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Workshop Synthesis

Towards Sustainable Environmental Quality: Priority Research Questions for the Australasian Region of Oceania

Sally Gaw,† Andrew Harford,‡ Vincent Pettigrove,§ Graham Sevicke-Jones,|| Therese Manning,# James Ataria,†† Tom Cresswell,‡‡ Katherine A Dafforn,§§ Frederic DL Leusch,|||| Bradley Moggridge,## Marcus Cameron,††† John Chapman,‡‡‡ Gary Coates,§§§ Anne Colville,|||||| Claire Death,### Kimberly Hageman,†††† Kathryn Hassell,§ Molly Hoak,‡‡‡‡ Jennifer Gadd,§§§§ Dianne F Jolley,|||||||| Ali Karami,#### Konstantinos Kotzakoulakis,‡‡‡‡‡ Richard Lim,|||||||| Nicole McRae,† Leon Metzeling,††††† Thomas Mooney,‡ Jackie Myers,§ Andrew Pearson,§§§§§ Minna Saaristo,||||||||| Dave Sharley,##### Julia Stuthe,†††††† Oliver Sutherland,‡‡‡‡‡‡ Oliver Thomas,§§§§§§ Louis Tremblay,†† |||||||||| Waitangi Wood,###### Alistair BA Boxall,††††††† Murray A Rudd,‡‡‡‡‡‡‡ and Bryan W Brooks§§§§§§§

†School of Physical and Chemical Sciences, University of Canterbury, Christchurch, New Zealand
‡Department of the Environment and Energy, Australian Government, Darwin, Australia
§Aquatic Environmental Stress Research Centre, RMIT University, Bundoora, Victoria, Australia
||Environment Southland Regional Council, Invercargill, New Zealand
#Environmental Risk Sciences, Sydney, Australia
††Cawthron Institute, Nelson, New Zealand
‡‡Australia's Nuclear Science and Technology Organisation, Lucas Heights, Australia
§§Department of Environmental Sciences, Macquarie University, North Ryde, Australia
||||Australian Rivers Institute and School of Environment and Science, Griffith University, Brisbane, Australia
##Institute for Applied Ecology, University of Canberra, Canberra, Australia
†††Auckland Council, Auckland, New Zealand
‡‡‡Office of Environment and Heritage, New South Wales, Australia
§§§Te Rūnanga o Ngāi Tahu, Christchurch, New Zealand
||||||School of Life Sciences, University of Technology Sydney, Sydney, Australia
###Faculty of Veterinary Science, University of Melbourne, Victoria, Australia
††††Department of Chemistry and Biochemistry, Utah State University, Logan, Utah, USA
‡‡‡‡School of Biosciences, The University of Melbourne, Parkville, Victoria, Australia
§§§§National Institute of Atmospheric and Water Research, Auckland, New Zealand
||||||||Faculty of Science, University of Technology Sydney, Sydney, Australia
####Environmental Futures Research Institute, Griffith University, Brisbane, Australia
†††††Environment Protection Authority, Victoria, Australia
‡‡‡‡‡Macquarie University, Sydney, Australia
§§§§§Ministry for Primary Industries, Wellington, New Zealand
|||||||||School of Biological Sciences, Monash University, Melbourne, Australia
#####Bio2Lab, Melbourne Innovation Centre, Greensborough, Australia
††††††CSIRO Publishing, Clayton, Victoria, Australia
‡‡‡‡‡‡Independent researcher, Nelson, New Zealand
§§§§§§School of Applied Chemistry and Environmental Science, RMIT University, Melbourne, Victoria, Australia
||||||||||School of Biological Sciences, University of Auckland, Auckland, New Zealand
######Tau Iho I Te Po Trust, Kaeo, New Zealand
†††††††Environment Department, University of York, York, United Kingdom
‡‡‡‡‡‡‡World Maritime University, Malmö, Sweden
§§§§§§§Baylor University, Waco, Texas, USA

SETAC Horizon Scanning™

ABSTRACT

Environmental challenges persist across the world, including the Australasian region of Oceania, where biodiversity hotspots and unique ecosystems such as the Great Barrier Reef are common. These systems are routinely affected by multiple stressors from anthropogenic activities, and increasingly influenced by global megatrends (e.g., the food–energy–water nexus, demographic transitions to cities) and climate change. Here we report priority research questions from the Global Horizon Scanning Project, which aimed to identify, prioritize, and advance environmental quality research needs from an Australasian perspective,

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* Address correspondence to sally.gaw@canterbury.ac.nz

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within a global context. We employed a transparent and inclusive process of soliciting key questions from Australasian members of the Society of Environmental Toxicology and Chemistry. Following submission of 78 questions, 20 priority research questions were identified during an expert workshop in Nelson, New Zealand. These research questions covered a range of issues of global relevance, including research needed to more closely integrate ecotoxicology and ecology for the protection of ecosystems, increase flexibility for prioritizing chemical substances currently in commerce, understand the impacts of complex mixtures and multiple stressors, and define environmental quality and ecosystem integrity of temporary waters. Some questions have specific relevance to Australasia, particularly the uncertainties associated with using toxicity data from exotic species to protect unique indigenous species. Several related priority questions deal with the theme of how widely international ecotoxicological data and databases can be applied to regional ecosystems. Other timely questions, which focus on improving predictive chemistry and toxicology tools and techniques, will be important to answer several of the priority questions identified here. Another important question raised was how to protect local cultural and social values and maintain indigenous engagement during problem formulation and identification of ecosystem protection goals. Addressing these questions will be challenging, but doing so promises to advance environmental sustainability in Oceania and globally. *Integr Environ Assess Manag* 2019;00:1–19. © 2019 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals, Inc. on behalf of Society of Environmental Toxicology & Chemistry (SETAC)

Keywords: Multiple stressors and mixtures Risk assessment Contaminants of emerging concern Indigenous knowledge Cultural values

INTRODUCTION

Achieving sustainable environmental quality and ecosystem integrity is a critical goal shared by diverse stakeholders around the world. Unimpaired and diverse ecosystems conserve biodiversity and provide essential ecosystem services, while being more resilient when natural and anthropogenic disasters occur (Alexander et al. 2016). The United Nations Sustainable Development Goals aim to protect the planet and realize prosperity for all people, including future generations (UN 2015). Within

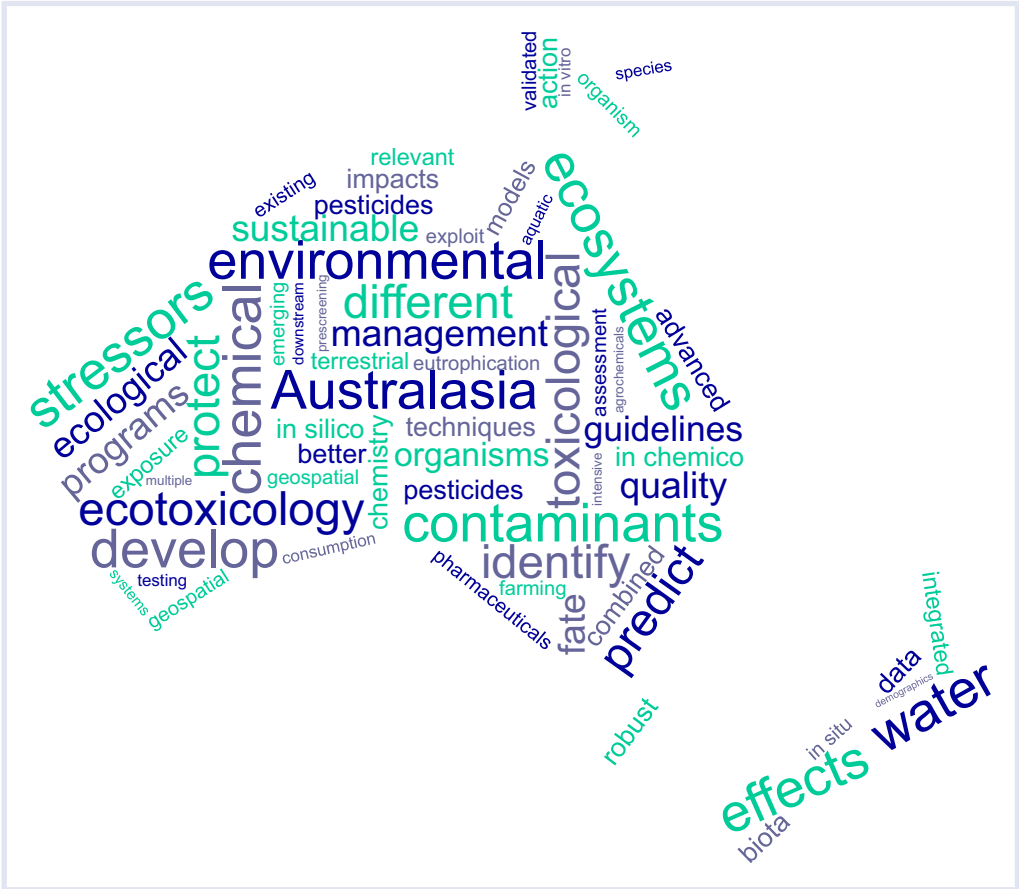

Figure 1. Word cloud of priority research questions from the Australasian Region of Oceania.

this framework are interconnected goals that inherently rely on achieving more sustainable environmental quality and ecosystem integrity. But achieving these goals depends on effective environmental management efforts informed by the best available scientific knowledge and technological advancements. Integration of robust environmental risk assessment with ecosystem protection goals is therefore critical in light of global megatrends (e.g., the food–energy–water nexus, demographic transitions to cities) and climate change that present unique challenges for policy makers and environmental and health professionals. These sustainable management challenges are complex, particularly given environmental, political, and economic contexts that exist among and within global regions.

Intersections of biodiversity, environmental variability, and anthropogenic stressors are pronounced in the Australasian region of Oceania. Countries in the region have iconic landscapes with unique flora and fauna. The ability to participate in outdoor activities, including hiking, camping, fishing, and swimming, is treasured in Australia and New Zealand and considered to be part of their national identities (Garner 2013; McCrone 2017). Biodiversity hotspots are prevalent, as are freshwater and marine ecosystems (e.g., the Great Barrier Reef), which are susceptible to stress from anthropogenic activities, including climate change (Adams et al. 2016). Interconnections among stressors from landscape development and urbanization across freshwater to marine gradients are widespread in this region (e.g., Mayer-Pinto et al. 2015; Weeks et al. 2016), where the vast majority of human populations reside within 50 km from the coast.

Climate change is significantly affecting the island nations of Oceania (Caritas 2018) and magnifying the importance of understanding how multiple physical and chemical stressors impact biodiversity and ecosystem services (Weeks et al. 2016). However, information on the influences of natural and anthropogenic stressors, particularly chemical contaminants, is scarce for native species. There are also relatively few ecotoxicology data sets of relevance to the tropical conditions of Papua New Guinea and much of the northern parts of Australia. Degradation of water quality is of particular concern for Māori and Aboriginal communities. There is growing appreciation of the spiritual and cultural values and developmental aspirations of indigenous communities; momentum is building to incorporate these in environmental policies and decision making (Bark et al. 2015; Harmsworth et al. 2016; Ataria et al. 2018). Unfortunately, identifying global priority environmental quality research needs to attain these ecosystem protection goals and effectively implement policy instruments has remained elusive on a regional scale. Horizon-scanning approaches for identifying key research questions may be part of developing sustainable solutions.

Horizon scanning using a key questions approach has emerged from the conservation sciences, public health, and other disciplines as an effective means to identify important research needs through engagement of diverse stakeholders (Sutherland and Woodroof 2009; Boxall et al. 2012; Rudd et al. 2018). The Global Horizon Scanning Project (GHSP) was initiated with the Society of Environmental Toxicology and Chemistry (SETAC) to identify priority research questions that advance understanding of how environmental stressors impact environmental quality (Brooks et al. 2013). This initiative is collecting and prioritizing the most important current and emerging research questions related to environmental quality as recognized by scientists and engineers from multiple disciplines working in government, academia, and business around the globe. For example, priority research questions were recently reported from Latin America (Furley et al. 2018), Europe (Van den Brink et al. 2018), and North America (Fairbrother et al. 2019). Here we specifically present results from the GHSP project focused on the Australasian region of Oceania. The scope of these questions was intended to be of relevance to Australasia, within a global context. We anticipate these priority research questions will be indispensable in informing and structuring research agendas by the government and business communities in the future.

METHODS

In the present study, we followed previously reported methods (Boxall et al. 2012; Furley et al. 2018; Van den Brink et al. 2018) to identify priority research questions. Prior to holding a workshop in Nelson, New Zealand, in 2015, members of SETAC and other scientists from Oceania were asked to submit research questions which, in their view, were priority environmental quality research needs to address. Consistent with methods employed in other studies (Sutherland et al. 2011) and SETAC geographic regions (Furley et al. 2018; Van den Brink et al. 2018), participants were provided criteria for an ideal question, which should address important gaps in knowledge; be answerable through a realistic research design; have a factual answer that does not depend on value judgments; cover a spatial and temporal scale that could realistically be addressed by a research team; not be answerable by "it all depends," "yes," or "no"; and if related to impact and interventions, the research question should contain a subject, an intervention, and a measurable outcome. In total, 78 questions were received and are presented in Supplemental Data.

Before the workshop, questions were partitioned among 6 themes, including contaminants of emerging concern; environmental chemistry: analysis, fate, and exposure; multiple stressors and mixtures; risk assessment, regulations, and guidelines; spotlight on Australasia; and tools for improving risk assessment. These 6 themes were used to structure an expert workshop held in Nelson, New Zealand as part of the SETAC Australasia meeting in 2015 at which the questions were discussed. During the workshop, 20 priority research questions were identified by participants from academic, business, the indigenous community, and government sectors. We specifically examine each of these priority research questions in the sections that follow (Table 1).

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Table 1. Top 20 priority research questions from the Australasian portion of the Global Horizon Scanning Project by theme

Themes and priority research questions
Contaminants of emerging concern
What are the most appropriate toxicological approaches to develop regulatory guidelines specifically for contaminants of emerging concern that address multimodes of action and sublethal effects?
How can we identify and prioritize contaminants (traditional and emerging stressors) for sustainable management of ecosystems within different biogeographic regions?
How can we identify and examine the environmental fate and toxicity of ingredients other than the stated "active" components in commercial formulations, individually and in chemical mixtures?
Environmental chemistry: Analysis, fate, and exposure
How can we develop robust chemical assays and models to replace, refine, and reduce biological testing?
How do we better understand the linkages between the structural and physicochemical properties of substances to predictively model fate and bioavailability in different environments?
How do we develop better broad-screening analytical and information-processing techniques that do not require preselection of target contaminants?
How do we use chemistry to better design sustainable waste management?
How can we ensure sustainable supplies of clean water, energy development, and food security while simultaneously minimizing ecological impacts and protecting environmental quality?
Multiple stressors and mixtures
What are the combined impacts of various agrochemicals (e.g., veterinary medicines, pesticides) and eutrophication from intensive terrestrial farming operations on the health of aquatic and terrestrial organisms?
What are the effects of changing demographics, economic development, consumption patterns, and climate (e.g., ocean acidity, water temperature) on chemical emissions, environmental fate, and ecotoxicology of contaminants and multiple stressors?
What are the combined effects of very low levels of multiple contaminants (e.g., pesticides, natural resource extraction contaminants, salinity, pharmaceuticals and personal care products, endocrine-disrupting chemicals) with different modes of action on aquatic and terrestrial organisms and ecosystems?
Risk assessment, regulations and guidelines
What water quality guidelines are needed to protect temporary waters and associated ecosystems from the influences of development?
What are the effects of short magnitude, frequency, and duration (e.g., intermittent, episodic) exposures to contaminants and other stressors, and how can these scenarios be effectively incorporated into water quality guidelines?
How can we measure ecosystem resilience to and recovery following exposure to stressors?
Spotlight on Australasia
Are there differences in toxicological thresholds among native and nonnative organisms, and how can species sensitivity information from nonresident species be used to predict adverse outcomes and protect our unique biota and ecosystems?
How do we incorporate and protect cultural and social values (relating to humans, biota, and ecosystems) to empower citizen, societal, and indigenous engagement in the research, management, and legislation of priority environmental contaminants?
Tools for improving risk assessment
How do we exploit, collate, and integrate existing environmental toxicology, chemistry, and geospatial data to help develop robust risk assessment?
How can prescreening techniques (e.g., in silico, in vitro) be developed, advanced, and validated to identify and predict whole organism effects?
How can ecotoxicology information be integrated more closely during interpretation of ecological data?
How do we advance ecotoxicology testing to be more relevant to ecological systems?

