Commentary

Prioritization of heterocyclic and substituted polycyclic aromatic compounds for toxicological assessment using new approach methodologies: A canadian perspective

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Abstract

Since the late 1970s, environmental monitoring and toxicological testing of polycyclic aromatic compounds (PACs) have focused overwhelmingly on the 16 polycyclic aromatic hydrocarbons (PAHs) designated as toxic substances under the Canadian Environmental Protection Act (1999). Advances in analytical chemistry now show that substituted and heterocyclic PACs, including alkylated, halogenated, nitrogen-, sulfur-, and oxygen-containing structures, are widespread in the environment, yet toxicological data for most of these compounds are unavailable or outdated. In this commentary, drawing on a Canadian perspective and data produced over the last 20 years, we identify 10 heterocyclic PACs that we propose should be prioritized for toxicological evaluation. The priority list was developed from multiple lines of evidence: environmental concentrations, detection frequencies, physicochemical properties, bioaccumulation potential, structural features indicative of aryl hydrocarbon receptor (AhR) binding, and toxicological data gaps. We argue that new approach methodologies (NAMs), integrating *in vitro* assays, high-throughput screening, and computational models, offer the most efficient and ethically responsible strategy for characterizing the toxicity of these structurally diverse compounds. We outline a tiered testing framework that begins with mechanistic *in vitro* assays targeting key toxicity pathways and integrates the resulting data with *in silico* tools such as quantitative structure–activity relationship (QSAR) models, graph neural networks, molecular docking, and read-across analyses. Combining high-throughput *in vitro* screening with artificial intelligence (AI)-enabled modeling provides a cost-effective and scientifically robust framework for prioritizing substituted and heterocyclic PACs for further toxicological evaluation and risk assessment, while reducing reliance on animal testing.

Keywords: Polycyclic aromatic compounds, heterocyclic PACs, new approach methodologies, chemical prioritization, aryl hydrocarbon receptor, *in silico* toxicology, quantitative structure-activity relationship, environmental monitoring

A PRIORITIZED LIST OF SUBSTITUTED AND HETEROCYCLIC PACS

In this commentary, we highlight 10 polycyclic aromatic compounds PACs [Table 1] that we propose for prioritized toxicological evaluation using new approach methodologies (NAMs) as the preferred strategy for toxicity assessment. This work is

timely given that the designated PACs list under the Canadian Environmental Protection Act (1999) was determined in the 1970s. Over the 50 years ago the analytical chemistry methods available to determine PACs in the environment have grown exponentially, which has also lead to a dramatic increase in the availability of environmental data to assess sources and effects in the environment.

Table 1. Candidate substituted and heterocyclic polycyclic aromatic compounds prioritized for toxicological assessment based on environmental exposure, bioaccumulation potential, and hazard criteria.

Compound	log Kow	COGRAD data	Other Data	Bioaccum. potential	Structural AhR concern	Tox-data availability	Data-gap concern
Benzo[b]naphtho[2,3-d]furan	5.5	Very High	Moderate	Moderate	Very High	None/Low	Very High
11H-Benzo[a]carbazole	4.6	Very High	Moderate	High	Very High	Moderate	High
Benz[c]acridine	4.5	Very High	High	Moderate	Very High	High	Moderate
9-Bromoacridine	4.4	High	None/Low	Moderate	Very High	None/Low	Very High
5-Bromoindole	3.0	Very High	None/Low	Very High	Moderate	None/Low	Very High
5-Chloroindole	2.8	Very High	None/Low	Very High	Moderate	None/Low	Very High
3,6-Dimethylcarbazole	4.3	High	High	Moderate	High	None/Low	Very High
1,2,3,4-Tetrahydrocarbazole	3.6	Very High	None/Low	High	Moderate	None/Low	Very High
2-Methylacridine (alkylated acridine)	3.9	High	Moderate	High	High	None/Low	High
2-Bromoquinoline (Halo-quinolines)	3.0	Moderate	None/ Low	Moderate	High	None/Low	High

log Kow, logarithm of the octanol-water partition coefficient; COGRAD, Centre for Oil and Gas Research and Development data and other data columns rank how frequently and at what levels each compound was detected in Canadian datasets (COGRADs and other regional datasets). Scores were assigned as follows: Very High — DF ≥ 80% in

one or more environmental matrices and/or recurring elevated concentrations (sediment median ≥ 40 ng/g dry weight or lipid-corrected tissue median ≥ 150 ng/g); High — DF 50-79% with detections across more than one matrix or study; Moderate — DF 10-49% or detections limited to a single matrix or localised point-source environment; None/Low — DF < 10% or compound not detected above analytical method detection limits in available datasets. A compound attains Very High combined occurrence only when both tiers independently support that designation; compounds with strong evidence in only one tier are capped at High. AhR, aryl hydrocarbon receptor. All qualitative hazard, bioaccumulation and data-gap rankings reflect a structural weight-of-evidence expert judgement to be quantitatively validate via the proposed tiered NAMs framework.

