Polycyclic aromatic compounds (PACs) in the Canadian environment: The challenges of ecological risk assessments

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Abstract

Ecological risk assessments (ERAs) of polycyclic aromatic compounds (PACs), as single congeners or in mixtures, present technical challenges that raise concerns about their accuracy and validity for Canadian environments. Of more than 100,000 possible PAC structures, the toxicity of fewer than 1% have been tested as individual compounds, limiting the assessment of complex mixtures. Because of the diversity in modes of PAC action, the additivity of mixtures cannot be assumed, and mixture compositions change rapidly with weathering. In vertebrates, PACs are rapidly oxygenated by cytochrome P450 enzymes, often to metabolites that are more toxic than the parent compound. The ability to predict the ecological fate, distribution and effects of PACs is limited by toxicity data derived from tests of a few responses with a limited array of test species, under optimal laboratory conditions. Although several models are available to predict PAC toxicity and rank species sensitivity, they were developed with data biased by test methods, and the reported toxicities of many PACs exceed their solubility limits. As a result, Canadian Environmental Quality Guidelines for a few individual PACs provide little support for ERAs of complex mixtures in emissions and at contaminated sites. The issues are illustrated by reviews of three case studies of PAC-contaminated sites relevant to Canadian ecosystems. Interactions among ecosystem characteristics, the behaviour, fate and distribution of PACs, and non-chemical stresses on PAC-exposed species prevented clear associations between cause and effect. The uncertainties of ERAs can only be reduced by estimating the toxicity of a wider array of PACs to species typical of Canada's diverse geography and environmental conditions. Improvements are needed to models that predict toxicity, and more field studies of contaminated sites in Canada are needed to understand the ecological effects of PAC mixtures.

Capsule: This review assesses the challenges of ecological risk assessments for polycyclic aromatic compounds due to complex interactions among a diversity of structures, exposures, and receptors in Canadian ecosystems.

Keywords: Polycyclic aromatic compounds (PACs); species sensitivity; environmental stressors; mixture interactions; ecological risk assessments (ERA).

Running Head: Ecological Risk Assessments of PACs

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1 Introduction

The present paper is one of a series that review the sources, distribution and potential effects of polycyclic aromatic compounds (PACs) emitted to Canadian ecosystems (Galarneau, *In prep. for this special issue*). The objective of the present contribution is to identify the obstacles to realistic ecological risk assessments of PAC emissions, research needs and new approaches to assessing risks in Canada at local and regional scales.

Marvin et al. (*In prep. for this special issue*) found clear evidence of environmental contamination by PACs in virtually every region of Canada (Table 1). The sources include petrogenic PACs from the extraction, refining and spills of fossil fuels, pyrogenic PACs from urban and industrial development, the combustion of fossil fuels, and forest fires, and biogenic synthesis from organic industrial wastes. These diverse sources emit a wide array of PACs comprised of polycyclic aromatic hydrocarbons (PAH), PAH with multiple alkyl substitutions, and heterocyclic aromatic compounds containing oxygen, nitrogen and sulfur; PACs containing halogens are not reviewed. Canada's Air Pollutant Emission Inventory estimated that 110,000 kgs of *only* four PACs (benzo[a]pyrene (BaP), benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene) were emitted from anthropogenic sources in 2017 (ECCC 2019), a small fraction of the total. Despite an overall reduction (ECCC 2019; Berthiaume et al. *In prep. for this special issue*), PAC emissions from some sectors are increasing, particularly Alberta's Oil Sands industries (Harner et al. 2018). This reflects a doubling of crude oil exports between 2009 and 2018, from 292 to 571,000 m^3d^{-1} (Canada 2019), and an increase in the risk of PAC contamination by oil spills. A moderate-sized spill of 1,000,000 L (1000 m^3) of crude oil containing 1% PACs by weight (mid-range; Wang et al. 2003) would introduce 10,000 kg (10 T) of PACs to a receiving environment.

Clearly, ERAs are needed for PAC emissions and contaminated sites, but it is likely that the environmental fate and effects of fewer than 1% of the PACs released to Canadian environments have ever been studied. There is ample evidence that emissions have contaminated aquatic and terrestrial species, particularly at sites of legacy industrial contamination and areas affected by bitumen mining (Marvin et al. 2020). In contrast, the environmental effects of PACs are less evident. The toxicity of PACs has only been studied with a relatively small number of Canada's plant and animal species and the range and nature of effects are not well known

(Wallace et al. 2020). Most toxicity tests are conducted under standard laboratory conditions. In contrast, there is little knowledge of how PAC fate and effects would vary among Canada's temperate and Arctic, desert and rainforest, and marine and freshwater ecosystems. There are also emerging concerns about potential interactions between climate change and the frequency of forest fires, and the expansion of Arctic resource extraction as global warming extends the shipping season.

Given the wide range of environmental conditions and natural resources in a country as large as Canada, there is an urgent need for ecological risk assessments (ERAs) to understand and limit the potential effects of PAC emissions. Their objective is to define PAC emission rates and concentrations in environmental media that may harm Canada's plant and animal species. The present review summarizes some of the challenges for ERAs of the diversity of PACs and PAC mixtures, aquatic and terrestrial species, and environmental conditions in Canada. These challenges are illustrated with three case studies of PAC-contaminated environments relevant to Canadian ecosystems. Although the emphasis is on aquatic environments, reflecting the predominance of literature on aquatic species, the conclusions are valid for all terrestrial and aquatic ecosystems.

2 Diversity of PACs

Pyrogenic PACs are generally unsubstituted and dominated by 4-6-ringed PAHs and heterocycles. In contrast, petrogenic PACs in crude and refined oils are mainly 2-4-ringed PAHs, with lower proportions of heterocycles, and higher proportions (80-95%) of alkyl-substituted PAHs (Wang et al. 2003). For heterocyclic PACs, the O, N and S atoms may substitute for carbon in ring structures (e.g., dibenzothiophene) or occur as side groups (e.g., aromatic amines); heterocycles may also contain alkyl substituents (Manzano et al. 2016; 2017).

The effects of PAC structures on exposure and toxicity are clear. Lipid solubility, bioaccumulation, retention in lipid-rich tissues and toxicity increase markedly with molecular size, with strong correlations to the logarithm of octanol-water partition coefficients (log K_{OW}) (Di Toro et al. 2000). These correlations become non-linear at log K_{OW} >6.5, largely because of low water solubility and steric hindrance of trans-membrane diffusion by large molecules (Oliver 1984). The chemical properties, environmental behaviour and toxicity of alkyl and heterocyclic PACs can deviate markedly from those of unsubstituted PAHs.

The diversity and complexity of PAC structures is evident in the large number of unique compounds in PAC mixtures. For example, alkyl substituents of phenanthrene can be located at one or more of the 10 available ring carbons. The permutations and combinations of C1, C2, C3, and C4 linear or branched substituents predict 575 different alkyl phenanthrenes; for alkyl-BaP, there are 2000 C1-C4 congeners (Johnson et al. 2018). However, toxicity data to support ERAs represent only a small fraction (< 1%) of the more than 100,000 possible PACs released to the environment, and toxicity varies widely among the few tested (Hodson 2017). Even fewer are included in Canadian environmental quality guidelines. For example, the current freshwater guidelines are derived from toxicity data for only three aquatic species and 12 pyrogenic PACs (CCME 1999; https://public.tableau.com/views/DRAFTPAHGuidelinesdashboard_v1/Dashboard1?:display_count=y&:origin=viz_share_link). Toxicity is usually measured by laboratory tests of individual compounds under standard conditions, but in the environment, PACs invariably occur in complex mixtures. The source of these mixtures (pyrogenic vs petrogenic) determines their composition, which changes rapidly with dilution and weathering. Therefore, it is unlikely that the ecological effects of PAC mixtures can be predicted successfully from the toxicity of a few and often unrepresentative congeners measured under standard conditions (Section 4.1).

2.1 Multiple modes of action

Wallace et al. (2020) reviewed numerous toxic effects caused by specific groups of PACs plus multiple effects of some individual PACs, reflecting multiple modes of action (MOA), some unique to the species affected. Mechanisms included those typical of acute lethality (narcosis, oxidative stress) and of chronic and sublethal toxicity. For vertebrates, commonly measured effects included carcinogenicity, embryotoxicity and endocrine disruption; much less is known about PAC toxicity to invertebrates.

The diversity of PAC structures enables interactions with a wide array of cellular receptors, particularly the aryl hydrocarbon receptor (AHR) protein, a ligand-activated nuclear transcription factor that is also activated by dioxin-like compounds (chlorinated PACs including dioxins, furans, and non-*ortho* substituted biphenyls). Activation of the AHR is frequently linked to toxicity, with more potent activators being more toxic. However, while AHR binding is the main pathway of toxicity for dioxin-like compounds, PAC mixtures act through multiple pathways

depending on the dose and the mixture components. For example, PACs affect the developing heart of fish embryos by AHR-independent mechanisms that disrupt ion regulation in cardiac myocytes (Incardona 2017).

Even when the AHR does not directly mediate PAC toxicity, AHR binding by some PACs triggers the synthesis of cytochrome P450 enzymes. These powerful enzymes oxygenate PACs to hydroxylated metabolites, changing their tissue distributions, tissue concentrations and rates of excretion according to their susceptibility to oxygenation (Wallace et al. 2020). However, some PAC metabolites are also highly toxic; e.g., diol-epoxide derivatives of BaP initiate cancers when they form covalent bonds with DNA and disrupt replication. Similarly, reactive oxygen species released by P450 enzyme activity can denature proteins and initiate lipid peroxidation and oxidative stress. Thus, PAC oxygenation may limit toxicity by accelerating excretion rates or increase toxicity by producing toxic metabolites (Wassenberg et al. 2005; Hodson et al. 2007; Scott & Hodson 2008; Mu et al. 2016). In mixtures, the toxicity of one PAC may depend on how much P450 enzyme activity is induced or inhibited by others such as 2-aminoanthracene (Scott et al. 2009), a PAC found in industrially-contaminated sediments (Nelson & Hites 1980).