CONTAMINANTS OF EMERGING CONCERN

What are the most appropriate toxicological approaches to develop regulatory guidelines specifically for contaminants of emerging concern (CECs) that address multimodes of action and sublethal effects?

Measures of effect are selected during problem formulation in ecological risk assessment to support assessment endpoints that are aligned with ecosystem protection goals (Suter 2006). Historically, these measures of effect include a limited number of model organisms and endpoints (survival, growth, reproduction) linked to adverse outcomes of importance to the population level and environmental management. Single-species ecotoxicity information for a specific chemical is then routinely utilized to develop species sensitivity distributions from which water quality criteria, standards, or guidelines are derived around the world (Posthuma et al. 2001). Recent revisions of Australia and New Zealand Environment and Conservation Council (ANZECC, now referred to as the Australian and New Zealand Governments [ANZG] 2018) guidelines, and the use of multiple lines of evidence in weight-of-evidence assessments, represent global steps forward consistent with global trends.

Although recent years have seen an increase in the use of chronic toxicity testing with Australasian species, a historical overreliance on a limited number of model organisms and endpoints has potentially undermined management activities related to sustainable environmental quality and ecosystem integrity. Much of the available ecotoxicology information has been primarily comprised of acute lethality responses of several species (e.g., *Daphnia* sp.) from the Northern Hemisphere. Sublethal responses to chemical stressors were primarily available for cladoceran reproduction, microalgal growth rate, and juvenile fish growth. Similar model organisms and endpoints also have been employed for whole effluent (aka "direct toxicity assessments") and ambient toxicity testing (USEPA 1991). However, assays based on these model organisms and endpoints were often not developed to account for mutagenicity, teratogenicity, and other adverse outcomes that result from diverse molecular initiation events (MIEs; Ankley et al. 2010). Other ecologically important endpoints, including developmental and behavioral responses, are increasingly receiving attention in terms of potential importance (e.g., Saaristo et al. 2018).

Early research with endocrine-disrupting and -modulating chemicals (EDCs) recognized some of the limitations of these traditional tools to assess environmental quality and to derive guideline values protective of aquatic systems. For example, a 6-order-of-magnitude difference exists between adverse effects on cladoceran (Clubbs and Brooks 2007) versus fish reproduction (Kidd et al. 2007) elicited by the human estrogen agonist 17α-ethinylestradiol because invertebrates do not possess a functional estrogen receptor (Ankley et al. 2016). After almost a decade of health and ecological research on EDCs, Ankley et al. (2007) identified that such lessons learned from these chemicals were important to understanding risks of pharmaceuticals in the environment. Subsequently, efforts such as the development of adverse outcome pathways (AOPs; Ankley et al. 2010), informed by comparative pharmacology and toxicology research (LaLone et al. 2016; Brooks 2018), have been advancing the use of pathway-based predictive approaches in ecological risk assessment.

In parallel, buoyed by release of Toxicity Testing in the 21st Century (NRC 2007), the Tox21 and ToxCast programs were launched (Dix et al. 2006). These have screened thousands of chemicals with hundreds of in vitro assays, largely adapted from drug discovery and safety testing programs, to identify likely MIEs associated with many untested chemicals. These and related next-generation risk assessment efforts are breaking new ground (Cote et al. 2016). For example, identification of diverse MIEs associated with chemical properties is supporting development of next-generation computational toxicology models to identify problematic (and useful) substances and to sustainably design less hazardous chemicals. More recent applications include employing these in vitro systems for prioritizing environmental assessments (Li et al. 2017) and performing cross-species extrapolation (LaLone et al. 2018), or tracking movements of multiple individuals simultaneously using ToxTrac (Rodriguez et al. 2018). Such efforts promise to continue to further advance environmental risk assessment practices (Villeneuve et al. 2019).

Integrating comparative toxicology information and mechanistic tools such as high-throughput assays with regulatory guideline development and environmental monitoring and assessment represents important research needs. In the case of pharmaceuticals, for example, short-term standardized ecotoxicity test model species and endpoints are often not adequate to define chronic toxicity (Brooks 2018). Herein, therapeutic hazard values (Brooks 2014) and minimal selective concentrations and associated predicted no-effect concentrations for the development of antibiotic resistance by microorganisms in particular (Bengtsson-Palme and Larsson 2016) represent recent approaches to identify water concentrations supporting more robust ecological and human health water quality assessments, respectively, and to further support environmental diagnostic applications. However, integrative, comparative, and predictive toxicology research must be advanced to understand ecologically important effects caused by new and poorly studied chemicals.

How can we identify and prioritize contaminants (traditional and emerging stressors) for sustainable management of ecosystems within different biogeographic regions?

Like other regions of the globe, Australasian ecosystems are subject to a variety of chemical and other stressors, which challenges stressor identification research and practice. However, due to the smaller economies of the Australasian region, it is especially not feasible to have ever-expanding monitoring lists for contaminants, and care is needed to avoid needless selection of priority contaminants based on data from different biogeographic regions of Australasia. Risk-based frameworks for identification and

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prioritization of contaminants that incorporate local ecosystem-specific vulnerability to contaminants and Australasia-specific use of chemicals are urgently needed. Factors contributing to Australasia-specific use of chemicals include regulatory decisions, patents, demographics, land use, and climate, along with human and animal disease and pest profiles (Daughton 2014; Kookana et al. 2014; Gaw and Brooks 2016). These factors will change over time, and prioritization schemes and ultimately regulatory and monitoring regimes will need to be sufficiently agile and adaptive to examine substances currently in commercial use. Solid waste and wastewater management practices in Australasia will also determine priority substances in the region. In addition to anthropogenic chemical contaminants, transformation products and endogenous biomolecules, including toxins from harmful algal blooms (HABs), need to be assessed. Other important stressors that also need to be taken into consideration include changing land use, urbanization, climate change, and biological stressors such as predation, overexploitation, and invasive species. Globally, the need for contaminant prioritization has been identified for pharmaceuticals and personal care products (Boxall et al. 2012), microplastics (Eerkes-Medrano et al. 2015), and pesticides and their transformation products (Sinclair et al. 2006). Ultimately, risk-based identification and prioritization frameworks for contaminants, which are currently used by Australasian chemical management authorities, need to be diligently updated to reflect contemporary uses and potential exposure. They also need to be further developed to be broader than single classes of contaminants and to incorporate nonchemical stressors.

How can we identify and examine the environmental fate and toxicity of ingredients other than the stated "active" components in commercial formulations, individually and in chemical mixtures?

Ecotoxicity testing is generally focused on known active components as pure substances rather than as components of commercial formulations and chemical mixtures. Many products contain ingredients other than the stated active components to enhance the stability or performance of the product. Examples include adjuvants added to pesticides, coloring agents and preservatives added to soaps, fragrances added to cleaning products, and a wide range of excipients added to pharmaceutical products. These "other" or "inert" ingredients have the potential to alter the environmental fate and toxicity of the active components in commercial formulations as well as in other contaminants and may also present their own inherent hazards and risks (Cox and Surgan 2006). For example, glyphosate formulations containing surfactants were more toxic than glyphosate on its own (Vincent and Davidson 2015). Such ingredients may not be listed, especially for proprietary formulations, making it difficult to identify and prioritize components of formulations for study. Identification of potentially problematic ingredients in products other than the active ingredient will lead to improved risk assessment and ultimately to safer products.

ENVIRONMENTAL CHEMISTRY: ANALYSIS, FATE, AND EXPOSURE

How can we develop robust chemical assays and models to replace, refine, and reduce biological testing?

Globally there is a focus on reducing biological testing to reduce the numbers of animals used in testing and to minimize the costs and time involved (e.g., Hutchinson et al. 2016). Additionally, the ever-increasing volume and classes of chemicals in widespread use makes comprehensive biological testing unfeasible. Consequently, in silico toxicology efforts that commonly employ quantitative structure–activity relationships (QSARs) have become critical for early tier assessments of industrial chemicals (Myatt et al. 2018). The AOP approach has been proposed as a tool to help assess the safety of chemicals that, when coupled with robust computational toxicology, will reduce reliance on biological testing (Burden et al. 2015). Importantly, more research efforts should be targeted at predictively identifying chemical properties that result in MIEs with adverse outcomes at the organism and population levels. Also as noted above, one such attempt is the United States Environmental Protection Agency's (USEPA) ToxCast program, which employs computational and high-throughput screening (HTS) tools for prioritizing environmental contaminants (Dix et al. 2006; Cote et al. 2016). In fact, molecular docking (McRobb et al. 2014) and quantum mechanics approaches are advancing the science beyond traditional log K_{ow} based QSAR approaches (Kostal 2018).

How do we better understand the linkages between the structural and physicochemical properties of substances to predictively model fate and bioavailability in different environments?

Structural and physicochemical properties of compounds are used in risk assessments to identify priority persistent and bioaccumulative compounds (Howard and Muir 2010). Many of the algorithms used in risk assessments were developed for hydrophobic organic compounds under temperate conditions. There is increasing evidence that these "rules of thumb" developed for neutral hydrophobic compounds may not be sufficiently predictive of the fate and bioavailability of hydrophilic compounds and do not predict the behavior of ionizable compounds. For example, the octanol–water partition coefficient log K_{ow} is used as an indicator of enhanced accumulation, with molecules that have log K_{ow} values greater than 3 predicted to accumulate. However, some uncharged molecules with low log K_{ow} values have also been shown to accumulate in organisms (e.g., Emnet et al. 2015). Similarly, K_{ow}-based approaches have limitations for ionizable chemicals such as pharmaceuticals and per- and polyfluoralkyl substances (PFAS), which partition by nonhydrophobic mechanisms (e.g., ion exchange, protein binding; Armitage et al. 2017). There is a need to undertake a metaanalysis of the available data on the linkages between the structural and physicochemical properties of substances and their environmental fate and bioavailability. Basic and applied research will be necessary to improve

predictive models for properties that fall outside of the mechanistic domain of historic hydrophobic contaminants.

How do we develop better broad-screening analytical and information-processing techniques that do not require preselection of target contaminants?

"You only find what you are looking for" is a truism of environmental monitoring (Waller and Allen 2008). Widely available analytical techniques require preselection of target analytes and commonly include extensive sample preparation. This approach means that environmental monitoring programs selectively include known contaminants for which robust analytical methods exist and may not provide data on the priority contaminants for a particular time or location (Daughton 2014; Gaw and Brooks 2016). Analysis costs associated with screening just 1 water sample, for example, can be prohibitive when using multiple traditionally available analytical methods for diverse classes of contaminants. In addition, it can be difficult to establish whether there are no data for a particular contaminant because it is not present in the environment or because there are no suitable analytical methods and standards. Although new approaches using high-resolution mass spectrometry are being developed to enable nontarget analysis of organic compounds (Samanipour et al. 2016; Hollender et al. 2017), these techniques are not yet routine and provide information only on organic classes of contaminants. In contrast, ecosystems are exposed to complex mixtures that contain nutrients and metals, in addition to synthetic and naturally produced organic compounds. Advancing development and availability of robust nontarget screening techniques would significantly enhance environmental protection and would specifically support a number of the other top 20 research questions identified here.

How do we use chemistry to better design sustainable waste management?

Global pollution is now recognized as being responsible for the loss of more human lives each year than all wars or cancers (Landrigan et al. 2018). Human population growth and urbanization results in product use and chemical consumption being concentrated in cities faster than environmental management systems and interventions are being developed (Brooks 2018). For example, solid waste generation, which is currently estimated at 10 billion tons per year in urban areas, will continue to grow and become increasingly concentrated, particularly in developing and middle-income countries (Wilson et al. 2015). In Australia, although per capita waste generation has decreased, the mass of solid waste produced continues to increase, with a 7 % increase over a recent 11-y period (over the period of 2006–2007 to 2016–2017; National Waste Report 2018). New Zealand is one of the highest generators of household waste in the Organisation for Economic Co-operation and Development (OECD 2019). Similarly, wastewater production is concentrated in cities, yet 80 % of the global sewage production is released untreated to the environment (WWAP 2017). Key sustainable development goals aim to increase sustainable cities and communities as well as responsible consumption (UN 2015), which will require development and implementation of innovative waste management programs. Advancing green engineering to reduce waste generation, increasing beneficial reuse and recovery from diverse waste streams, and stimulating sustainable molecular design of chemical ingredients and products that maintain function but are less hazardous and degrade faster (Coish et al. 2016) represent important opportunities to meet sustainability goals while stimulating innovation and reducing chemical risks to public health and the environment. In fact, designing a future without waste and associated environmental pollution was recently identified as a grand challenge for environmental engineering (NASEM 2018). To realize this challenge, environmental toxicology, chemistry, and engineering will need to advance transdisciplinary research cooperation with ecology, public health, and other disciplines.

How can we ensure sustainable supplies of clean water, energy development, and food security while simultaneously minimizing ecological impacts and protecting environmental quality?

This question represents perhaps the grandest challenge of the 21st century. Increasing populations and levels of development across the globe are driving the need for sustainable supplies of clean water, energy development, and food security (UN 2015). In fact, the US National Academy of Science also identified the production of sustainable supplies of food, energy, and water as a grand challenge for environmental engineering in the 21st century (NASEM 2018). However, there is a need to ensure that any new technological advances to address a particular issue do not result in risk trade-offs that have adverse impacts to environmental quality and ecosystem integrity. For example, sources of clean energy are being heavily promoted to mitigate climate change and poor air quality. In 2018 six solar panels were installed every minute in Australia, with 1 of every 5 households hosting rooftop solar generation (CER 2019). Over the next 10 y the use of solar technologies is expected to accelerate, and improved solar energy capture and storage materials are being developed. There is the potential for these materials to become sources for CECs and to enter waste streams as they are decommissioned and replaced. Therefore, as we move toward a circular economy, we must be mindful of the implications of new technologies for environmental quality. Better integration of robust predictive and comparative toxicology within life cycle assessment represents an important research opportunity.

MULTIPLE STRESSORS AND MIXTURES

What are the combined impacts of various agrochemicals (e.g., veterinary medicines, pesticides) and eutrophication from intensive terrestrial farming operations on the health of aquatic and terrestrial organisms?

Primary industry is a key economic driver in the Australasian region of Oceania. Intensive and industrial agricultural

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practices have resulted in increased levels of pollutants being discharged to the environment increasing the potential to impact associated ecosystems and adjacent landscapes. Agrochemicals and veterinary medicines often co-occur in nutrient-enriched ecosystems, yet ecotoxicology studies of these contaminants across nutrient gradients are rare (Brooks et al. 2008). Traditionally, ecological risk assessment of agrochemicals has been conducted on a chemical-by-chemical basis, but the cumulative effects of these chemicals with veterinary medicines, with other stressors (Gustavsson et al. 2017), or within eutrophic systems (Baxter et al. 2016) has not been robustly addressed. Common ecotoxicity assays with plants and algae often employ media with nutrient-enriched concentrations and stoichiometric conditions that deviate from environmentally relevant conditions (Brooks et al. 2015). Further, nutrient-enriched conditions can promote development of HABs and associated production of algal toxins, which are now recognized to confound stressor identification approaches for anthropogenic contaminants (Brooks et al. 2016).

More complex laboratory and (semi)controlled field studies are needed to assess the potential additive, antagonistic, or synergistic effects of these complex stressor mixtures. As one example, Taylor et al. (2018) recently demonstrated the usefulness of employing coupled field studies with experimental stream mesocosm experiments to identify ecological thresholds associated with P enrichment. Unfortunately, similar studies have rarely examined influences of agrochemicals or veterinary medicines, a number of which are actually pesticidal, on stream ecosystems across nutrient gradients. Aided by answering other priority research questions identified in the current paper, developing fundamental understanding of the specific Mode of Action (MOAs) of these chemicals will help determine their combined effects. However, the data generated need to be supported within ecological risk assessment models that are able to accurately predict cumulative effects, including ecosystems services (Syberg et al. 2017). Herein, future research at the intersections of ecological stoichiometry and toxicology (i.e., how nutrition can affect the toxicity of contaminants, how contaminants can influence nutrient dynamics, or how nutrients can influence toxins production) promises to support an understanding of interactive effects of anthropogenic contaminants and algal toxins in nutrient-enriched systems (Conine and Frost 2016). Similarly, advances in ecological genomics are poised to support environmental assessment of complex stressors in the field (Yang et al. 2018; Zhang et al. 2018).

