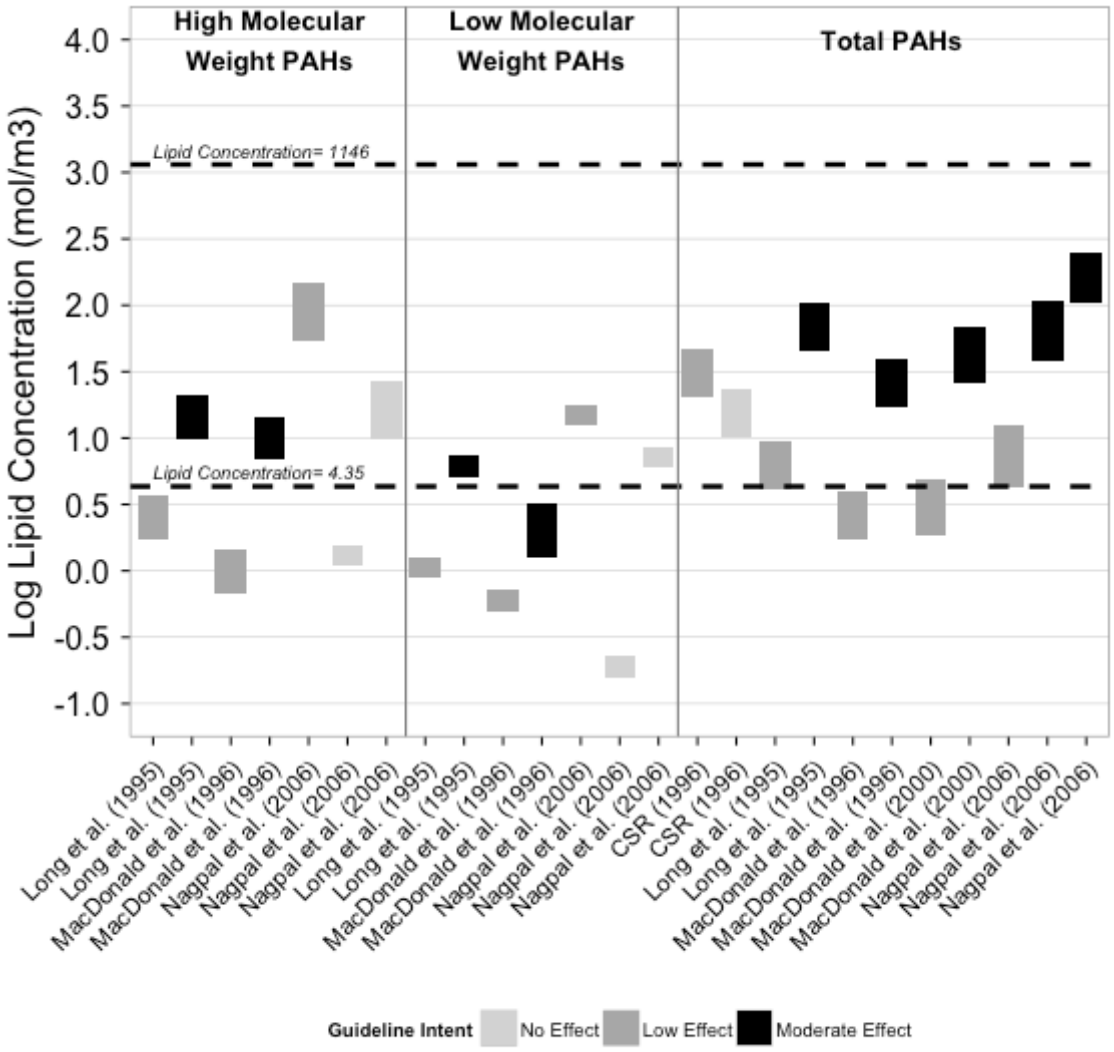


Figure 4.20. Sediment quality guidelines for PHC mixtures from British Columbia environmental quality guidelines (Nagpal et al., 2006), British Columbia Contaminated Sites Regulation (CSR, 1996), Long et al. (1995), and MacDonald et al. (1996, 2000) expressed as total concentration of PHCs in lipid phase.

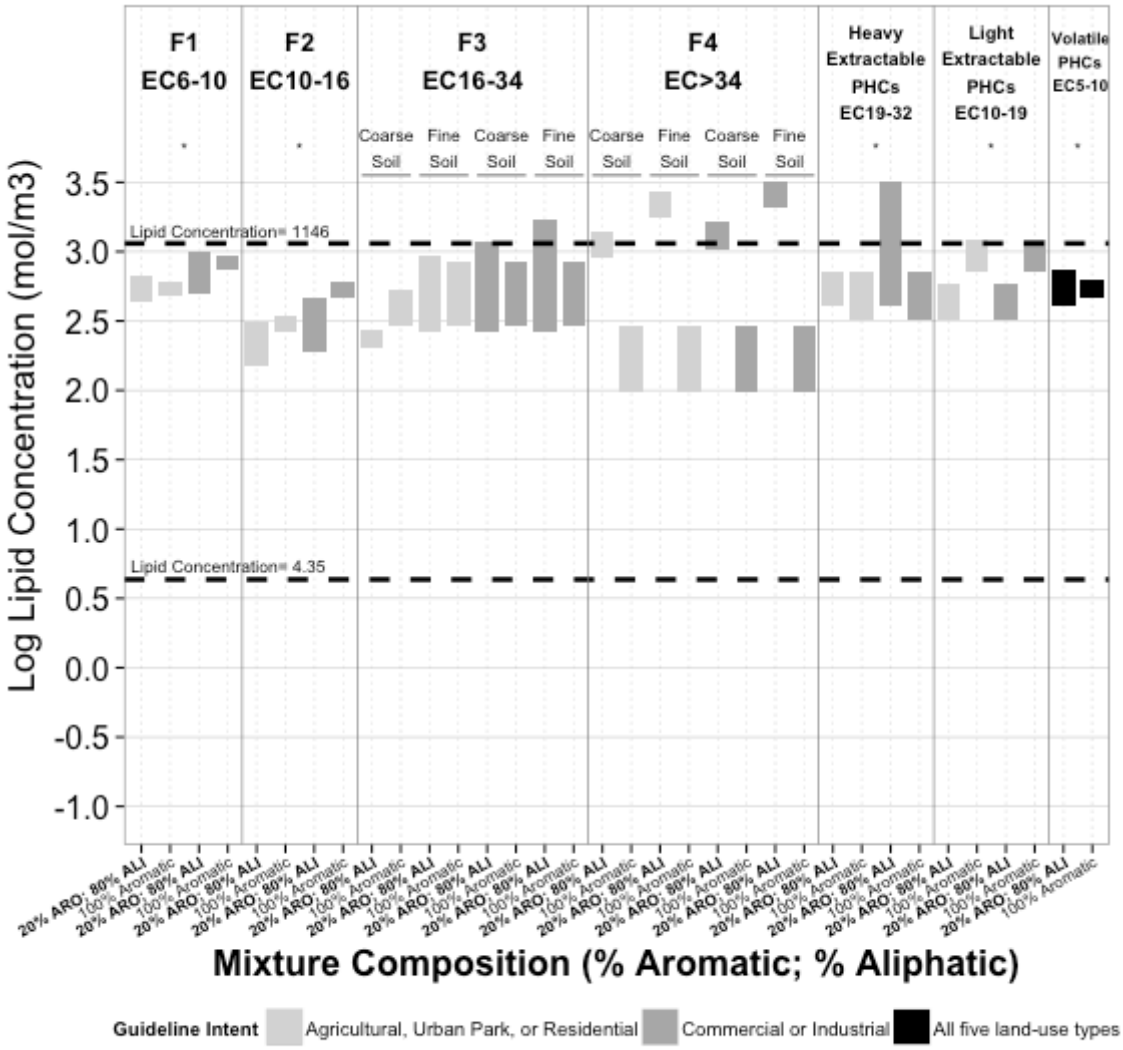


Figure 4.21. Soil quality guidelines for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996; heavy and light extractable PHCs, and volatile PHCs) and Canadian Council of Ministers of the Environment (CCME, 2008; F1, F2, F3, F4) expressed as total concentration of PHCs in lipid phase.

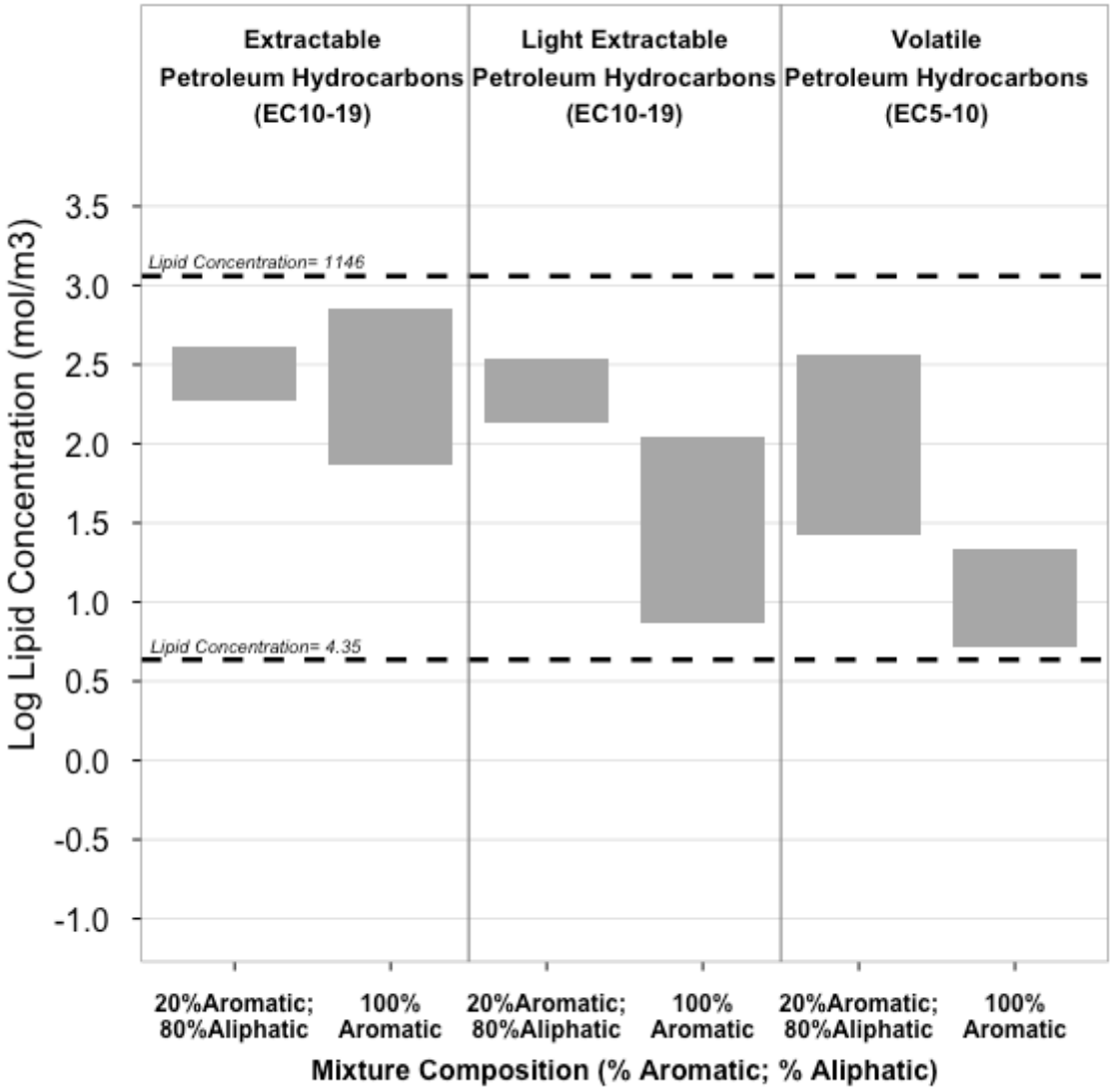


Figure 4.22. Water quality standards for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996) expressed as total concentration of PHCs in lipid phase.

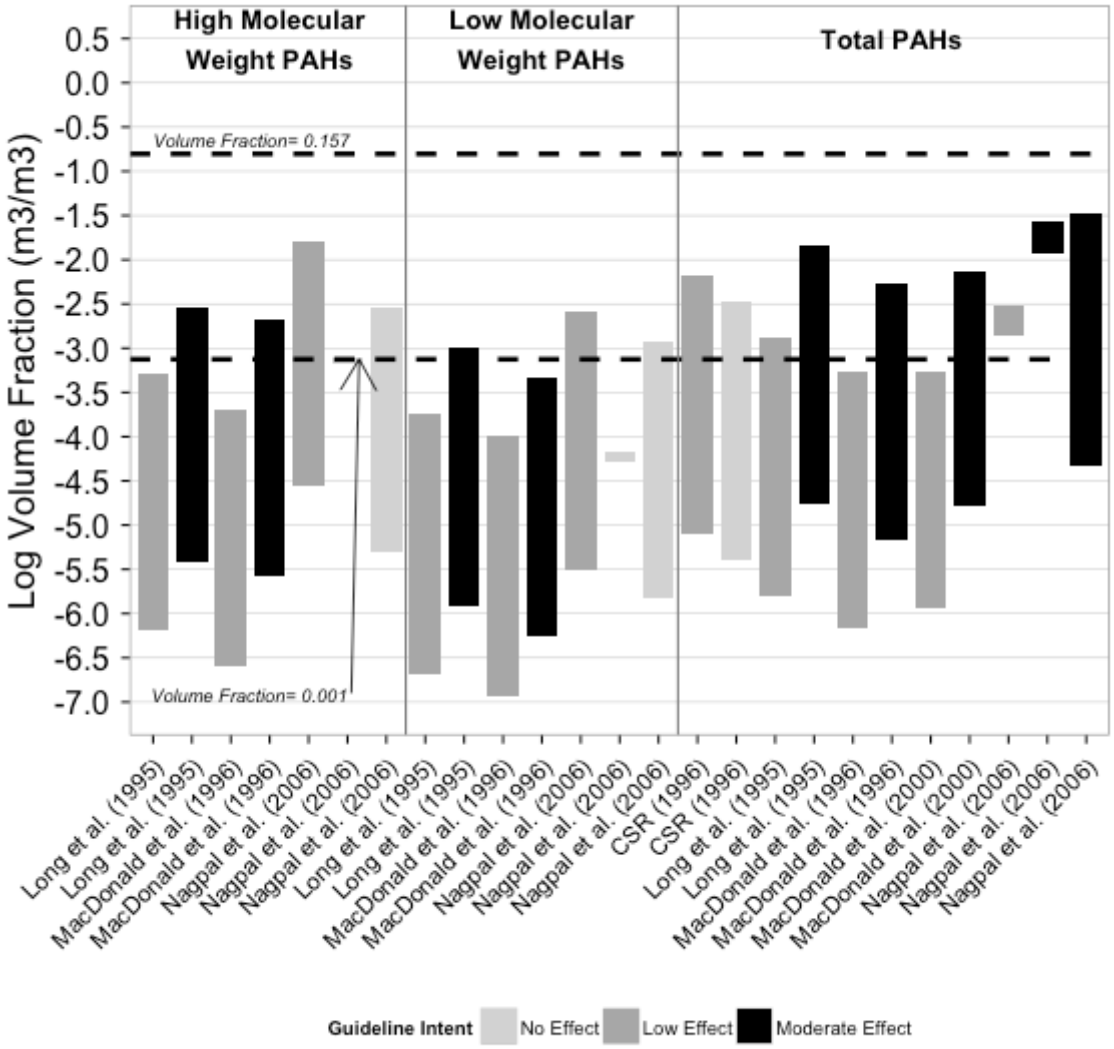


Figure 4.23. Sediment quality guidelines for PHC mixtures from British Columbia environmental quality guidelines (Nagpal et al., 2006), British Columbia Contaminated Sites Regulation (CSR, 1996), Long et al. (1995), and MacDonald et al. (1996, 2000) expressed as total volume fraction of PHCs in lipid.