Overall, the variability in tissue distribution, pharmacokinetics and toxicity among PACs presents challenges for ERAs of mixtures, particularly using models that assume common MOAs (Section 2.2). Nevertheless, some mixtures may share a common MOA despite different origins and chemical compositions. Pyrogenic PACs extracted from industrially contaminated sediments and petrogenic PACs from light crude or heavy fuel oil caused similar effects on zebrafish (*Danio rerio*) when added to their diet (Larcher et al. 2014; Vignet et al. 2014). After nine months, all diets caused an increased frequency of deformities and pre-neoplastic and neoplastic lesions of bile duct epithelium. However, PACs from heavy fuel oil were most toxic and uniquely reduced long-term survival. Compared to lighter oils, their higher toxicity may reflect their higher concentrations of 3-6 ringed PACs (Larcher et al. 2014; Vignet et al. 2014), or other hydrocarbons co-extracted with PACs (Meador & Nahrgang 2019).

Other factors affecting ERAs are the very small number of PACs whose toxicity has been measured, the complexity of PAC mixtures, and rapid changes in mixture composition caused by weathering (e.g., volatilization; biodegradation; photo-oxidation). Given these obstacles, ERAs of mixtures may be most successful if based on toxicity tests of the whole mixture, although

177 such ERAs may be quite specific to the mixture studied, and not easily applied to other mixtures
178 (Heys et al. 2016). The alternative is to model risks from toxicity data for each component
179 measured in the mixture, or those that are most potent (Section 2.2). Experimentally, the most
180 toxic components can be identified by effects-driven chemical fractionation, an approach that
181 identified 3-5-ringed PACs as the most likely components of crude oil causing embryotoxicity to
182 fish (Adams et al. 2014).

183 **2.2 Tools for predicting the toxicity of PAC mixtures**

184 Several models predict the toxicity of PACs or PAC mixtures from their physical properties or
185 the toxicity of reference compounds. Each assumes that all PACs modeled share a common
186 MOA, a potential flaw for such a large and structurally diverse group of compounds.

187 **2.2.1 Toxic Unit Models**

188 Toxic unit (TU) models compute the toxicity of mixtures of compounds from the sum of their
189 fractional toxicities, i.e., the ratios of measured concentrations in a mixture to the concentrations
190 causing a given effect (e.g., LC50). Barron et al. (2004) applied a TU model to the measured
191 concentrations of PACs in test solutions of crude oil to identify the MOA that best predicted the
192 observed fish embryotoxicity. Assuming additivity, the best predictor was a model that summed
193 the TUs for the embryotoxicity of alkyl phenanthrenes. Models for a wider array of PACs based
194 on LC50s for narcosis, EC50s for AHR binding, or a combination of mechanisms, were less
195 successful. The poor results for these latter models may reflect a lack of toxicity data for
196 petrogenic alkyl PAC or, more likely, the assumption of a common MOA among PACs that
197 cause many different effects (Wallace et al. 2020).

198 **2.2.2 Target Lipid Model**

199 The most widely used tool for estimating the acute lethality of mixtures of hydrophobic
200 organic compounds is the target lipid model (Di Toro et al. 2000). It assumes that narcosis
201 (physical disruption of lipid membrane structure and function) is the MOA. Lethality occurs
202 when hydrophobic compounds accumulate in lipid membranes to a toxic concentration, termed
203 the critical body burden, or median lethal dose (μmol g^{-1} lipid). The critical body burden is
204 assumed to vary little among compounds but may vary considerably among species (McCarty &
205 Mackay 1993). Each component of a mixture is also assumed to partition from water to tissue
206 lipids in proportion to its K_{OW}. Hence, at any given concentration in water, the molar

concentration of that compound in tissue lipid can be calculated from its K_{OW}, and *vice versa*. The aqueous concentration corresponding to an LD50 (i.e., the critical body burden) would be the LC50 (i.e., 1.0 TU). For mixtures, the model calculates the sum of the molar lipid concentrations of each component that corresponds to their water concentrations, expressed as TUs. If the sum of TUs in tissues equals or exceeds 1.0, mortality would be expected (Di Toro et al. 2007). The target lipid model successfully predicts the lethality of PAC mixtures, although a correction factor is needed for the 'additional' toxicity of PACs compared to 'baseline' narcotics. Error limits on predictions are often wide, perhaps because some PACs have multiple MOAs and cannot be grouped as narcotics. As well, the model was developed from published reports of the measured toxicities of PACs, which can vary widely among labs because of different test methods (Section 4.1).

Incardona (2017) challenged the application of the target lipid model to chronic toxicity, particularly as a model for PAC-induced cardiotoxicity. He characterized narcosis as over-simplified because it does not account for PAC interactions with cellular receptors, ion channels and receptors governing membrane function. The relationships between fish embryotoxicity and log K_{OW} also vary considerably. A regression for closely-related PACs (alkyl-phenanthrenes; $r^2 \approx 0.94$; $n = 6$) (Turcotte et al. 2011) was less variable than one for diverse structures (alkyl anthracenes, phenanthrenes, chrysenes, benzo[a]anthracenes, plus C0-BaP; $r^2 \approx 0.73$; $n = 16$) (Hodson 2017). All data were collected in one laboratory using the same test method. Hence, the greater variance of the 4-family model may reflect multiple receptor interactions driven by the number, size and substitution patterns of alkyl side chains and the number and shape of fused benzene rings. Regardless of MOA, the target lipid model highlights the critical role of water-lipid partitioning in determining tissue dose, a primary driver of toxicity.

2.2.3 Toxic Equivalency Factors

The toxicity of mixtures of compounds can be described by a Toxic Equivalents (TEQs) model (Van den Berg et al. 1998). For each mixture component, a Toxic Equivalency Factor (TEF) is the ratio of its measured toxicity to that of a more widely tested reference compound, and its TEQ represents its TEF multiplied by its concentration in the mixture. When the sum of TEQs for all mixture components exceed the toxicity of the reference compound, toxicity should occur.

A fundamental assumption of TEQ models is that all components share a common MOA, (e.g., binding to the AHR) and that mixture interactions are additive, not antagonistic or synergistic. Although a common MOA may characterize dioxin-like compounds, Incardona (2017) concluded that many PACs act through AHR-independent mechanisms, causing a wide array of different effects (Wallace et al. 2020). The TEQ approach has been useful for assessing the potency of some PAC mixtures. For example, a TEF model successfully predicted the mammalian carcinogenicity of mixtures containing non-alkylated PAHs with ≥ 4 rings; the reference PAC was BaP (Nisbet and LaGoy 1992). However, the model over-estimated toxicity for mixtures of smaller PAHs that were less carcinogenic. Congeners with <4 rings also have a lower affinity for the AHR (Billiard et al. 2002). Although the TEF approach shows promise for PAHs, it must be refined to improve its predictive capacity and to deal with multiple MOAs and families of PACs.

2.2.4 Grouping PACs to assess risks of mixtures

In Canada, some ERAs have grouped PACs into classes to assess mixture toxicity because there are too few data or guidelines to evaluate each compound individually. For example, the ERA for a Teck Frontier oil sands mine grouped more than 50 PACs into nine categories for ERAs of water, sediment, and air (Teck 2015). Groups contained 3-11 PACs, including an indicator compound whose toxicity or guideline represented the entire group. Although grouping simplified ERAs, it likely underestimated mixture risks because all members of each group were assumed to have the same MOA and toxicity as the indicator PAC. For example, the chronic toxicity of anthracene represented a limited array of anthracenes/phenanthrenes, despite evidence that alkyl substitution increases phenanthrene toxicity (Hodson, 2017) and that C0-C4 petrogenic anthracenes/phenanthrenes likely include >1000 possible congeners. Although this approach might be attractive in the absence of data, the estimated risks will be highly uncertain, likely underestimated to an unknown degree, and an inadequate basis for decision-making.

2.2.5 Other approaches for assessing ecological risk

In vitro tests of AHR binding affinities have been used to assess the risks of dioxin-like toxicity to vertebrates. A rat hepatoma assay demonstrated additivity of AHR binding for mixtures of unsubstituted, alkylated, and oxygenated PACs (Lam et al. 2018). Although methylated PACs were often more potent than unsubstituted PACs, predictions of mixture potency supported

additivity, particularly with increasing numbers of components. For the *in vitro* SOS chromotest, the genotoxicity of complex mixtures of unsubstituted PACs was effectively additive at lower concentrations, but far less than additive at high concentrations (White 2002).

These *in vitro* results were partially consistent with P450 induction *in vivo*. Rainbow trout (*Oncorhynchus mykiss*) exposed to mixtures of potent inducers (e.g., benzo[k]fluoranthene; benzo[b]fluoranthene) showed additivity of induction, as did mixtures of weak inducers (e.g., C1-C3 phenanthrenes) (Basu et al. 2001). However, combinations of strong and weak inducers caused an 8- to 9-fold more-than-additive induction of P450s, high-lighting interactions among induction potency, P450 activity, and rates and products of PAC metabolism that would not be evident *in vitro*.

Overall, the interactions of different PAC structures with many molecular and physiological processes suggest that PAC mixture toxicity might best be described by combining models. Pharmacokinetic models (e.g., water-lipid partitioning; P450 metabolism; excretion) coupled with mechanistic models (interactions among PAC structures, receptor binding, and toxic effects) could improve risk predictions and highlight interactions that are poorly understood.

3 Species Sensitivity

The total number of species in global ecosystems is estimated at approximately 8,700,000 (Mora et al. 2011). In contrast, the number included in toxicity tests is likely much less than 0.01% of this total and the number used routinely is even smaller. The life cycle of each species may also include several life stages, each with its own environmental requirements, interactions with other species, and sensitivity to PAC toxicity. Biodiversity highlights a critical challenge for ERAs: environmental protection relies on a poorly-tested assumption that toxicity data for a few lab-tolerant species can adequately predict the risk for >8,700,000 species of unknown sensitivity.

There are many reports of PAC toxicity to marine and freshwater fish and invertebrates, yet few conclusions can be drawn about which taxa are consistently most sensitive. For example, BaP LC50 and EC50s for zooplankton ranged from 5 - 58 $\mu g\ L^{-1}$, whereas the range for phytoplankton was 1 - 4000 $\mu g\ L^{-1}$ (Behera et al. 2018). For an aquatic ecosystem in China, BaP acute LC50s for three invertebrate species ranged from 1 - 2 $\mu g\ L^{-1}$ compared to LC50s of 4 - 30 $\mu g\ L^{-1}$ for three fish and one amphibian species (Wu et al. 2016). In contrast, fish from the Canadian oil sands watershed were quite sensitive to PACs, in contrast to invertebrates

296 (*Hyalella*; mussel larvae; *Daphnia*) (McMaster et al., 2018a; Parrott et al. 2018). The toxicity of
297 PACs also vary considerably within taxa. For example, dibenzothiophene was embryotoxic to
298 zebrafish (Incardona et al. 2004) and Japanese medaka (*Oryzias latipes*, (Rhodes et al. 2005), but
299 non-toxic to zebrafish (Peddinghaus et al. 2012) and killifish (*Fundulus heteroclitus*)
300 (Wassenberg et al. 2005). The apparent contradiction for zebrafish suggests either differences in
301 dibenzothiophene sensitivity among populations of the same species or in test methods among
302 laboratories (Section 4.1).