What are the effects of changing demographics, economic development, consumption patterns, and climate (e.g., ocean acidity, water temperature) on chemical emissions, environmental fate, and ecotoxicology of contaminants and multiple stressors?

Anthropogenic stressors, including increased population, economic activity, and changing consumption patterns, are contributing to rapid environmental change (Steffen et al. 2015). The identified global megatrends of increased urbanization, diverging population trends, changing disease burdens, and accelerating technological growth will determine the types and quantities of chemicals released regionally (e.g., Kookana et al. 2014). Our current paradigms for environmental fate and toxicity of contaminants will be challenged by the anticipated increase in environmental pollution (EEA 2015) and the consequences of climate change. Global climate change is anticipated to alter both the environmental variables (e.g., temperature, precipitation, salinity, pH) that determine the environmental fate and toxicity of chemicals as well as the resilience of organisms to cope with exposure to chemical stressors (Hooper et al. 2013). Risk assessment tools and environmental surveillance systems will need to be sufficiently adaptive to identify and prioritize emerging threats, particularly those that arise due to a combination of chemical and physical stressors, some of which will be driven by global climate changes (Landis et al. 2013). Given the inherent difficulties in replicating "real world" conditions for experiments, our predictive modeling tools will need to be refined to ensure that a precautionary approach can be taken to managing risk in a rapidly changing world.

What are the combined effects of very low levels of multiple contaminants (e.g., pesticides, natural resource extraction contaminants, salinity, pharmaceuticals and personal care products, endocrine-disrupting chemicals) with different modes of action on aquatic and terrestrial organisms and ecosystems?

Understanding environmental consequences of chemical mixtures remains one of the most challenging issues in achieving sustainable environmental quality (Van den Brink et al. 2018; Fairbrother et al. 2019). With increasing urbanization, multiple land uses are interfacing in peri-urban watersheds, which inherently increases the likelihood of diverse contaminants from urban, agricultural, and industrial activities that co-occur in complex mixture scenarios. Guidelines derived for individual stressors may not be sufficiently protective when ecosystems are exposed to multiple stressors. For example, changes in benthic community distributions have been reported at concentrations below individual metal guideline values (Tremblay et al. 2017). Salinization is particularly relevant to regions in Australasia, yet influences of salinity gradients on contaminants with diverse modes of action are poorly understood among species (Canedo-Arguelles et al. 2018). Various toxicity identification evaluation (TIE) protocols, response-directed fractionation procedures, and effects-directed analyses have been developed to identify causative chemical stressors within surface waters and sediments. However, it is particularly important to define strengths and limitations of historical bioassays employed for such activities, particularly when low levels of biologically active contaminants with diverse MIEs are considered. In recent years, bioassay tools with increasing mechanistic specificity have become important for diagnostic applications (Escher et al. 2014) beyond the

traditional morphometric aquatic toxicity responses introduced above that are employed in TIEs (USEPA 1991). Unprecedented opportunities are emerging with use of high-throughput in vitro, transgenic fish lines, and in situ toxicogenomic platforms when coupled with targeted and nontargeted chemical analyses (Bradley et al. 2017) in the field (Blackwell et al. 2017; Bradley et al. 2017; Perkins et al. 2017). However, metabolic transformation of contaminants and other basic scientific limitations remain when extrapolating in vitro to in vivo effects and even comparing responses among the 2 most common fish models (Corrales et al. 2016; Steele et al. 2018). Advancing AOP efforts for mixtures and predictive modeling of these complex low-level constituents will be important. The funnel hypothesis (Warne and Hawker 1995) postulates that, as the numbers of chemicals present at equipotent concentrations increases, the likelihood of additive combined effects increases. Efforts are needed to identify whether, when, and what specific MOAs drive divergence from such theoretical constructs of low-level mixture toxicity. It is thus not surprising that understanding the environmental implications of chemical mixtures was also identified as a priority research question in GHSP efforts from Europe (van den Brink et al. 2018), Latin America (Furley et al. 2018), and North America (Fairbrother et al. 2019). Clearly, this area deserves future attention.

RISK ASSESSMENT, REGULATIONS, AND GUIDELINES

What water quality guidelines are needed to protect temporary waters and associated ecosystems from the influences of development?

Temporary waters (i.e., intermittent, ephemeral, and seasonal) are common in temperate, arid, and semiarid landscapes of Australia and many other regions around the world. Sheldon and Fellows (2010) reported that up to 95 % of Australia's river channels are temporary, while a large proportion of the standing inland waters are also classified as temporary. Consequently, when these waters are present they are an extremely important source of water for the ecosystems of inland Australia and other regions. To date, much of the research has focused on the effects of extraction and sustainable use of temporary waters (Acuña et al. 2014; Datry et al. 2014), provision of their ecosystem services (Boulton 2014), and the importance of wetting and drying cycles for ecosystem health (Leigh 2013). However, there is a recognized need to better address changes in water quality arising from urbanization, agriculture, and mining (e.g., Queensland, Ramsay et al. 2012; South Australia, Botwe et al. 2015).

Due to the nature of these temporary waters, they are likely to experience pulse-exposure scenarios, but there are limited data sets that are useful for determining water quality guideline values for episodic exposures to contaminants. Moreover, many temporary waters have been converted to perennial or near-perennial waters by effluent discharges (Brooks et al. 2006), which represent important systems for environmental management with changing climatic conditions (Luthy et al. 2015). Although there are controls in Australia and some other countries on water quality in discharges and/or receiving waters for perennial or near-perennial waters, no specific guidance exists in any set of guidelines or regulations on the combined impact of conversion from temporary to nontemporary status together with alteration of water quality. Understanding and managing environmental quality impairments in these temporary waters represents a timely research need for parts of the Australasian region of Oceania and other global systems experiencing urbanization and climate change.

What are the effects of short magnitude, frequency, and duration (e.g., intermittent, episodic) exposures to contaminants and other stressors, and how can these scenarios be effectively incorporated into water quality guidelines?

Water quality criteria, standards, and guidelines are developed to protect various uses of surface waters. Through these efforts, threshold concentrations of contaminants (e.g., metals, pesticides, ammonia) and other stressors (e.g., depressed dissolved O, increased temperature) are identified and then applied, particularly in developed countries. Such regulatory "bright lines," representing specific concentrations of individual contaminants, have historically been intended to be protective of, and ideally predictive of, ecological integrity. Presently, these numeric values are most commonly derived from probabilistic analyses of results from single-species toxicity assays, which are intended to identify concentration–response thresholds, instead of individual species or community effects from episodic exposures that inherently vary in magnitude, frequency, and duration (Posthuma et al. 2001). For example, King et al. (2016) recently reported ecological structure and function responses to environmentally realistic episodic pulses of a common herbicide using outdoor stream mesocosms. Clearly, an advanced understanding of responses to episodic and intermittent chemical exposures is needed. Such information, while requiring innovative mechanistic coupling of toxicokinetics and toxicodynamics, and ecological genomics in the field, promises to reduce uncertainties associated with laboratory-to-field extrapolation during derivation of water quality guidelines.

How can we measure ecosystem resilience to and recovery following exposure to stressors?

Stochastic events influence ecosystem services and biodiversity, which are among the most common protection goals identified during problem formulation of ecological risk assessments. Such stochasticity inherently affects interpretation of stressor-response observations in the field and implementation of environmental management decisions. Although the diversity–stability hypothesis and functional redundancies have long been considered, both theoretically and empirically, and debated (McCann 2000) in ecology and ecotoxicology, identifying functional traits within

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assemblages and other ecosystem characteristics that impart resilience to natural and anthropogenic stressors remains decidedly challenging. In fact, 2017 has been described as the year of the disaster, with numerous billion-dollar events reported throughout the world (NOAA 2018). Herein, ecosystem services, when not compromised, represent key management objectives for disaster risk reduction and climate change adaptation (Monty et al. 2016; Renaud et al. 2016), and are appropriately included in the United Nation's Sendai Framework for Disaster Risk Reduction for 2015 to 2030 (UNDRR 2015). For example, rapid global declines of terrestrial and aquatic species present a profound manifestation of cumulative threats to biodiversity. In Australasia, degradation of the Great Barrier Reef has prompted extensive efforts to define cumulative stressors and advance resilience-based management (Anthony et al. 2013). In New Zealand, large earthquakes in the Canterbury region resulted in loss of habitat and measurable stress on aquatic organisms (Potter et al. 2015; Chandurvelan et al. 2016). Similarly, the Rena oil spill, New Zealand's largest maritime environmental disaster, impacted hundreds of kilometers of coastline in 2011 (Schiel et al. 2016). In such cases, influences of rare species on ecosystems functions require additional study (Leitao et al. 2016). With the prospects of climate change further compounding multiple stressor effects on aquatic and terrestrial ecosystems, it appears clear that developing an advanced understanding of ecosystem resiliency prior to and following disasters and in the face of cumulative stressors has never been more important.

SPOTLIGHT ON AUSTRALASIA

Are there differences in toxicological thresholds among native and nonnative organisms, and how can species sensitivity information from nonresident species be used to predict adverse outcomes and protect our unique biota and ecosystems?

The iconic aquatic and terrestrial species unique to Oceania in general and Australasia in particular hold deep cultural significance to indigenous communities and are important to the recreational, commercial, and conservation sectors. However, most of the toxicity estimates are derived from studies that use North American and European species; very little toxicity data exist using Oceania species, with some notable exceptions. Consequently, the Australian and New Zealand Water Quality Management Strategy (ANZECC 2000), and the new revised guidelines took the pragmatic approach of deriving Default Water Quality Guideline Values using any available data that passed predefined quality control criteria. However, this approach makes the considerable assumption that native Oceania species are of a similar sensitivity to that of nonnative species. This assumption has not been comprehensively tested because there have been no broad-scale systematic comparisons on toxicity data from native Oceania species and nonnative species. It is important to note that a similar question was recently identified from Latin America (Furley et al. 2018). Advancing comparative and predictive toxicology research promises to help us understand differences among species sensitivities to contaminants with diverse mechanisms of action (Brooks 2018).

There have been many toxicity tests developed for native species in Australasia. The earliest of these native-species suites were developed to satisfy the research needs for controversial issues. For example, in the early 1990s, the National Pulp Mills Research Programme identified a number of temperate Australian species to assess the toxicity of pulp mill effluents and test "greener" technology options (Crossland and Abel 1992; Stauber et al. 1994). In New Zealand, a standard suite of 3 marine and 4 freshwater tests on native species was developed by the National Institute of Water and Atmospheric Research (Hall and Golding 1998), and sensitivities of these species were compared with those of nonnative species for 4 reference toxicants. A suite of standardized tropical freshwater toxicity tests was developed by the Environmental Research Institute of the Supervising Scientist for the regulation of the Ranger Uranium Mine, which is adjacent to the World Heritage–listed Kakadu National Park (Riethmuller et al. 2003). Both of these industries were faced with significant public opposition, but the development of native-species toxicity tests helped decision makers reassure the public that environmental issues were being addressed appropriately.

In more recent years, a member of the business community invested in the development of a suite of toxicity tests using native tropical marine species to improve the environmental management of their industrial effluents by using biological effects data (van Dam et al. 2018). The motivation for this was to address a gap that existed for tropical species because most toxicity tests were developed by first-world nations in temperate environments (van Dam et al. 2008). Such research investments have subsequently benefited other industries that have capitalized on the availability of the tropical tests (e.g., Gissi et al. 2018), which has enabled valuable tropical-versus-temperate comparisons (Peters et al. 2019). Ad hoc toxicity testing using culturally significant fishes (e.g., Inanga, *Galaxis maculatus;* McRae et al. 2018) and invertebrates (e.g., freshwater mussels, clams, and crayfish; Clearwater et al. 2014) has been developed in New Zealand and Australia (e.g., Markich and Camilleri 1997). The sensitivities of native and nonnative species to certain contaminants have been compared in some cases. For example, Hagen and Douglas (2014) asked this question but could find sufficient data for only 3 chemicals, that is, 4-chlorophenol, phenol, and ammonia. They concluded that there were no differences in species sensitivity that warranted the application of safety factors. However, until a sufficient Oceania data set for a broader set of chemicals is available, this question will remain unaddressed. Here again, advancing comparative ecotoxicology research in this area is a priority.

How do we incorporate and protect cultural and social values (relating to humans, biota, and ecosystems) to empower citizen, societal, and indigenous engagement in the research, management, and legislation of priority environmental contaminants?

Indigenous peoples are key to many environmental management projects and decisions globally, where their status ranges from disadvantaged minorities to the dominant cultural group within their respective communities and country. Indigenous peoples carry with them distinctive and localized cultural and environmental knowledge, based on thousands of years' experience (Stevenson 1996). However, mechanisms to incorporate their indigenous knowledge, cultural values, and traditional management systems into decision-making processes remain poorly formulated in most global legislatures, business decisions, and academic programs. This is the case despite numerous international and regional, legally and nonlegally binding instruments (Convention on Biological Diversity 1992; UN 1992, 2007) and statutory national obligations (legislative and policy level; Palmer 2008) requiring appropriate and meaningful indigenous peoples' involvement. Further, ignorance of inherent challenges around the application of indigenous knowledge, existing power relations, and contextual nuances of Indigenous knowledge have also hampered access to, and an articulation of indigenous knowledge in, environmental management and decision-making processes (Briggs 2005).

Oceania, like other global regions, has a diverse range of indigenous peoples, each with their own unique history, experiences, and challenges with respect to articulating their voice around environmental contaminants. Unfortunately, indigenous knowledge and values (IK&V) are not well represented in assessment and management approaches in environmental issues. Applying an indigenous knowledge lens considers the whole of environmental change in determining the impact of contaminants (Kookana et al. 2013). In addition to considering the impact of contaminants to indigenous people's environments, biodiversity, and culture (Ataria et al. 2016), the impact of practices that disrupt ecological patterns and services are also critical to consider, particularly for those communities that are reliant on natural resources for their physical and cultural existence.

The collaboration of traditional knowledge and research is needed between communities and indigenous peoples. Advancing forward it will be imperative to manage environmental quality as both strive to advance their knowledge systems to protect environmental quality and natural resources. Engagement protocols differ across all indigenous peoples globally. However, the environmental science and engineering communities can assist in cocreating protocols in close consultation with the relevant indigenous peoples that are specific to regions, are equitable, empower mutual benefit, and are enduring. Indigenous people assert an inherent expectation to be involved in caring for, protecting, and rejuvenating their traditional land, freshwater, marine, and atmospheric environments. To some it is a cultural obligation as custodians, whereas to others it is a means of maintaining their identity by reinstating and retaining their cultural practice and heritage and by empowering their developmental aspirations for future generations. Here we call for concerted global research efforts to integrate IK&V during problem formulation and, more specifically, identification of ecosystem protection goals within environmental risk assessment and management efforts.

TOOLS FOR IMPROVING RISK ASSESSMENT

How do we exploit, collate, and integrate existing environmental toxicology, chemistry, and geospatial data to help develop robust risk assessment?

Natural ecosystems are increasingly degraded as a result of exposure to multiple stressors that vary over space and time. We now know that the global reach of anthropogenic stressors is beyond what was previously predicted, with persistent pollutants such as PCBs, polybrominated diphenyl ethers (PBDEs), and microplastics found in the remote Arctic and deep sea trenches (Schlining et al. 2013; Van Cauwenberghe et al. 2013; Obbard et al. 2014; Jamieson et al. 2017). To address these challenges, we have increasing access to physical, biological, and chemical measurements from new remote sensing tools and their integration into geographical information systems (Dafforn et al. 2016). Moreover, advances in molecular analysis have allowed us to capture more holistic information about the health of entire ecosystems, from microbial to macrobiotic scales, and to go beyond impacts on structure to understand consequences for ecosystem function and services (Chariton et al. 2016). The advent of real-time technologies such as the MinION for DNA/RNA sequencing and the microfluidic lab-on-a-chip provides us with more opportunities for improved spatiotemporal analyses (Campana and Wlodkowic 2018). The availability of these data and new geospatial and ecogenomic bioassessment tools has the potential to increase our capacity for ranking and understanding stressor impacts and crucially to allow us to differentiate stressors impacts when present in combination.