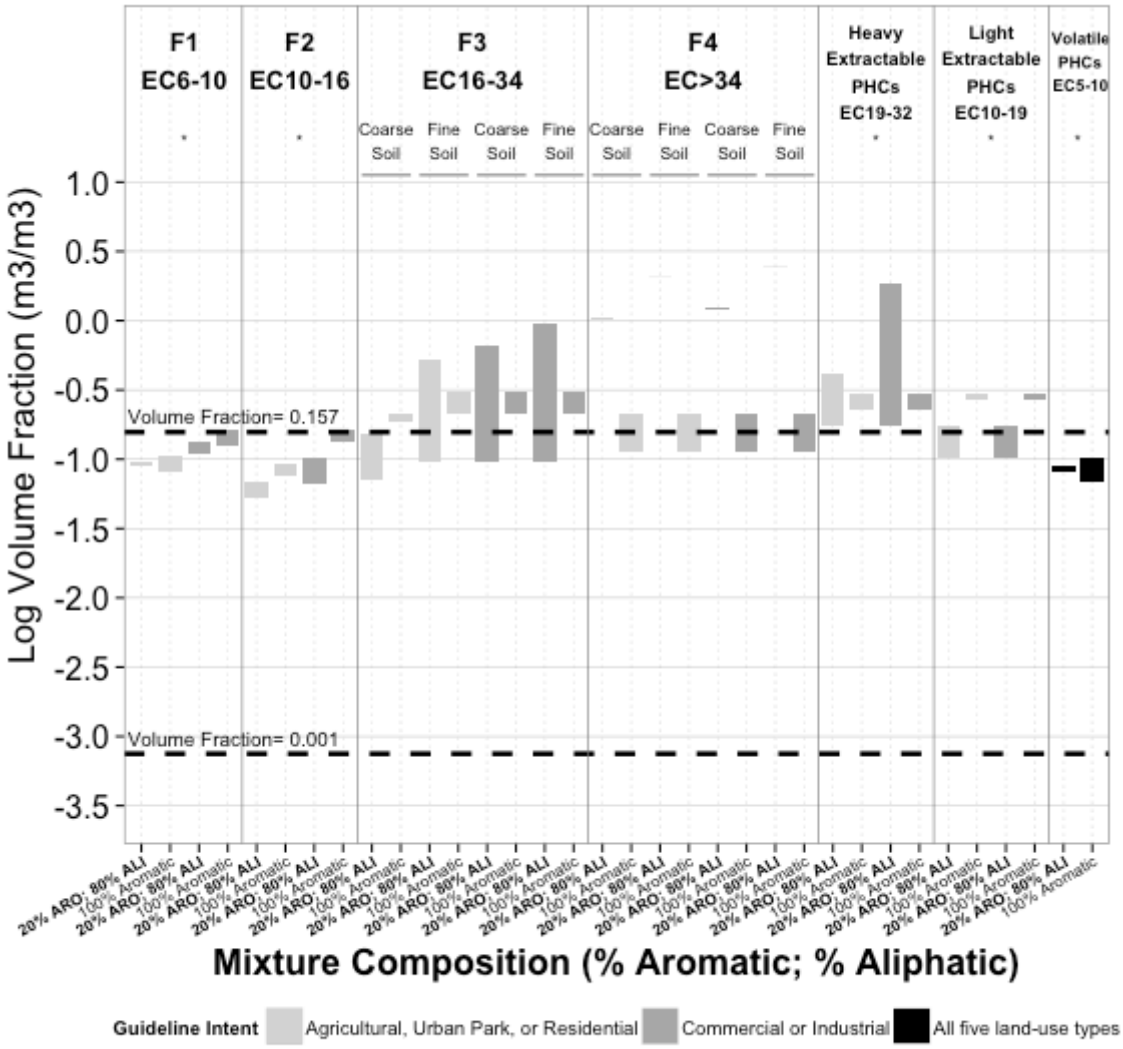


Figure 4.24. Soil quality guidelines for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996; heavy and light extractable PHCs, and volatile PHCs) and Canadian Council of Ministers of the Environment (CCME, 2008; F1, F2, F3, F4) expressed as total volume fraction of PHCs in lipid.

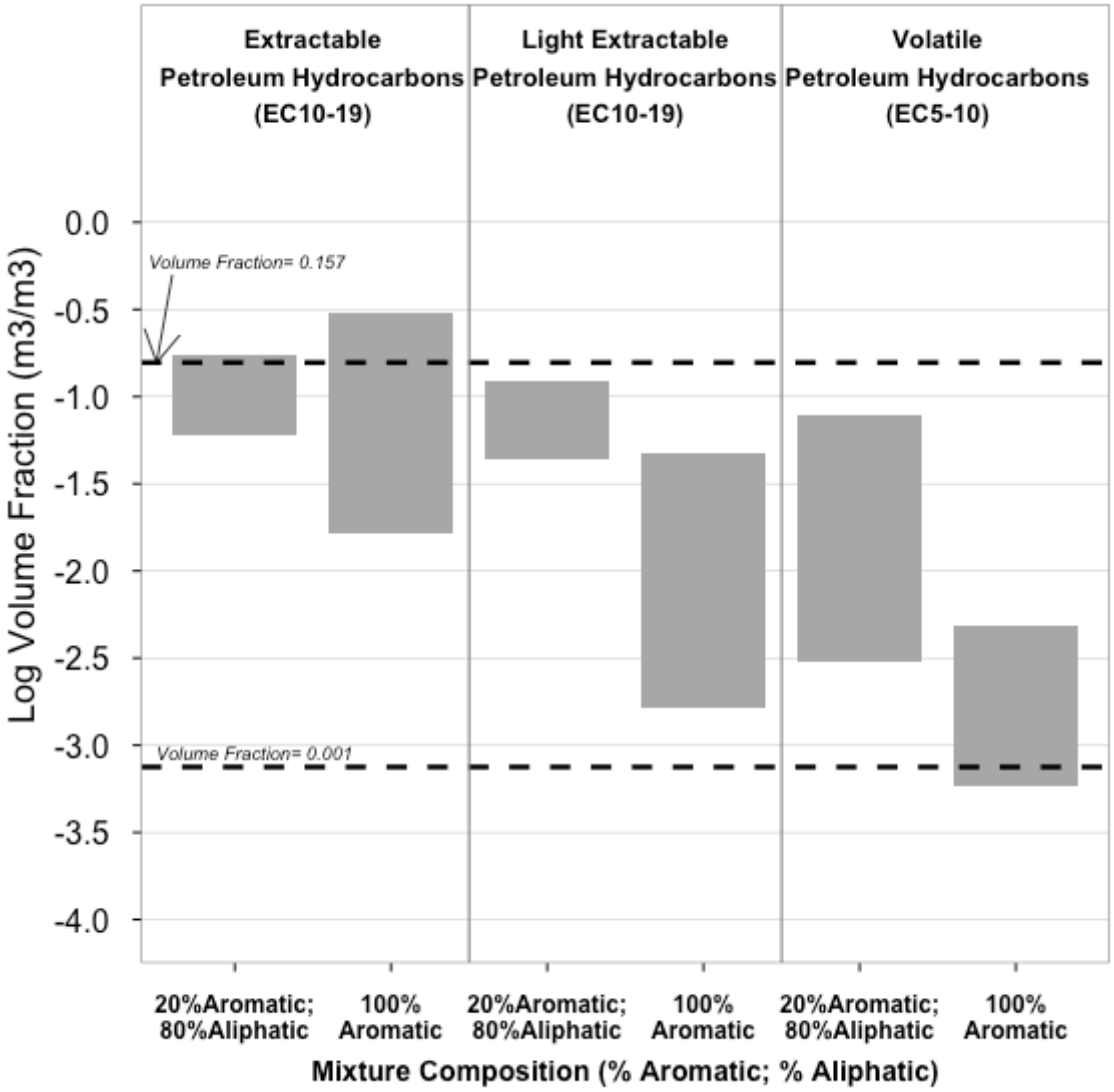


Figure 4.25. Water quality standards for PHC mixtures from British Columbia Contaminated Sites Regulation (CSR, 1996) expressed as total volume fraction of PHCs in lipid.

The lipid-normalized concentration at equilibrium with soil or water-based PHC mixtures at concentrations equal to current guidelines may not be meeting intended protection goals of these guidelines applied to wildlands. The lipid-normalized concentrations at equilibrium with soil-based PHC mixture guidelines range from 96.6 to 3250 mol/m3 (Figure 4.21) which is greater than the range of lipid-normalized concentration calculated for LC50s of PHC mixtures in soil (22 to 334 mol/m3; Figure 4.12c). The lipid-normalized concentrations at equilibrium with water-based PHC mixture guidelines range from 5.2 to 710 mol/m^3 (Figure 4.22), and are greater than the

range of lipid-normalized concentrations calculated for LC50s of PHC mixtures in water (4.6 to 74 mol/m^3; Figure 4.12c). Similarly, the ranges in volume fractions of PHC mixtures in lipid at equilibrium with soil and water guidelines (Figure 4.24, Figure 4.25) exceed or overlap the range of volume fractions associated with toxicity of PHCs and PHC mixtures.

Sediment was the only media where the lipid-normalized concentrations (Figure 4.20) or volume fractions (Figure 4.23) calculated for the guidelines included a range that fell below the 5^{th} percentile of LC50 toxicity data for single PHCs. The sediment quality guidelines may have a higher incidence of false positive (or Type I) errors. However, from an environmental protection perspective, the sediment quality guidelines are more likely than the guidelines for soil and water to fulfill the British Columbia Ministry of Environment responsibility to protect wildlands ecosystems from adverse effects associated with PHC mixture exposure.

In order to minimize risk of Type II (or false negative) errors associated with environmental quality guidelines applied to wildlands, I recommend activity-based guidelines for any and all media, built from the lower limit of the distribution of all PHC toxicity data. Guidelines for PHCs (either individuals or mixtures) in water, soil, and sediment that are set using an activity = 0.003 will protect multiple species from adverse effects associated with exposure to multiple petroleum hydrocarbons.

4.4. Proposed Application of Activity-based Approach to Risk Assessment and Criteria Development

Activity can be applied to help address some of the data limitations currently facing petroleum hydrocarbon risk assessments and guideline development. Current data limitations include an absence of toxic effects data for many aquatic and terrestrial wildlands-relevant species, including endangered species. There is also an absence of toxicity data for all individual PHC components present in environmentally-relevant mixtures and for all unique PHC mixtures that wildlands organisms may be exposed to (e.g., weathered versus fresh oil products). In the absence of comprehensive chemical-specific, media-specific, species-specific, and PHC mixture-specific toxicity data, an

activity-based approach may be applied to fill in these data-gaps since activity associated with effects from PHC exposure is hypothesized to be similar across chemicals, media, species, and effects.

Both risk assessments and criteria development involve identifying a single value or range of values that represent toxic effects to organisms resulting from chemical exposure. For risk assessments, exposure concentrations from any environmental medium, including sediment, water, soil, and tissue can be expressed as activity and compared to a single distribution of toxic effects data for PHCs also expressed as activity. Similarly, a single distribution of toxic effects data for PHCs expressed as activity can be converted back to media-specific concentrations to develop guidelines in any medium. Figure 4.26 illustrates the conceptual model of how activity can integrate a broad range of toxic effects data for PHCs and be applied for risk assessments and guideline development of PHCs.

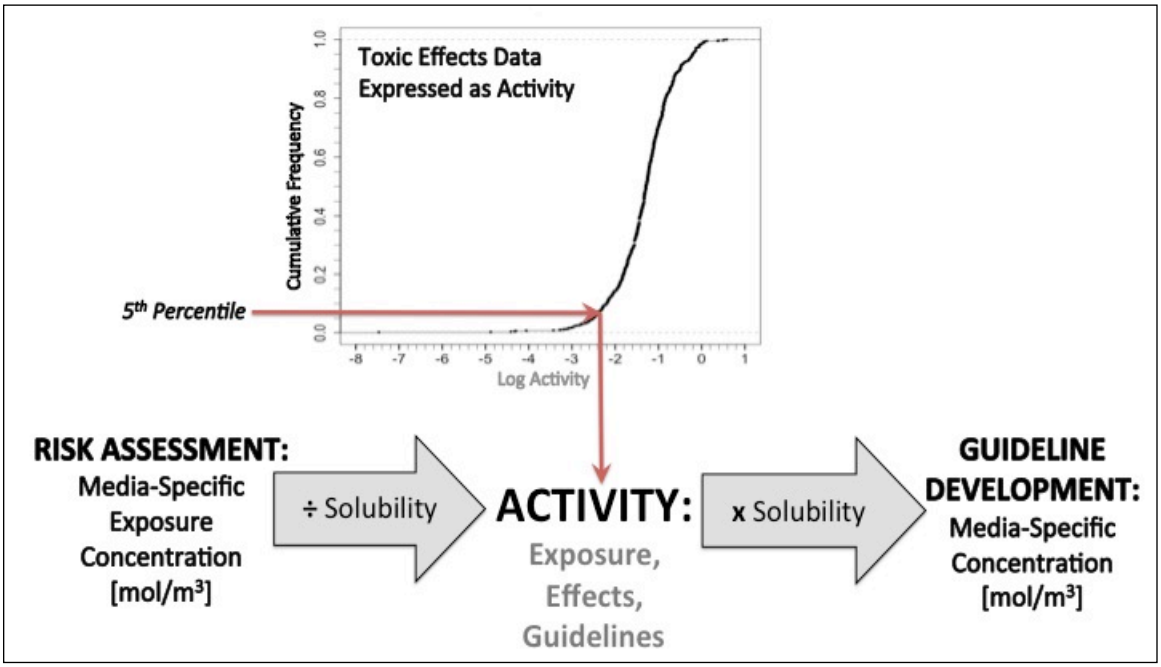


Figure 4.26. Conceptual model of activity applied to risk assessment and guideline development.

Toxicity expressed as activity (and consequently equilibrium-based lipid-normalized concentration and lipid-volume fraction calculated using activity) are similar across PHC chemicals, media, species, and effects (Section 4.1). Furthermore, the

activity associated with effects from single PHC chemicals (Section 4.1) can be used to evaluate toxic effects from PHC mixtures (Section 4.2). Therefore, one distribution of all available PHC toxicity data can be applied to any medium, species, chemical, or chemical mixture of any composition. The lower 5th percentile of toxicity data (activity = 0.003) was used in this research as an illustrative, but relevant endpoint for risk assessment and guideline development. Applying a value at the lower end of the distribution (such as the 5th percentile) help set an acceptable frequency of false negatives. A false negative in risk assessments or criteria development would determine exposure to PHCs is safe but in actuality, exposure to PHCs is hazardous to wildlands ecosystems.

Risk assessments ultimately involve comparing environmental exposure concentrations to concentrations associated with adverse effects to the plants and animals living in those ecosystems. Using the chemical composition of a PHC mixture measured in any environmental abiotic or biotic exposure medium (e.g., water, sediment, soil, or tissue), the activity of individual PHCs (a_i), and $\sum a_i$ of all PHC mixture components can be calculated using methodology presented in Section 3.2. This calculation then allows environmental exposures in any medium to be expressed as activity and directly compared to a breadth of effects data representing multiple chemicals, species, media, and effects which are also expressed in unitless activity (e.g., activity = 0.003). Similarly, any environmental exposure concentration of PHC mixtures measured in any medium can be expressed as a lipid-normalized concentration [C_L ; units of mol/m^3_{lipid}] or as a volume fraction [V_C/V_L ; units of $m^3_{chemical}/m^3_{lipid}$]). The environmental exposure can then be directly compared to C_L = 4.35 mol/m^3 or V_C/V_L = 0.008 $m^3_{chemical}/m^3_{lipid}$, the 5th percentiles of LC50s across a variety of PHCs and species in a variety of media.

Activity-based methodologies can also be applied to environmental quality guideline development to help address some of the toxicity data limitations. Derivation of environmental quality guidelines ultimately involves extrapolating toxicity data measured under laboratory conditions to potential effects in an actual ecosystem. Since activity (and consequently lipid-normalized concentration and lipid-volume fraction calculated from activity) is similar across PHC chemicals, media, species, and effects

(Section 4.1), a single activity value at the lower range of this distribution of toxicity data, for example the 5th percentile, can be applied as an activity-based guideline that will protect multiple species from multiple effects in any medium, from any chemical, and from PHC mixtures of any chemical composition. In order to facilitate meaningful application of this activity-based guideline, it can be converted back to medium-specific concentrations for individual chemicals in any medium by re-arranging the equation for activity (activity = concentration ÷ solubility ; Equation 3.5) to solve for concentration:

$$C_{GUIDELINE} = a_{GUIDELINE} \bullet S_{PHC} \qquad \text{…Equation 4.2}$$

Where $C_{GUIDELINE}$ is the concentration of singe PHC in any media (in mol/m^3); $a_{GUIDELINE}$ is the activity value selected for guideline development that represents toxic effects data (unitless; e.g., a=0.003); and S_{PHC} = media-specific solubility for individual PHC for which guideline is being developed (in mol/m^3; See Table 3.4 for media-specific solubility equations).

Media-specific guidelines for PHC mixtures can also be built from a single activity value and expressed in terms of concentrations:

$$C_{GUIDELINE\text{-}MIXTURE} = \sum C_i = \left(\frac{a_{GUIDELINE}}{n} \right) \bullet \sum S_{PHC\text{-}i} \qquad \text{…Equation 4.3}$$

Where $C_{GUIDLINE\text{-}MIXTURE}$ is the media-specific concentration of all PHC components in the mixture (in mol/m^3); C_i is the media specific concentration of an individual component in the PHC mixture (in mol/m^3); $a_{GUIDELINE}$ is the activity value selected for guideline development that represents toxic effects data (unitless; e.g., a=0.003); n is the number of individual components in the PHC mixture for which a guideline is being developed; and $S_{PHC\text{-}i}$ is the media-specific solubility of an individual component in the PHC mixture. Equation 4.3 assumes that the composition of the mixture is equimolar between all components. A range of media-specific concentration-based guidelines can be calculated from a single activity-based guideline, by incorporating different mixture compositions. Site-specific, media-specific guidelines can also be calculated from a single activity-based guideline by incorporating site-specific PHC mixture compositions:

$$C_{GUIDELINE\text{-}MIXTURE} = \sum C_i = a_{GUIDELINE} \bullet \sum(\phi_i \bullet S_{PHC\text{-}i}) \qquad \text{…Equation 4.4}$$

Where ϕ_i = the fraction of the component "i" in the total PHC mixture (unitless).