The priority list was developed using multiple lines of evidence, including emerging PAC environmental concentration data, compound detection frequencies, physicochemical properties, bioaccumulation potential, structural parameters indicative of AhR binding potential, and current toxicological data gaps. Environmental concentrations and detection frequencies were determined using data primarily from Canadian studies found using Web of Science and SciFinder-n literature searches. The search (2000-2026) paired compound- and class-name terms (e.g., “heterocyclic PAC”, “alkylated PAH”, “halogenated PACs”) with environmental-matrix and Canadian-location terms; primary studies reporting measured environmental concentrations were retained. Experimental or estimated log K_{ow} values were obtained from the ECOSAR database.

Aryl hydrocarbon receptor (AhR) binding was emphasized because AhR activation is a well-characterized initiating event in PAC toxicity that can be inferred directly from molecular structure; it is, however, one of several relevant pathways, and estrogen-, thyroid-, and other receptor-mediated effects are captured by the tiered assays described below. AhR activation potential was scored from structural features such as the degree of planarity, aromatic ring number, heteroatom type and position, based on experimental potency data for structurally related compounds[1-3]. The candidate data set was compiled and organized with the assistance of the Artificial Intelligence (AI) tool (Claude Opus 4.8; Anthropic, 2025), and a structured and thorough literature search in Web of Science and SciFinder-n was then conducted to map toxicological data gaps. Compounds with no or low (< 10 studies) existing *in vivo*, *in vitro*, and/or *in silico* toxicity data were identified and used to refine the priority list. Compounds were identified by a weight-of-evidence integration of seven criteria [Table 1]. Those scoring High or Very High for environmental exposure (concentration/detection) and for toxicological data gaps, and at least Moderate for bioaccumulation or AhR concern, were prioritized. Several compounds that were prioritized from exposure and structural criteria were excluded from the list due to the availability of toxicity data (e.g., C2-chrysene, 9-methylcarbazole, 2-methyldibenzofuran, carbazole and benzo(h)quinline).

A TIERED FRAMEWORK FOR FIT-FOR-PURPOSE TOXICITY TESTING

In vivo toxicity assessments using fish and mammalian models, such as zebrafish (*Danio rerio*) and rodents, remain the benchmark for toxicity characterization. However, these experiments are costly, time-consuming, ethically constrained, and impractical for evaluating the thousands of structurally diverse pollutant classes such as PACs[4]. Integration of NAM *in vitro* assays, computational models, and high-throughput screening approaches generate mechanistic toxicity data while substantially reducing the reliance on animal testing[5]. A tiered testing framework could begin with *in vitro* assays targeting key toxicological pathways, including AhR reporter assays that experimentally confirm the structure-based AhR predictions used for priorization, alongside estrogen- and thyroid-receptor transactivation assays, oxidative stress assays, genotoxicity assays, cytotoxicity assays, and assays using metabolically competent human-relevant 2D and 3D cell models including hepatic, reproductive, immune and other target tissues. Together, these mechanistic assays can rapidly identify compounds with the greatest potential for adverse biological effects while substantially reducing reliance on animal testing[6-8].

INTEGRATING *IN VITRO* SCREENING WITH AI-ENABLED *IN SILICO* MODELING

Experimental data generated from the above described assays can be integrated with *in silico* approaches to strengthen toxicity predictions and guide future chemical prioritization. Recent advances in AI and machine learning have significantly improved computational toxicology by enabling accurate prediction of receptor-ligand interactions, toxicological endpoints, and modes of action from molecular structure alone. Such approaches, including quantitative structure-activity relationship (QSAR) models, graph neural networks, molecular docking, and read-across analyses, can predict endpoints such as receptor-binding affinity, mutagenicity, carcinogenicity, endocrine-disrupting potential, and bioaccumulation. *In silico* screening has already been applied to heterocyclic PACs to help fill physicochemical and hazard data gaps[9]. By combining high-throughput *in vitro* screening with AI-enabled *in silico* modeling, researchers can establish an efficient, cost-effective, and scientifically robust framework for prioritizing the compounds that most warrant *in vivo* testing and inclusion in future CEPA risk-assessments[10].

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the commentary, data compilation, and interpretation, and drafted the manuscript: Vitharana NN, Tomy GT. Contributed to data interpretation, critical revision of the manuscript for important intellectual content, and approved the final version: Stetefeld J, Marvin C, Thomas PJ, Dupuis-Smith R, Provencher JF, Fisk AT, Holloway AC

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

The candidate compound data set was compiled and organized with the assistance of an AI tool (Claude Opus 4.8; Anthropic, 2025), as described in the main text. All AI-assisted outputs were verified by the authors against primary literature, and the authors take full responsibility for the content of this commentary.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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