At the same time, we are experiencing technological advances and associated information booms, with many decades of ecotoxicological testing and biomonitoring information collected and added to databases following regulatory requirements. Numerous databases around the world hold information about different chemical stressors as well as potential biological responses. For example, the Pesticide Properties DataBase has approximately 2300 pesticide active substances and >700 metabolites stored alongside response metrics related to human and environmental health (Lewis et al. 2016). Other large collections of biological data such as GENBANK (Benson et al. 2010), TRY (Kattge et al. 2011), D3 (Hintze et al. 2013), COMADRE, and COMPADRE (Salguero-Gómez et al. 2015) offer information related to genetics, functional plant ecology, grassland

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ecology, and plant and animal demography alongside metadata from, for example, ecoregions that can be used to ask globally relevant questions (Salguero-Gómez et al. 2015) and be integrated within risk assessment frameworks.

Machine learning techniques could be used to harness the power of such extensive data sets into risk assessment. For example, molecular tools such as transcriptomics have been integrated with machine learning techniques to identify and classify priority EDCs (Ornostay et al. 2013). Similarly, artificial neural networks have been used to select biomarkers on the basis of key response variables (Bradley 2012). Decision tree models based on environmental metadata have been used to predict benthic macroinvertebrate distributions (D'Heygere et al. 2003). Environmental metadata using a Random Forests machine learning algorithm have likewise been used to reveal nonlinear relationships and critical thresholds for cyanobacterial blooms (Nelson et al. 2018), which is significant because HABs now represent the greatest water quality threat in some ecosystems (Brooks et al. 2017).

Overall, our predictive power has exponentially increased, allowing us to move beyond the current norm of single-stressor assessments, done at small spatial scales and with few receptors, to enhanced risk assessment (Van den Brink et al. 2016). However, there are still hurdles to overcome before we can harness and exploit this Big Data to its fullest. We need to 1) improve our techniques for data validation to remove errors in, for example, specimen identifications for DNA barcoding; 2) improve the availability of data not just through openness but also by targeting underrepresented taxonomic and geographic groupings; 3) improve standardization so that data are comparable over space and time; and 4) invest in real-time technologies that provide direct measures of impact rather than providing proxies (Dafforn et al. 2016).

How can prescreening techniques (e.g., in silico, in vitro) be developed, advanced, and validated to identify and predict whole organism effects?

The rate of discovery and synthesis of new chemicals has grown exponentially in the last decades, exceeding our ability to empirically determine the toxicity of new compounds using conventional (whole animal) toxicity testing methods. This means that more and more chemicals are put into global circulation without a thorough understanding of their potential toxic impacts. Too often, the chemicals substituted for problematic substances display unacceptable toxicity profiles (Rosal et al. 2010; Björnsdotter et al. 2017). Unfortunately, conventional toxicity testing provides too narrow a funnel (in terms of time, cost, and ultimately, throughput) to assess the risk of the vast number of new compounds designed daily by chemical, pharmaceutical, and agricultural industries. Clearly, a higher throughput approach is required.

This is where in silico modeling and in vitro pretesting methods offer a way forward. Using these HTS techniques, which can screen thousands of chemicals every day, toxicity testing can be prioritized and focused on those molecules most likely to pose a threat to humans and/or ecosystems (Collins et al. 2008). This is the paradigm shift foreshadowed in the Tox21 vision for toxicity testing in the 21st century (NRC 2007), and which relies on the AOP concept (Ankley et al. 2010) to translate a key initiating event at the molecular or cellular level (either modeled in silico or measured in vitro) to the adverse outcome of consequence (e.g., survival, reproduction, development, behavior) that is our focus of concern (Ankley et al. 2016).

Although tremendous progress has been achieved in adapting and validating in vitro tools to environmental monitoring and risk assessment (e.g., in Australasia, Coleman et al. 2008; Mispagel et al. 2009; Chinathamby et al. 2013; Bain et al. 2014; Escher et al. 2014; Leusch et al. 2014; Scott et al. 2014; Roberts et al. 2015; Boehler et al. 2017; Neale, Achard et al. 2017; Neale, Altenburger et al. 2017; Chen et al. 2018; Leusch et al. 2018), some fundamental questions still need to be systematically addressed before these techniques can become reliable predictors of whole animal level effects:

1) Refine quantitative in vitro to in vivo extrapolation (QIVIVE): Although there is a clear correlation between in vitro response and in vivo effects for some endpoints such as acute toxicity (Kaiser 1998; Tanneberger et al. 2013; Natsch et al. 2018) and receptor-mediated endocrine effects (Sonneveld et al. 2006, 2011; Henneberg et al. 2014), toxicokinetic factors (absorption, distribution, metabolism, and excretion) still pose a difficult challenge for QIVIVE (Blaauboer 2015; Meek and Lipscomb 2015), although groundbreaking studies suggest that this may soon be within reach (Rotroff et al. 2010; Wetmore 2015).
2) Fully map relevant AOPs: There is still much work to be done to map key events (KEs) to connect the dots between the molecular or cellular initiating event and the ultimate apical consequence to produce comprehensive AOPs, for both humans and ecosystems (Ankley et al. 2016). In combination with QIVIVE, this mapping would ultimately allow us to produce quantitative AOPs.
3) How much is too much? In vitro assays are often exquisitely sensitive and able to detect activity even in clean samples. In whole organisms, a small amount of dysfunction at the molecular and cellular level can often be compensated for by defense and repair mechanisms to avoid any higher level consequence. Until we can quantitatively extrapolate from in vitro to in vivo (steps 1 and 2 above) and quantify the repair ability for each type of dysfunction, it will be difficult to accurately link an in vitro response to an in vivo adverse effect. In the meantime, several different approaches have been proposed to produce effects-based trigger (EBT) values, including reading across from current chemical guidelines (Escher et al. 2015, 2018) or de novo derivation (Brand et al. 2013; Jarošová et al. 2014) and how to use them in a practical context (Leusch and Snyder 2015; Ron et al. 2017).

Clearly, there are still some unanswered questions in how we use in silico models and in vitro bioassays. But these new

tools also offer a unique and necessary solution to overhaul the single-chemical risk assessment approach that relies on the traditional aquatic models and endpoints discussed above and to properly screen the sheer number of chemicals that make our modern lifestyles possible without negatively impacting human health and the environment. Further, advancing these diagnostic tools, particularly when coupled with nontarget analytical methods, promises to support efforts to answer other priority research questions identified here.

How can ecotoxicology information be integrated more closely during interpretation of ecological data?

Two closely related questions focus on the necessity of more closely integrating research among ecology and ecotoxicology, which in many parts of the world remain separate fields of study. Whereas basic ecology studies in terrestrial and aquatic systems are fundamentally important for conservation, including understanding ecosystem services and biodiversity, translational ecological efforts remain critical for environmental assessment and management (Saaristo et al. 2018). Interpretation of field data sets can be challenging due to ecosystems commonly being exposed to multiple stressors, which may be known or unknown. Subsequently, identifying underlying causative relationships among complex stressors requires multidisciplinary perspectives. For example, failure to consider chemical stressors beyond nutrient enrichment during basic ecological and biogeochemical studies in systems influenced by agriculture and urbanization can confound interpretation of findings. For decades, researchers have called for close integration among ecology and ecotoxicology research pursuits (Cairns 1988; Zala and Penn 2004; Melvin and Wilson 2013; Arnold et al. 2014).

More recent contributions in community and stream ecology (Rohr et al. 2006; Rosi-Marshall and Royer 2012; Bernhardt et al. 2017), behavioral ecology (Saaristo et al. 2018), and ecophysiology (Cooke et al. 2013) consistently echo these earlier sentiments. Beyond applied studies aimed at stressor identification, anthropogenic chemicals, particularly specifically acting contaminants (e.g., pesticides, pharmaceuticals), can serve as experimental scalpels to dissect basic structural and functional relationships. For example, mesocosm studies by Fairchild et al. (1994) with pesticides partitioned direct from indirect community interactions. Environmental studies with pharmaceuticals have yielded unique comparative ecophysiology information (Owen et al. 2007). Addressing several of the questions identified in earlier sections aimed at advancing integrated research in ecological threshold analyses, environmental genomics, quantitative AOPs, and integrative, comparative, and predictive toxicology, when coupled within mainstream experimental and theoretical ecology, promises reciprocal and transformational basic and applied benefit, particularly as global ecosystems continue to be influenced by complex stressors.

How do we advance ecotoxicology testing to be more relevant to ecological systems?

Prospective ecotoxicology assays are employed by businesses and government agencies to assess the safety of substances prior to their introduction to the market or to assess contaminants of potential concern before they are released to the environment. Industrial operations have also been required to synthesize predicted effluents for safety assessments when changing their waste treatment or introducing new ones. Historical products in commerce may also be prioritized for more detailed safety assessment. Whereas retrospective ecotoxicological studies often include in vitro and in vivo models to examine field-collected water, sediment, or soil in laboratory settings, in situ studies with caged organisms, and surveillance of biological conditions in the field, micro- and mesocosm studies are employed for both prospective and retrospective efforts in an attempt to bridge laboratory-to-field information. For decades, researchers have noted challenges from lower to higher scales of biological complexity due to increasing endpoint variability (and societal relevance) and environmental stochasticity as one moves from the laboratory model to ecosystem-level perturbations (Dickson et al. 1992; La Point and Waller 2000).

Predictive coupling of laboratory with field perturbations remains a grand challenge in environmental science. However, it remains important to ensure the quality of data produced from standardized model systems, while advancing innovative and exploratory ecotoxicological research that may not be intended or amenable to directly be integrated within environmental assessments (Moermond et al. 2017). Such challenges were considered during a recent SETAC Pellston Workshop on "Improving Usability of Ecotoxicology in Regulatory Decision Making, August 2015" which has documented the need for ecotoxicological data sets that are reliable and relevant (Rudén et al. 2017). Beyond the traditional biological indices approaches, recent progress in ecological threshold analysis (Baker and King 2010), ecological genomics (Zhang et al. 2018), and species traits (Van den Brink et al. 2013) are improving field studies. Future research in mechanistic and comparative ecotoxicology, if integrated with ecology, is poised to support more robust experimental designs and extrapolations across levels of biological organization, although uptake of recent advances within prospective and retrospective regulatory activities remains differential around the world. Therefore, employing reasonable and defensible weight-of-evidence approaches will remain important (Suter 2016).

CONCLUSIONS

The Australasian region of Oceania faces increasingly diverse environmental challenges associated with multiple stressor influences on environmental quality. The current analysis represents an initial attempt within Oceania to develop a research agenda aimed at advancing toward more sustainable environmental quality and ecosystem integrity. Through a transparent, bottom-up, multidisciplinary, and

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multistakeholder process, we identified 20 priority questions to support future environmental research. As noted recently (Van den Brink et al. 2018), step changes are needed for basic and applied studies of environmental stressors, and their management, if we are to achieve the United Nation's Sustainable Development Goals (UN 2015). We agree, as evidenced by the interconnections among priority research questions reported herein.

Several questions identified the need to improve predictive environmental exposure and toxicology tools for risk assessment and to reduce and replace animal testing. Similarly, the development of robust nontarget analytical screening techniques to determine priority contaminants in ecosystems exposed to complex mixtures was identified as an urgent need. Strategically advancing these areas will assist in addressing other questions related to multiple stressors (e.g., chemicals, salinity, acidification), susceptibility of regional flora and fauna, management of unique ecosystems (e.g., ephemeral water bodies), and stress from global megatrends (e.g., urbanization, the food–energy–water nexus) and climate change. The importance of understanding the comparative sensitivities of regionally unique species was also reported from Latin America (Furley et al. 2018). Incorporating and protecting cultural and social values to empower citizens, especially indigenous peoples' engagement during research, management, and policy development, was further identified as a key research opportunity. In this regard, ongoing efforts within Australasia are incorporating cultural knowledge during identification of ecosystems protection goals (i.e., the Whanganui River and other systems in New Zealand have been granted the same legal rights as a person), which represents an interesting model that could benefit elsewhere.

We expect the top 20 questions identified here will be complementary to and assist advancement of national prioritization efforts such as the Australian Science and Research Priorities and Practical Challenges (Australian Government 2015) and the New Zealand National Science Challenges (MBIE 2016). For example, 5 of the 11 Australian Science Research Priorities (e.g., Environmental Change, Energy, Soil, Water, Food) include Practical Challenges to address sustainable environmental quality and ecosystem integrity. Similarly in New Zealand, Science Challenges relevant to sustainable environmental quality include Biological Heritage, The Deep South, Sustainable Seas, and Our Land and Water. Expertise and capacity within the Australasia chapter of SETAC and other scientific disciplines in Oceania are well positioned to support these efforts (a brief history of SETAC Australasia can be found in the Supplemental Data). Answering the 20 priority research questions will not be trivial, but will support basic and applied research innovation and advancement of robust practices to achieve more sustainable environmental quality within the region and other parts of the world.

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SUPPLEMENTAL DATA

Background information and the full set of questions submitted:

1) A Brief History of the SETAC Australasia Chapter
2) Author-submitted Questions

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Per- and Polyfluoroalkyl Substances

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemical compounds that are of emerging concern to environmental health. A recent count has identified over 4,700 individual PFAS species. PFAS all share the characteristic of being “chains” that contain “links” made of carbon fluorine bonds (C-F). PFAS encompass a big universe of different substances that vary in state from gas to liquid or solid, all with vastly different properties.

PFAS substances have unique characteristics—resistance to heat, water, oil and stains—that make them useful in a variety of industrial applications and popular in consumer goods such as waterproof outdoor gear, non-stick cookware and stain-resistant upholstery. Many PFAS are stable and long-lasting in the environment, acquiring the name “forever chemicals.” Industrial use

In this section

of some of these compounds has been halted; however, many derivatives are still in commerce and more are under development. PFAS are now found in many compartments of the environment.

There is a pressing need to identify an approach to characterize and measure PFAS routinely, as well as assess their potential effects on human and ecological health. To do so, there is a lot of research directed at understanding the sources of PFAS, their fate and transport in the environment, and their potential toxicity to humans and wildlife. SETAC scientists are heavily involved in this work, some of which is presented below.

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- Metals and Metalloids
- Nanomaterials
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- Environmental Risk Assessment of PFAS
  Virtual Issue (2019)

- A Critical Review of the Application of Polymer of Low Concern and Regulatory Criteria to Fluoropolyers
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- Fate and Effects of Poly- and Perfluoroalkyl Substances in the Aquatic Environment
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Critical Review

Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research

Suzanne E. Fenton,[a] Alan Ducatman,[b] Alan Boobis,[c] Jamie C. DeWitt,[d] Christopher Lau,[e] Carla Ng,[f] James S. Smith,[g] and Stephen M. Roberts[h,*]

[a]National Toxicology Program Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA
[b]West Virginia University School of Public Health, Morgantown, West Virginia, USA
[c]Imperial College London, London, United Kingdom
[d]Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA
[e]Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, North Carolina, USA
[f]Departments of Civil and Environmental Engineering and Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[g]Navy and Marine Corps Public Health Center, Portsmouth, Virginia, USA
[h]Center for Environmental & Human Toxicology, University of Florida, Gainesville, Florida, USA

Abstract: Reports of environmental and human health impacts of per- and polyfluoroalkyl substances (PFAS) have greatly increased in the peer-reviewed literature. The goals of the present review are to assess the state of the science regarding toxicological effects of PFAS and to develop strategies for advancing knowledge on the health effects of this large family of chemicals. Currently, much of the toxicity data available for PFAS are for a handful of chemicals, primarily legacy PFAS such as perfluorooctanoic acid and perfluorooctane sulfonate. Epidemiological studies have revealed associations between exposure to specific PFAS and a variety of health effects, including altered immune and thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, adverse reproductive and developmental outcomes, and cancer. Concordance with experimental animal data exists for many of these effects. However, information on modes of action and adverse outcome pathways must be expanded, and profound differences in PFAS toxicokinetic properties must be considered in understanding differences in responses between the sexes and among species and life stages. With many health effects noted for a relatively few example compounds and hundreds of other PFAS in commerce lacking toxicity data, more contemporary and high-throughput approaches such as read-across, molecular dynamics, and protein modeling are proposed to accelerate the development of toxicity information on emerging and legacy PFAS, individually and as mixtures. In addition, an appropriate degree of precaution, given what is already known from the PFAS examples noted, may be needed to protect human health. *Environ Toxicol Chem* 2021;40:606–630. © 2020 SETAC

Keywords: Per- and polyfluoroalkyl substances; Perfluorooctane sulfonate; Perfluorooctanoic acid; Persistent compounds; Contaminants of emerging concern

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in environmental media because of their prolific use in a variety of industrial and consumer products and processes (Jian et al. 2018; Sunderland et al. 2019). Widespread human exposure to PFAS in water, food, and air coupled with the lengthy environmental persistence and biological half-lives of some PFAS have led to measurable PFAS in the blood of nearly the entire population in developed countries, with health effects reported globally (Kato et al. 2011; Khalil et al. 2016; Stubleski et al. 2016; Jian et al. 2018). Information needed to evaluate the potential risk of harm from PFAS includes the types of adverse health effects that might occur at environmentally relevant exposures, especially in sensitive life stages. Information is also needed regarding the mode(s) of action for

* Address correspondence to smroberts@ufl.edu
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PFAS toxicity, PFAS toxicokinetics in both humans and laboratory animal models, and dose–response relationships. Risk estimates can be used to inform public health exposure limits that will determine the need for exposure mitigation and environmental cleanup.