Media-specific solubilities in Equations 4.2, 4.3, and 4.4 for concentration-based guidelines can be calculated using either generalized media properties (e.g., organic carbon content = 1%) to develop generic guidelines for individual media with set characteristics. Or, solubility can also be calculated using more specific properties (e.g., organic carbon content measured in sediment at a particular site) to develop more site-specific PHC guidelines. Depending on the specific desired narrative intent of an environmental quality guideline, different points along the toxicity distribution curve expressed as activity (Figure 4.2a; e.g., 10^{th} percentile, instead of the 5^{th} percentile) can be interpolated and expressed as a concentration in any medium.

Activity provides a useful tool for risk assessment and guideline development because the same activity value (e.g., activity = 0.003) can be applied in any medium to protect multiple species from multiple effects resulting from exposure to any individual PHC, or PHC mixtures of any composition. This single activity value can be applied to directly compare effects and exposure by either translating it into a concentration in any medium, or by directly comparing it to an exposure in any medium that is also expressed as activity. Lipid-concentration, and lipid-volume fraction concentration calculated from activity (Table 3.2) can also be applied to risk assessments and guideline development in the same way as activity, since lipid concentration and volume fraction are similar across chemicals, media, species, and effects. The lower 5^{th} percentile of lipid-phase concentration (4.35 mol/m^3), and of volume-fraction (0.0008 m^3/m^3) associated with LC50s for single PHC chemicals (Table 4.1; Figure 4.2) are useful tools because they are perhaps easier to conceptualize than activity which is a slightly more abstract concept. These two metrics are also in more familiar concentration units (mol/m^3_{lipid}, or $m^3_{chemical}/m^3_{lipid}$), and align with established concepts currently used in risk assessment and guideline development such as the critical body burden and tissue residue approach (Meador et al., 2006). However, these two metrics are still subject to the same limitations and uncertainties around chemical property data as activity-based methodology, and also introduce additional uncertainty in the lipid solubility term and

molar volume term (in cm^3/mol) that are required for calculations (Table 3.4). Therefore, activity is highlighted in this research as a more useful method than C_L and V_C/V_L for the purposes of enhancing the usability of the variety of exposure and effects data that are currently available, and addressing some of the data gaps presently facing risk assessments and guideline development of petroleum hydrocarbon mixtures.

5. Recommendations & Conclusions

Activity of PHC mixtures associated with 50% mortality typically falls within the same range of activities of single PHC chemicals associated with 50% mortality, regardless of the media, species, chemical, or mixture composition. Concentration and volume fraction in the lipid-phase can also be calculated from activity and also are similar between single chemicals and sum of mixtures' chemical components. Therefore, activity, lipid-concentration and volume fraction appear to be additive for complex, environmentally relevant mixtures of PHCs. Fugacity was similar for chemicals with the same physical-chemical properties (i.e., log K_{OW}), but varied widely across different chemicals. Therefore fugacity does not provide a very helpful tool for describing toxicity of PHC mixtures. Toxicity datapoints falling below the 5^{th} percentile of activity are likely a result of toxic metabolites and more specific modes of toxic action. Toxicity datapoints above the 95^{th} percentile, and greater than or equal to one likely represent toxicity through physical impacts, rather than non-polar narcosis. Some improvements to the additive activity model that could be explored in future research include corrections to account for non-equilibrium based on varying kinetics of uptake rates between biotic and abiotic media (e.g., BCF, BSAF, time to equilibrium); and corrections to account for enhanced solubility of PHC mixture components in the presence of multiple other mixture components.

Activity-based methodology provides a tool for risk assessment and guideline development that allows available data from different media, chemicals, species, and effects to be directly compared. Toxic units, hazard quotients and other similar ratio-type metrics used in risk assessments are essentially normalizing an exposure to an effect, thereby integrating an exposure assessment and effect assessment all into one number. These types of calculations require exposure data and effects data to be expressed in the same units and in the same medium. For example, sediment exposure data in milligrams per gram organic carbon must be compared to effects data (either

toxicity data, or a developed sediment quality guideline) also expressed in the same medium-specific units of milligrams per gram organic carbon. In contrast, the effects data for PHCs explored in this research (primarily LC50s) show similar activity across media, across species, across different individual chemicals, and across PHC mixtures of varying compositions. Therefore risk assessments using an activity-based approach can apply one activity value (e.g., activity = 0.003, the lower 5^{th} percentile of activities associated with LC50s) representing the effects of individual PHCs or PHC mixtures to evaluate risk associated with exposure data from any medium. By applying an activity-based approach, risk assessments can directly incorporate a broader range of toxic effects data than would be available if risk assessment was limited to data from only a single medium at a time, for specific mixture compositions and specific species. Similarly, an activity-based approach can enhance the depth of information available to inform development of environmental quality guidelines and criteria that are protective of multiple species in multiple media from multiple effects from multiple chemicals.

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Appendix A.

Chemical Properties

Table A1. Physical-Chemical properties of Petroleum Hydrocarbons

	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
Chemical	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3• mol^{-1}	na	na	°C	na	cm^3• mol^{-1}		
ALIPHATIC PETROLEUM HYDROCARBONS											
<C10 aliphatic	137.2	3.09E-03	3.60E+02	1.16E+05	5.59		na	na	218.3	1	
>C10-C11 aliphatic	151.2	2.04E-04	1.14E+02	5.57E+05	6.12		na	na	240.5	1	
>C11-C12 aliphatic	165.3	5.37E-05	3.60E+01	6.69E+05	6.65		na	na	262.7	1	
>C12-C13 aliphatic	179.4	1.38E-05	1.68E+01	1.22E+06	7.18		na	na	284.9	1	
>C13-C14 aliphatic	193.5	3.55E-06	7.34E+00	2.07E+06	7.71		na	na	307.1	1	
>C14-C15 aliphatic	207.5	9.12E-07	3.20E+00	3.51E+06	8.24		na	na	329.3	1	
>C15-C16 aliphatic	221.6	2.40E-07	1.40E+00	5.83E+06	8.77		na	na	351.5	1	
>C16-C17 aliphatic	235.7	6.17E-08	6.11E-01	9.90E+06	9.30		na	na	373.7	1	
>C17-C18 aliphatic	249.7	1.58E-08	2.67E-01	1.68E+07	9.83		na	na	395.9	1	
>C18-C19 aliphatic	263.8	1.58E-08	1.16E-01	7.34E+06	10.36		na	na	418.1	1	
>C19-C20 aliphatic	277.9	1.58E-08	5.08E-02	3.20E+06	10.89		na	na	440.3	1	
>C20-C21 aliphatic	291.9	1.58E-08	2.22E-02	1.40E+06	11.42		na	na	462.5	1	
>C21-C22 aliphatic	306.0	1.58E-08	9.68E-03	6.11E+05	11.95		na	na	484.7	1	
>C22-C23 aliphatic	320.1	1.58E-08	4.22E-03	2.67E+05	12.48		na	na	506.9	1	
>C23-C24 aliphatic	334.2	1.58E-08	1.84E-03	1.16E+05	13.01		na	na	529.1	1	
>C24-C25 aliphatic	348.2	1.58E-08	8.05E-04	5.08E+04	13.54		na	na	551.3	1	
>C25-C26 aliphatic	362.3	1.58E-08	3.51E-04	2.22E+04	14.07		na	na	573.5	1	
>C26-C27 aliphatic	376.4	1.58E-08	1.53E-04	9.68E+03	14.60		na	na	595.7	1	
>C27-C28 aliphatic	390.4	1.58E-08	6.69E-05	4.22E+03	15.13		na	na	617.9	1	
>C28-C29 aliphatic	404.5	1.58E-08	2.92E-05	1.84E+03	15.66		na	na	640.1	1	
>C29-C30 aliphatic	418.6	1.58E-08	1.28E-05	8.05E+02	16.19		na	na	662.3	1	
>C30-C31 aliphatic	432.6	1.58E-08	5.57E-06	3.51E+02	16.72		na	na	684.5	1	
>C31-C32 aliphatic	446.7	1.58E-08	2.43E-06	1.53E+02	17.25		na	na	706.7	1	
>C32-C33 aliphatic	460.8	1.58E-08	1.06E-06	6.69E+01	17.78		na	na	728.9	1	
>C33-C34 aliphatic	474.9	1.58E-08	4.63E-07	2.92E+01	18.31		na	na	751.1	1	
>C34-C35 aliphatic	488.9	1.58E-08	2.02E-07	1.28E+01	18.84		na	na	773.3	1	
>C35-C36 aliphatic	503.0	1.58E-08	8.83E-08	5.57E+00	19.37		na	na	795.5	1	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
>C36-C37 aliphatic	517.1	1.58E-08	3.85E-08	2.43E+00	19.90		na	na	817.7	1	
>C37-C38 aliphatic	531.1	1.58E-08	1.68E-08	1.06E+00	20.43		na	na	839.9	1	
>C38-C39 aliphatic	545.2	1.58E-08	7.34E-09	4.63E-01	20.96		na	na	862.1	1	
>C39-C40 aliphatic	559.3	1.58E-08	3.20E-09	2.02E-01	21.49		na	na	884.3	1	
1-hexene	84.2	5.94E-01	2.48E+04	4.17E+04	3.39	2.41	-139.8	1	133.2	2	
1,1-dimethylcyclohexane	100.8	1.08E-01	3.03E+03	2.81E+04	4.06		-33.3	1	162.6	3	5
1,1,2,2-tetrachloroethane	167.9	1.69E+01	7.93E+02	4.70E+01	2.31		-42.4	1	135.4	2	
1,2-cis-dimethylcyclohexane	112.2	5.35E-02	1.93E+03	3.61E+04	4.39		-49.8	1	162.6	2	
1,2-dimethylcyclohexane	112.2	5.35E-02	1.93E+03	3.61E+04	4.39		-49.8	1	162.6	2	6
1,3-dimethylcyclohexane	100.8	1.16E-01	2.35E+03	2.03E+04	4.06		-90.1	1	162.6	3	5
1,4-dimethylcyclohexane	112.2	3.42E-02	3.02E+03	8.83E+04	4.39		-36.9	1	162.6	2	8
1,4-trans-dimethylcyclohexane	112.2	3.42E-02	3.02E+03	8.83E+04	4.39		-36.9	1	162.6	2	
C10 aliphatic	144.2	2.04E-04	2.02E+02	9.90E+05	5.85		na	na	229.4	1	
C11 aliphatic	158.3	5.37E-05	6.39E+01	1.19E+06	6.38		na	na	251.6	1	
C12 aliphatic	172.4	1.38E-05	2.02E+01	1.46E+06	6.91		na	na	273.8	1	
C13 aliphatic	186.4	3.55E-06	1.11E+01	3.13E+06	7.44		na	na	296.0	1	
C14 aliphatic	200.5	9.12E-07	4.85E+00	5.32E+06	7.97		na	na	318.2	1	
C15 aliphatic	214.6	2.40E-07	2.12E+00	8.83E+06	8.50		na	na	340.4	1	
C16 aliphatic	228.6	6.17E-08	9.24E-01	1.50E+07	9.03		na	na	362.6	1	
C17 aliphatic	242.7	1.58E-08	4.03E-01	2.55E+07	9.56		na	na	384.8	1	
C18 aliphatic	256.8	1.58E-08	1.76E-01	1.11E+07	10.09		na	na	407.0	1	
C19 aliphatic	270.8	1.58E-08	7.69E-02	4.85E+06	10.62		na	na	429.2	1	
C20 aliphatic	284.9	1.58E-08	3.36E-02	2.12E+06	11.15		na	na	451.4	1	
C21 aliphatic	299.0	1.58E-08	1.46E-02	9.24E+05	11.68		na	na	473.6	1	
C22 aliphatic	313.1	1.58E-08	6.39E-03	4.03E+05	12.21		na	na	495.8	1	
C23 aliphatic	327.1	1.58E-08	2.79E-03	1.76E+05	12.74		na	na	518.0	1	
C24 aliphatic	341.2	1.58E-08	1.22E-03	7.69E+04	13.27		na	na	540.2	1	
C25 aliphatic	355.3	1.58E-08	5.32E-04	3.36E+04	13.80		na	na	562.4	1	
C26 aliphatic	369.3	1.58E-08	2.32E-04	1.46E+04	14.33		na	na	584.6	1	