303 For birds, few embryotoxicity data for PACs are available. However, several studies
304 suggest that large inter-species differences in sensitivity among other AHR ligands (e.g., dioxin-
305 like compounds) are not evident for PACs. For example, the embryotoxicity of
306 benzo[k]fluoranthene differed little among chicken (*Gallus gallus domesticus*), turkey
307 (*Meleagris gallopavo*), and domestic duck (*Anas platyrhynchos domesticus*), three species with
308 dramatically different sensitivities to dioxin-like compounds (Brunström et al. 1990; Franci et al.
309 2018). Similarly, Louisiana crude oil applied directly onto chicken and mallard eggshells caused
310 equivalent embryo lethality to both species (Hoffman 1978), suggesting an equal sensitivity to
311 petrogenic PACs.

312 Variations among species in the nature of responses to single PAC or PAC mixtures may
313 reflect their unique genetics, developmental stage, physiology, life histories, and habitat. For
314 insects and amphibians, there are tectonic changes in these characteristics as one life stage
315 transitions to the next, with each uniquely susceptible to PAC exposure and toxicity. For all
316 species, embryonic stages appear particularly sensitive to PAC, likely due to the many target
317 genes that are expressed (or suppressed) during critical developmental processes. Sensitivity may
318 also reflect changes in PAC exposures. Red sea bream (*Pagrosomus major*) larvae were more
319 sensitive than embryos to BaP, phenanthrene, or pyrene exposures, possibly the protective
320 chorion is lost at hatch (Zhao et al. 2017). Species that develop slowly may be more sensitive
321 than those developing quickly (Raine et al. 2018) due to longer exposure times. For example,
322 early-season copepods (*Calanus glacialis*) and larval sculpin (*Myoxocephalus sp.*) were more
323 sensitive to dispersed crude oil than more rapidly developing late-season copepods and juvenile
324 Arctic cod (*Boreogadus saida*) (Gardiner et al. 2013).

Species sensitivity to PACs is also determined by exposure route. The toxicity of oil to zebrafish embryos is a function of dissolved PAC concentrations in test solutions, not the oil droplets introduced during oil-water mixing (Carls et al. 2008). In contrast, the accumulation of oil droplets on the chorions of Atlantic haddock (*Melanogrammus aeglefinus*) eggs likely increased their exposure to PACs compared to Atlantic cod (*Gadus morhua*) eggs that did not accumulate droplets (Sørensen et al. 2017). Bird and mammal embryos can also be exposed to high concentrations of PACs *in ovo* or *in utero* by maternal transfer (Ramesh & Archibong 2011). However, in harp seals (*Pagophilus groenlandicus*) from the Northwest Atlantic, PAC concentrations were lowest in foetuses, highest in juveniles, and intermediate in mature adults (Hellou et al. 1991).

3.1 Species Sensitivity Distributions

In the absence of toxicity data for all species in an ecosystem, species sensitivity distributions (SSDs) are often used to predict chemical concentrations that are toxic or hazardous to the 5th percentile of tested species (HC_5). This threshold is somewhat arbitrary, but statistically supportable for a limited array of species (Bejarano & Mearns 2015). The critical assumption is that SSDs for a few species represent the range of sensitivity among all. To construct an SSD, species are ranked by their sensitivity to a chemical; the most sensitive (e.g., lowest EC50) is ranked first. Ranks are converted to proportions that are compared to measured EC50s, and the concentration toxic to the 5th percentile species is calculated by regression analysis. The SSDs can be applied within taxa (e.g., fish species) or more broadly among taxa (Bejarano & Mearns 2015).

Species sensitivity distributions can improve ERAs for PACs, but few have been published due to a paucity of toxicity data (Section 2), although databases can be augmented by toxicity values estimated from interspecies correlations (Dyer et al. 2008). For example, BaP water quality guidelines in China were developed from SSDs bolstered by LC50s and EC50s for eight Chinese species computed from interspecies correlation models (Wu et al. 2016). For the present review, two SSD plots were constructed to illustrate how sensitivity to waterborne BaP or Cold Lake Blend diluted bitumen (dilbit; contains a mixture of PACs) varies among taxa (Figure 1; Table SI-2). Benzo[a]pyrene is among the most tested PAC for international regulations and dilbit is the most studied source of petrogenic PACs in Canada. Invertebrates and fish from

different habitats are well represented in these SSDs, but only two amphibian species were included, and data for species exposed to BaP or dilbit in non-aqueous media were not included.

The predicted HC_5 for BaP was 0.55 µg L^{-1}, similar to the values of 0.39 and 0.51 µg L^{-1} reported by Wu et al. (2016). These concentrations are 25 - 37-fold higher than the 2015 guideline of 0.015 µg L^{-1} for the protection of freshwater life recommended by the Canadian Council of Ministers of the Environment (CCME 1999). The guideline was calculated with a correction factor of 0.01 applied to the lowest LC50 found in a literature review, i.e., 1.5 µg L^{-1}. There is no CCME guideline for petrogenic total PAC concentrations.

Figure 1a demonstrates that LC50s for BaP vary over 10,000-fold among taxa, although no taxon is consistently more sensitive than the others. The variations among LC50s generated a relatively wide confidence interval about the HC_5 of 0.55 µg L^{-1}, perhaps due to species characteristics such as life stage tested (Table SI-2). However, the wide interval more likely reflects the diversity among publications of test methods that influence the composition, stability and toxicity of PAC solutions (Section 4.1). The reported LC50s for 14 of 20 species (70%) in Figure 1a exceeded the BaP solubility limit of 4 µg L^{-1} (Pearlman et al. 1984), creating considerable uncertainty about the validity of the regression and the estimated HC_5. While informative, the computed HC_5 and CCME Guideline are insufficient for ERAs of BaP or complex mixtures containing BaP.

Given the instability of oil solutions, dilbit toxicity should also be highly variable. However, the predicted HC_5, expressed as the sum of all measured PACs, was 12.1 µg/L with a coefficient of variation (cv; 13%) 6.5-fold lower than for BaP (Figure 1b). Most data for the dilbit SSD were from a single source (Barron et al. 2018) that applied standard methods for preparing, testing and characterizing solutions of dilbit. Thus, the most precise and useful SSDs will be derived by consistently applying standard test methods in one laboratory and calculating toxicity from measured concentrations of PACs in test solutions, not applied concentrations (Hodson et al. 2019).

3.2 Adaptations to PAC exposure

Sensitivity to PACs varies not only among taxa, but also among conspecific populations. Adaptive and non-adaptive responses to long-term exposure may change phenotypic expression, giving exposed populations a broader range of tolerance (Wallace et al. 2020). Estimates of PAC

toxicity depend on the source population and the degree to which individuals have accommodated PAC exposures through physiological, epigenetic, or genetic adaptations. Toxicity data from tolerant populations would bias ERAs of the toxicity of PACs to non-adapted populations.

The metabolism of PACs by P450 enzymes plays an important role in modifying toxicity. During ongoing dietary exposures of areolate grouper (*Epinephelus areolatus*) to 12.5 $\mu g\ g^{-1}$ BaP, hepatic P450 activity peaked in week 2, then declined to background by week 4. The apparent resistance likely reflected the activation of detoxification/depuration mechanisms to restore homeostasis, reducing or even eliminating PAC toxicity (Wu et al. 2003). However, PAC metabolism can also increase toxicity if metabolites are more toxic than the parent compound, as observed for trout embryos exposed to retene (Hodson et al. 2007). Although some PACs do not induce P450 enzymes, all may be metabolized when present in mixtures that include inducing compounds.

Non-heritable adaptations to PACs may still translate across at least one generation. Killifish sampled from the PAC-contaminated Elizabeth River (VA, USA) were less sensitive to P450-inducing compounds than reference fish (Meyer et al. 2002). Resistance extended to the F1 generation, but was lost in F2 and F3 generations, indicating a non-genetic basis for resistance. Conversely, F1 and F2 generations from resistant killifish had lower rates of teratogenesis following exposure to benzo[k]fluoranthene or fluoranthene than those bred from reference fish (Clark et al. 2014), suggesting an inherited response. Despite large differences in PAC toxicity between F1 embryos from resistant and reference populations, the differences involved the regulation of comparatively few genes (Bozinovic et al. 2013). The mechanisms of inherited resistance to PACs also differed among phenotypic traits. Prolonged exposure to PACs may favour certain genotypes, altering population gene frequencies and reducing the sensitivity of exposed populations to chemical stressors.

Compared to fish from non-contaminated sites, Atlantic tomcod (*Microgadus tomcod*) from the highly contaminated Hudson River (USA) were resistant to toxicity and P450 induction by dioxin-like compounds, but not by PACs. Wirgin et al. (2011) identified heritable genomic alterations in the AHR2 receptor of Hudson River tomcod, suggesting a rapid evolution in response to selective pressure from dioxin-like compounds. The number of populations that have

adapted to PAC exposure, through either a permanent change to population gene frequencies or more transient epigenetic responses, is unknown.

3.3 Standard vs 'non-standard' test species

Most regulatory and research tests of PAC toxicity involve a few standard species (e.g., Table SI-2). These species are easily obtained, easily cultured in large numbers, and of a size and behaviour that suits laboratory culture and standard test protocols (e.g., EC 2007; Busquet et al. 2013). However, their selection for testing bears no relationship to their chemical sensitivity which can vary markedly among species and compounds tested.

Non-standard species are tested less frequently because they are available only seasonally or may be difficult and expensive to culture and test. Among 39 subarctic Alaskan species of marine fish and invertebrates exposed to crude and refined oils, there were significant challenges in collecting early life stages; many species were available as gametes or embryos for only brief periods. Within these limitations, the most sensitive species were shrimp and pelagic fish embryos (Rice et al. 2005). Nevertheless, studies with non-standard species are highly valued because their adaptations to different environments (e.g., pelagic vs benthic) may influence their sensitivity to PAC exposure. The geographic overlap of the sources and distributions of PACs and the distribution of highly valued species should dictate priorities for test species.