There are several challenges in obtaining the information needed to assess human health risk from the large number of PFAS with a wide range of structures and chemical properties (Buck et al. 2011; Wang Z et al. 2017; Organisation for Economic Co-operation Development 2018). Data on the identity, composition, and quantity of PFAS used in products and processes are often treated as confidential business information, hampering efforts to estimate exposure sources and routes. The Organisation for Economic Co-operation and Development's (OECD's) chemical inventory reports over 4000 substances that contain at least one perfluoroalkyl (–CnF2n–) moiety (Organisation for Economic Co-operation Development 2018), and the US Environmental Protection Agency (USEPA) has a curated list of over 8000 PFAS included, based on structure (US Environmental Protection Agency 2018) from the CompTox Chemicals Dashboard (Williams et al. 2017). The USEPA estimates that more than 600 PFAS are currently in commercial use (US Environmental Protection Agency 2019). Experimental studies of PFAS have been limited by funding and the availability of analytical standards, confounded by the prevalence of background contamination in laboratory materials, and challenged by physicochemical properties such as high surface activity that can interfere with and complicate measurements. Consequently, sufficient information to conduct quantitative risk assessment is currently available for only a relative few PFAS (Post 2020). Further, although typical human exposures involve various combinations of PFAS (Centers for Disease Control and Prevention 2017), only a few efforts address interactions of PFAS mixtures; and a well-founded, scientific basis on which to evaluate their combined toxic potential does not yet exist (Carr et al. 2013; Wolf et al. 2014; Zhou et al. 2017; Hoover et al. 2019; US Environmental Protection Agency 2020).

The Society of Environmental Toxicology and Chemistry (SETAC) North America held the focused topic meeting and workshop "Environmental Risk Assessment of PFAS" on 12 to 15 August 2019, covering a wide range of topics related to the characterization of health risks posed by PFAS. The overarching purpose of the meeting was to begin a scientific discussion on how best to approach studying, grouping, and regulating the large number of PFAS to which people and other species are potentially exposed (for charge questions and other details, see Johnson et al. 2020). We refer to these PFAS as "legacy" (those perfluoroalkyl acids for which there are accumulating health data but that may be phased out or decreased in use) and "emerging" (those which are being used as replacements, often with minimal health effects data). The objectives of the Human Health Toxicity section were to provide an assessment of the state of the science in understanding toxicological effects of PFAS and to explore and discuss strategies for advancing knowledge on the toxicity of individual and groups of PFAS.

CURRENT KNOWLEDGE OF PFAS TOXICITY IN HUMANS

Like other chemicals, PFAS are potentially capable of producing a wide range of adverse health effects depending on the circumstances of exposure (magnitude, duration, and route of exposures, etc.) and factors associated with the individuals exposed (e.g., age, sex, ethnicity, health status, and genetic predisposition). Aspects to consider when establishing the health effects of greatest concern are 1) effects for which evidence is the strongest (strength of evidence can come from consistency of effect across studies, strength of effect associations in epidemiological studies, and species concordance, as examples), and 2) effects for which potential impact is greatest (factors contributing to impact can include severity of effect, functional impairment, persistence, and specific age groups that are susceptible, as examples). Brief summaries of candidate PFAS health effects from human and experimental reports are provided in this section (Figure 1).

Immune function

Epidemiological studies have explored relationships between PFAS exposure and laboratory biomarkers of immunomodulation, such as vaccine responses. A doubling of perfluorooctane sulfonate (PFOS) in maternal serum was associated with a 39% ($p < 0.001$) reduction in diphtheria antibody concentration in children (age 5 yr), with increased odds of falling below clinically protective values against diphtheria and tetanus at age 7 yr. The authors noted that a "2-fold greater concentration of major PFCs [perfluorinated compounds] in child serum was associated with a difference of −49% (95% CI, −67% to −23%) in the overall antibody concentration" (Grandjean et al. 2012). Decreased immunological response persisted at age 13 yr (Grandjean et al. 2017). Adverse associations were also noted for responses to rubella, mumps, and *Hemophilus* influenza vaccinations in children and to vaccinations in adults (Granum et al. 2013; Looker et al. 2014; Stein et al. 2016; Abraham et al. 2020). In a single study, modest down-regulation of C-reactive protein response, a marker of human systemic inflammation, was also reported to be associated with perfluorooctanoic acid (PFOA) blood levels (Genser et al. 2015).

Disease outcomes linked with immunosuppression such as clinician-recorded diagnoses of childhood infections have also been associated with prenatal exposures to PFOS and perfluorohexane sulfonate (PFHxS) (Goudarzi et al. 2017). A pregnancy cohort study prospectively detected increased risk of airway and throat infections and diarrhea in children through age 10 yr, correlated with cord-blood PFAS measurements (Impinen et al. 2018, 2019). A recent review concluded that exposure to PFAS in infancy and childhood resulted in an immunosuppressive effect characterized by an increased incidence of atopic dermatitis and lower respiratory tract infections (Kvalem et al. 2020). Some of the immunological effects were sex-specific, but the authors cautioned that there were inconsistencies across studies (Kvalem et al. 2020).

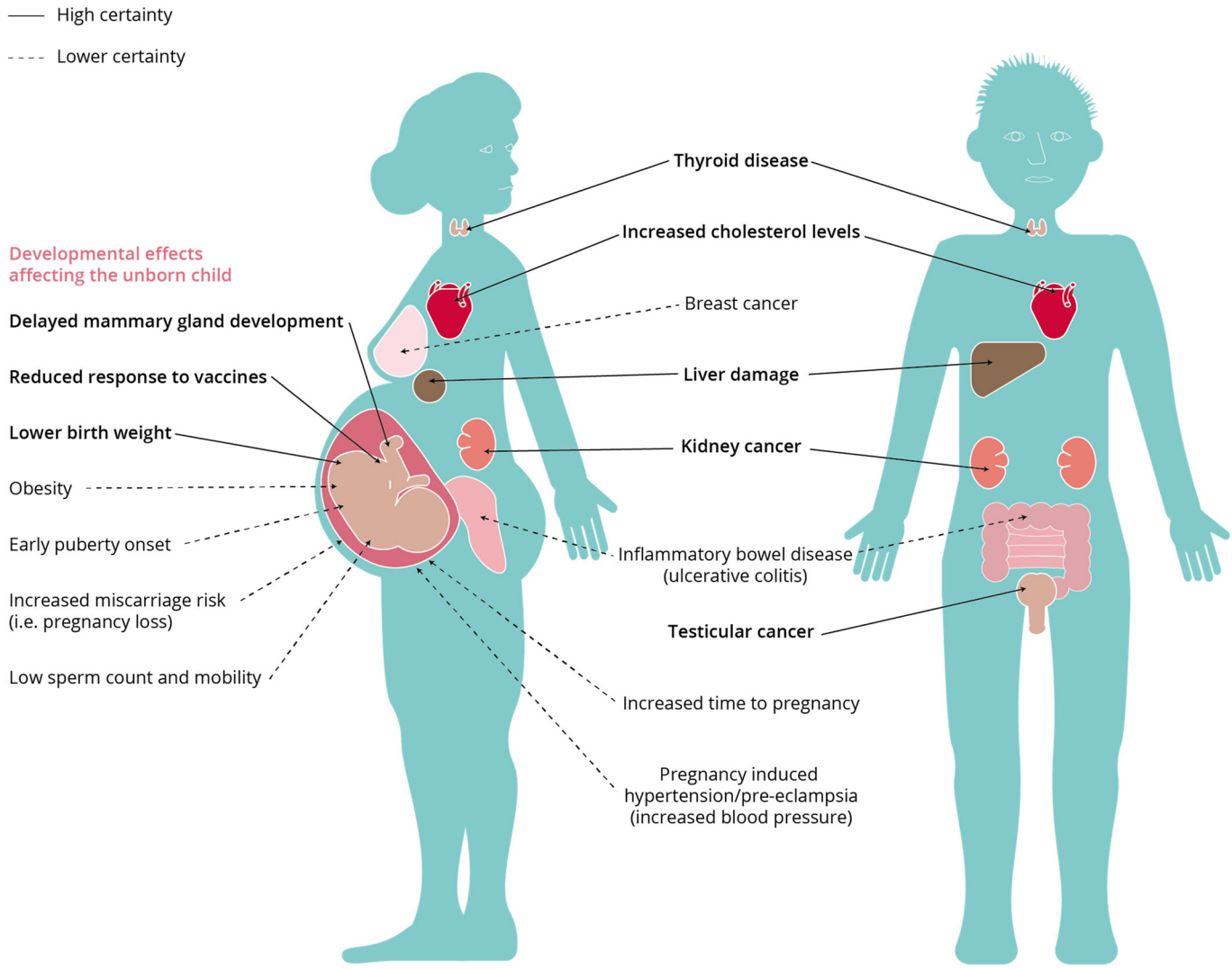

FIGURE 1: Effects of per- and polyfluoroalkyl substances on human health. Used with permission from European Environment Agency (2019). Original sources for this figure: National Toxicology Program (2016), C8 Science Panel (2012), IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2017), Barry et al. (2013), Fenton et al. (2009), and White et al. (2011b).

Overall, available data provide strong evidence that PFAS exposure can suppress the human immune response.

Population studies of immune hyperreactive diseases have resulted in mixed findings. Studies on childhood allergy and asthma outcomes have shown no association with PFAS (Impinen et al. 2018, 2019), whereas others have found substantial effects, including provocative evidence that subgroups of individuals not adequately immunized may be at an increased risk for disease a priori (Qin et al. 2017; Timmermann et al. 2017a). For example, a case–control study of Taiwanese children compared the first and fourth quartiles of serum measurements for 11 PFAS with asthma and other immune markers and reported confidence intervals well above 1.0 for PFOA and others (Qin et al. 2017). However, review articles concerning PFAS and childhood allergy and asthma offer nuanced, age- and sex-specific interpretations and advise against firm conclusions (Kvalem et al. 2020).

Chronic autoimmune outcomes, including thyroid disease (see section *Thyroid function*) and inflammatory bowel disease (IBD), have also been considered. A study in contaminated communities ($n = 32\,254$) detected an association between both prevalence and incidence of ulcerative colitis (UC) and PFOA exposure (linear trend $p = 0.0001$ [Steenland et al. 2013]). A worker study ($n = 3713$) found a higher prevalence ($p = 0.01$) and incidence ($p < 0.05$) of UC with increasing log PFOA serum concentrations (Steenland et al. 2015). A case–control study of children and young adults from a background exposure community in Atlanta, Georgia, USA, also found higher serum PFOA levels in patients with UC (Steenland et al. 2018b). In contrast to PFOA-related associations in US populations, a study of a contaminated community in Sweden ($n = 63\,074$) did not show a consistent association of IBD with any PFAS exposure (Xu et al. 2020b).

Recent, thorough reviews (National Toxicology Program 2016; DeWitt et al. 2019; Pachkowski et al. 2019) emphasize some key concepts: 1) there is concordance between animal studies and human epidemiological observations that PFAS modify the immune response, and 2) there are noted

complexities in assuming dose–response continuums, including possible differences in life-stage vulnerability. Authors of these reviews note uncertainty about which outcome will be of most importance but agree that immunotoxicity should be included among sensitive human PFAS toxicity endpoints.

Thyroid function

The C8 Science Panelists concluded that there is a "probable link" of PFOA exposure to thyroid disease, with sex-specific outcomes in women (for hyperthyroid disease) versus men (hypothyroid disease) (C8 Science Panel 2012). Subsequent reviews drew attention to hypothyroid outcomes in women and children and to the possibility that populations with a priori circulating antithyroid peroxidase antibodies may be at additional risk (Coperchini et al. 2017). A broad childhood disease review noted "some evidence" that PFAS cause childhood hypothyroidism and characterized the number of studies as "limited" for childhood disease conclusions (Rappazzo et al. 2017). A meta-analysis of 12 child and adult studies that excluded populations with higher exposures noted that PFAS exposure is negatively associated with serum total thyroxine levels and that "PFAS could induce thyroid dysfunction and disease" (Lee and Choi 2017).

Human thyroid disease is mostly the result of an autoimmune response and is 5 to 10 times more prevalent in women than men (Tadic et al. 2018). Concerning PFAS and clinically diagnosed outcomes, women in the highest quartile of PFOA exposure (>5.7 ng/mL) reported clinical hypothyroid disease (odds ratio 2.2, 95% confidence interval [CI] 1.4–3.7) over 3 cycles of National Health and Nutrition Examination Survey (NHANES) data (1999–2006, $n = 3974$ adults), with similar findings in men (Melzer et al. 2010). The C8 Science Panel studies (median serum PFOA 26.1 ng/mL) found thyroid disease hazard ratios of 1.00, 1.24, 1.27, 1.36, and 1.37 across cumulative exposure quintiles in women (log-linear trend $p = 0.03$ [Winquist and Steenland 2014b]), with parallel hypothyroid findings in children aged 1 to 17 yr (Lopez-Espinosa et al. 2012). The Ronneby, Sweden, population experienced excess risk of thyroid disease in a discrete time period (1984–2005) among women (hazard ratio 1.29, 95% CI 1.05–1.57) that did not persist over time despite higher cumulative PFAS exposure (Andersson et al. 2019). The authors did not link exposure to hypothyroid outcome, noting a nonmonotonic dose–response relationship (Andersson et al. 2019).

Human population studies augment experimental data that PFAS interact with thyroid hormone binding proteins (Berg et al. 2015; Ren et al. 2016; Zhang J et al. 2016), one of several mechanisms by which PFAS can perturb feedback relationships between free thyroid hormone and the hypothalamic–pituitary–thyroid axis. Exposures to PFAS also interfere with thyroid peroxidase (TPO) enzyme activity in vitro (Song et al. 2012). Several PFAS studies have pursued this putative mechanism, finding that maternal and neonatal thyroid hormone outcomes were more readily detected in those with a priori abnormally high circulating anti-TPO antibodies (Webster et al. 2014, 2016). One case–control study investigated congenital hypothyroidism, a rare condition. Serum concentrations of PFOA (5.40 vs 2.12 ng/mL; $p < 0.01$), perfluorononanoic acid (PFNA; 1.93 vs 0.63 ng/mL; $p < 0.001$), perfluorodecanoic acid (PFDA; 0.52 vs 0.30 ng/mL; $p < 0.005$), and perfluoroundecanoic acid (0.98 vs 0.44 ng/mL; $p < 0.005$) were higher in the diagnosed newborns; and levels of several PFAS, including PFOA and PFHxS, were correlated with thyroid autoantibodies (Kim et al. 2016).

Thyroid disease is not the only concern. Clinicians are concerned about subclinically elevated thyroid-stimulating hormone (TSH) in early pregnancy because it may be associated with several possible adverse maternal and fetal outcomes (Forhead and Fowden 2014). This general concern has prompted numerous PFAS-exposure evaluations of corresponding TSH in maternal serum, cord blood, and newborns. A review of maternal and child biomarkers with PFAS exposure noted that higher TSH has been reported in 4 second-trimester studies (Ballesteros et al. 2017), but there are also conflicting findings. Studies measuring PFAS in the first trimester have also found associations between PFAS exposure and altered TSH levels in newborns, including nonmonotonic patterns of dose response that mirror the marked alterations of thyroid hormone levels during pregnancy (Inoue et al. 2019).

From the available studies, PFAS definitively alter human thyroid hormones and potentially contribute to thyroid autoimmunity but do not so far appear to be a cause of thyroid cancer (Barry et al. 2013; Vieira et al. 2013). Also, thyroid cancer is usually survived; thus, morbidity rather than mortality studies are useful.