Chemical	MW g•mol^{-1}	S_W mol•m^{-3}	P_L Pa	H Pa•m^3•mol^{-1}	Log K_{OW} na	Log K_{OA} na	T_M °C	F na	V_M cm^3•mol^{-1}	Reference	Comment
C27 aliphatic	383.4	1.58E-08	1.01E-04	6.39E+03	14.86		na	na	606.8	1	
C28 aliphatic	397.5	1.58E-08	4.42E-05	2.79E+03	15.39		na	na	629.0	1	
C29 aliphatic	411.5	1.58E-08	1.93E-05	1.22E+03	15.92		na	na	651.2	1	
C30 aliphatic	425.6	1.58E-08	8.43E-06	5.32E+02	16.45		na	na	673.4	1	
C31 aliphatic	439.7	1.58E-08	3.68E-06	2.32E+02	16.98		na	na	695.6	1	
C32 aliphatic	453.8	1.58E-08	1.61E-06	1.01E+02	17.51		na	na	717.8	1	
C33 aliphatic	467.8	1.58E-08	7.01E-07	4.42E+01	18.04		na	na	740.0	1	
C34 aliphatic	481.9	1.58E-08	3.06E-07	1.93E+01	18.57		na	na	762.2	1	
C35 aliphatic	496.0	1.58E-08	1.34E-07	8.43E+00	19.10		na	na	784.4	1	
C36 aliphatic	510.0	1.58E-08	5.83E-08	3.68E+00	19.63		na	na	806.6	1	
C37 aliphatic	524.1	1.58E-08	2.55E-08	1.61E+00	20.16		na	na	828.8	1	
C38 aliphatic	538.2	1.58E-08	1.11E-08	7.01E-01	20.69		na	na	851.0	1	
C9 aliphatic	130.1	3.09E-03	6.39E+02	2.07E+05	5.32		na	na	207.2	1	
commercial hexane	86.2	1.10E-01	2.02E+04	1.83E+05	3.90	2.44	-95.4	1	140.6	2	9
cyclohexane	84.2	6.89E-01	1.30E+04	1.89E+04	3.44	2.71	6.6	1	118.2	2	
cyclopentane	70.1	2.37E+00	4.24E+04	1.79E+04	3.00		-93.4	1	99.5	2	
cyclopentene	70.1	7.85E+00	5.07E+04	6.46E+03	3.00	2.05	-135.0	1	92.1	2, 3 (Log K_{OA})	
docosane	310.6	1.03E-09	2.65E-04	2.58E+05	11.15		72.2	0.341	495.8	3	
dodecane	170.3	2.20E-05	1.80E+01	8.19E+05	6.80		-9.6	1	273.8	2	
dotriacontane	450.9	6.64E-15	7.02E-05	1.06E+10	16.06		69.7	0.361	717.8	3	
eicosane	282.5	8.46E-10	1.61E-03	1.90E+06	8.92		36.6	0.769	451.4	2	
ethylcyclohexane	112.2	3.53E-02	1.71E+03	4.83E+04	4.56		-111.3	1	162.6	2	
heneicosane	296.6	7.04E-09	1.16E-02	1.65E+06	10.65		40.5	0.702	473.6	3	
hentriacontane	436.9	2.30E-14	4.96E-09	2.16E+05	15.57		67.9	0.376	695.6	3	
heptacosane	380.8	3.39E-12	8.22E-05	2.42E+07	13.60		59.5	0.456	606.8	3	
heptadecane	240.5	5.82E-06	6.15E-02	1.06E+04	7.68		22.0	1	384.8	2	
hexacosane	366.7	1.80E-13	6.60E+01	3.68E+14	11.40		56.1	0.495	584.6	2	
hexadecane	226.4	2.19E-07	1.91E-01	8.74E+05	7.26		18.1	1	362.6	2	
hexane	86.2	1.10E-01	2.02E+04	1.83E+05	4.11	2.44	-95.4	1	140.6	2	9
methylcyclohexane	98.2	1.54E-01	6.18E+03	4.02E+04	3.88		-126.6	1	140.4	2	
n-hexane	86.2	1.10E-01	2.02E+04	1.83E+05	4.11	2.44	-95.4	1	140.6	2	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
n-nonane	128.3	1.70E-03	5.71E+02	3.36E+05	5.65	3.51	-53.5	1	207.2	2 3 (Log K_{OA})	
nonacosane	408.8	2.79E-13	1.38E-07	4.94E+05	14.58		63.7	0.414	651.2	3	
nonadecane	268.5	9.41E-08	7.87E-03	8.36E+04	9.67		32.1	0.851	429.2	3	
octacosane	394.8	9.11E-13	5.24E-07	5.75E+05	14.09		64.5	0.407	629.0	3	
octadecane	254.5	1.48E-08	1.90E-02	1.28E+06	8.13		28.2	0.930	407.0	2	
octane	114.2	5.80E-03	1.80E+03	3.10E+05	5.15		-56.8	1	185.0	2	
pentacosane	352.7	4.25E-11	3.90E-04	9.19E+06	12.62		54.0	0.516	562.4	3	
pentadecane	212.4	2.88E-04	5.76E-01	2.00E+03	6.78		10.0	1	340.4	2	
pentane	72.2	5.34E-01	6.84E+04	1.28E+05	3.45		-129.7	1	118.4	2	
phytane	282.6	5.94E-08	4.45E-01	7.50E+06	9.87		22.2	1	451.4	3	
pristane	268.5	1.97E-07	5.84E-01	2.96E+06	9.38		12.1	1	429.2	3	
tetracosane	338.7	9.64E-13	2.37E-05	2.46E+07	10.50		50.4	0.563	540.2	2	
tetradecane	198.4	1.66E-06	1.80E+00	1.08E+06	8.00		5.8	1	318.2	2	
tetratriacontane	478.9	9.94E-16	2.91E-05	2.93E+10	17.04		47.6	0.598	762.2	3	
triacontane	422.8	8.01E-14	9.22E-09	1.15E+05	15.07		65.8	0.395	673.4	3	
tricosane	324.6	5.42E-10	3.88E-03	7.16E+06	11.64		47.6	0.598	518.0	3	
tridecane	184.4	1.07E-03	6.68E+00	6.22E+03	6.05		-5.4	1	296.0	2	
tritriacontane	464.9	1.90E-15	1.56E-08	8.23E+06	16.55		72.0	0.343	740.0	3	
undecane	156.3	2.60E-05	5.22E+01	2.01E+06	6.51		-25.5	1	251.6	2	
AROMATIC PETROLEUM HYDROCARBONS											
[1,1'-biphenyl]-4-ol	154.2	1.39E+00	8.70E+00	6.25E+00	3.69		166.0	0.041	192.0	2	10
<C10 aromatic	121.3	6.92E-01	3.60E+02	5.20E+02	3.19		na	na	160.8	1	
>C10-C11 aromatic	127.6	6.92E-01	1.14E+02	1.64E+02	3.34		na	na	179.3	1	
>C11-C12 aromatic	134.0	6.92E-01	3.60E+01	5.20E+01	3.49		na	na	197.8	1	
>C12-C13 aromatic	140.4	1.78E-01	1.68E+01	9.46E+01	3.64		na	na	201.3	1	
>C13-C14 aromatic	146.7	1.78E-01	7.34E+00	4.13E+01	3.79		na	na	219.8	1	
>C14-C15 aromatic	153.1	1.78E-01	3.20E+00	1.80E+01	3.94		na	na	238.3	1	
>C15-C16 aromatic	159.4	4.57E-02	1.40E+00	3.06E+01	4.09		na	na	256.8	1	
>C16-C17 aromatic	165.8	4.57E-02	6.11E-01	1.34E+01	4.24		na	na	275.3	1	
>C17-C18 aromatic	172.2	4.57E-02	2.67E-01	5.83E+00	4.39		na	na	293.8	1	
>C18-C19 aromatic	178.5	1.20E-02	1.16E-01	9.68E+00	4.54		na	na	297.3	1	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
>C19-C20 aromatic	184.9	1.20E-02	5.08E-02	4.22E+00	4.69		na	na	315.8	1	
>C20-C21 aromatic	191.2	1.20E-02	2.22E-02	1.84E+00	4.84		na	na	334.3	1	
>C21-C22 aromatic	197.6	1.20E-02	9.68E-03	8.05E-01	4.99		na	na	352.8	1	
>C22-C23 aromatic	204.0	3.09E-03	4.22E-03	1.37E+00	5.14		na	na	371.3	1	
>C23-C24 aromatic	210.3	3.09E-03	1.84E-03	5.97E-01	5.29		na	na	389.8	1	
>C24-C25 aromatic	216.7	3.09E-03	8.05E-04	2.60E-01	5.44		na	na	393.3	1	
>C25-C26 aromatic	223.0	7.94E-04	3.51E-04	4.42E-01	5.59		na	na	411.8	1	
>C26-C27 aromatic	229.4	7.94E-04	1.53E-04	1.93E-01	5.74		na	na	430.3	1	
>C27-C28 aromatic	235.8	7.94E-04	6.69E-05	8.43E-02	5.89		na	na	448.8	1	
>C28-C29 aromatic	242.1	2.04E-04	2.92E-05	1.43E-01	6.04		na	na	467.3	1	
>C29-C30 aromatic	248.5	2.04E-04	1.28E-05	6.25E-02	6.19		na	na	485.8	1	
>C30-C31 aromatic	254.8	2.04E-04	5.57E-06	2.73E-02	6.34		na	na	489.3	1	
>C31-C32 aromatic	261.2	2.04E-04	2.43E-06	1.19E-02	6.49		na	na	507.8	1	
>C32-C33 aromatic	267.6	5.37E-05	1.06E-06	1.98E-02	6.64		na	na	526.3	1	
>C33-C34 aromatic	273.9	5.37E-05	4.63E-07	8.62E-03	6.79		na	na	544.8	1	
>C34-C35 aromatic	280.3	5.37E-05	2.02E-07	3.76E-03	6.94		na	na	563.3	1	
>C35-C36 aromatic	286.6	1.38E-05	8.83E-08	6.39E-03	7.09		na	na	581.8	1	
>C36-C37 aromatic	293.0	1.38E-05	3.85E-08	2.79E-03	7.24		na	na	585.3	1	
>C37-C38 aromatic	299.4	1.38E-05	1.68E-08	1.22E-03	7.39		na	na	603.8	1	
>C38-C39 aromatic	305.7	3.55E-06	7.34E-09	2.07E-03	7.54		na	na	622.3	1	
>C39-C40 aromatic	312.1	3.55E-06	3.20E-09	9.03E-04	7.69		na	na	640.8	1	
1-ethylnaphthalene	156.2	6.85E-02	6.96E+00	1.02E+02	4.40		-13.9	1	192.0	3	
1-methyl-7-(1-methylethyl) phenanthrene	234.3	2.05E-04	1.93E-01	9.45E+02	6.35		101.0	0.177	288.0	3	
1-methylchrysene	242.3	1.07E-02	6.05E-04	5.64E-02	6.07		256.5	0.005	273.0	3	
1-methyl dibenzothiophene	198.3	7.08E-03	5.12E-02	7.23E+00	4.84		99.7	0.182	213.5	3	
1-methylfluorene	180.2	2.46E-02	1.36E-01	5.53E+00	4.18		87.0	0.246	210.1	2	
1-methylnaphthalene	142.2	1.97E-01	8.84E+00	4.49E+01	3.87		-30.4	1	169.8	2	
1-methylphenanthrene	192.3	1.29E-02	1.86E-02	1.44E+00	5.14		123.0	0.109	221.4	2	
1,2-dibromobenzene	235.9	3.17E-01	2.57E+01	8.10E+01	3.56		7.1	1	142.6	2	
1,2-dichlorobenzene	147.0	9.52E-01	1.70E+02	1.79E+02	3.31		-17.0	1	137.9	2	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
1,2-diethylbenzene	134.2	5.30E-01	1.40E+02	2.64E+02	3.72		-31.2	1		2	
1,2-dimethylchrysene	256.3	2.79E-04	3.15E-04	1.13E+00	6.62		153.9	0.053	295.2	3	
1,2-dimethyl naphthalene	156.2	9.51E-02	8.70E-01	9.15E+00	4.31		0.8	1	192.0	2, 3 (S_W)	
1,2-dimethyl phenanthrene	206.3	2.34E-03	1.63E-02	6.95E+00	5.44		108.9	0.148	247.1	3	
1,2,3-trichlorobenzene	181.4	2.10E-01	5.07E+01	2.42E+02	3.98		51.3	0.552	158.7	2	
1,2,3-trimethyl phenanthrene	220.3	7.34E-04	8.58E-03	1.17E+01	5.99		115.6	0.127	265.8	3	
1,2,3,4-tetrachlorobenzene	215.9	6.00E-02	6.64E+00	1.11E+02	4.64		47.5	0.602	179.6	2	
1,2,3,4-tetramethyl naphthalene	184.3	2.59E-03	2.88E-01	1.11E+02	5.36		72.0	0.343	236.4	3	
1,2,3,4-tetramethyl phenanthrene	234.3	2.63E-04	4.53E-03	1.73E+01	6.53		128.0	0.096	288.0	3	
1,2,4-trichlorobenzene	181.4	2.20E-01	4.00E+01	1.81E+02	4.00		16.9	1	158.7	2	
1,2,4-trimethylbenzene	120.2	4.74E-01	2.70E+02	5.70E+02	3.70		-43.8	1	162.6	2	
1,2,4,5-tetramethylbenzene	134.2	2.60E-02	6.60E+01	2.54E+03	4.10		79.3	0.293	184.8	2	
1,3-dimethylnaphthalene	156.2	5.12E-02	1.48E+00	2.88E+01	4.42		-6.0	1	192.0	2	
1,3-dimethylphenanthrene	206.3	2.34E-03	1.63E-02	6.95E+00	5.44		108.9	0.148	247.1	3	
1,3,5-trimethylbenzene	120.2	4.16E-01	3.25E+02	7.81E+02	3.58		-44.7	1	162.6	2	
1,4-dibromobenzene	235.9	3.48E-01	3.82E+01	1.10E+02	3.55		87.4	0.244	142.6	2	
1,4-dichlorobenzene	147.0	1.03E+00	2.45E+02	2.39E+02	3.24		53.1	0.530	137.8	2	
1,4-difluorobenzene	114.1	1.07E+01	9.58E+03	8.94E+02	2.11		-23.6	1	106.0	2	
1,4-dimethylnaphthalene	156.2	7.30E-02	2.27E+00	3.11E+01	4.37		7.6	1	192.0	2	
1,4,5-trimethylnaphthalene	170.3	2.91E-02	1.61E+00	5.53E+01	5.00		63.0	0.424	214.2	2	
1,5-dimethylnaphthalene	156.2	7.19E-02	1.93E+00	2.68E+01	4.38		82.0	0.276	192.0	2	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
1,6-dimethylchrysene	256.3	2.79E-04	3.15E-04	1.13E+00	6.62		153.9	0.053	295.2	3	
1,6,7-trimethylnaphthalene	170.3	1.40E-02	5.75E-01	4.12E+01	4.81		55.6	0.498	214.2	3	
2-ethylfluorene	194.3	2.65E-02	2.81E-01	1.06E+01	5.14		68.5	0.371	232.3	3	
2-ethylnaphthalene	156.2	5.13E-02	4.21E+00	8.21E+01	4.38		-7.4	1	192.0	3	
2-methylanthracene	192.3	9.94E-03	2.07E-02	2.08E+00	5.15		209.0	0.016	218.9	2	
2-methylchyrsene	242.3	9.13E-04	6.05E-04	6.63E-01	6.07		148.4	0.060	273.0	3	
2-methyl dibenzothiophene	198.3	7.08E-03	5.12E-02	7.23E+00	4.84		99.7	0.182	213.5	3	
2-methylnaphthalene	142.2	2.18E-01	1.12E+01	5.13E+01	3.86		34.6	0.805	169.8	2	
2-methylphenanthrene	192.3	6.95E-03	1.76E-02	2.53E+00	5.15		93.6	0.209	221.4	3	
2,3-dimethylnaphthalene	156.2	9.76E-02	6.10E+00	6.25E+01	4.40		105.0	0.164	192.0	2	
2,3,5-trimethylnaphthalene	170.3	1.40E-02	6.03E-01	4.32E+01	4.81		55.6	0.498	214.2	3	11
2,3,9-trimethylfluorene	208.3	1.01E-04	5.14E-02	5.09E+02	5.24		94.4	0.206	254.5	3	
2,4,6-trimethylfluorene	208.3	1.24E-03	4.23E-02	3.40E+01	5.66		101.4	0.175	254.5	3	
2,6-dimethylnaphthalene	156.2	7.77E-02	1.00E+01	1.29E+02	4.31		112.0	0.140	192.0	2	
2,7,9-trimethylfluorene	208.3	2.38E-03	5.15E-02	2.16E+01	5.24		94.4	0.206	254.5	3	
3-methylchrysene	242.3	9.13E-04	6.05E-04	6.63E-01	6.07		148.4	0.060	273.0	3	
3-methyl dibenzothiophene	198.3	7.08E-03	5.12E-02	7.23E+00	4.84		99.7	0.182	213.5	3	
3-methylphenanthrene	192.3	6.95E-03	1.76E-02	2.53E+00	5.15		93.6	0.209	221.4	3	
3,6-dimethyl phenanthrene	206.3	5.32E-03	1.63E-02	3.05E+00	5.44		145.0	0.065	247.1	3	
4-methylchrysene	242.3	1.07E-02	6.05E-04	5.64E-02	6.07		256.5	0.005	273.0	3	
4-methyl dibenzothiophene	198.3	7.08E-03	5.12E-02	7.23E+00	4.84		99.7	0.182	213.5	3	
4-methylphenanthrene	192.3	1.31E-02	1.87E-02	1.43E+00	5.08		123.0	0.107	221.4	3	
5,6-dimethylchrysene	256.3	1.05E-03	3.15E-04	3.00E-01	6.62		129.3	0.093	295.2	3	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
6-ethylchrysene	256.3	3.32E-04	3.15E-04	9.48E-01	6.56		156.7	0.050	295.2	3	
7,8,12-trimethyl benz(a)anthracene	284.4	9.19E-05	1.63E-04	1.77E+00	7.16		164.9	0.041	298.7	3	
9-isopropylfluorene	208.3	2.03E-04	7.83E-02	3.85E+02	5.06		79.7	0.288	254.5	3	
9-isopropyl phenanthrene	220.3	7.79E-04	1.26E-02	1.62E+01	5.80		102.2	0.172	265.8	3	
9-methylanthracene	192.3	4.87E-03	8.03E-03	1.65E+00	5.07		81.5	0.279	218.9	2	
9-methylfluorene	180.3	4.14E-03	2.21E-01	5.34E+01	4.15		46.5	0.613	210.1	3	
9,10-diethylphenanthrene	234.3	2.71E-04	4.53E-03	1.67E+01	6.42		119.7	0.116	288.0	3	
acenaphthene	154.2	1.06E-01	1.41E+00	1.33E+01	3.92		93.4	0.213	173.1	2	
acenaphthylene	152.2	4.50E-02	4.14E+00	9.20E+01	4.00		91.8	0.221	165.7	2	
anthracene	178.2	1.88E-02	7.46E-02	3.96E+00	4.54		215.8	0.013	196.7	2	
benz(a)anthracene	228.3	1.03E-03	5.98E-04	5.81E-01	5.91		160.5	0.047	248.3	2	
benzene	78.1	2.28E+01	1.27E+04	5.57E+02	2.13	2.80	5.5	1	96.0	2	
benzo(a)pyrene	252.3	5.12E-04	2.38E-05	4.65E-02	5.73		181.1	0.029	262.9	2	
benzo(b)fluoranthene	252.3	1.50E-04	1.30E-06	8.67E-03	5.80		168.0	0.040	268.9	2	
benzo(e)pyrene	252.3	5.43E-04	2.53E-05	4.66E-02	6.44		181.4	0.029	262.9	2	
benzo(ghi)perylene	276.3	2.52E-04	2.25E-05	8.93E-02	6.50		272.5	0.004	277.5	2	
benzo(k)fluoranthene	252.3	2.42E-04	3.97E-09	1.64E-05	6.00		217.0	0.013	268.9	2	
biphenyl	154.2	1.22E-01	3.50E+00	2.86E+01	4.01		68.9	0.371	184.6	2	
C0 alkyl dibenzothiophene	184.3	3.14E-02	1.40E+00	4.45E+01	4.38		98.2	0.191	191.3	2	12
C0 alkylfluorene	166.2	8.56E-02	6.82E-01	7.97E+00	4.18		114.8	0.132	187.9	2	13
C0 alkylnaphthalene	128.2	8.43E-01	3.62E+01	4.30E+01	3.37	5.10	80.3	0.287	147.6	2	14
C0 alkylphenanthrene	178.2	3.30E-02	1.07E-01	3.24E+00	4.36		99.2	0.187	199.2	2	15
C0 chrysene	228.3	1.60E-03	1.07E-04	6.69E-02	5.73		255.5	0.005	250.8	2	16
C1 alkyl dibenzothiophene	198.3	7.08E-03	5.12E-02	7.23E+00	4.84		99.7	0.182	213.5	4	17
C1 alkylfluorene	180.2	1.44E-02	1.79E-01	1.24E+01	4.17		66.8	0.429	210.1	4	18
C1 alkylnaphthalene	142.2	2.08E-01	1.00E+01	4.83E+01	3.87		2.1	0.903	169.8	4	19
C1 alkylphenanthrene	192.3	9.96E-03	1.81E-02	1.82E+00	5.13		108.3	0.159	221.4	4	20
C1 chrysene	242.3	7.45E-03	6.05E-04	8.12E-02	6.07		220.5	0.023	273.0	4	21