4 Other challenges in applying PAC toxicity data to ERAs

An assumption inherent in many ERAs is that estimates of PAC toxicity are absolute and unaffected by environmental conditions. However, the opposite is true: PAC exposures and toxicity not fixed but depend highly on test methods, interactions with other chemicals, and the environmental conditions of lab tests and the ecosystem where risk is being assessed.

4.1 Test methods and data quality

Predictions of the ecological risks of PACs are only as good as the toxicity data applied in the assessment. Much of the variance in measured toxicity is derived from test methods, including test conditions, preparation of test solutions, and the extent to which non-steady state exposures are recognized and characterized, especially for aquatic tests (Bejarano et al. 2014; Hodson et al. 2019). Most PACs are relatively insoluble in water (log K_{OW} ranges from <4 to >7) and typically added to test solutions in miscible solvents, creating a potential for PAC-solvent interactive

toxicity. Hydrophobic PACs disappear rapidly from aqueous solutions due to uptake by test organisms, partitioning to test containers and particulates, evaporation, biodegradation and photolysis (Section 4.4) (Peddinghaus et al. 2012; Hodson et al. 2019). These issues are particularly critical when test solutions are not renewed (static protocols) or renewed periodically (e.g., static with daily renewal). Concentrations of PACs can decline to non-detectable within hours or days, especially when initial concentrations exceed solubility limits. Under these conditions, concentrations that best represent the actual exposures of test organisms are unknown and end points calculated from added (nominal) concentrations under-estimate toxicity and bias subsequent ERAs (Hodson et al. 2019) (e.g., Section 3.1).

One solution to this dilemma is passive dosing, i.e., the partition-controlled delivery of PACs to aqueous solutions from solid substrates, such as polydimethylsiloxane films loaded with the test chemical. When partitioning between polydimethylsiloxane and water reaches an equilibrium, aqueous concentrations remain constant, providing stable gradients of exposure within the solubility limits of each PAC and reliable estimates of toxicity (e.g., Kiparissis et al. 2003; Turcotte et al. 2011; Lin et al. 2015; Butler et al. 2016; Jahnke et al. 2016;).

Another strategy is to calculate PAC toxicity from tissue dose (e.g., LD50s), although measuring dose is technically challenging for small organisms (e.g., embryos). However, dose can be estimated from K_{OW} with pharmacokinetic models, or by passive samplers added to test solutions as surrogates of bioaccumulation (biomimetic extraction; McConville et al. 2018). Passive samplers integrate time-varying concentrations of PACs in water, but not those associated with particulates or oil droplets that bias chemical analyses of test solutions. In 96-h lethality tests, passive samplers demonstrated that sablefish (*Anoploma fimbria*; deep sea species) are among the most sensitive of marine species to Alaska North Slope Crude oil, toluene, 2-methylnaphthalene and phenanthrene (McConville et al. 2018). In any aquatic test of PAC toxicity, it is essential that PAC concentrations be measured in test solutions to characterize variations in PAC exposures and incorporate them into calculated toxicity (Hodson et al. 2019).

Test methods for non-aquatic species raise related concerns. Although tests of PAC toxicity to bird embryos avoid issues of water solubility, it is difficult to define exposures (dose) because birds metabolize PACs rapidly. For example, 94% of a mixture of PACs injected into the yolk of chicken eggs on embryonic day four was metabolized within two weeks (Näf et al.

1992), indicating a non-steady-state exposure. Individual embryos vary substantially in their capacity for P450 induction and PAC metabolism (Head & Kennedy 2019), increasing the variance of measured doses in wild birds and their apparent sensitivity to PAC exposure.

The two main routes of bird embryo exposure are maternal transfer during egg formation, and external contamination (e.g., by transfer of oil from contaminated feathers of parents). These routes can be modelled by injecting PACs directly into eggs or by applying oil droplets to eggshells (Albers 2006). Both routes are environmentally realistic, although the injection site (air cell, yolk, or albumen) can generate widely different results (Heinz et al. 2006). Factors such as the developmental stage of embryos when injected, the carrier solvents used, and injection location also complicate data interpretation (Heinz et al. 2006; Henshel et al. 1997). In general, embryos were more sensitive to PACs injected through the air cell than into the yolk, possibly because the air cell interacts with the chorioallantoic membrane (Albers 2006). This vascularized membrane could modulate embryo exposure by metabolizing PACs to compounds that differ in toxicity from the parent compound (Granberg et al. 2003). For PAC mixtures applied to eggs, the fraction that crosses the shell and membrane varies with the nature of the mixture (e.g., solvent type; weathered vs unweathered oil), increasing the variability of measured toxicity (Hoffman & Gay 1981; Albers 2006). Absorption of PACs from the yolk represents a more natural exposure route, although yolk may dilute reduce PAC exposures (Henshel et al. 1997) unless the test period incudes yolk absorption. Hence, interactions between exposure methods and the physiology of eggs must be considered when comparing sensitivity among PACs for ERAs.

4.2 Interactions of PACs with other contaminants

In ecosystems near urban, industrial and agricultural development, PAC emissions will inevitably be mixed with other contaminants, including pulp mill and mining effluents, pesticides from agriculture or forestry, and components of sanitary and storm sewage, such as pharmaceuticals. The toxicity of PACs in binary mixtures with other contaminants such as metals may be synergistic or antagonistic, depending on the specific PACs and metals (Fleeger et al. 2007; Wang et al. 2008). A review of metal-PAC interactions found additivity in 50% of cases or fewer, and synergism was quite common (Table 2), particularly when one mixture component occurred at non-toxic concentrations (Gauthier et al. 2014). The mechanisms

underlying additivity were complex and numerous, including direct competition for receptor sites and effects on membrane structure and function. Indirect mechanisms included interactions with the pharmacokinetics and disposition of test compounds or metabolites, the generation of reactive intermediates (e.g., oxyradicals), and the expression of genes responding to oxidative stress. For example, metals induce the hypoxia inducible factor HIF-α which modulates AHR signaling or inhibits P450 enzymes by binding to active sites; both mechanisms would slow the depuration of PACs and prolong their toxic actions. Inhibiting P450 enzymes could shift PAC metabolism to less efficient pathways with different metabolites having different effects and toxicity (Hodson et al. 2007). Both metals and PACs inhibit key cell functions such as adenosine triphosphate ion pumps, suggesting that combined MOAs influence toxicity. Mixtures of arsenic and BaP did not affect P450 enzyme activity of mouse hepatoma Hepa-1 cells but increased the levels of DNA adducts by disrupting glutathione homeostasis (Maier et al. 2002). These MOAs may be unique to each PAC or metal, so that predicting interactions in real-world mixtures is quite complex (Gauthier et al. 2014).

The toxicity of PACs is also influenced by organic compounds with similar MOAs. A mixture of BaP and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) caused a dose-dependent increase in toxicity to Pacific oyster (*Crassostrea gigas*) embryos. However, the mixture was not as potent as either contaminant separately, indicating a less-than-additive toxicity (Xie et al. 2017). Compared to either compound alone, mixtures of benzo[k]fluoranthene and 3,3',4,4',5-pentachlorobiphenyl (PCB-127) injected into chicken embryos increased mortality additively. However, the mixture did not induce more P450 activity than the highly potent PCB, suggesting a maximum capacity for induction (Brunström et al. 1990; Brunström 1992) or that PCB-127 out-competed benzo[k]fluoranthene for AHR binding. Exposures of turbot (*Scophthalmus maximus*) to both BaP and PCB-77 induced the formation and persistence of BaP diol epoxide metabolites; these adducts were not present when fish were exposed only to BaP (Gunawickrama et al. 2008).

4.3 PAC toxicity and environmental stressors

Each ecosystem does not represent a single set of environmental conditions. They encompass mosaics and gradients of conditions that determine the presence, abundance and distribution of species, the distribution, fate and effects of PACs, and subsequent ecological effects. Each

534 species is also subject to physiological stress due to seasonal environmental extremes (e.g.,
535 temperature; food availability; predator-prey interactions), and seasonal cycles of sexual
536 maturation, reproduction, and migration. The disparity between toxicity measured under standard
537 conditions and under actual environmental conditions is largely unknown and creates significant
538 uncertainties in estimates of ecological risk. Thus, it is not surprising that ERAs of PACs present
539 far greater challenges than human health risk assessments, which focus on one species living in
540 relatively controlled environments.

541 Except for hypoxia and temperature, there are few studies of the interactions between
542 PAC toxicity and environmental variables. Hypoxia alone had no effect on pericardial edema in
543 zebrafish embryos co-exposed to β-naphthoflavone or BaP, despite inhibiting P450 activity by
544 up to 60%. In contrast, fluoranthene or α-naphthoflavone caused severe edema and spinal
545 curvatures when combined with moderate hypoxia, even though these PACs are not typically
546 embryotoxic (Matson et al. 2008). Embryotoxicity to sheepshead minnow (*Cyprinodon*
547 *variegatus v.*) was also enhanced following co-exposure to hypoxia and oil from the Deepwater
548 Horizon spill (Hedgpeth & Griffitt 2016).

549 Many species are subjected to extremes of temperature, either seasonally or during
550 migrations, but no studies were found of PAC toxicity at the limits of a species' temperature
551 tolerance. However, for rainbow trout, no change in retene toxicity was observed within the
552 normal temperature range for larval development (5 to 11 °C) (Honkanen et al. 2020). Toxicity
553 was evident only when larvae reached swim-up, i.e, when they were ready to feed. At 11 °C,
554 swim-up and higher rates of mortality and deformities occurred after 16-18 d exposure. At 5 °C,
555 larvae showed the same signs of toxicity and identical EC50s only after 32 d exposure. The
556 difference in time-to-toxicity was eliminated by expressing exposure times as thermal units or
557 'degree-days', a critical factor determining larval development rates. Thus, temperature affected
558 both the rates of larval development and the timing of retene toxicity, but not the concentrations
559 causing toxicity. For ERAs of aquatic ecosystems with variable temperatures, the effects of
560 PACs on any aquatic species should be assessed at its limits of thermal tolerance as well as
561 within its optimal range. At lower temperatures, it is essential to prolong exposures to
562 encompass all sensitive developmental stages to ensure that exposure times are sufficient to
563 express toxicity.

Overall, a critical research need is to assess the physical and chemical characteristics of each ecosystem that determine the form, distribution and concentration of each PAC. These factors must be measured and included in models of their environmental fate and effects.

4.4 Phototoxicity of PACs

The composition and concentration of PAC mixtures derived from oil spills or industrial emissions change rapidly as weathering by evaporation, dissolution, and adsorption to particulates removes lighter or more hydrophobic components. In air, water or on surfaces, PACs can also be degraded (photolysis) by the interactions of ultraviolet (UV) light, particularly UVA (wavelengths of 320-400 nm), with the double bonds of benzene rings to release excited state electrons.