Liver disease and cancer

The liver is a primary target organ for long-chain PFAS storage, and accompanying experimental evidence of toxicity includes hepatocyte fat infiltration, specific P450 (CYP) pathway induction, apoptosis, hepatocellular adenomas and carcinomas, and disrupted fatty acid trafficking that can be peroxisome proliferator–activated receptor alpha (PPARα)–dependent or –independent and present across species (Maestri et al. 2006; Cui et al. 2009; Wan et al. 2012; Huang et al. 2013; Perez et al. 2013; Filgo et al. 2015; Xu et al. 2016, 2020a; Yao et al. 2016; Zhang L et al. 2016b; Hui et al. 2017; Li et al. 2017a; Guillette et al. 2020; National Toxicology Program 2020a).

Population studies demonstrate significant associations of long-chain PFAS (>6 fluorinated carbons) exposure to higher liver enzymes, such as alanine aminotransferase in adults and adolescents (Sakr et al. 2007a; Gallo et al. 2012; Yamaguchi et al. 2013; Gleason et al. 2015; Attanasio 2019; Nian et al. 2019), including in longitudinal studies (Sakr et al. 2007b; Darrow et al. 2016). Following low-dose exposures, these associations may be more evident in obese participants (Lin et al. 2010; Gallo et al. 2012; Jain and Ducatman 2019e).

Based on experimental data (Martin et al. 2007; Wan et al. 2012; Wang et al. 2013; Das et al. 2017), nonalcoholic

fatty liver disease (NAFLD) has been investigated as a clinical outcome of PFAS exposure mediating consistent population PFAS-altered liver enzyme findings. Studies with NAFLD cytokeratin C18 biomarkers have provided supportive evidence for PFAS inducing steatosis (Bassler et al. 2019). Metabolomic studies have been directed at potentially explanatory human glycerophosphocholine and fatty acid profiles (Kingsley et al. 2019; Salihovic et al. 2019; Wahlang et al. 2019). Processes which favor steatosis promote advanced liver disease including liver cancer in humans (Massoud and Charlton 2018; National Toxicology Program 2020a). Associations of PFAS with advanced human liver disease and liver cancer are technically hard to study for reasons including (and not limited to) lethality, selection of comparison populations, and alterations of excretion mechanics associated with disease states. In a clinic-based study, mostly obese (85%) children aged 7 to 19 yr with biopsy-proven NAFLD had more advanced disease associated with PFOS and PFHxS exposure as well as associations with lipid and amino acid pathways linked to NAFLD pathogenesis (Jin et al. 2020). However, an adult study reported that serum PFHxS was inversely associated with hepatic lobular inflammation in morbidly obese bariatric surgery patients (Rantakokko et al. 2015). A study of heavily exposed workers ($n = 462$, geometric mean serum PFOA of 4048 ng/mL) detected significantly increased incident mortality for cirrhosis (relative risk = 3.87, 95% CI 1.18–12.7) and liver cancer (relative risk = 6.69, 95% CI 1.71–26.2) compared to a regional population (Girardi and Merler 2019), whereas no PFAS association to cancer or advanced liver disease was reported in a 3M worker cohort or in the C8 Health study population (Lundin et al. 2009; Barry et al. 2013; Vieira et al. 2013).

Emerging animal toxicology and histology and human population data provide mechanistic clues that PFAS disrupt hepatic metabolism, leading to increased bile acid reuptake and lipid accumulation in liver (Salihovic et al. 2020; Schlezinger et al. 2020). A review of NAFLD and toxicant exposure concluded that PFAS are associated with early steatosis ("fatty liver"), the preclinical stage of NAFLD (Armstrong and Guo 2019).

Lipid and insulin dysregulation

Cross-sectional and longitudinal investigations indicate that PFAS increase serum total and low-density lipoprotein cholesterol in adults and children (Steenland et al. 2009; Frisbee et al. 2010; Nelson et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Fitz-Simon et al. 2013; Geiger et al. 2013; Fu et al. 2014; Starling et al. 2014; Winquist and Steenland 2014a; Skuladottir et al. 2015; Zeng et al. 2015; Koshy et al. 2017; Convertino et al. 2018; He et al. 2018; Seo et al. 2018; Dong et al. 2019; Lin et al. 2019; Li et al. 2020; Liu G et al. 2020), including clinically defined high cholesterol (Steenland et al. 2009; Winquist and Steenland 2014a; Lin et al. 2019). Studies of large populations, featuring wide exposure ranges, demonstrate that serum lipids rapidly increase beginning at background (1–10 ng/mL) serum concentration and then are followed by attenuating ("plateaued") cholesterol measurements as (log-transformed) exposures to long-chain PFAS increase (Steenland et al. 2009; Frisbee et al. 2010; Li et al. 2020). These findings suggest partially saturable mechanisms; thus, the cholesterol dose response at pharmacologic or acutely toxic doses should be viewed with caution; associations can be missed or may be misleading when an environmental range of exposure is absent. At background exposure levels, residual associations may be more detectable in obese participants (Timmermann et al. 2014; Jain and Ducatman 2019d), a finding congruent with experimental PFAS outcomes in rodents fed "Western" or high-fat diets (Tan et al. 2013; Quist et al. 2015; Rebholz et al. 2016). Human gene expression pathways provide support for an interaction of obesity and PFAS exposures and suggest possible sex differences (Fletcher et al. 2013). A pharmacokinetic model predicts that approximately half of the PFOS-exposed population would experience a >20% rise in serum cholesterol (Chou and Lin 2020). Risk-assessment implications for low-PFAS dose increases in cholesterol have been noted (New Jersey Drinking Water Quality Institute Health Effects Subcommittee 2017; Li et al. 2020), and a review of population and toxicity data concluded that dyslipidemia is the strongest metabolic outcome of PFAS exposure (Sunderland et al. 2019).

Human PFAS lipid findings may be related to experimental findings of induced adipogenesis, impaired bile acid metabolism/synthesis, strongly decreased CYP7A1 enzyme activity, altered fatty acid transport, and intracellular lipid accumulation with steatosis, including in PPAR-α-null or PPAR-α-humanized animals (Guruge et al. 2006; Lau et al. 2007; Bijland et al. 2011; Bjork et al. 2011; Wang et al. 2014; Filgo et al. 2015; Das et al. 2017; Salihovic et al. 2019; Zhang et al. 2019; Behr et al. 2020a; Liu S et al. 2020b; Schlezinger et al. 2020). Independent of PFAS exposure, similar alterations in metabolic pathways have been related to disrupted fatty acid beta-oxidation and increased free cholesterol in toxicology studies (Perla et al. 2017).

Cross-sectional studies of diabetes outcomes can be misleading for reasons discussed in the renal section (see section *Kidney disease, uric acid, and kidney cancer*). Emerging longitudinal and diabetes clinical trial data indicate that PFAS may increase human insulin resistance, associated with dysregulated lipogenesis activity (Alderete et al. 2019; Lin et al. 2019). Longitudinal studies of clinically diagnosed diabetes patients have sometimes associated PFAS exposures with diabetes (Sun et al. 2018) or with small changes in glycemic markers (Cardenas et al. 2017); however, diabetes associations to date are not consistent (Karnes et al. 2014; Cardenas et al. 2017; Donat-Vargas et al. 2019). Future studies should consider whether PFAS may instigate autoimmune diabetic outcomes in humans, as shown in experimental studies (Bodin et al. 2016). Experimental data reveal that PFAS activate G protein–coupled receptor 40, a free fatty acid–regulated membrane receptor on islet ß cells, stimulating insulin secretion (Qin et al. 2020; Zhang L et al. 2020).

Kidney disease, uric acid, and kidney cancer

Extended human half-lives of long-chain PFAS are attributed to active renal tubular reabsorption. Of concern, legacy PFAS

such as PFOA and PFOS are concentrated in renal tissues, and histopathologic, molecular, oxidative stress, and epigenetic studies provide evidence of potential nephrotoxicity (Wen et al. 2016; Stanifer et al. 2018; Sakuma et al. 2019; Rashid et al. 2020). In addition, the strong influence of kidney reabsorption on the extended half-lives of long-chain PFAS is consistent with both human protein binding and experimental PFAS excretion data.

Human studies have associated legacy PFAS exposure to diminished glomerular filtration and/or defined chronic kidney disease in adults and children (Shankar et al. 2011; Watkins et al. 2013; Kataria et al. 2015; Blake et al. 2018). However, this outcome may be due to reverse causation (Watkins et al. 2013; Dhingra et al. 2017). Some reviews of the available epidemiologic and toxicologic evidence suggest causative links between PFAS and diminished kidney function and chronic kidney disease (Stanifer et al. 2018; Ferrari et al. 2019); these authors also note several knowledge gaps and uncertainty about which proposed mechanisms of action are most important. A propensity score approach to NHANES data (Jain and Ducatman 2019c; Zhao et al. 2020) and a study with repeated PFAS and health measures over an 18-yr period (Blake et al. 2018) recently concluded that PFAS exposure likely causes diminished renal glomerular filtration.

Uric acid, a biomarker of increased risk for renal disease (Obermayr et al. 2008), is also consistently associated with PFAS exposure in adults and children (Steenland et al. 2010; Geiger et al. 2013; Gleason et al. 2015; Kataria et al. 2015; Qin et al. 2016; Zeng et al. 2019), including a visible dose–response curve that begins at or near historic background levels in human populations (Steenland et al. 2010; Zeng et al. 2019). Serum PFAS concentrations exhibit an inverted U-shaped pattern related to glomerular filtration, initially exhibiting a modest accumulation as glomerular filtration begins to decrease and then decreasing in advancing renal disease, likely due to failure of normal strong reabsorption mechanisms in moderate to severe kidney disease (Jain and Ducatman 2019c). This finding is more dramatic across stages of glomerular filtration when there is also albuminuria (Jain and Ducatman 2019b). Studies suggest that the association of PFAS to uric acid is not due to reverse causation and is underestimated because the failing kidney excretes long-chain PFAS but retains uric acid. An implication is that population outcomes that occur in the presence of either albuminuria or moderate to severe renal disease such as hypertension (Jain 2020) increasing presence of and uric acid (a biomarker of renal disease; Jain and Ducatman 2019a; Zeng et al. 2019) can be underestimated in cross-sectional studies; in other words, the link between these health outcomes and PFAS exposure is obscured in these studies because of enhanced PFAS excretion patterns in the presence of either albuminuria or moderate to severe kidney disease. Furthermore, the strong influence of renal reabsorption on the long half-lives of long chain PFAS is consistent with both human protein binding of PFAS and experimental PFAS excretion rates in high-dose rodent studies (Cheng and Ng 2017).

Kidney cancer diagnoses have been increasing since 1975, a finding that is partially independent of improved detection, with 5-yr cancer-specific survival of approximately 80% (Gandaglia et al. 2014). The C8 Health studies noted longitudinal ($n = 32\,254$) increases of kidney cancer (hazard ratio = 1.10, 95% CI 0.98–1.24) and kidney cancer mortality (Steenland and Woskie 2012; Barry et al. 2013; Vieira et al. 2013). A review of 6 published studies found long-chain PFAS exposure associated with kidney cancer or kidney cancer mortality, with risks ranging from 1.07 to 12.8 (Stanifer et al. 2018). Subsequent preliminary data from the heavily exposed Veneto, Italy, population also suggest a significant increase in kidney cancer mortality with PFAS exposure (Mastrantonio et al. 2018). Evidence is accumulating for PFAS as a cause of chronic disease and kidney cancer. Study designs must consider the peculiar PFAS excretion mechanics involved in and associated with kidney disease.

Reproductive and developmental outcomes

Exposure to PFOA impairs human sperm motility and sperm penetration into viscous media (Sabovic et al. 2020; Yuan et al. 2020) and is longitudinally associated with lower sperm concentration and count and higher adjusted levels of luteinizing and follicle-stimulating hormones in young men (Joensen et al. 2009; Vested et al. 2013; Song et al. 2018). Serum concentrations of PFAS are also cross-sectionally associated with deleterious markers of semen quality (Louis et al. 2015; Pan et al. 2019).

Legacy and emerging PFAS have been found in follicular fluid (Kang et al. 2020). They appear to alter endometrial regulation such as progesterone activity in young women (Di Nisio et al. 2020b) and possibly menstrual cycle length (Lum et al. 2017). Associations with menarche and menopause may be substantially due to reverse causation because menstruation is a route by which women eliminate PFAS (Dhingra et al. 2017), partially explaining why men have higher PFAS levels than women in the same communities. Women on birth control and who do not menstruate or with poor cyclicity because of age, activity level, or disease may have elevated PFAS levels in comparison with menstruating women. Exposure to PFAS has been associated with endometriosis in the United States and in China (Louis et al. 2012; Campbell et al. 2016; Wang B et al. 2017a), but the specific PFAS associated with this effect vary among studies.

Time-to-pregnancy (fecundity) studies provide indirect evidence of changes in fertility. Methodologic considerations include maternal and paternal age, parity (which in turn affects serum PFAS), and health status. Among 1240 women in the Danish National Birth Cohort, PFOS exposure was associated with decreased fecundity (median serum PFOS 35.5 ng/mL; Fei et al. 2009). Reverse causation may explain this finding because it is duplicated in parous, but not among nonparous, women (Whitworth et al. 2012; Bach et al. 2015). Prospective odds of actual infertility in the Maternal–Infant Research on Environmental Chemicals cohort ($n = 1743$) at low-dose exposures were associated with PFOA (geometric mean 1.66 ng/mL; odds ratio = 1.31, 95% CI 1.11–1.53) and PFHxS

(odds ratio = 1.27, 95% CI 1.09–1.48; Velez et al. 2015). The reported fertility rate improved following water filtration in a PFAS-contaminated community (incidence rate ratio 0.73, 95% CI 0.69–0.77 prior to filtration) along with measures of birth weight (Waterfield et al. 2020).

Per- and polyfluoroalkyl substances reliably move across the placenta and enter breast milk (Gyllenhammar et al. 2018; VanNoy et al. 2018); serum PFAS levels in young children generally exceed maternal serum concentrations (Fromme et al. 2010; Papadopoulou et al. 2016; Eryasa et al. 2019). Population studies provide evidence that breastfeeding duration and milk quantity are adversely affected by PFAS exposure (Romano et al. 2016; Timmermann et al. 2017b; Rosen et al. 2018).

A systematic review reported that PFOA exposure was associated with a small decrease in infant birth weight; the meta-analysis estimated that a 1-ng/mL increase in PFOA was associated with an approximately 19-g reduction (95% CI −29.8 to −7.9 g) in birth weight (Lam et al. 2014). The authors noted similarities in experimental studies (Johnson et al. 2014; Koustas et al. 2014) and concluded that there was "sufficient" human and corroborative toxicology evidence of a detrimental effect of PFOA on birth weight (Johnson et al. 2014; Koustas et al. 2014; Lam et al. 2014). However, another meta-subpopulation analysis, focused on early pregnancy or the time shortly before conception, detected only a small and nonsignificant association, which was less subject to bias (Steenland et al. 2018a). Different approaches to the possible confounding role of shifting glomerular filtration rates in pregnancy can affect interpretations; evidence suggests this consideration can, at most, only partially explain associations of PFAS exposure to decreased birth weight (Interstate Technology and Regulatory Council 2020; Wikstrom et al. 2020). A recent review of mostly prospective cohort studies ($n = 24$ studies) noted PFAS associated with altered fetal and postnatal growth measures, such as lower birth weight. Many ($n = 22$) of the relevant studies suggest developmental and childhood immunomodulatory effects, whereas 21 studies concerning neurodevelopment were inconclusive (Liew et al. 2018). The authors of the review noted methodologic challenges of developmental and newborn epidemiology, including consideration of critical exposure windows for developmental effects, the effects of breastfeeding and parity on maternal PFAS levels, and the variety of possible mechanistic explanations for growth outcomes, such as disruption of glucocorticoid and thyroid hormone metabolism in utero (Liew et al. 2018). Recent Faroe Island studies report that prenatal PFAS effects on thyroid hormone status do not support a causal relationship (Xiao et al. 2020).

Review articles suggest that prenatal exposure to PFOA may increase risk of subsequent childhood adiposity, noting that steroid hormones, retinoid X receptor, and other pathways may be contributing to this effect (Halldorsson et al. 2012; Hall and Greco 2019). Prospective evidence supports this relationship in adults with a high risk of diabetes (Cardenas et al. 2017). However, some well-performed community studies do not support this outcome in adults or children (Barry et al. 2014; Martinsson et al. 2020).