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
C1-fluorenes	180.2	1.44E-02	1.79E-01	1.24E+01	4.17		66.8	0.429	210.1	4	18
C1-naphthalenes	142.2	2.08E-01	1.00E+01	4.83E+01	3.87		2.1	0.903	169.8	4	19
C2 alkyl dibenzothiophene	212.3	2.52E-03	2.59E-02	1.03E+01	5.36		109.4	0.146	235.7	4	22
C2 alkylfluorene	194.3	2.65E-02	2.81E-01	1.06E+01	5.14		68.5	0.371	232.3	4	23
C2 alkylnaphthalene	156.2	7.77E-02	3.77E+00	4.85E+01	4.37		50.2	0.597	192.0	2	24
C2 alkyl phenanthrene	206.3	3.34E-03	1.63E-02	4.88E+00	5.44		121.0	0.120	247.1	2	25
C2 chrysene	256.3	4.85E-04	3.15E-04	6.49E-01	6.61		148.4	0.062	295.2	2	26
C2-fluorenes	194.3	2.65E-02	2.81E-01	1.06E+01	5.14		68.5	0.371	232.3	4	23
C2-naphthalenes	156.2	7.77E-02	3.77E+00	4.85E+01	4.37		50.2	0.597	192.0	4	24
C3 alkyl dibenzothiophene	226.3	8.56E-04	1.36E-02	1.59E+01	5.93		122.0	0.110	257.9	4	27
C3 alkylfluorene	208.3	9.83E-04	5.59E-02	5.68E+01	5.30		92.5	0.219	254.5	4	28
C3 alkylnaphthalene	170.3	2.15E-02	1.09E+00	5.08E+01	4.91		59.3	0.461	214.2	4	29
C3 alkyl phenanthrene	220.3	7.57E-04	1.06E-02	1.40E+01	5.90		108.9	0.150	265.8	4	30
C3 chrysene	284.4	9.19E-05	1.63E-04	1.77E+00	7.16		164.9	0.041	298.7	4	31
C3-fluorenes	208.3	9.83E-04	5.59E-02	5.68E+01	5.30		92.5	0.219	254.5	4	28
C3-naphthalenes	170.3	2.15E-02	1.09E+00	5.08E+01	4.91		59.3	0.461	214.2	4	29
C4 alkylnaphthalene	184.3	2.59E-03	2.88E-01	1.11E+02	5.36		72.0	0.343	236.4	4	
C4 alkylphenanthrene	234.3	2.46E-04	6.74E-02	2.74E+02	6.43		116.2	0.129	288.0	4	32
C4-naphthalenes	184.3	2.59E-03	2.88E-01	1.11E+02	5.36		72.0	0.343	236.4	4	33
chrysene	228.3	1.60E-03	1.07E-04	6.69E-02	5.73		255.5	0.005	250.8	2	
dibenz (a,h)anthracene	278.4	5.40E-04	9.27E-08	1.72E-04	6.75		269.5	0.004	299.9	2	
dibenzothiophene	184.3	3.14E-02	1.40E+00	4.45E+01	4.38		98.2	0.191	191.3	2	
dimethyl dibenzothiophene	212.3	2.47E-03	2.57E-02	1.04E+01	5.39		111.0	0.141	235.7	3	
ethylbenzene	106.2	1.43E+00	1.27E+03	8.87E+02	3.13		-95.0	1	140.4	2	
ethyl dibenzothiophene	212.3	2.57E-03	2.61E-02	1.02E+01	5.33		107.9	0.151	235.7	3	
fluoranthene	202.3	8.81E-03	8.42E-03	9.56E-01	5.20		110.2	0.146	217.3	2	
fluorene	166.2	8.56E-02	6.82E-01	7.97E+00	4.18		114.8	0.132	187.9	2	

Chemical	MW	S_W	P_L	H	Log K_{OW}	Log K_{OA}	T_M	F	V_M	Reference	Comment
	g•mol^{-1}	mol•m^{-3}	Pa	Pa•m^3•mol^{-1}	na	na	°C	na	cm^3•mol^{-1}		
hexachlorobenzene	284.8	1.76E-03	2.30E-01	1.31E+02	5.50		228.8	0.010	221.4	2	
Indeno (1,2,3-cd)pyrene	276.3	5.87E-04	2.30E-07	3.92E-04	6.72		162.0	0.045	283.5	2	
isopropylbenzene	120.2	4.16E-01	6.10E+02	1.47E+03	3.63	3.98	-96.0	1	162.6	2	
m-xylene	106.2	1.51E+00	1.10E+03	7.30E+02	3.20		-47.8	1	140.4	2	
naphthalene	128.2	8.43E-01	3.62E+01	4.30E+01	3.37	5.10	80.3	0.287	147.6	2	
o-xylene	106.2	2.07E+00	1.17E+03	5.65E+02	3.15		-25.2	1	140.4	2	
p-xylene	106.2	2.02E+00	1.17E+03	5.78E+02	3.18		13.3	1	140.4	2	
pentachlorobenzene	250.3	1.03E-02	8.73E-01	8.48E+01	5.32		86.0	0.252	200.5	2	
perylene	252.3	4.79E-04	4.23E-09	8.83E-06	6.25		277.8	0.003	262.9	2	
phenanthrene	178.2	3.30E-02	1.07E-01	3.24E+00	4.36		99.2	0.187	199.2	2	
propyl benzene	120.2	4.33E-01	4.50E+02	1.04E+03	3.69		-99.6	1	162.6	2	34
pyrene	202.3	1.29E-02	1.19E-02	9.23E-01	5.18		150.6	0.059	213.8	2	
styrene	104.1	2.40E+00	8.80E+02	3.67E+02	3.05		-30.7	1	133.0	2	
toluene	92.1	5.59E+00	3.80E+03	6.80E+02	2.69		-95.0	1	118.2	2	
trimethyl dibenzothiophene	226.3	8.56E-04	1.36E-02	1.59E+01	5.93		122.0	0.110	257.9	3	
xylene, m-, o-, p-	268.5	3.59E-04	2.83E+00	7.89E+03	9.71		22.1	1	429.2	2	35

MW = molecular weight ; S_W = aqueous water solubility of liquid or super-cooled liquid; P_L = vapour pressure of liquid or super-cooled liquid; H = Henry's Law constant; K_{OW} = octanol-water partition coefficient; K_{OA} = octanol-air partition coefficient; T_M = melting temperature; F = fugacity ratio (calculated using Walden's Rule); V_M = molar volume.

References for Physical-Chemical Properties:

1 QSAR (Table 3.5)

2 MacKay et al., (2006)

3 EpiSuite (2013)

4 Representative Chemical(s) (Table 3.6)

5 V_M is same as 1,2 and 1,4 isomers

6 Applied properties for 1,2-cis-dimethylcyclohexane

7 V_M is same as 1,2 and 1,4 isomers

8 Applied properties for 1,4-trans-dimethylcyclohexane

9 Applied properties for n-hexane

10 Applied properties for 4-phenylphenol (synonym)

11 Applied properties for 1,6,7-trimethylnaphthalene

12 Applied properties for dibenzothiophene
13 Applied properties for fluorene
14 Applied properties for naphthalene
15 Applied properties for phenanthrene
16 Applied properties for chrysene
17 Applied properties for 1-methyldibenzothiophene
18 Applied averaged properties for 1-methylfluorene and 9-methylfluorene
19 Applied averaged properties for 1-methylnaphthalene and 2-methylnaphthalene
20 Applied averaged properties for 1- ; 2- ; 3- ; 4- methylphenanthrene
21 Applied averaged properties for 1- ; 3- ; 4- methylchrysene
22 Applied averaged properties for dimethyldibenzothiophene and ethyldibenzothiophene
23 Applied properties for 2-ethylfluorene
24 Applied averaged properties for 1,2- ; 1,3- ; 1,4 ; 1,5 ; 2,3 ; 2,6- dimethylnaphthalenes
25 Applied averaged properties for 1,2- ; 1,3- ; 3,6- dimethylphenanthrene
26 Applied averaged properties for 1,2- ; 1,6- ; 5,6- dimethylchrysene ; 6-ethylchrysene
27 Applied properties for trimethyldibenzothiophene
28 Applied averaged properties for 2,3,9-trimethylfluorene and 9-isopropylfluorene
29 Applied averaged properties for 1,4,5- ; 1,6,7- trimethylnaphthalene
30 Applied averaged properties for 1,2,3-trimethylphenanthrene and 9-isopropylphenanthrene
31 Applied properties for 7,8,12-trimethylbenz(a)anthracene
32 Applied averaged properties for 1-methyl-7-(1-methylethyl)phenanthrene ; 1,2,3,4-tetramethylphenanthrene ; 9,10-diethylphenanthrene
33 Applied properties for 1,2,3,4-tetramethylnaphthalene
34 Applied properties for n-propyl benzene
35 Applied averaged properties for m-, o-, p- xylene

Appendix B.

Supplementary Data Files

Description:

The accompanying csv files contains the following data, expressed as activity, fugacity, lipid-normalized concentration (C_L) and lipid-normalized volume fraction (V_C/V_L):

- Toxicity of individual petroleum hydrocarbons
- Toxicity of mixtures of petroleum hydrocarbons (both total sum for mixtures, as well as individual components of mixtures)
- Current guidelines for PHC mixtures

Description of the column headings in the PHC toxicity datafile:

- "datatype" – either individual chemical ("individual") or individual component in a mixture ("mix_component")
- "mix_id" – for mixture components only, a label for the PHC mixture that was tested
- "aro_ali" – either an "aliphatic" or "aromatic" petroleum hydrocarbon
- "chemical" – the name of the individual component or mixture component
- "inex" – describes if the reported concentration was measured either "internal" to the test organism (i.e., biotic media), or "external" (i.e., abiotic media)
- "medium" – the phase in which the chemical's concentration was reported
- "spp_category" – either amphibian ("Amph"), freshwater invertebrate ("Inv-FW"), freshwater fish ("F-FW"), saltwater invertebrate ("Inv-SW"), saltwater fish ("F-SW"), mammal ("M"), or soil invertebrate ("Inv-Soil")
- "spp_sci" – reported scientific species name
- "effect" – the type of esponse observed in toxicity test organisms
- "endpoint_type" – the level of quantification of the effect (e.g., LC50)
- "test_day_duration" – duration of toxicity test in days
- "reference" – source of the toxicity data
- "diss_f" – calculated fraction of chemical bound to dissolved or suspended organic carbon
- "mol_conc_m3" – molar concentration of chemical in mol/m^3

- "activity" – unitless activity calculated for the chemical
- "fugacity" – in pascals
- "lipid_conc" – lipid concentration (calculated) in mol/m^3_{LIPID}
- "vf_lipid" – volume fraction in lipid phase (calculated ($m^3_{CHEMICAL}/m^3_{LIPID}$)

Description of the column headings in the PHC mixture guideline datafile:

- "medium" – the phase for which the guideline is defined
- "reference" – the source of the guideline (e.g., BC Contaminated Sites Regulation)
- "phc_mixture_description" – the mixture of petroleum hydrocarbons for which the guideline is defined
- "narrative intent" – a description of the protection goals behind the guideline
- "molar_concentration_min_2080", "a_min_2080", "f_min_2080", "cl_min_2080", and "vf_min_2080" each describe the molar concentration (mol/m^3), activity (unitless), fugacity (pascals), lipid-phase concentration (mol/m^3_{LIPID}), and lipid-phase volume fraction ($m^3_{CHEMICAL}/m^3_{LIPID}$) associated the guideline, assuming that the PHC mixture is composed of 20% of the lightest possible aromatic PHCs in the guideline's stipulated composition, and 80% of the lightest possible aliphatic PHCs in the guideline's stipulated composition.
- molar_concentration_min_2080", "a_max_2080", "f_max_2080", "cl_max_2080", and "vf_max_2080" each describe the molar concentration (mol/m^3), activity (unitless), fugacity (pascals), lipid-phase concentration (mol/m^3_{LIPID}), and lipid-phase volume fraction ($m^3_{CHEMICAL}/m^3_{LIPID}$) associated the guideline, assuming that the PHC mixture is composed of 20% of the heaviest possible aromatic PHCs in the guideline's stipulated composition, and 80% of the heaviest possible aliphatic PHCs in the guideline's stipulated composition.
- molar_concentration_min_100aro", "a_min_100aro", "f_min_100aro", "cl_min_100aro", and "vf_min_100aro" each describe the molar concentration (mol/m^3), activity (unitless), fugacity (pascals), lipid-phase concentration (mol/m^3_{LIPID}), and lipid-phase volume fraction ($m^3_{CHEMICAL}/m^3_{LIPID}$) associated the guideline, assuming that the PHC mixture is composed of 100% of the lightest possible aromatic PHCs in the guideline's stipulated composition.
- molar_concentration_min_100aro", "a_max_100aro", "f_max_100aro", "cl_max_100aro", and "vf_max_100aro" each describe the molar concentration (mol/m^3), activity (unitless), fugacity (pascals), lipid-phase concentration (mol/m^3_{LIPID}), and lipid-phase volume fraction ($m^3_{CHEMICAL}/m^3_{LIPID}$) associated the guideline, assuming that the PHC

mixture is composed of 100% of the heaviest possible aromatic PHCs in the guideline's stipulated composition.

Filenames:

i) CrawfordMeara_MRM699_datafile_PHCtoxicity.csv

ii) CrawfordMeara_MRM699_datafile_PHCmixtureguidelines.csv

Appendix C.

Species Represented in Individual PHC Toxicological Effects Dataset

Table C.1. List of species represented in the toxicological effects dataset for individual PHCs.

Species Category (spp. count in category)	Scientific Species Name	Common Species Description
Freshwater Amphibian (2)		
	Ambystoma mexicanum	Mexican axolotl
	Xenopus laevis	South african clawed frog
Freshwater Fish (23)		
	Carassius auratus	Goldfish
	Catostomus commersoni	White sucker
	Clarias lazera	Catfish
	Cottus cognatus	Slimy sculpin
	Danio rerio	Zebra danio
	Gambusia affinis	Western mosquitofish
	Gasterosteus aculeatus	Threespine stickleback
	Ictalurus punctatus	Channel catfish
	Lepomis macrochirus	Bluegill
	Lepomis sp.	Sunfish
	Micropterus salmoides	Largemouth bass
	Oncorhynchus gorbuscha	Pink salmon
	Oncorhynchus kisutch	Coho salmon
	Oncorhynchus mykiss	Rainbow trout
	Oncorhynchus nerka	Sockeye salmon
	Oncorhynchus tshawytscha	Chinook salmon
	Oryzias latipes	Medaka, high-eyes
	Pimephales promelas	Fathead minnow
	Poecilia reticulata	Guppy
	Salmo trutta	Brown trout
	Salvelinus malma	Dolly varden
	Thymallus arcticus	Arctic grayling
	Tilapia mossambica	Mozambique tilapia

Species Category (spp. count in category)	Scientific Species Name	Common Species Description
Freshwater Invertebrate (33)		
	Aplexa hypnorum	Snail
	Asellus aquaticus	Aquatic sowbug
	Brachionus calyciflorus	Rotifer
	Ceriodaphnia dubia	Water flea
	Chironomus attenuatus	Midge
	Chironomus riparius	Midge
	Chironomus thummi	Midge
	Corixa punctata	Water boatman
	Daphnia cucullata	Water flea
	Daphnia magna	Cladoceran
	Daphnia pulex	Cladoceran
	Gammarus fossarum	Amphipod, scud
	Gammarus minus	Amphipod, scud
	Gammarus pseudolimnaeus	Amphipod, scud
	Gammarus pulex	Amphipod, scud
	Hyalella azteca	Amphipod, scud
	Hydra americana	Hydra
	Hydra oligactis	Hydra
	Hydra sp.	Hydra
	Limnodrilus hoffmeisteri	Oligochaete; worm
	Lumbriculus variegatus	Worm, oligochaete
	Lymnaea stagnalis	Great pond snail
	Macrobrachium kistnensis	Shrimp
	Moina macrocopa	Water flea
	Ophiogomphus sp.	Dragonfly
	Palaemonetes sp.	Grass shrimp
	Paratanytarsus sp.	Midge
	Peltoperla maria	Stonefly
	Physa gyrina	Pouch snail
	Physa heterostropha	Pond snail, pneumonate snail
	Physella virgata	Snail
	Streptocephalus proboscideus	Fairy shrimp
	Tanytarsus dissimilis	Midge

Species Category (spp. count in category)	Scientific Species Name	Common Species Description
Saltwater Fish (10)		
	Clupea harengus pallasi	Pacific herring
	Cyprinodon variegatus	Sheepshead minnow
	Menidia beryllina	Inland silverside
	Menidia menidia	Atlantic Silverside
	Morone saxatilis	Striped bass
	Mugil curema	White mullet
	Platichthys flesus	Starry, european flounder
	Pleuronectes americanus	Winter flounder
	Solea solea	Dover sole
	Therapon jarbua	Tigerfish, crescent perch
Saltwater Invertebrate (43)		
	Americamysis bahia	Opossum shrimp
	Ampelisca abdita	Amphipod
	Arbacia punctulata	Purple-spined sea urchin
	Artemia salina	Brine shrimp
	Callinectes sapidus	Blue crab
	Cancer magister	Dungeness Crab
	Corophium insidiosum	Amphipod, scud
	Corophium spinicorne	Corophiid amphipod
	Crangon septemspinosus	Sand shrimp
	Crangon franciscorum	Bay shrimp
	Crassostrea gigas	Pacific oyster
	Crepidula fornicata	Slipper limpet
	Cyclops viridis	Cyclopoid copepod
	Diaptomus forbesi	Calanoid copepod
	Dinophilus gyrociliatus	Archiannelid
	Elasmopus pectinicrus	Scud
	Emerita analoga	Pacific sand crab
	Eohaustorius estuarius	Amphipod
	Eualis suckleyi	Kelp shrimp
	Eualus sp.	Shrimp
	Eurytemora affinis	Calanoid copepod
	Excirolana vancouverensis	Isopod
	Grandidierella japonica	Amphipod, scud

Species Category (spp. count in category)	Scientific Species Name	Common Species Description
	Hemigrapsus nudus	Shore crab
	Homarus americanus	American lobster
	Katelysia opima	Marine bivalve
	Leptocheirus plumulosus	Amphipod
	Mulinia lateralis	Coot clam
	Mya arenaria	Soft-shell clam
	Mypsidopsis bahia	Opossum shrimp
	Mytilus edulis	Blue mussel
	Nassarius obsoletus	Mud snail
	Neanthes arenaceodentata	Annelid worm
	Neomysis americana	Opossum shrimp
	Nereis arenaceodentata	Polychaete worm
	Nitocra spinipes	Harpacticoid copepod
	Palaemonetes pugio	Daggerblade grass shrimp
	Pandalus goniurus	Humpy shrimp
	Paqurus longicarpus	Hermit crab
	Penaeus aztecus	Brown shrimp
	Portunus pelagicus	Blue Crab
	Rhepoxynius abronius	Amphipod
	Scylla serrata	Crab

Appendix D.