Photolysis will reduce the exposure of biota to PACs if the photoproducts are diluted in air or water before they are bioaccumulated. The photolysis of airborne PACs depends on day length, light intensity (time of year, cloud cover) and sun angle as it reflects off the water's surface (Barron 2017). At any latitude, the risk of phototoxicity is greatest in the summer when light intensities and the angle of incidence are highest. In water, the maximum depth of UVA penetration with sufficient intensity to cause photolysis ranges from <1 m (turbid waters) to 78 m (ultra-clear alpine lakes); penetration depths in the ocean range from 8 to 37 m (Roberts et al. 2017). In many Canadian lakes, UV penetration is limited by humic substances (Weinstein & Oris 1999) and turbidity caused by suspended solids. Under these conditions, PACs will not be photodegraded and may ultimately be transported to sediments.

In contrast, PACs that have been bioaccumulated by semi-transparent biota cause tissue necrosis and rapid mortality (phototoxicity) when photolysis occurs *in vivo* (Vehniainen et al. 2003; Barron 2017). Cell death and necrosis occur when reactive products, including excited-state PACs or singlet oxygen, interact with double bonds to cause lipid peroxidation, protein denaturation, and DNA damage (Barron 2017; Roberts et al. 2017). The severity of phototoxicity varies with tissue PAC concentrations and UV exposure; low concentrations and high UV intensities cause the same toxicity as high concentrations and low UV intensities (Roberts et al. 2017). Unpigmented early life stages of inland silverside (*Menidia beryllina*), sheepshead minnow, Gulf killifish (*Fundulus grandis*) and mysid shrimp (*Americamysis bahia*) showed enhanced sensitivity to fluoranthene under UV light (Finch & Stubblefield 2016). In

fact, most zooplankton species are highly sensitive to phototoxicity (Roberts et al. 2017). Larvae of Lahontan redside minnow (*Richardsonius egregious*) were more heavily pigmented and less affected by exposure to ambient levels of UV light or UV light plus fluoranthene than bluegill larvae (*Lepomis macrochirus*). Nevertheless, for both species, mortality due to UV light was significantly enhanced by co-exposure to 0.05 µg L^{-1} of fluoranthene, a component of motorboat exhaust (Gevertz et al. 2012).

Those PACs most susceptible to photolysis include 3-5-ringed compounds and their alkylated congeners (Huovinen et al. 2001; Hakkinen et al. 2003; Roberts et al. 2017). Phototoxicity to fish embryos is associated with the 3- to 5-ringed PACs derived from crude oils, middle distillates, and heavy fuel oils (Barron 2017; Hatlen et al. 2010), likely due to their higher content of PACs. Aliphatics, mono- and di-aromatics, and asphaltenes are not considered phototoxic (Barron 2017). Thus, refined fuels low in PACs would not be phototoxic, in contrast to pyrogenic (Gevertz et al. 2012) or petrogenic PACs (Barron 2017). For 3-d-old mysid shrimp, phototoxicity increased with increasing PAC methylation (Finch et al. 2017), and the acute lethality of a mixture of three phototoxic PACs (fluoranthene, pyrene, anthracene) to mysid shrimp and inland silverside minnows was additive (Finch & Stubblefield 2019). Rates of mortality and malformations of olive flounder (*Paralichthys olivaceus*) embryos exposed for five days under natural UV light to seawater contaminated by a diesel spill increased with concentrations of polar compounds from photooxidation (Kim et al. 2019). However, UV alone can induce P450 and heat shock proteins in whitefish (*Coregonus lavaretus*), but not in vendace (*Coregonus albula*) (Vehniainen et al. 2003), perhaps due to photolysis of endogenous compounds that resemble PACs.

Many aquatic species are protected from phototoxicity by habitat selection (deep water benthic species) and avoidance (diurnal vertical migrations; sheltering in shaded areas) (Roberts et al. 2017). Nevertheless, by virtue of their habitat, many aquatic species (>30) are sensitive to environmentally realistic concentrations of PACs and UV intensities (Gevertz & Oris 2014; Alloy et al. 2016; Alloy et al. 2017). For example, the mass mortality of Pacific herring embryos (*Clupea pallasii*) spawned in an intertidal zone of San Francisco Bay (USA) followed exposure to a heavy fuel oil spill and UV light at low tide (Incardona et al. 2011).

623 Amphibians are also affected by phototoxicity. Northern leopard frog (*Lithobates pipiens*)
624 larvae experienced phototoxicity just after hatch when exposed to fluoranthene and natural
625 sunlight (Hatch & Burton 1998) or full intensity UV light (Monson et al. 1999). The mortality of
626 common frog embryos (*Rana temporaria*) exposed to UV light and a mixture of naphthalene,
627 phenanthrene, and pyrene increased slightly when the jelly that surrounds the eggs was removed,
628 suggesting it limits exposures to PACs and UV radiation (Marquis et al. 2006). Common frog
629 tadpoles were unaffected by UVB alone or BaP alone, but a combination of UVB and 250 - 500
630 $\mu g\ L^{-1}$ BaP was acutely lethal within 48 h (Marquis et al. 2009). The differences among species
631 demonstrate the need to recognize unique aspects of the biology of each species when assessing
632 PAC toxicity.

633 **5 Field studies of PAC exposures and effect**

634 Despite many reports of PAC toxicity to plants and animals, it is difficult to associate cause and
635 effect in field studies of contaminated sites, which questions whether ERAs of PAC exposure
636 can be validated. It is even more challenging to link PAC exposure and toxic effects to changes
637 in population structure. Although uncommon, field experiments are particularly valuable when
638 some environmental variables can be eliminated or controlled. For example, whitefish
639 (*Coregonus clupeaformis*) eggs held in cages on heavy fuel oil-contaminated sediments in
640 Wabamun Lake (AB, Canada) showed a higher prevalence and severity of deformities when
641 hatched than eggs held on reference sediments within the same lake. The connection between
642 PAC exposures and effects was established through multiple passive samplers installed adjacent
643 to egg cages (Debruyn et al. 2007).

644 Case studies that do link cause and effect are highly valued, and three are presented below as
645 evidence of PAC toxicity under conditions highly relevant to Canadian aquatic ecosystems. The
646 first reviews the response of brown bullhead (*Ameiurus nebulosus*), a benthic fish that feeds on
647 sediment invertebrates, to PACs in sediments of Great Lakes tributaries contaminated by steel
648 mill effluents. This case is highly relevant to Canada because steel mills have historically
649 discharged effluents to the St. Mary's River at Sault Ste Marie (between Lakes Superior and
650 Huron) and Hamilton Harbour (L. Ontario). The second summarizes research on the effects of
651 the Exxon Valdez oil spill on Pacific herring and pink salmon (*Oncorhynchus gorbuscha*) in
652 Prince William Sound AK, a marine area similar to coastal British Columbia. The third

describes the impacts of bitumen mining and processing on fish species in the Athabasca River watershed (AB, Canada).

5.1 Case Study 1: PAC-induced cancers in brown bullhead

Some of the earliest reports of PAC effects on fish focussed on the carcinogenicity of pyrogenic PACs in freshwater and marine sediments contaminated by industrial emissions. To assess the strength of proposed cause-effect relationships, Rafferty et al. (2009) reviewed 18 studies comparing the prevalence of pre-cancerous and cancerous lesions to PAC concentrations in sediments of tributaries to Lake Erie. At sites contaminated by steel mills, bullhead exhibited external (skin, lips, barbels) and internal (liver, bile duct) tumour-like lesions, and measurable concentrations of PACs and PAC metabolites in tissue and bile. Liver lesions ranged from cellular changes typical of pre-neoplastic tumours to liver and bile duct carcinomas. Their prevalence and severity increased with fish age, typical of PAC toxicity. The associations between skin lesions and PAC exposures were not as clear cut as for liver cancer. Non-cancerous tumours were associated with infections (bacteria, parasites, viruses), environmental stressors (temperature, organic enrichment), complex mixtures of other contaminants, and population demographics (age, size, sex, sexual maturation) (Rafferty et al. 2009).

Cause-effect associations were reinforced experimentally by pre-neoplastic lesions in bullhead fed laboratory diets containing extracts of PAC-contaminated sediments (Rafferty et al. 2009). However, some associations were weakened because PACs co-occurred with raw or treated sewage containing pharmaceuticals and metals (e.g., As, Cr) as well as potent P450 inducers (e.g., dioxin-like compounds) that could accelerate the oxygenation of PACs to carcinogenic metabolites. The plausibility of associations was strengthened by species differences in the inducibility of P450 enzymes and the prevalence of cancer. For closely-related channel catfish (*Ictalurus punctatus*), there was less induction of P450 and epoxide hydrolase enzymes, more resistance to oxidative stress, fewer BaP proximate carcinogens (e.g., BaP-7,8-dihydrodiol), and a lower prevalence of cancer than in bullheads (Rafferty et al. 2009).

Some correlations between PAC contamination and cancer prevalence were weakened by flaws in survey designs related to characterizing chemical exposures, confirming cancers histologically, identifying or eliminating alternative causes, and controlling for confounding factors. When Rafferty et al. (2009) applied seven epidemiological criteria for causality to 18

683 published studies of cancer in bullheads, the case for a chemical cause of skin neoplasms was
684 relatively weak, but the case for liver neoplasms was strong. The evidence was strongest for
685 biological and technical plausibility and strength of association, moderate for consistency of
686 association, temporal sequence, dose-response and experimental evidence, and weakest for
687 specificity of the relationship. The weak specificity was not surprising given the number of
688 chemicals in contaminated sediments and the small proportion tested for carcinogenicity to fish.

689 While the association between cancer and PAC contamination was plausible, the
690 ecological consequences were less well understood. A multi-year study of bullhead exposed to
691 PAC-contaminated sediments in the Black River, OH (USA) near steel and coking mills
692 demonstrated a high prevalence of liver cancer. Cancer rates declined after the mills closed,
693 increased again when dredging re-distributed contaminated sediments, and declined thereafter to
694 rates typical of reference sites (Baumann & Harshbarger 1995). In all years, the prevalence and
695 severity of liver cancer increased with age; when the mills were operating, the age-distribution of
696 bullheads was truncated, with few fish older than 6+ years (Baumann & Harshbarger 1998).
697 Following the closure, the number of older fish increased, suggesting lower rates of cancer-
698 induced mortality. Nevertheless, even when cancer was most prevalent, bullhead survived past
699 the age of first reproduction. Unfortunately, these studies did not assess critical indicators of
700 ecological effects such as abundance, the role of immigration from uncontaminated areas in
701 sustaining abundance, or changes in the fish community structure.