Based on several preliminary findings, supported by longitudinal follow-up studies (Stein et al. 2009; Savitz et al. 2012; Darrow et al. 2013; Avanasi et al. 2016a, 2016b), the C8 Science Panel concluded that PFOA is probably linked to pregnancy-induced hypertension or preeclampsia. Population-level evidence implicating additional PFAS having this effect has included studies with longitudinal designs (Huang et al. 2019; Wikstrom et al. 2019; Borghese et al. 2020). Experimental support includes PFAS effects on human trophoblast migration in vitro (Szilagyi et al. 2020) and recent evidence of PFOA and GenX (or hexafluoropropylene oxide dimer acid) effects on mouse placenta, as well as excessive gestational weight gain (Blake et al. 2020). However, a recent longitudinal study did not find an association of PFAS with pregnancy-associated hypertension (Huo et al. 2020).

The possibility that circulating PFAS may reduce bone mineral density has been investigated. Cross-sectional and practical trial associations have been found in adults (Lin et al. 2014; Hu et al. 2019; Di Nisio et al. 2020a), and there is emerging longitudinal evidence from a mother and child pair study indicating that children may also be affected (Cluett et al. 2019).

Testicular cancer diagnoses are increasing steadily, a trend unrelated to improved detection (Cheng et al. 2018; Park et al. 2018). Most patients diagnosed (>90%) will be cured and die of other causes; mortality studies therefore provide little help in understanding disease risk factors. The C8 Science Panel detected longitudinal evidence for increased testicular cancer risk (1.35, 95% CI 1.00–1.79) for cumulative PFOA exposure (Barry et al. 2013). There are ample supportive data of testicular damage following PFAS exposure, including strong evidence of endocrine disruption; but the cell-specific associations are different in humans (germ cell) than the outcomes in rodents (stromal).

Per- and polyfluoroalkyl substances have deleterious effects on conception, pregnancy, and infant development. The underlying birth weight data are mostly supportive, although the subsequent growth and adiposity literature is mixed. The most sensitive reproductive and developmental outcomes are a topic of ongoing discussion.

Outcomes replicated across populations, such as perfluorocarboxylate (PFCA) and perfluorosulfonate (PFSA) exposures associated with down-regulation of immune response; increases in cholesterol, liver enzymes, and uric acid; alterations in thyroid hormone binding proteins; growth deficits; and effects on breast milk and lactation, indicate priority areas for understanding mechanisms and health implications.

CURRENT KNOWLEDGE OF PFAS TOXICITY IN EXPERIMENTAL MODELS

Animal studies have focused most intensely on PFOA and PFOS, using laboratory rodents and, more recently, zebrafish as models. Perfluoroalkyl acids of varied carbon-chain lengths as well as a few replacement chemicals with ether linkages in the carbon backbone (such as GenX and 3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid], or ADONA) have also

been examined, with outcome profiles thus far generally consistent with legacy chemicals. The varying extent of responses is likely related to toxicokinetic disposition (excretion or half-life) and relative potency and affinity of the individual chemical for binding to receptor proteins. Some PFAS (i.e., PFHxS, PFOA, and PFNA) have longer half-lives in mice than rats and typically much longer half-lives in humans (Table 1). These differences in elimination kinetics complicate the cross-species evaluation of toxicity. In addition, some PFAS (such as PFOA and PFNA) exhibit a profound sex difference in the rate of chemical elimination and bioaccumulation in the rat: females eliminate them much faster than males (Table 1). Sex differences in half-lives, although important, are much smaller in humans and have a different explanation. The mouse also typically has more limited sex-based PFAS elimination differences, making this species more amenable for extrapolation to humans, especially for mechanistic and toxicity evaluations.

In general, human health effects associated with PFOA and PFOS exposure (described in section *Current Knowledge of PFAS Toxicity in Humans*) have also been reported in animal models: hepatic/lipid metabolic toxicity, developmental toxicity, immune suppression, tumor induction, endocrine disruption, and obesity. These findings are often derived from well-controlled laboratory experiments in more than one species using wide dose ranges that are often orders of magnitude higher than typical human exposure, to account for differences in half-life across species. Some of the phenotypic findings are supported by in vitro mechanistic investigation and/or molecular queries on target tissues. Our understanding of the toxicologic properties of PFAS other than PFOA and PFOS is notably less advanced and, in the case of emerging replacements and by-products, completely unexplored.

Hepatic and metabolic toxicity

In rodent studies, dose-dependent increases in liver weight, in hepatocellular hypertrophy associated with vacuole formation, and with or without increased peroxisome proliferation have been observed with a significant body burden of PFAS, especially for the most persistent and potent long-chain homologs. Hepatocyte proliferation, necrosis, and apoptosis are outcomes occurring at relatively low doses. This is also true for a new replacement chemical, GenX, which altered liver histopathology and function and increased apoptosis in mice and fish (Blake et al. 2020; Guillette et al. 2020). Correspondingly, transcriptional activation of mouse and, to a lesser extent, human PPARα-related genes in liver was detected in adult-exposed models; activation of other nuclear receptors such as PPARγ, constitutive androstane receptor (CAR), and pregnane X-receptor (PXR) has also been reported. These nuclear receptors, metabolic sensors that regulate lipid and glucose metabolism and transport and inflammation, tend to be more responsive in tissues of rodents than in humans (Wolf et al. 2012; Rosen et al. 2017). Recent work using developmental models reports that

TABLE 1: Per- and polyfluoroalkyl substances serum half-life estimates in rat, mouse, monkey, and humans

	PFBS (C4)		PFHxS (C6)		PFOS (C8)		PFBA (C4)		PFHxA (C6)		PFHpA (C7)		PFOA (C8)		PFNA (C9)		PFDA (C10)		F-53B		GenX	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Rat	0.6–4.0 h	2.1–4.5 h	1.8 d	6.8 d	62–71 d	38–41 d	1.0–1.8 h	6–9 h	0.4–0.6 h	1.0–1.7 h	1.2 h	2.4 h	2–4 h	4–6 d	1.4–6.4 d	31–55 d	59–75 d	40–80 d			8 h	3 h
Mouse	4.5 h	5.8 h	25–2 d	28–3 d	31–3 d	36–4 d	3 h	12 h	~1.2 h	~1.6 h			16 d	22 d	26–6 d	34–6 d					18 h	20 h
Cynomolgus Monkey	3.5 d	4.0 d	87 d	141 d	110 d	132 d	1.7 d		2.4 h	5.3 h			30 d	21 d								
Human	28 d		5.3–8.5 yr		3.4–5.0 yr		3 d		32 d		1.2–2.5 yr		2.1–3.8 yr		2.5–4.3 yr		15.3 yr					

GenX = hexafluoropropylene oxide dimer acid; PFBA = perfluorobutanoic acid; PFBS = perfluorobutanesulfonic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate.

mitochondrial dysfunction is associated with hepatocellular hypertrophy in young adult mice (Quist et al., 2015) and that other fatty acid metabolism pathways are activated (Jones et al. 2003; Shabalina et al. 2016). Steatosis is also a common feature of PFAS chronic exposure in rodents. Exposure in rodent models typically decreases serum cholesterol, whereas elevations of circulating cholesterol levels have been reported in humans. The mode of action concerning serum cholesterol is debatable. For example, PFOA exposure increased liver weight, increased liver enzymes, and led to persistent histopathological changes (particularly damage to the bile duct) in livers of wild-type and PPARα-null rodent strains (reviewed in Division of Science and Research, New Jersey Department of Environmental Protection 2019). Many of these effects are reversible on cessation of PFAS exposure, and this observation has been interpreted by some as evidence of "adaptive" responses to exposure. However, this reversibility is irrelevant to ongoing environmental PFAS exposure (for instance, from drinking water) because exposure will persist until contamination is remediated. In summary, there is a strong confluence of animal toxicology and histology and human population data that PFAS disrupt hepatic metabolism and lead to lipid accumulation in liver, although the mechanism(s) is unclear. Effects on bile acid metabolism, mitochondrial perturbation, and cholestatic mechanisms deserve further investigation at human-relevant exposures.

Reproductive and developmental toxicity

Only a few reproductive toxicity studies of males and females are available, primarily focusing on long-chain PFAS. Profound developmental toxicity has been described following gestational and lactational exposure to PFOS, PFOA, and PFNA in mice (Thibodeaux et al. 2003; Lau et al. 2006; Das et al. 2015) and in mice and rats gestationally exposed to GenX (Conley et al. 2019; Blake et al. 2020). Neonatal morbidity and mortality were seen with exposure to high doses of legacy PFAS; growth deficits and developmental delays were noted in offspring exposed to lower doses. Evidence of lactation impairment was seen in mice at doses of 5 mg PFOA/kg body weight (White et al. 2007), leading to increased offspring mortality (Lau et al. 2006); recent studies have indicated a role of placental dysfunction in these adverse developmental outcomes (Blake et al. 2020). Deficits of mammary gland development were also observed in mice exposed to PFOA (doses of 1 mg/kg body wt and lower) during gestation, which persisted into adulthood, although these exposure levels did not alter body weight, lactational function, or neonatal growth of offspring (F1 or F2 mice; Macon et al. 2011; White et al. 2011b; Tucker et al. 2015). Systematic reviews support a relationship between in utero exposure to PFOA and PFOS and reduced fetal growth in animals and humans, and the relationship between PFOA and reduced fetal growth in mice was recently validated (Koustas et al. 2014; Blake et al. 2020). Also, PFAS are reported to have reproductive effects such as ovulation failure in mice (Zhang Y et al. 2020).

Immunotoxicity

A few long-chain PFAS (PFOS, PFOA, PFNA, and PFDA) have been shown to alter immune status in rodents and nonhuman primates. Effects are predominantly immunosuppressive and include reductions in thymus and spleen weights and associated immune cell populations, in numbers of circulating immune cells, in certain aspects of innate immunity (i.e., natural killer cell cytotoxicity), in infectious disease resistance, and in antibodies produced in response to an antigen (i.e., analogous to the vaccine response in humans). In their 2018 draft *Toxicological Profile for Perfluoroalkyls*, the US Agency for Toxic Substances and Disease Registry (ATSDR) noted changes to the aforementioned immune parameters observed in experimental rodents exposed to PFOA, PFOS, PFNA, PFHxS, PFDA, perfluorobutanesulfonic acid (PFBS), or perfluorobutanoic acid (PFBA; Agency for Toxic Substances and Disease Registry 2018). The US National Toxicology Program conducted a systematic review of the immunotoxicological literature for PFOA and PFOS and concluded that PFOA and PFOS were presumed to be immune hazards to humans based on a high level of evidence for suppression of antibody responses in experimental animals and a moderate level of evidence for suppression of antibody responses in humans (National Toxicology Program 2016). The ATSDR (Agency for Toxic Substances and Disease Registry 2018) also included a decreased antibody response to vaccines (PFOA, PFOS, PFHxS, and PFDA) and increased risk of asthma diagnosis (PFOA) among the list of adverse health effects in PFAS-exposed humans. Reduction in the antibody response to a vaccine, an adaptive immune function, is a well-accepted measure of immunotoxicity, is consistent with the mode of action for the effects of fatty acids on immune system function (Fritsche 2006), and is compelling evidence that the immune system is a sensitive target of PFAS.

Tumor induction

Per- and polyfluoroalkyl substances are not known to be directly mutagenic; PFOA, PFOS, and other tested PFAS show little or no evidence for induction of gene mutation, clastogenicity, or aneuploidy in vitro or in vivo by a direct mode of action (see EFSA Panel on Contaminants in the Food Chain [2020] for details). There is evidence that PFAS can induce DNA damage, such as strand breaks, and other genotoxic effects, secondary to oxidative stress (EFSA Panel on Contaminants in the Food Chain 2020). This occurs at concentrations or doses that are high relative to human environmental exposures to PFAS, and the mechanism is such that their dose–response will be sublinear. Hence, PFAS are unlikely to be of mutagenic concern in exposed populations.

In adult-exposed rodents and fish, PFOA and PFOS have been shown to induce tumors. Liver adenomas, pancreatic acinar cell tumors, and testicular Leydig cell adenomas have been detected in rats treated chronically with PFOA (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2017) as well as its replacement, GenX (Caverly Rae et al. 2015). Following

gestational and chronic exposure to PFOA, 58% of male rats demonstrated pancreatic tumors at the lowest dose administered (National Toxicology Program 2020b). This finding has spurred Minnesota and California policymakers to consider cancer as an endpoint in risk assessment, whereas the European Food Safety Authority (EFSA Panel on Contaminants in the Food Chain 2020) has the opinion that there is not adequate evidence for a link between exposure to PFAS and cancer risk in humans. This "tumor triad" profile has been associated with the PPARα-mediated molecular signaling pathway in rats exposed to high doses of PFAS. Consequently, liver tumors involving this mode of action are not considered relevant to humans at equivalent PFAS exposures (Post et al. 2017). The human relevance of PPARα-mediated pancreatic tumors in rodents remains to be determined. Liver lesions evident in PPARα-null mice exposed to PFOA during pregnancy and lactation (Filgo et al. 2015) suggest a non-PPARα-mediated liver response. Induction of liver tumors mediated by estrogen receptor (ER) activation has also been reported in fish (Tilton et al. 2008), and several non-PPARα-mediated hypotheses, including increased reactive oxygen species formation, oxidative stress, and mitochondrial dysfunction; decreased tumor cell surveillance by the immune system; and diminished gap junction cellular communication, are documented (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2017; New Jersey Drinking Water Quality Institute Health Effects Subcommittee 2017).

Endocrine disruption

The primary evidence for the endocrine-disrupting potential of PFAS involves induction of hypothyroxinemia and reduction of serum testosterone in rats. An early review of PFAS endocrine-disrupting properties in humans concluded that the "thyroid may be one axis significantly affected by PFOA exposure while the animal toxicology literature is less certain due to technical issues" (White et al. 2011a).

The effects of PFAS on thyroid hormone status detected in animal studies differ from classical hypothyroidism, in that reduction of circulating total thyroxine is not accompanied by a compensatory increase of TSH. A possible mechanism for these effects may be related to the propensity of protein binding of legacy PFAS, which could lead to displaced total thyroxine binding to its carrier proteins (transthyretin and thyroxine-binding globulin). Human population studies augment animal data showing that PFAS interact with thyroid hormone binding proteins (Berg et al. 2015; Ren et al. 2016; Zhang J et al. 2016a), one of several mechanisms by which PFAS can perturb feedback relationships between free thyroid hormone available to cells (free total thyroxine) and the hypothalamic–pituitary axis. Some estrogenic effects of PFAS have also been illustrated by in vitro studies, although there is no evidence of direct transactivation of estrogen, androgen, or glucocorticoid receptors (Behr et al. 2018, 2020b).

The evidence for PFAS affecting ER signaling in humans and animals is mixed. Although studies have identified some PFAS as being without estrogenic activity (Behr et al. 2018; Borghoff et al. 2018; Gogola et al. 2019), others suggest an ability of PFAS to modulate or even activate ER-mediated effects (Benninghoff et al. 2010; Kjeldsen and Bonefeld-Jørgensen 2013; Wang et al. 2018; Bjerregaard-Olesen et al. 2019; Qiu et al. 2020), with some effects only observed in aquatic organisms (Wei et al. 2009; Chen et al. 2016, 2018). Microarray analyses of human primary hepatocytes confirmed that PFOA activated the ER pathway (Buhrke et al. 2015).

Neurotoxicity

Potential adverse effects of PFAS on the nervous system and functions have not been widely investigated. A few studies reported neurotoxicity of PFOS, PFHxS, and PFOA in cell culture systems (Slotkin et al. 2008), as well as altered behavioral responses (Goulding et al. 2017) and deficits in learning and memory ability in rodents (Viberg et al. 2013). In contrast, no significant developmental neurotoxic effects were seen from prenatal exposure to PFOS in USEPA guideline–based studies with rats (Butenhoff et al. 2009).

Obesity

Numerous cell-based assays in human and mouse preadipocytes and animal studies with and without high-fat diets have consistently shown that some PFAS have the potential to increase lipid production by adipocytes and fat pads (van Esterik et al. 2016). Exposure of pregnant mice to low doses of PFOA produced obesity in young adult female offspring (Hines et al. 2009; van Esterik et al. 2016), a finding that was recapitulated in Danish women exposed in utero to PFOA (Halldorsson et al. 2012). Both PFOA and GenX increased weight gain of pregnant mice (Blake et al. 2020), an effect also seen in women during pregnancy (Ashley-Martin et al. 2016), although discordant results have been reported in other studies (Barry et al. 2014; Ngo et al. 2014). These apparently disparate findings in experimental models may be associated with differences among mouse strains examined, exposure periods, statistical methodology, and/or the rodent diets used.