Duration of Toxicity Tests for Individual Petroleum Hydrocarbons

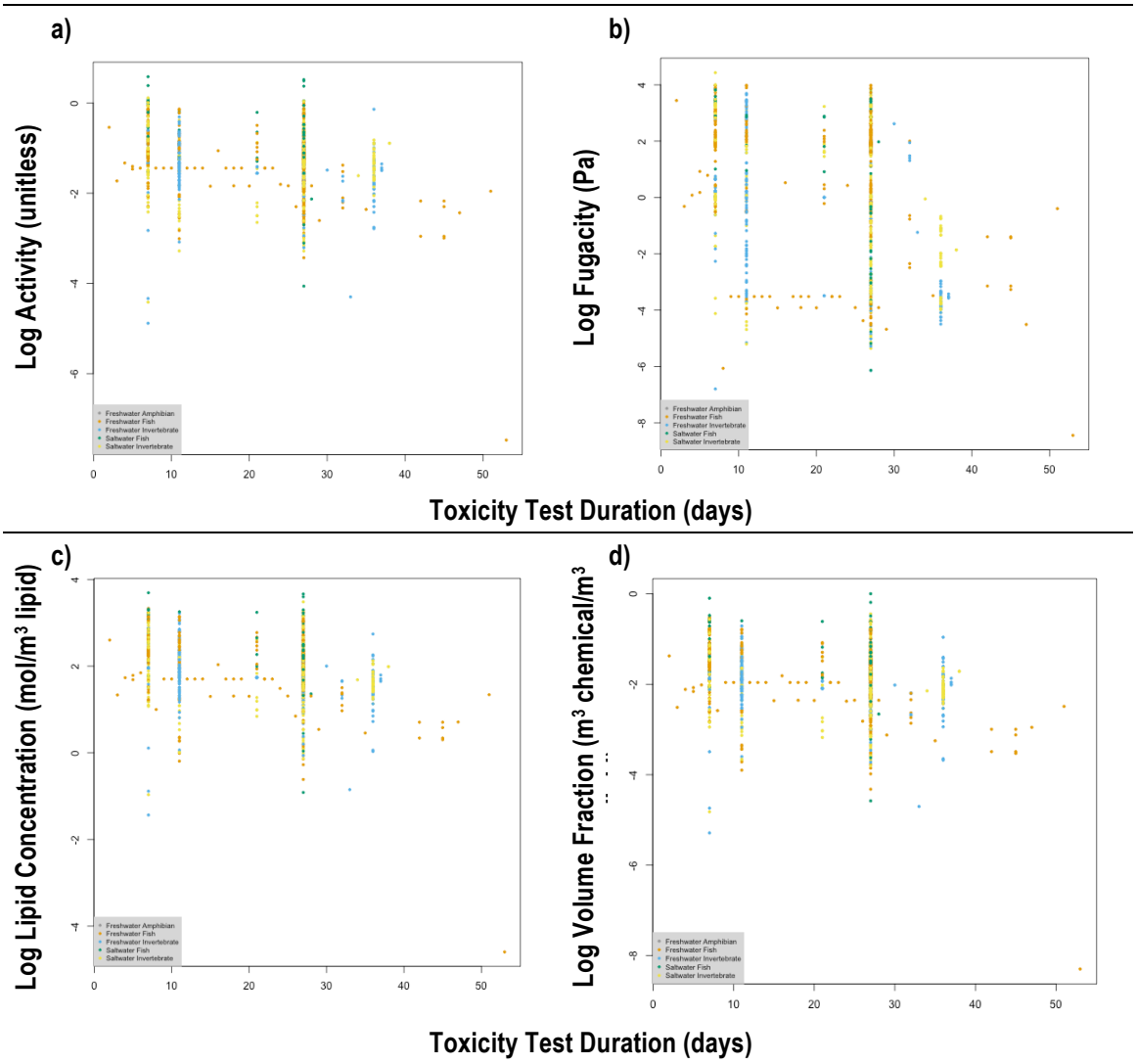


Figure D1. **Toxicity test duration and variation in LC50s expressed as a) activity, and b) fugacity across different test species categories (amphibians, fish, invertebrates).**

Appendix E.

Across Media Comparison of Individual PHC Toxicity

Table E1. Comparison of PHC toxicity (LC50s) measured across different media-types expressed as activity, fugacity, and lipid-phase concentration and volume fraction.

	Log Activity				Log Fugacity			
Summary Statistic	**Water**	**Pore-water**	**Sediment**	**Lipid**	**Water**	**Pore-water**	**Sediment**	**Lipid**
n	882	21	23	27	882	21	23	27
$Mean_A$	-1.33	-1.18	-1.63	-1.41	0.23	-2.30	-3.35	0.13
95% CI	-1.377 to -1.278	-1.278 to -1.074	-1.806 to -1.45	-1.56 to -1.27	0.059 to 0.4	-2.75 to -1.85	-3.771 to -2.93	-0.44 to 0.7
5th to 95th %iles	-2.53 to -0.195	-1.45 to -0.883	-2.15 to -1.198	-1.98 to -0.804	-3.94 to 3.44	-3.48 to -0.73	-4.22 to -1.17	-2.98 to 1.58
Minimum	-7.47	-1.62	-2.30	-2.32	-8.44	-3.70	-4.37	-4.12
Maximum	0.585	-0.821	-0.138	-0.538	4.43	-0.67	-1.11	3.44
Standard Deviation	0.732	0.234	0.427	0.375	2.52	1.03	1.00	1.48
	Activity (unitless)				**Fugacity (Pa)**			
$Mean_A$	0.141	0.076	0.055	0.056	515.8	0.041	0.010	108
95% CI	0.121 to 0.161	0.059 to 0.093	-0.006 to 0.116	0.033 to 0.079	404 to 627	0.011 to 0.071	-0.0002 to 0.02	-96.8 to 313
$Mean_G$	0.047	0.067	0.024	0.039	1.69	0.005	0.0004	1.35
95% CI	0.042 to 0.053	0.053 to 0.084	0.016 to 0.035	0.028 to 0.054	1.15 to 2.505	0.002 to 0.014	0.0002 to 0.0012	0.363 to 5.05
5th to 95th %iles	0.003 to 0.64	0.035 to 0.13	0.007 to 0.06	0.011 to 0.17	0.0001 to 2781	0.0003 to 0.184	0.0001 to 0.068	0.008 to 41.01
Minimum	3.36E-08	2.38E-02	5.06E-03	4.84E-03	3.60E-09	0.0002	4.26E-05	0.0001
Maximum	3.84	0.15	0.73	0.29	27028	0.213	0.078	2774
Standard Deviation	0.297	0.039	0.147	0.060	1657	0.069	0.024	533
ANOVA: Is there a difference in mean LC50 between media:								
F (p)	1.78 (0.179)				22.7 (**<0.001**)			
Tukey HSD Multiple Comparisons of Means:								
	a	a	a	a	a	b	b	a

Significance denoted in bold for p<0.05.

Table continued next page…

Table continued…

Summary Statistic	Log Lipid Concentration				Log Volume Fraction			
	Water	Pore-water	Sediment	Lipid	Water	Pore-water	Sediment	Lipid
n	882	21	23	27	882	21	23	27
$Mean_A$	1.93	1.84	1.48	1.84	-1.898	-1.866	-2.200	-1.978
95% CI	1.88 to 1.98	1.72 to 1.95	1.33 to 1.62	1.7 to 1.98	-1.95 to -1.848	-1.98 to -1.751	-2.34 to -2.057	-2.12 to -1.841
5th to 95th %iles	0.54 to 3.08	1.43 to 2.23	1 to 1.69	1.17 to 2.48	-3.16 to -0.794	-2.27 to -1.43	-2.66 to -1.994	-2.55 to -1.415
Minimum	-4.60	1.43	0.85	0.94	-8.296	-2.273	-2.814	-2.924
Maximum	3.69	2.26	2.74	2.60	0.002	-1.404	-0.960	-1.304
Standard Deviation	0.77	0.26	0.35	0.37	0.742	0.264	0.344	0.358
	Lipid Concentration ($mol \cdot m^{-3}$)				**Volume Fraction ($m^3_{CHEMICAL} \cdot m^{-3}_{LIPID}$)**			
$Mean_A$	258	80.6	52.0	97.2	0.036	0.016	0.011	0.014
95% CI	227 to 289	60.2 to 101	6.44 to 97.5	61.8 to 133	0.032 to 0.041	0.012 to 0.021	0.002 to 0.0197	0.0097 to 0.018
$Mean_G$	84.6	68.5	30.0	69.3	0.013	0.014	0.006	0.011
95% CI	75.1 to 95.3	52.8 to 88.7	21.4 to 42.1	49.8 to 96.5	0.011 to 0.014	0.0104 to 0.018	0.005 to 0.009	0.008 to 0.014
5th to 95th %iles	3.43 to 1195	26.96 to 171.1	10.02 to 49.1	15.3 to 310	0.001 to 0.161	0.005 to 0.037	0.002 to 0.0101	0.003 to 0.039
Minimum	2.54E-05	26.8	7.06	8.64	5.06E-09	5.33E-03	1.53E-03	1.19E-03
Maximum	4928	182	550	400	1.00	0.039	0.110	0.050
Standard Deviation	460	46.7	109	92.1	0.070	0.0100	0.0217	0.0114
ANOVA: Is there a difference in mean LC50 between media:								
F (p)	2.89 (**0.035**)				1.43 (0.233)			
Tukey HSD Multiple Comparisons of Means:								
	a	ab	b	ab	a	a	a	a

Significance denoted in bold for p<0.05.

Appendix F.

Across Species Comparison of Individual PHC Toxicity

Table F1. Summary and comparison of PHC toxicity (LC50s) measured for different species categories expressed as activity, fugacity, and lipid-phase concentration and volume fraction.

Summary Statistic	Log Activity					Log Fugacity				
	Freshwater			Saltwater		Freshwater			Saltwater	
	Inv	Fish	Amph	Inv	Fish	Inv	Fish	Amph	Inv	Fish
n	209	410	5	259	70	209	410	5	259	70
$Mean_A$	-1.44	-1.32	-1.08	-1.37	-0.957	-0.913	0.875	2.01	-0.547	0.629
95% CI	-1.53 to -1.35	-1.39 to -1.25	-1.60 to -0.562	-1.46 to -1.29	-1.16 to -0.751	-1.26 to -0.567	0.647 to 1.10	0.243 to 3.77	-0.848 to -0.25	-0.015 to 1.27
5th to 95th %iles	-2.47 to -0.641	-2.52 to -0.212	-1.71 to -0.622	-2.53 to -0.272	-2.33 to 0.382	-4.18 to 3.3	-3.91 to 3.48	-0.15 to 3.48	-4.06 to 3.28	-3.74 to 3.66
Minimum	-4.88	-7.47	-1.71	-4.42	-4.06	-6.80	-8.44	-0.153	-5.36	-6.13
Maximum	-0.138	0.046	-0.607	0.115	0.585	3.69	3.98	3.50	4.43	3.87
Std.Dev.	0.644	0.723	0.578	0.677	0.861	2.50	2.31	1.97	2.42	2.69
	Activity (unitless)					Fugacity (Pa)				
$Mean_A$	0.075	0.127	0.140	0.116	0.436	256	569	1684.2	391.9	877.1
95% CI	0.061 to 0.088	0.107 to 0.147	0.041 to 0.24	0.091 to 0.14	0.247 to 0.625	146 to 365	407 to 731	298 to 3071	151 to 632	468 to 1286
$Mean_G$	0.037	0.048	0.083	0.042	0.110	0.12	7.49	101.62	0.28	4.26
95% CI	0.03 to 0.045	0.04 to 0.056	0.025 to 0.274	0.035 to 0.051	0.069 to 0.177	0.055 to 0.271	4.44 to 12.7	1.75 to 5895	0.14 to 0.567	0.97 to 18.764
5th to 95th %iles	0.003 to 0.229	0.003 to 0.613	0.019 to 0.239	0.003 to 0.53	0.005 to 2.41	0.0001 to 1998	0.0001 to 3033	0.7037 to 3040	0.0001 to 1919	0.0002 to 4632
Minimum	1.31E-05	3.36E-08	1.94E-02	3.85E-05	8.71E-05	1.60E-07	3.60E-09	7.04E-01	4.40E-06	7.33E-07
Maximum	0.727	1.11	0.247	1.30	3.84	4866	9484	3140	27028	7374
Std.Dev.	0.099	0.198	0.112	0.200	0.791	791	1639	1550	1935	1710
ANOVA: Is there a difference in mean LC50 between media:										
F (p)	6.52 (**<0.001**)					26.1 (**<0.001**)				
Tukey HSD Multiple Comparisons of Means:										
	b	b	ab	b	a	b	a	ab	b	a

Note: Bold denotes significance at alpha = 0.05.
Inv = invertebrate; Amph = amphibian; CI = confidence interval; Std.Dev. = standard deviation; HSD = honest significant difference.

Table continued…

Summary Statistic	Log Lipid Concentration					Log Volume Fraction				
	Freshwater			Saltwater		Freshwater			Saltwater	
	Inv	Fish	Amph	Inv	Fish	Inv	Fish	Amph	Inv	Fish
n	209	410	5	259	70	209	410	5	259	70
$Mean_A$	1.77	1.99	2.33	1.81	2.26	-2.015	-1.869	-1.611	-1.981	-1.548
95% CI	1.68 to 1.86	1.91 to 2.06	1.72 to 2.94	1.72 to 1.89	2.07 to 2.46	-2.10 to -1.93	-1.94 to -1.79	-2.13 to -1.09	-2.06 to -1.9005	-1.74 to -1.351
5th to 95th %iles	0.692 to 2.78	0.541 to 3.105	1.58 to 2.87	0.716 to 2.89	1.15 to 3.31	-3.00 to -1.20	-3.15 to -0.799	-2.25 to -1.15	-3.13 to -0.875	-2.86 to -0.483
Minimum	-1.43	-4.60	1.58	-0.967	-0.915	-5.287	-8.296	-2.247	-4.820	-4.578
Maximum	3.22	3.33	2.88	3.48	3.69	-0.710	-0.521	-1.137	-0.445	0.002
Std.Dev.	0.676	0.795	0.684	0.657	0.821	0.638	0.765	0.581	0.649	0.822
	Lipid Concentration ($mol \bullet m^{-3}$)					Volume Fraction ($m^3_{CHEMICAL} \bullet m^{-3}_{LIPID}$)				
$Mean_A$	142	274	423	177	616	0.020	0.036	0.041	0.027	0.100
95% CI	111 to 174	235 to 314	106 to 740	135 to 218	374 to 857	0.016 to 0.023	0.031 to 0.041	0.012 to 0.071	0.021 to 0.033	0.057 to 0.143
$Mean_G$	58.9	96.8	214.7	64.0	183.1	0.010	0.014	0.024	0.010	0.028
95% CI	47.5 to 73.1	80.8 to 115.9	52.5 to 877.6	53 to 77.3	116.5 to 287.6	0.008 to 0.012	0.011 to 0.016	0.007 to 0.081	0.009 to 0.013	0.018 to 0.045
5th to 95th %iles	4.93 to 607	3.48 to 1273	38.36 to 736	5.2 to 776	14.95 to 2022	0.001 to 0.063	0.001 to 0.159	0.006 to 0.071	0.001 to 0.133	0.001 to 0.329
Minimum	3.68E-02	2.54E-05	3.84E+01	1.08E-01	1.22E-01	5.16E-06	5.06E-09	5.66E-03	1.51E-05	2.64E-05
Maximum	1648	2146	760	3037	4928	0.195	0.301	0.073	0.359	1.00
Std.Dev.	228	398	355	337	1009	**0.026**	0.052	**0.033**	**0.047**	0.179
ANOVA: Is there a difference in mean LC50 between media:										
F (p)	8.67 (**<0.001**)					6.87 (**<0.001**)				
Tukey HSD Multiple Comparisons of Means:										
	c	b	abc	c	a	b	b	ab	b	a

Bold denotes significance at alpha = 0.05.