702 **5.2 Case Study 2: Herring and salmon embryotoxicity from an oil spill**

703 The 1989 Exxon Valdez Oil Spill (EVOS) in Prince William Sound, AK created a new and
704 broader understanding of the effects of oil spills on fish reproduction. The new insights
705 included a recognition of the long-term persistence of oil and its effects on aquatic species, and
706 the great sensitivity of fish embryos to petrogenic PACs (Rice et al. 2001; Rice 2009). Prior to
707 the EVOS, studies of oil spills were focused on the acutely lethality of low molecular weight
708 (<12 carbons) alkanes and mono- and diaromatic compounds. Their partitioning from oil to
709 water could create acutely lethal concentrations of hydrocarbons that act additively (Di Toro et
710 al. 2007). However, the rapid spreading of oil and evaporation of low molecular weight
711 compounds limited the risk of lethality to a matter of hours or days. For the EVOS, 20% of the
712 oil was lost quickly by weathering, enriching the concentrations of residual 3-5-ringed PACs,

components that are chronically toxic to fish embryos (Rice et al. 2001; Adams et al. 2014). While juvenile and adult fish might avoid oil exposure, embryos of many species are sessile, and buried in sediments or attached to substrates contaminated by stranded oil. Embryos also occur at high densities; when large numbers are exposed at once, toxicity can impair recruitment and the abundance of adult fish.

Following the EVOS, research focused on the embryos of Pacific herring that spawned on submerged vegetation at the time of the spill, and of pink salmon which had already spawned in tributaries crossing the shorelines of Prince William Sound. Waterborne concentrations of PACs where herring spawned and in contaminated tributaries supporting salmon embryos corresponded to concentrations causing toxicity in laboratory studies (0.4 to 1.0 $\mu g\ L^{-1}$ total PAC). These concentrations could explain the missing 1989 year-class of herring at recruitment time and elevated mortality rates of salmon embryos for several years following the spill (Rice 2010). However, demonstrating PAC effects on the subsequent abundance of these species was challenging (Rice et al. 2001). Field studies to connect cause and effect were hindered by difficulties in measuring embryo exposures due to complex exposure pathways. Field sampling and *in situ* experiments were complicated by multiple biological and environmental factors that interact with PAC toxicity (see above), and the need for very large surveys to generate statistical power in highly variable environments.

In the years following the EVOS, ecological damage from the oil spill was attributed to the collapse of the herring population followed by a lower abundance of their predators, the stellar sea lion (*Eumatopius jubatus*) (Thorne & Thomas 2008). However, links between oil exposure and PAC effects on herring were obscured by an epizootic of viral haemorrhagic septicemia. This disease was attributed to an over-population of herring, a reduced food supply in previous years, the poor condition of adults, and an increased susceptibility to disease (Pearson et al. 1999). The counter argument that the EVOS did not affect herring was strengthened by population declines in other populations due to over-fishing and environmental stress caused by changes in ocean temperature and salinity. A more recent analysis concluded that herring decline was not associated with mass mortalities that typify an epizootic (Thorne & Thomas 2008). Instead, the decline occurred over five years, consistent with long-term effects on adult herring of floating oil encountered when surfacing at night to gulp air, a unique behaviour of herring.

744 For pink salmon, PAC concentrations in spawning shoals of streams that crossed oil-
745 contaminated beaches were sufficient to induce cytochrome P450 enzymes in caged fry,
746 demonstrating the multi-year bioavailability of PACs (Rice et al. 2001). Not surprisingly, these
747 PAC exposures were sufficient to cause embryotoxicity (Carls et al. 2003). Delayed effects on
748 salmon populations were also possible. When embryos that survived experimental exposures to
749 low oil concentrations were released to the ocean to feed and mature, there was a 15% decrease
750 in marine survival when the adults returned to spawn (Heintz et al. 2000). Nevertheless, the
751 effects of the EVOS on the numbers of adult salmon returning to Prince William Sound was
752 obscured by the large-scale release and good survival of hatchery-reared salmon (Rice 2009).

753 Applying the epidemiological criteria for plausibility (Rafferty et al. 2009), the case for
754 impacts of petrogenic PACs on pink salmon fisheries appears strongest for biological and
755 technical plausibility, dose-response, experimental evidence, and specificity of the relationship.
756 However, it was weaker for field studies of temporal sequence, strength of association, and
757 consistency of association. The case for PAC impacts on the herring fishery was similar to that
758 for pink salmon except that the specificity of the relationship was weaker. In both cases, a focus
759 on PACs may have over-looked the potential effects of other constituents of oil (Meador &
760 Nahrgang 2019).

761 **5.3 Case Study 3: PAC mixtures from the Alberta oil sands**

762 The oil sands of northern Alberta, Canada, present an important opportunity to understand the
763 effects of petrogenic PACs in freshwater environments. As with crude oil, PACs from oil sands
764 are diverse; most are alkylated, with only a small percentage unsubstituted (Wang et al. 2014). In
765 contrast to oil spills, PACs in the oil sands area occur naturally. Aquatic organisms are exposed
766 to PACs in river sediments containing eroded bitumen (Headley et al. 2001; Conly et al. 2002;
767 Akre et al. 2004; Glozier et al. 2018) and in some areas to waterborne PACs derived from
768 bitumen mining and processing (Tetreault et al. 2003; Colavecchia et al. 2004; Colavecchia et al.
769 2006; Evans et al. 2016; Droppo et al. 2019; Evans et al. 2019). Industrial sources of oil sands
770 PACs in abiotic matrices can be discriminated from natural sources by the spectrum of PACs
771 measured (Culp et al. 2018; McMaster et al. 2018a; McMaster et al. 2018b). However,
772 discriminating between these sources in biota is more difficult due to PAC metabolism and
773 excretion.

774 Forage fish in tributaries of the Athabasca River accumulated tissue concentrations of 100
775 to 280 ng g^{-1} (ww) total PACs; of 44 PACs measured, naphthalene and alkylated naphthalenes,
776 fluorenes and phenanthrenes were most concentrated (Evans et al. 2019). At reference sites, PAC
777 concentrations in slimy sculpin (*Cottus cognatus*) were highly variable despite low
778 concentrations of sediment and waterborne PACs. Monitoring fish health provides reliable
779 baselines for tracking future impacts of the growing oil sands industries (McMaster et al.,
780 2018b). Responses to PAC exposures of sculpin sampled near oil sands mines included larger
781 livers, smaller gonads, and higher P450 activities than at upstream reference sites.

782 Tailings ponds contain oil sand process water that can slowly leach into groundwater and
783 rivers (Ferguson et al. 2009; Fennell & Arciszewski 2019). It is a complex mixture of PACs,
784 naphthenic acids, heavy metals, and dissolved ions that impairs fish and invertebrate
785 development and reproduction, similar to the effects of PACs (Li et al. 2017). In general,
786 concentrations of hydrophobic PACs in tailings pond leachates are low, perhaps because they are
787 sorbed to solids and trapped in pond sediments or filtered out by fine substrates as the leachates
788 pass through (Ferguson et al. 2009; Frank et al. 2014; Roy et al. 2016).

789 The mining and processing of bitumen also create PAC-contaminated dusts and air
790 emissions that enter aquatic and terrestrial ecosystems directly or via contaminated rain and
791 snowfall (Kelly et al. 2009; Kurek et al. 2013). Air, snow, and lake sediments from the oil sands
792 region contain many PACs not typically measured in analyses of the 16 conventional PAHs and
793 35 alkyl PAHs (Manzano et al. 2016; Manzano et al. 2017). These include PACs containing Cl,
794 N, O, or S, detected only by high resolution two-dimensional gas chromatography-mass
795 spectrometry (Manzano et al. 2012; Manzano et al. 2013; Ahad et al. *In prep for this special*
796 *issue*). The potential ecological risks of airborne PACs are suggested by the contamination of
797 tadpoles in isolated wetland ponds near oil sands industries; the summed concentrations of 75
798 PACs ranged from 110 to 190 ng g^{-1} dry weight (Mundy et al. 2019). As well, the meltwater of
799 PAC-contaminated snow collected within seven km of oil sands mines was lethal to larval
800 fathead minnows (Parrott et al. 2018).

801 Although Harner et al. (2018) provide a thorough analysis of the sources, distribution, and
802 fate of PACs in the region, there is no equivalent review of the nature, distribution and trends of
803 effects. A detailed review is needed of research on the impacts of oil sands development to

assess potential cause-effect relationships for the observed levels of PAC contamination and to provide directions for future research in boreal ecosystems.

6 Modelling to predict the ecological risks of PAC emissions to air, land and water

The significance of Canada's PAC emissions, environmental contamination and toxicity is only apparent when assessed on a regional or site-specific basis. Modelling provides a rapid appreciation of the potential risks of ecological impacts and the information gaps that hinder understanding and appropriate management. Nationally, Canadian industries report their annual point-source emissions of PACs to the National Pollutant Release Inventory (NPRI) (Berthiaume et al. *In prep. for this special issue*). Although the NPRI details measured or estimated point source releases to air, water and land, the data are not combined across industries within a region to illustrate what resources are at risk of exposure and toxicity. The Risk Assessment IDentification And Ranking (RAIDAR) model, developed by Arnot et al. (2006) and Arnot and Mackay (2008), provides a screening-level exposure and risk assessment of chemical emissions. It combines mass balance, environmental fate and food web bioaccumulation models in a regional scale (100,000 km^2) environmental model representative of temperate Canada. Its objective is to better understand the connection between pollutant emissions, distributions, and corresponding ecological risks. The model is publicly available (https://arnotresearch.com/raidar/) and provides full details on physical compartments, representative organisms, food webs and other key parameters.