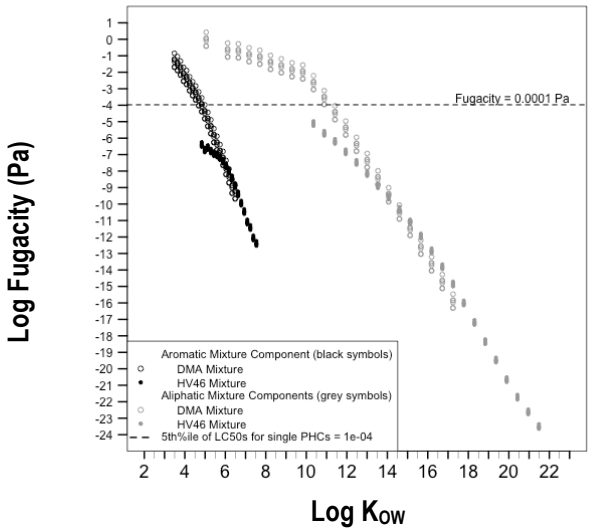
Inv = invertebrate; Amph = amphibian; CI = confidence interval; Std.Dev. = standard deviation; HSD = honest significant difference.

Appendix G.

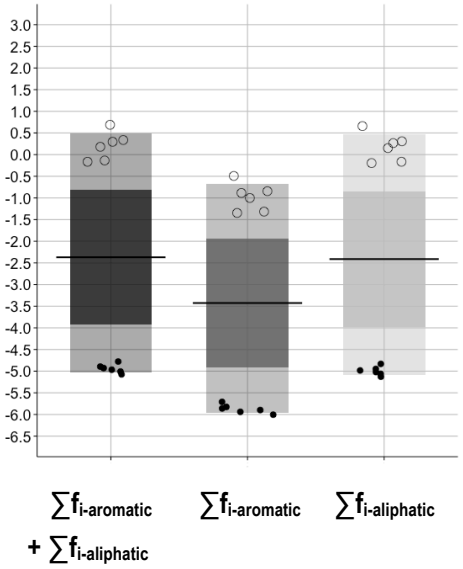
Fugacity of PHC Mixture components

a) Lipid-based PHC Mixture

Fugacity of Individual PHC Mixture Components (f_i)

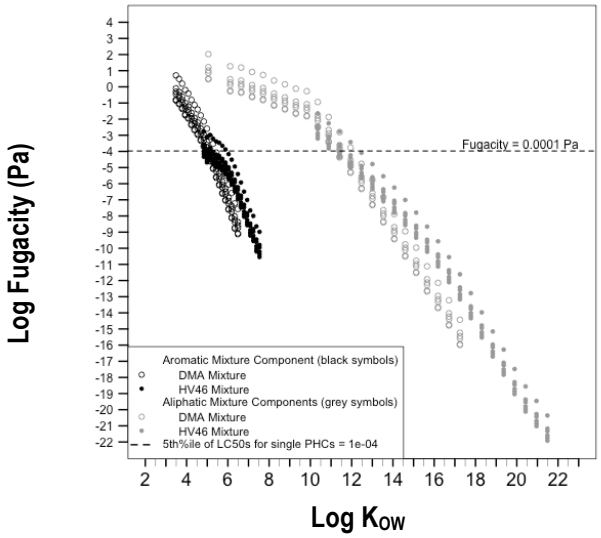


∑Fugacity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures

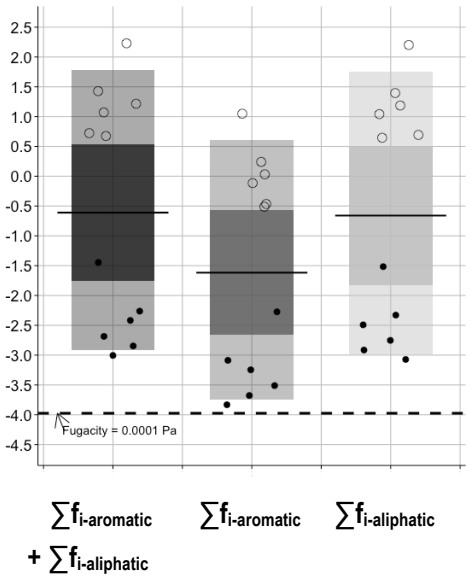


b) Sediment-based PHC Mixture

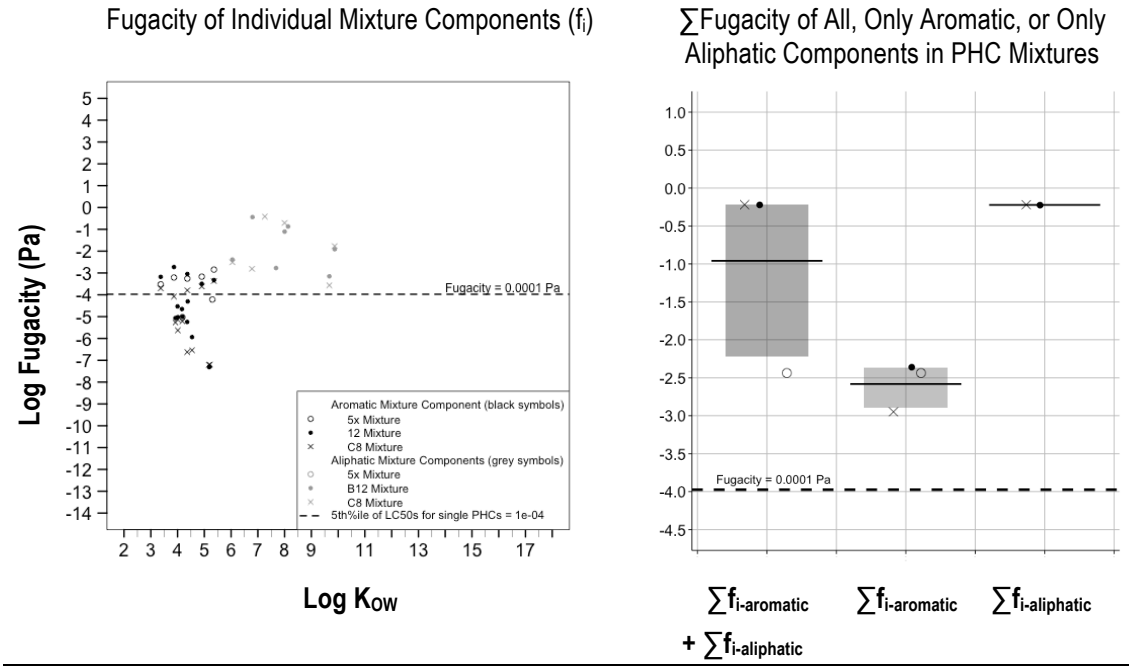
Fugacity of Individual Mixture Components (f_i)



∑Fugacity of All, Only Aromatic, or Only Aliphatic Components in PHC Mixtures



c) Water-based PHC Mixture



d) Soil-based PHC Mixture

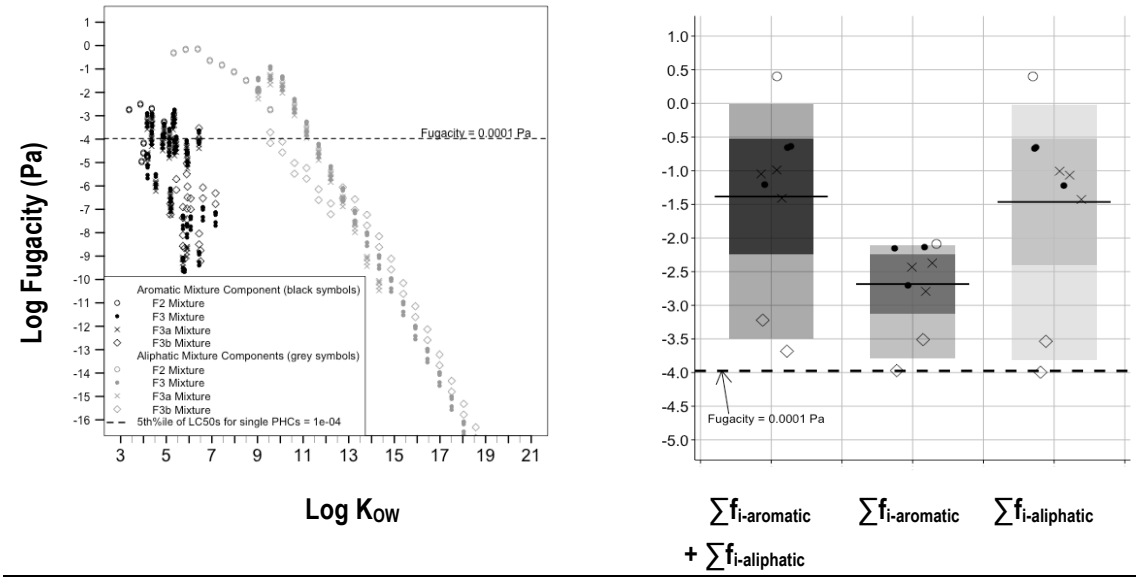


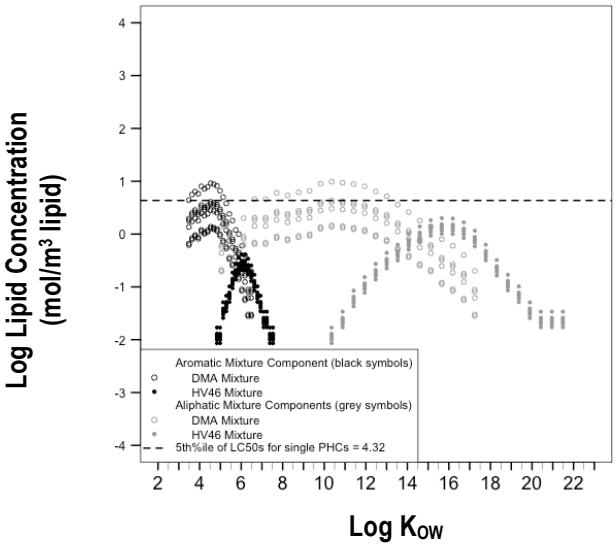
Figure G1. Fugacity of individual components (left-hand side), and sum activity of aromatic-only, aliphatic-only, and all components (right-hand side) of PHC mixtures at toxic concentrations (LC50s).

f_i = fugacity of individual mixture component.

Appendix H.

Lipid-phase Concentration of PHC Mixture Components

a) C_L of Individual Mixture Components in Lipid



b) $\sum C_L$ of Mixture Components in Lipid

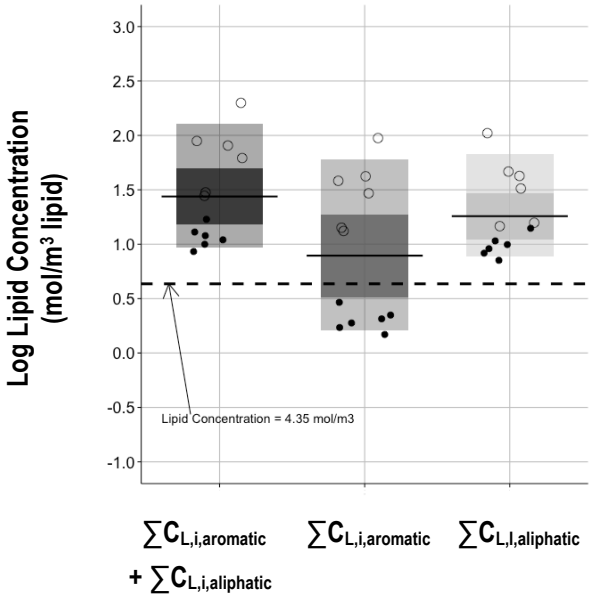
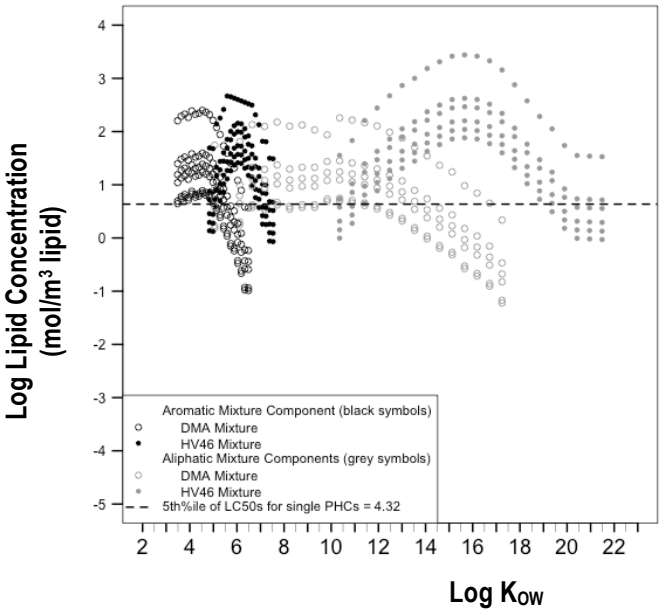


Figure H1. Lipid-phase concentration (C_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in lipid at toxic concentrations (LC50s).

$C_{L,i}$ = lipid-phase concentration of individual mixture component.

a) C_L of Individual Mixture Components in Sediment



b) $\sum C_L$ of Mixture Components in Sediment

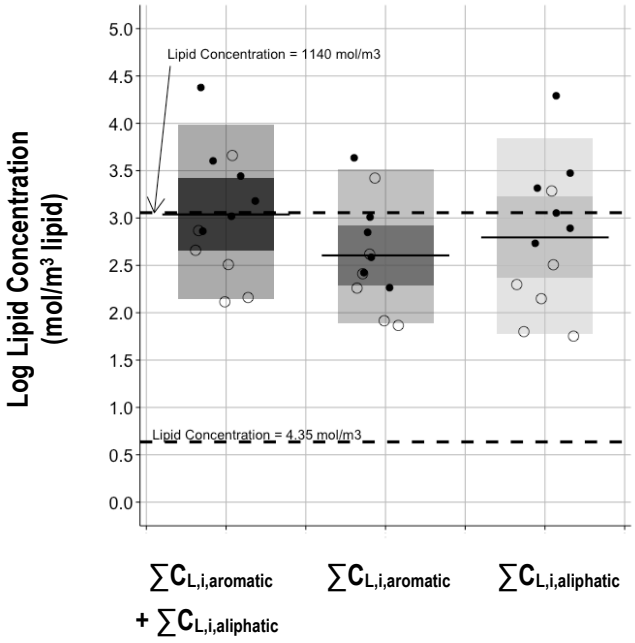
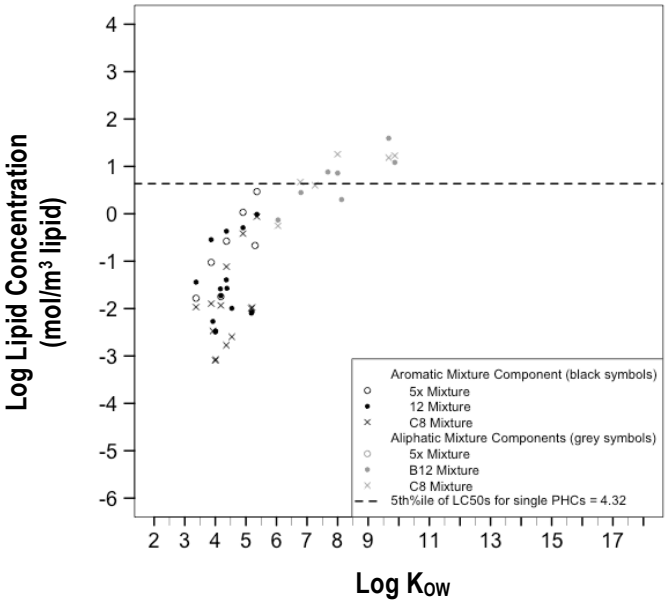


Figure H2. Lipid-phase concentration (C_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in sediment at toxic concentrations (LC50s).