A customized version of RAIDAR (version 2.985) was developed to estimate the risk of point source emissions of PACs (Berthiaume et al. *In prep. for this special issue*). For the 32 PACs reported to Canada's NPRI, model inputs included physical-chemical properties, chemical degradation half-lives, biotransformation half-lives in fish and mammals, and toxicity values (ARC 2014). As a first step, RAIDAR evaluated the exposure potential of individual PACs in a 'unit emission scenario' (assumes that each PAC is emitted at 1.0 kg. h^{-1}) using Level III multimedia chemical fate, food web, and bioaccumulation models (i.e., open systems in steady-state). Predicted whole-body concentrations of PACs in representative organisms for this 'unit' emission scenario were compared to critical effects concentrations from the EnviroTox database (HESI 2018; Arnot & Toose 2019; Connors et al. 2019). The comparison yielded a hazard assessment factor (HAF), i.e., the ratio between the expected dose calculated from 'unit emission

834 rates' and the toxic dose. The HAFs reflect the toxicity, persistence and bioaccumulation of each
835 PAC (Arnot & Mackay 2008; Arnot & Toose 2019) and can be compared and ranked by most
836 vulnerable organism or by organism of interest. When PACs from the 2017 NPRI data were
837 ranked by their predicted HAFs for the most vulnerable organism (Figure 2–A2), 1-nitropyrene
838 was most hazardous (greatest likelihood of toxicity in a 'unit' emission scenario), with plankton
839 the most likely species to be affected.

840 The NPRI emissions data were integrated into RAIDAR as actual emission rates to predict
841 PAC concentrations in representative organisms. The ratios of actual NPRI emissions to unit
842 emissions were used to scale the HAF to a risk assessment factor (RAF), i.e., the ratio between
843 the expected dose and the toxic dose. Figures 2-C and 2-E show the NPRI releases, ranked by
844 total quantity, and the resulting RAFs calculated by RAIDAR, ranked by the most vulnerable
845 receptor. When viewing Figure 2 by 'most vulnerable organism', the summed RAF values for
846 individual NPRI PACs are each far lower than one. This means that the conservative and
847 hypothetical scenario in which all 2017 NPRI emissions Canada-wide are released in a 100,000
848 km^2 regional environment do not expose the most vulnerable organism to PAC concentrations
849 greater than critical effect thresholds. The same conclusion was reached for all other years of
850 NPRI data examined (2008-2016). An alternative view is shown in Figure 2-F, where the
851 emphasis is on RAFs in all receptor organisms, not just the most vulnerable. Each PAC is
852 presented as an individual entity, and assuming additivity, the sum of RAFs for all NPRI PACs
853 per receptor organism (red star) is still far less than 1.0.

854 Although the RAIDAR scenario provides a perspective on risk, the computed RAFs must
855 still be interpreted with caution. Tevlin et al. (*in prep. for this special issue*) identified
856 exceedances of air quality guidelines for PACs in the oil sands area, suggesting a real risk of
857 PAC emissions. The conflict with the RAIDAR predictions could be explained two ways.
858 Either the risks of PAC emissions are truly low and Canada's air quality guidelines are overly
859 conservative, or the risks were under-estimated because significant diffuse sources such as
860 transportation were not included in the NPRI (Berthiaume et al. *In prep. for this special issue*).
861 The RAIDAR scenario also distributes PAC depositions evenly over 100,000 km^2 and over
862 seasons. Steep concentration gradients radiating from bitumen mines and urban point sources
863 (Harner et al. 2018; Whaley et al. 2018) are ignored, as well as seasonal pulses of PAC released
864 in spring snowmelt (Parrott et al. 2018). Synergistic and antagonistic interactions are also not

865 considered (Section 2.2), a weakness shared with air quality guidelines. The estimated risks of
866 complex mixtures would likely be higher if based on larger numbers of PACs; like all ERAs,
867 RAIDAR assessments are limited to a very small number of test species and environmental
868 interactions.

869 Further research and model enhancements are needed to better understand these results
870 and to apply the model more widely. For example, the hypothetical conditions of RAIDAR's
871 evaluative environment (e.g., meteorological, vegetative, land cover, size, etc.) could be more
872 specific and realistic, a task underway for human health risk assessment (ARC 2019). Replacing
873 the Level III steady-state fugacity modelling with non-steady-state modelling would address
874 questions related to the effects on PAC distribution of temporal and geographical gradients,
875 among others. Filling emission information gaps (Berthiaume et al. *In prep. for this special*
876 *issue*) will provide more realistic risk estimates than presented here. Recent comprehensive
877 reviews of the emissions and distribution of PACs from oil sands production (Harner et al. 2018)
878 could spur the development of models that provide a more focused and local understanding of
879 ecological risks. Finally, expanding the scope of the model to include known and fugitive PAC
880 emissions in solid and liquid wastes would provide a more complete view of ecological risks.

881 **7 Summary and research needs**

882 Despite the complexity and technical challenges associated with estimating the ecological risks
883 of PACs, there is abundant evidence that PAC emissions damage ecosystems. Nevertheless, the
884 toxicity of most alkylated and heterocyclic PACs typical of complex mixtures are still unknown,
885 limiting the development and utility of structure-activity models. Models predicting mixture
886 toxicity are often based on unrealistic assumptions, including additivity of effects among PACs
887 and single rather than multiple MOAs. Interactions of PACs with co-occurring contaminants
888 from urban, industrial, agricultural, and forestry activities are little known, increasing the
889 uncertainty of site-specific ERAs. Not surprisingly, current Canadian environmental quality
890 guidelines are limited to a small fraction of the thousands of different PAC congeners. There is
891 an urgent need to update the guidelines to include a wider array of PACs and to improve models
892 for estimating the ecological risks of complex mixtures.

893 To support more comprehensive guidelines and more realistic ERAs, research is needed to
894 expand our knowledge of the effects of diverse PAC structures and properties on their

environmental distribution, fate and effects. A focus is needed on PAC metabolism and degradation, interactions with cellular receptors and MOAs, structure-activity relationships and mixture interactions.

ERAs are also weakened by significant but unquantified errors in aquatic toxicity data when toxicity is estimated from nominal PAC concentrations in test solutions. To reduce uncertainty, it is essential that passive dosing and sampling methods be adopted in standard protocols to characterize PAC exposures. Improved test methods will increase the accuracy and precision of predictive models for ERAs of PAC emissions.

More site-specific research is needed to identify ecosystems that are particularly sensitive to PAC loadings and to establish priorities for managing regional impacts. Research at PAC-contaminated sites and 'spills of opportunity' is essential to understand ecological impacts of PACs under different environmental conditions and the significance of species that are hyper-sensitive or resistant to PAC exposures. Lack of access to contaminated sites limits ecological research, and ERAs will only be improved if collaborations among governments, industries and universities are facilitated. Although the present review focused primarily on aquatic ecosystems, the issues raised apply equally well to terrestrial ecosystems.

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9 Declaration of Interests.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Important sources of PAC contamination in Canada's provinces and territories (summarized from Marvin et al. *In prep for this special issue*). NL – Newfoundland and Labrador; NS – Nova Scotia; PEI – Prince Edward island; NB – New Brunswick; QC – Quebec; ON – Ontario; MB – Manitoba; SK - Saskatchewan; AB – Alberta; BC – British Columbia; NT – Northwest Territory; YT – Yukon Territory; NU – Nunavut.

Sources of PACs	NL	NS	PEI	NB	QC	ON	MB	SK	AB	BC	NT	YT	NU
Atmospheric transport	x	x	x	x	x	x	x	x	x	x	x	x	x
Forest fires	x	x		x	x	x	x	x	x	x	x	x	
Fossil fuel combustion and leakage from fuel storage	x	x	x	x	x	x	x	x	x	x	x	x	x
Coal mining		x							x	x			
Oil, gas and bitumen extraction	x					x		x	x	x	x		
Coastal oil refineries	x	x		x	x	x				x			
Crude & refined oil and gas transportation: By pipeline		x		x	x	x	x	x	x	x	x	x	
By rail		x		x	x	x	x	x	x	x			
By truck	x	x	x	x	x	x	x	x	x	x	x	x	
By Ship	x	x		x	x	x				x			X
Petcoke production									x				
Steel mills		x			x	x							
Aluminum production					x					x			
Biogenic production from pulp mill wastes	x	x		x	x	x			x	x			
Contaminated sediments in industrial harbours		x		x	x	x				x			
Demonstrated contamination of biota		x			x	x			x	x			

1458 **Table 2.** The percentage of mixture interactions that showed more-than-additive, additive, or
1459 less-than additive effects on mortality, accumulation of metals and production of reactive oxygen
1460 species, summarized from Gauthier et al. (2014). The interactions represented 63 combinations
1461 of four PACs (phenanthrene, fluoranthene, benzo[a]pyrene; phenanthrenequinone) and four
1462 metals (Cd, Cu, Ni, Zn) reported in nine studies of six species of fish, invertebrates and
1463 microbes.

Effect	**More-than-additive**	**Additive**	**Less-than-additive**
Mortality	44.7	44.7	10.6
Metal Accumulation	46.6	26.7	26.7
Production of reactive oxygen species	37.5	50	12.5

1464

1465

Figure Captions

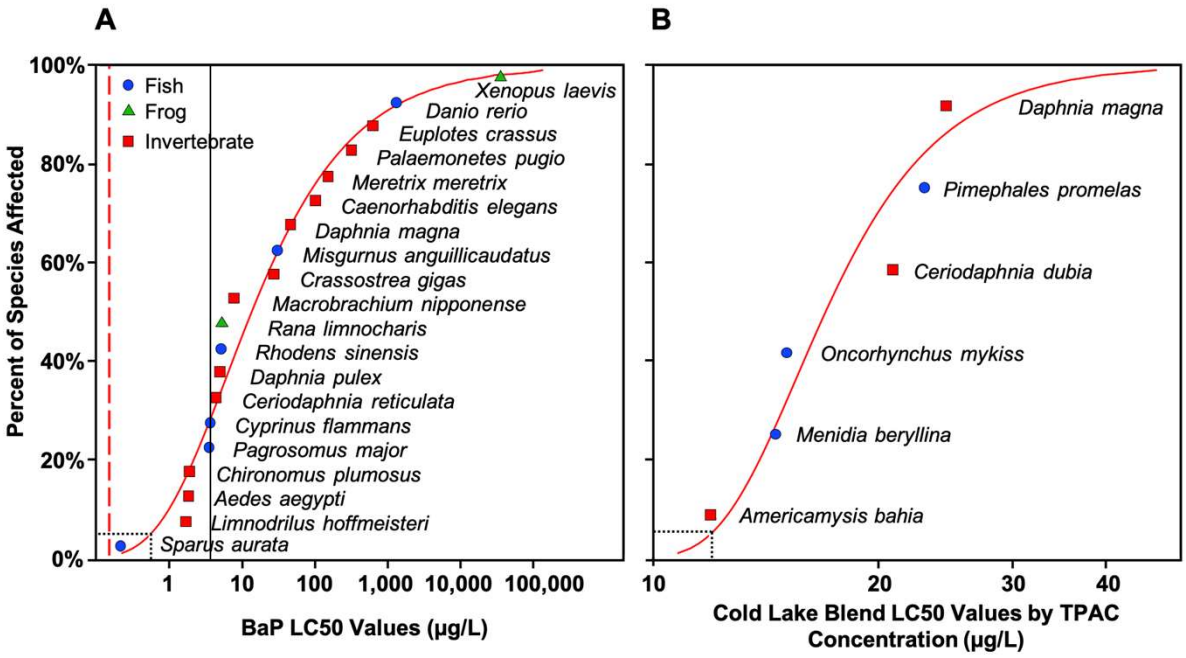
Figure 1. Species sensitivity distributions of LC50s for: A. waterborne benzo[a]pyrene (BaP) (20 species), and B. total concentrations of polycyclic aromatic compounds (TPAC) measured in test solutions of Cold Lake Blend dilbit (6 species). Dotted black lines define the HC_5 (concentration hazardous to the 5th percentile species): BaP = 0.55 ± 0.47 µg L^{-1} (95% CI = 0.22 – 2.0; cv = 85%); dilbit TPAC = 12.1 ± 1.55 µg L^{-1} (10.3 – 16.3; cv = 13%). The dashed red line represents Canada's BaP guideline for the protection of freshwater life (0.015 µg L^{-1}; CCME 1999). The solid black line represents the BaP solubility limit of 4 µg L^{-1}. LC50 data (Table SI-1) were fitted to a Log-Gumbel distribution based on goodness-of-fit tests; confidence limits were predicted from 10,000 bootstrap samples. Geometric means were used when multiple LC50s were available for one species.