$C_{L,i}$ = lipid-phase concentration of individual mixture component.

a) C_L of Individual Mixture Components in Water



b) $\sum C_L$ of Mixture Components in Water

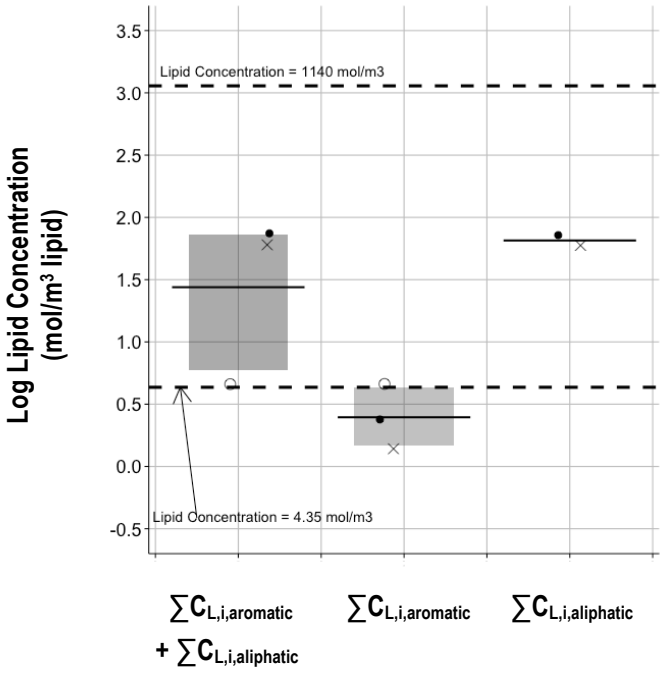
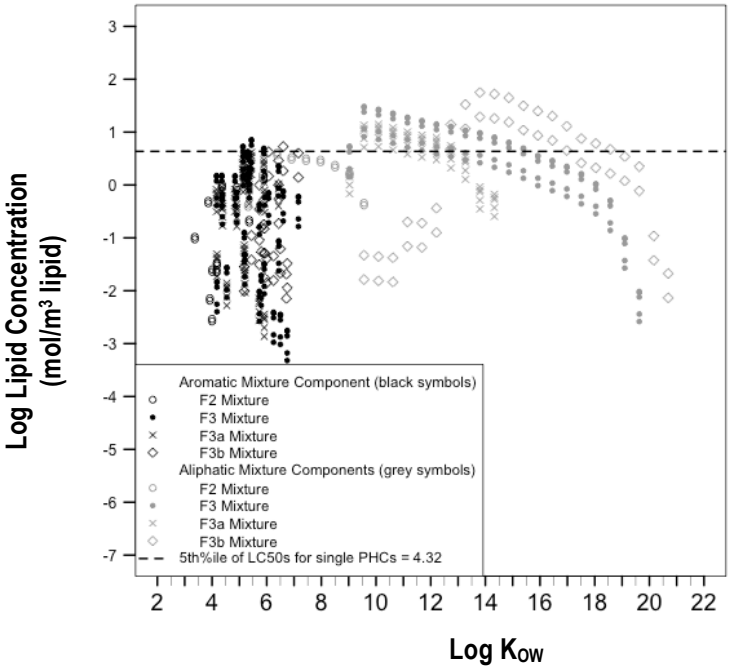


Figure H3. Lipid-phase concentration (C_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in water at toxic concentrations (LC50s).

$C_{L,i}$ = lipid-phase concentration of individual mixture component.

a) C_L of Individual Mixture Components in Soil



b) $\sum C_L$ of Mixture Components in Soil

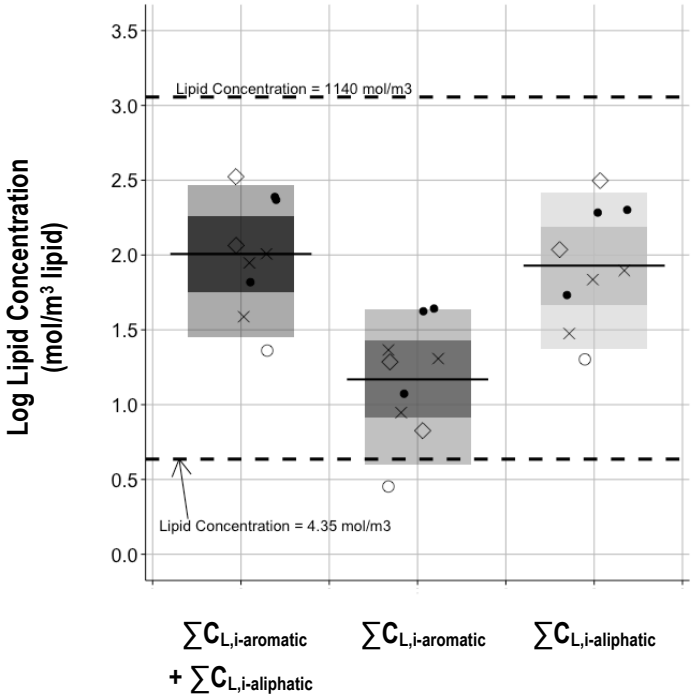


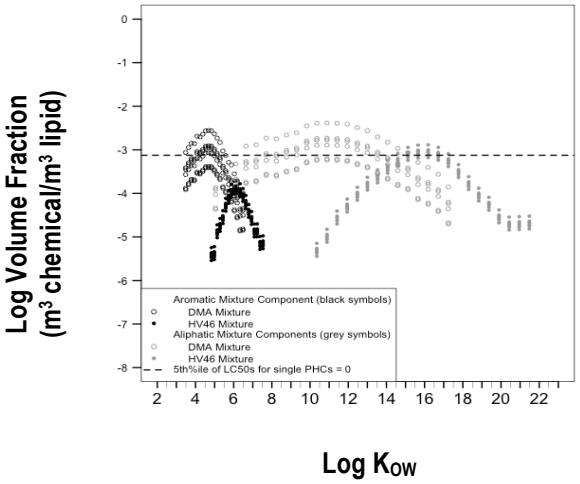
Figure H4. Lipid-phase concentration (C_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in soil at toxic concentrations (LC50s).

$C_{L,i}$ = lipid-phase concentration of individual mixture component.

Appendix I.

Lipid-phase Volume Fraction of PHC Mixture Components

a) V_C/V_L of Individual Mixture Components in Lipid



b) $\sum V_C/V_L$ of Mixture Components in Lipid

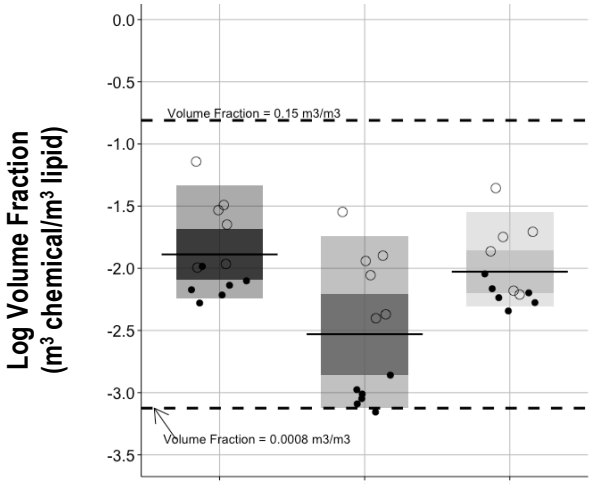
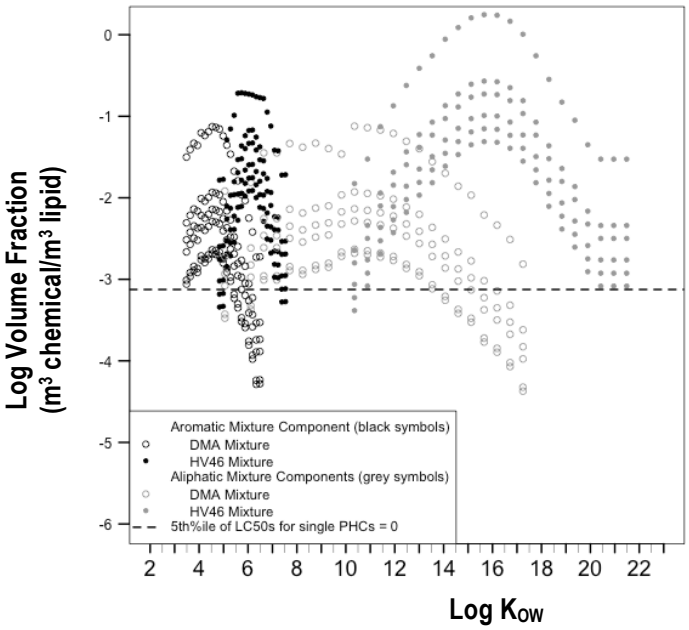


Figure I1. **Lipid-phase volume Fraction (V_C/V_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in lipid at toxic concentrations (LC50s).**

a) V_C/V_L of Individual Mixture Components in Sediment



b) $\sum V_C/V_L$ of Mixture Components in Sediment

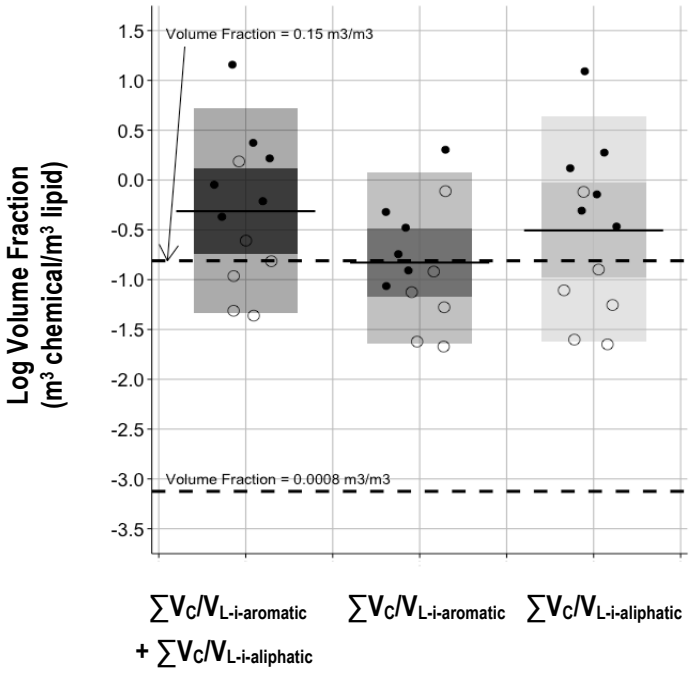
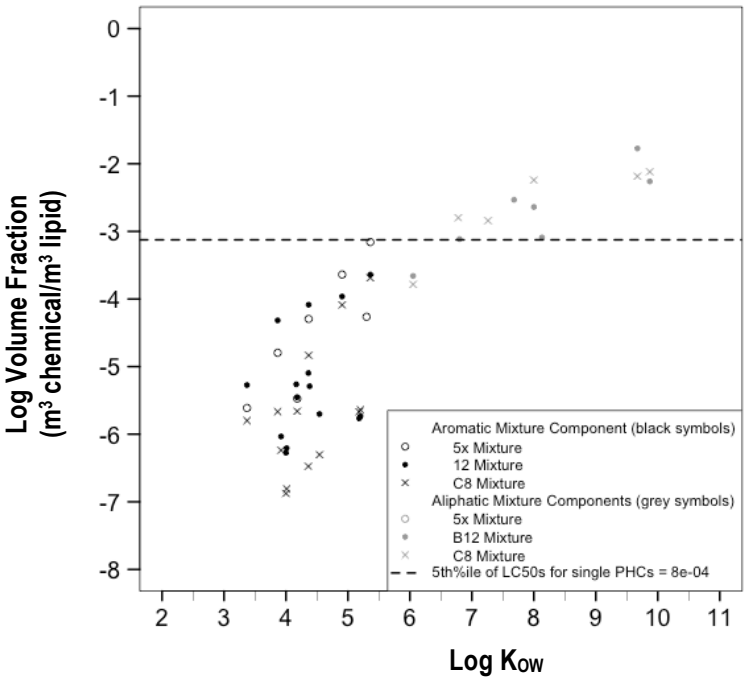


Figure I2. **Lipid-phase volume Fraction (V_C/V_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in sediment at toxic concentrations (LC50s).**

a) V_C/V_L of Individual Mixture Components in Water



b) $\sum V_C/V_L$ of Mixture Components in Water

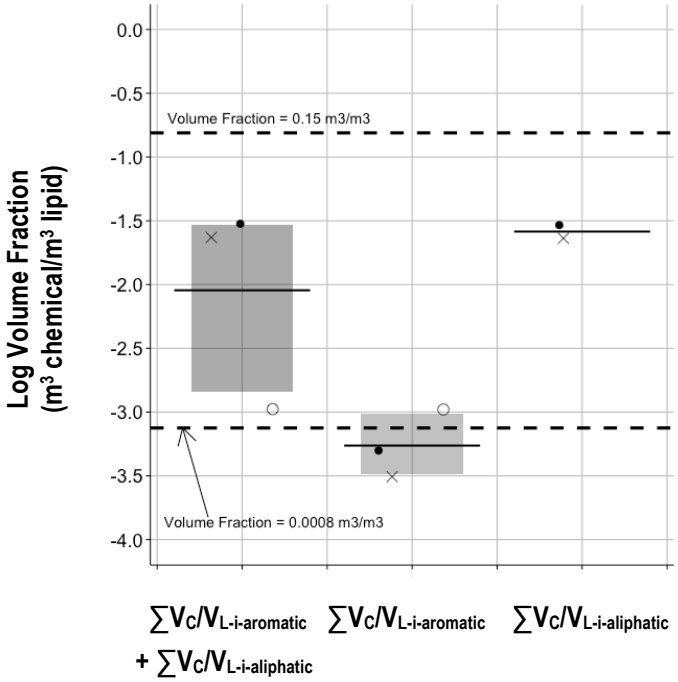
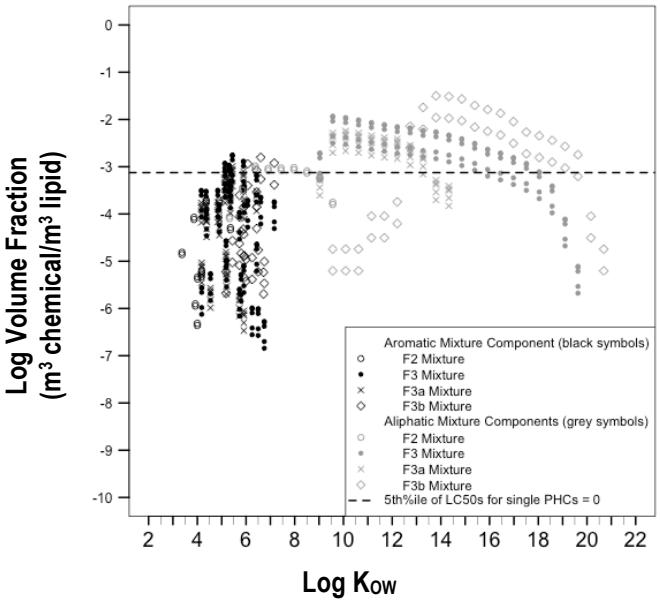


Figure I3. Lipid-phase volume Fraction (V_C/V_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in water at toxic concentrations (LC50s).

a) V_C/V_L of Individual Mixture Components in Soil



b) $\sum V_C/V_L$ of Mixture Components in Soil

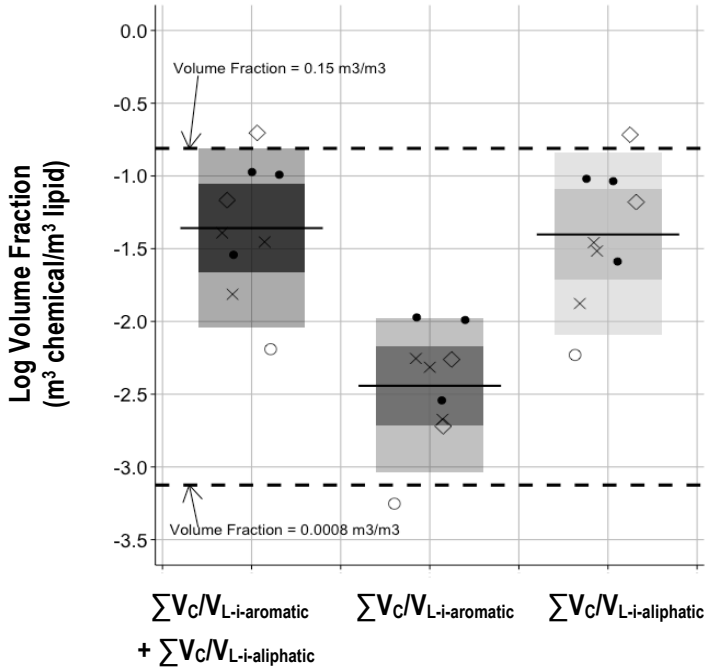


Figure I4. Lipid-phase volume Fraction (V_C/V_L) of (a) individual components and (b) sum activity of aromatic-only, aliphatic-only, and all components of PHC mixtures in soil at toxic concentrations (LC50s).

$(V_C/V_L)_i$ = lipid-phase volume fraction of individual mixture component.