Figure 2. RAIDAR model outputs for 2017 NPRI PAC emissions. **Panel 2a.** A1, A2: Hazard assessment factors (HAF) (unitless) for each PAC; B1,B2: Release type (environmental compartments); C1, C2: Release quantities (T); D: Species most vulnerable to PAC exposure; E1, E2: Predicted and ranked risk assessment factors (RAFs). Panel 2b. F: the sum of RAFs for all PACs, ranked by species sensitivity. Interactive versions of Figure 2 with reports filtered by year, substance, receptor, or environmental compartment are available at:

2.a: https://public.tableau.com/views/Draft-PAHsMostsensitiveorganismRAIDARanalysis_ForTableaupublic/Dashboard1?:display_count=y&:origin=viz_share_link

2.b: https://public.tableau.com/views/Draft-PAHs2008-2017RAIDARAllecoreceptororganisms_ForTableaupublic/Dashboard1?:display_count=y&publish=yes&:origin=viz_share_link

Figure 1 is a 2-column image



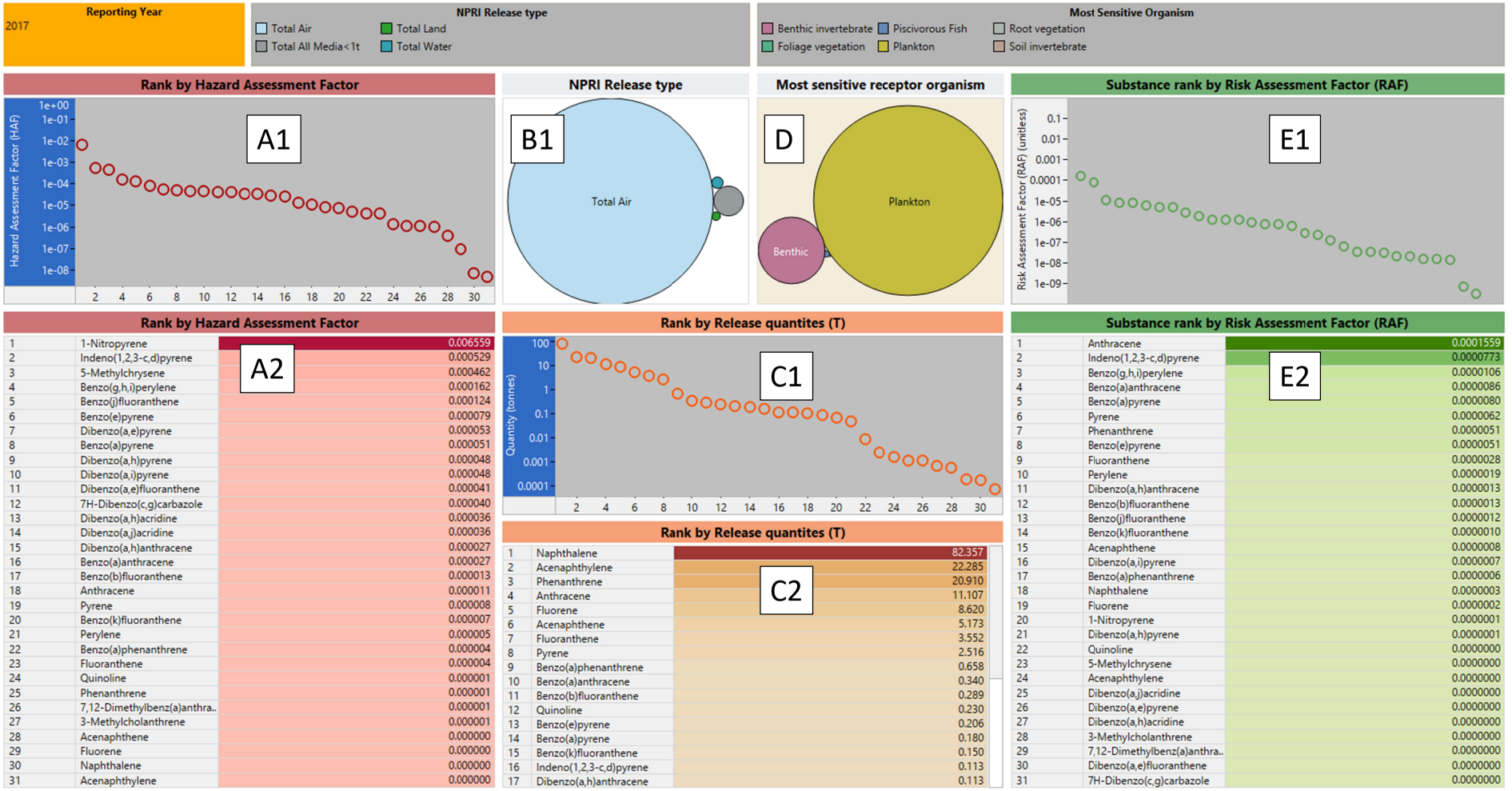
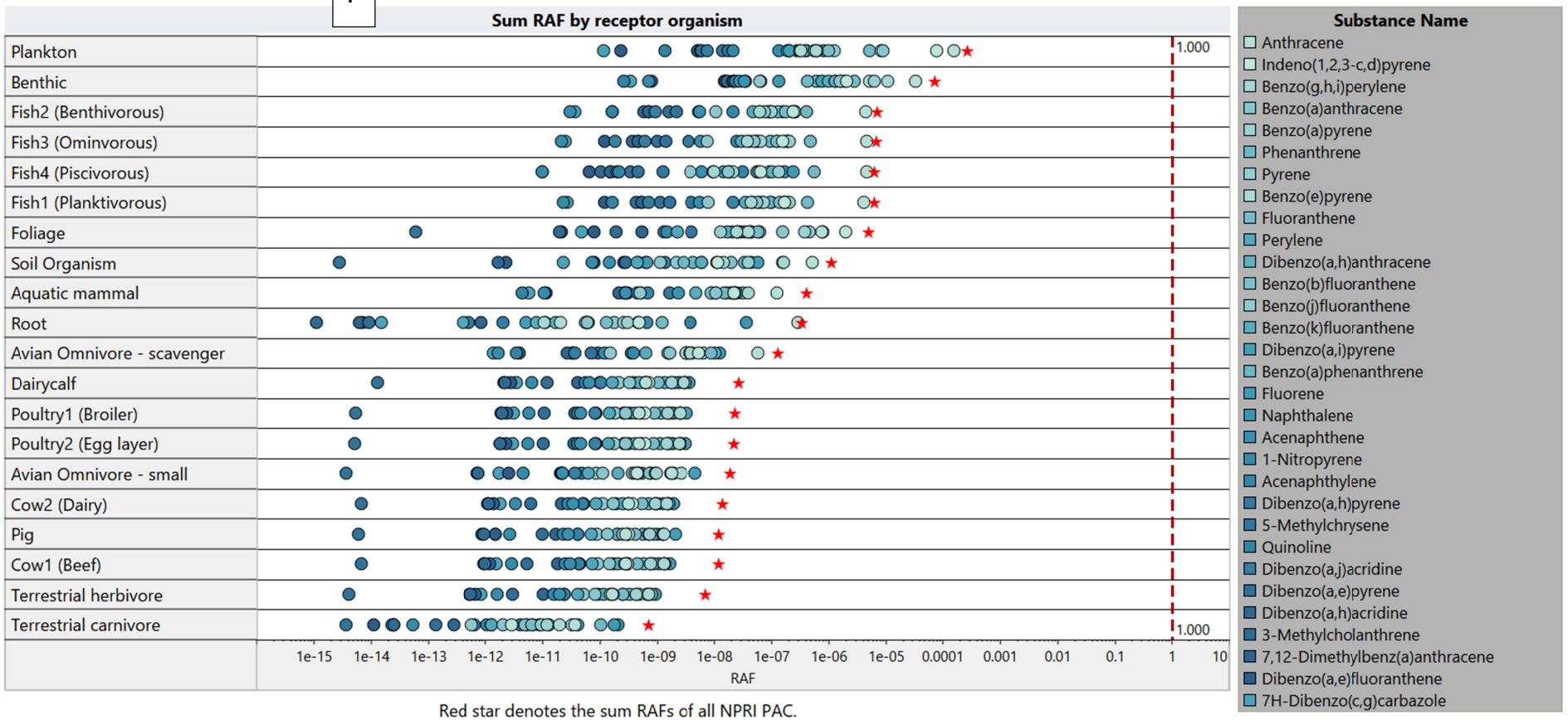


Figure 2a (Figure 2 is a 2-page image)

Reporting Year
2017

Substance Name
All

F



Red star denotes the sum RAFs of all NPRI PAC.
Red dotted line denotes a RAF = 1.

Figure 2b (Figure 2 is a 2-page image)

Polycyclic aromatic compounds (PACs) in Canada: The challenges of ecological risk assessments

Ecological Risk α *ƒ*((hazard, receptor, exposure) x environment)