There are specific differences in human and rodent health outcomes that deserve further investigation: 1) cholesterol metabolism, 2) thyroid effects, 3) mode of action for liver effects (different or same), and 4) kidney transporter or other mode of action leading to large differences in half-life. However, species concordance in the 6 human health effects discussed in the present review supports a weight of evidence for these effect for the handful of extensively studied PFAS.

Human health advisory and guidance values for a few PFAS have been issued to date by the USEPA, the ATSDR, several individual state environmental agencies or health departments, as well as regulatory agencies in Canada and Europe that are largely (but not exclusively) based on toxicological findings in animal models. However, risk-assessment scientists have not reached consensus in selecting a singular apical endpoint as the basis for a point of departure for assessments. Three

toxicological features of PFAS that have been commonly highlighted, based on their sensitivity (low dose effect), strength of evidence (robust corroborating studies with mechanistic support for human relevance), and corresponding findings noted in epidemiological investigation, are hepatotoxicity (and alterations in lipid metabolism), developmental toxicity, and immunotoxicity. It should be noted that apical endpoints that drive risk assessments often differ among individual PFAS, perhaps highlighting the complexity of these chemicals and the family of PFAS, in general.

IMPORTANCE OF TOXICOKINETICS IN UNDERSTANDING PFAS TOXICITY

Species and sex differences

Few of the substantial number of structurally diverse PFAS have been tested for toxicological effects. Some available toxicological information has come from studies in animals, where marked species and (in rat) sex differences in half-life for some PFAS (Table 1) have been observed and the relevance to humans is uncertain. These differences are due to toxicokinetic and toxicodynamic factors. There are also differences in mean PFAS serum levels between men and women in the same communities. Children may have elevated serum levels compared to parents, even with the same exposures (Emmett et al. 2006; Daly et al. 2018; Graber et al. 2019), for reasons relating to transplacental transfer, breastfeeding, and body mass (Emmett et al. 2006; Daly et al. 2018; Graber et al. 2019; Blake et al. 2020). Transplacental transfer of PFAS confers a substantial burden to the newborn infant. Because the infant has a smaller overall mass and blood volume, PFAS are concentrated, increasing PFAS per volume (Koponen et al. 2018). In addition, transfer of PFAS is common through lactation, and the longer a child breastfeeds, the higher the body burden (Gyllenhammar et al. 2018; VanNoy et al. 2018).

Effects of comorbidity on PFAS toxicokinetics

Factors affecting renal function can influence PFAS toxicokinetics. As discussed, opposing types of causation should be considered. Human toxicokinetics appear to vary bidirectionally with changing renal function, leading to nonmonotonic dose–response relationships and, depending on the study goal, possibly to errors in estimating disease associations. As progress is made in the field of PFAS toxicokinetics, new chemistries may have different clearance factors and nuances that vary by PFAS group or structures, and that will need to be investigated to accurately model half-lives in different exposure subgroups.

Sources of information on toxicokinetics in humans: strengths and limitations of studies

Some PFAS half-life data in humans were obtained from retired industry workers, particularly those who worked with PFOS, PFOA, and PFHxS (Olsen et al. 2007). Since then, these estimates have been modified slightly or confirmed with longitudinal data and modeling from contaminated communities once uncontaminated water options were provided (Bartell et al. 2010; Li et al. 2018). Other contemporary PFAS estimates are derived from biomonitoring studies of production workers, blood donors, study participants, and/or occupationally exposed cohorts (Olsen et al. 2009, 2017; Russell et al. 2013; Zhang et al. 2013). Some caution must be taken in using these data because variables affecting PFAS clearance may not be taken into consideration (age, sex, menstruation, disease, and medication status) and may contribute to confounding.

The challenge in determining a reliable human half-life in these types of studies is that exposure does not end with a clean water source, retirement, or a change of job and that continued exposures vary over potential depuration periods. Model components may also vary in subclasses. Children (small blood volumes and a large fraction of exposures comes from drinking), pregnant women (large increase in blood volume and water intake), parous women (transfer to fetus and breast milk), and athletes (water intake elevated) are examples of subpopulations with expected variation in half-life compared to adult men (Post et al. 2017). There will be more human estimates of PFAS forthcoming that involve variations in half-life (Post et al. 2017). Realistic computational modeling can help, so long as it clearly characterizes exposures and applicable populations. The continued goal should be to provide predictive values for those PFAS lacking actual measurements, based on chemical structures and trusted physiological parameters.

Physiologically based pharmacokinetic/toxicokinetic modeling in different-aged populations

In the blood and other tissues, PFAS toxicokinetics are influenced by their interactions with proteins (Andersen et al. 2006; Katakura et al. 2007; Nakagawa et al. 2008; Weaver et al. 2009; Figure 2). Certain toxicokinetic features are saturable, and thus dosing in toxicokinetic studies is of profound importance. Studies of renal reabsorption mechanisms in mammals show that reduced activity of transporters such as organic anion transporting polypeptide 1a1, through inactivation (e.g., genetic manipulation, castration, treatment with estrogen) or by saturation at increasing doses, leads to substantial reductions in half-lives of PFOA and PFOS (Andersen et al. 2006; Nakagawa et al. 2008; Weaver et al. 2009; Yang et al. 2009).

These protein-associated toxicokinetic processes were recently incorporated into a model for PFOA in the male Sprague-Dawley rat (Cheng and Ng 2017), which provides a useful platform to explore how changes in protein interactions might affect estimates of PFAS half-life (Figure 3). At high doses, it is typical to see clear biphasic behavior with rapid initial clearance, during which the serum half-life appears to be shorter especially at high enough doses that processes such as renal reabsorption are saturated, followed by a much longer tail

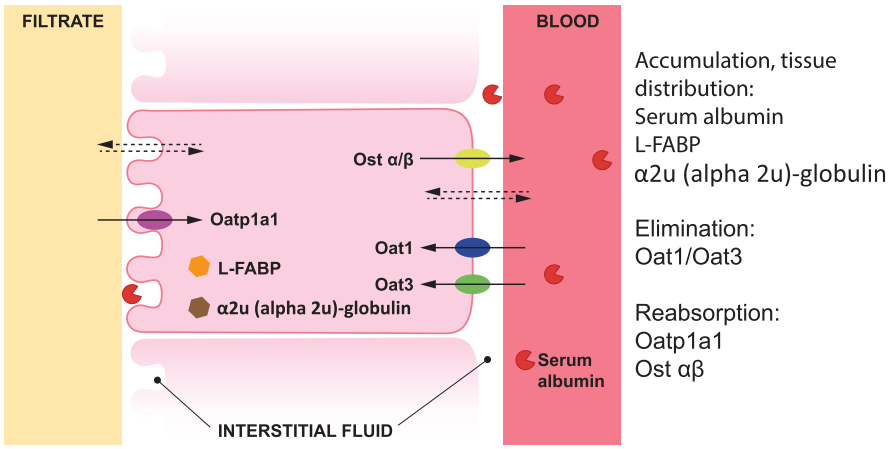

FIGURE 2: Example of proteins that are known to influence per- and polyfluoroalkyl substance toxicokinetics through binding (which affects tissue distribution and accumulation) and facilitation of membrane transport (which affects clearance and reabsorption). Illustrated for kidney and blood. L-FABP = liver fatty acid binding protein; Oat1 = organic anion transporting 1; Oatp1a1 = organic anion transporting polypeptide 1a1; Ost = organic solute transporter.

(Figure 3A). In a similar fashion, the magnitude of internal dose and rate of serum clearance can be profoundly influenced by proteins known to bind PFAS, such as serum albumin (Figure 3B). Increasing and decreasing the extent of reabsorption in the kidney increases and decreases the serum half-life, respectively (Figure 3C). Finally, the effect of saturating reabsorption is magnified when the half-life is longer because of increased serum binding (Figure 3D). In this case, taking an initial slope to calculate the serum half-life at high doses would lead to a profound underestimation.

Differences in protein expression, circulating levels, and even protein type across populations, sex, and species could lead to important species and sex differences in PFAS biological half-lives (Han et al. 2012); such differences should be

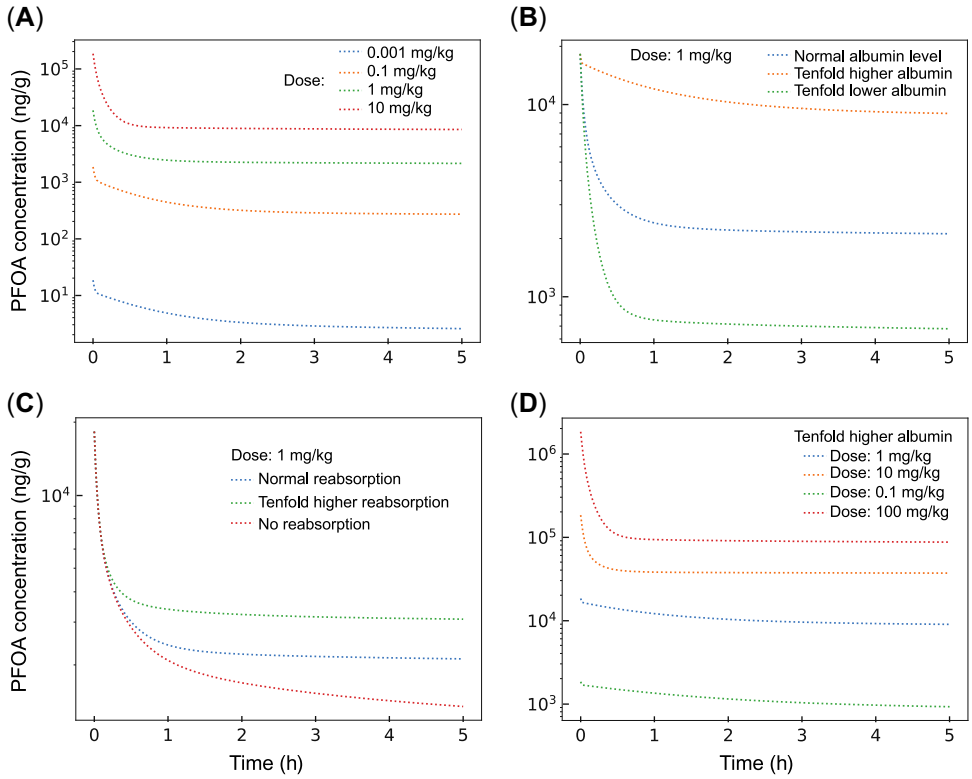

FIGURE 3: Simulations based on Cheng and Ng (2017), perfluorooctanoic acid (PFOA) toxicokinetic model for Sprague-Dawley rats. (**A**) Effect of dose on initial half-life. (**B**) Effect of higher and lower levels of serum albumin, which binds to PFOA, on serum clearance dynamics. (**C**) Effect of extent of reabsorption in kidney on serum half-life, based on organic anion transporting polypeptide 1a1 activity. (**D**) Effect of dose on elimination kinetics when half-life is longer because of higher albumin binding. Oat1 = organic anion transporting 1; Oat3 = organic anion transporting 3; Ost = organic solute transporter.

investigated and taken into account in the extrapolation to human equivalent doses. Because expression of proteins may change at different life stages, clearance factors and toxicokinetics may also change.

Given the large number of species-, sex-, and age-specific differences that have been observed, coupled with the lack of data for many PFAS, the parameterization of complex physiologically based toxicokinetic models remains a persistent challenge. Therefore, lower-resolution models (e.g., one-compartment or few-compartment models) may be more appropriate for species and settings where insufficient data are available for reasonably accurate parameterization. Alternatively, in silico and in vitro methods are under development that could aid in parameterization in the absence of in vivo data, as discussed in the section *New approaches for developing PFAS toxicity information*.

SO MANY PFAS, SO LITTLE TIME: ACCELERATING THE PACE OF DISCOVERY

Importance of determining mode of action and adverse outcome pathways

Information on modes of action and/or adverse outcome pathways (AOPs) is invaluable in 1) establishing human relevance of experimental evidence, 2) assessing causality in epidemiological studies, 3) applying "read-across" to PFAS for which there is little toxicological information, 4) assessing risks from mixtures, 5) guiding development and interpretation of new approach methodologies, 6) informing the development of biomarkers in epidemiologic investigation, and 7) identifying potentially vulnerable subpopulations and life stage–specific effects (Meek et al. 2014; LaLone et al. 2017). Verified modes of action and AOPs can inform risk assessment based on intermediate effects and enable development of new methodology-based approaches to assess PFAS safety (Meek et al. 2014).

Postulated modes of action/AOPs for PFAS

Mechanistic studies have been performed on only a few PFAS. These have been shown to activate a range of putative molecular initiating targets, among which are the nuclear receptors PPARα, PPARγ, PPARβ/δ, CAR, PXR, liver X receptor α, and ERα (Bijland et al. 2011; Bjork et al. 2011; Rosen et al. 2017; Li et al. 2019). However, modes of action verified by agreed procedures (World Health Organization 2020) have been established for few reported effects of PFAS, and those that have been interrogated involve activation of PPARα and, at higher doses, CAR as molecular initiating events (Klaunig et al. 2012; Rosen et al. 2017). Several AOPs involving these molecular targets are in various stages of development (Organisation for Economic Co-operation Development 2020), but few have been endorsed by the OECD following its agreed procedures (Organisation for Economic Co-operation Development 2017). Demonstration of receptor activation alone is insufficient to establish involvement of a mode of action or AOP in an observed effect, for which an overall weight-of-evidence approach is necessary (World Health Organization 2020).

Andersen et al. (2007) provide a useful, albeit dated, review of possible PFAS modes of action. Established modes of action are restricted largely to the liver and include species-specific hepatic hyperplasia and liver tumors (Butenhoff et al. 2012; Elcombe et al. 2012; Corton et al. 2018). Available studies on PFBS, PFHxS, perfluorohexanoic acid, PFNA and PFDA suggest that they share molecular targets with similar consequences, albeit with differences in potency, in part due to differences in their excretion and protein-interaction kinetics (Zeilmaker et al. 2018). However, studies in vitro have established intrinsic differences in potency among PFAS analogues. Potency in activating PPARα showed some relationship with PFAS chain length (Wolf et al. 2008). A mode of action or AOP provides a causal chain of key events between chemical exposure and outcome. The established modes of action for PFOS and PFOA provide a causal explanation for development of liver tumors observed in rodents on exposure to these compounds, through activation of PPARα, and the possible relevance to humans. However, this does not mean that other effects of PFAS are due to activation of PPARα or that other pathways might not lead to liver tumors in humans, such as secondary to the primary effect of steatosis.

Until recently, there has been little study of modes of action/AOPs for effects of PFAS other than hepatic outcomes in rodents, particularly for critical effects, such as immunosuppression and developmental toxicity, and from PFAS other than PFOS and PFOA (EFSA Panel on Contaminants in the Food Chain 2020; Temkin et al. 2020). The ability of various PFAS to interact with and modify lipid metabolism is, however, an intriguing hypothesis (Xu et al. 1999; Jones et al. 2003; Andersen et al. 2007; Tan et al. 2013; Pouwer et al. 2019). Other putative molecular initiating/key events for PFAS, in addition to nuclear receptor activation, include gap junctional inhibition to disrupt cell–cell communication, mitochondrial dysfunction, interference of protein binding, partitioning into lipid bilayers, oxidative stress, altered calcium homeostasis, and inappropriate activation of molecular signals controlling cell functions. Many of these effects are consistent with a nonspecific action of PFAS on the cellular lipid membrane (Spector and Yorek 1985; Bourre et al. 1989; Dodes Traian et al. 2012; Casares et al. 2019). However, these alternative events lack robust evidence to support a specific pathophysiological role in the multifaceted effects of PFAS. A better characterization of the modes of action/AOPs for PFAS toxicities remains an important area of future investigation, necessary to improve our understanding of PFAS impacts on human health.

At present, there is insufficient evidence to determine which of, and to what extent, these molecular interactions play a pathophysiological role in observed adverse outcomes of PFAS (Michigan PFAS Science Advisory Panel 2018). Hence, there is a need to integrate such mechanistic information into a weight-of-evidence framework, first by establishing the mode of action or AOP linking a proposed chain of key events to an adverse outcome and then by demonstrating that at human exposure levels of PFAS the established AOP or mode of action is causal in the adverse outcome observed. The substantial advantage

offered by such an approach is the ability to read across from representative members of appropriate PFAS groupings, based on quantitative information from new approach methodologies and exposure estimates. Hence, better characterization of the modes of action/AOPs for PFAS toxicities remains a critical area of future investigation and will allow us to understand which adversely PFAS-modified pathways must be interrogated prior to new chemicals joining this class. Predicting PFAS activity in the body should be the goal prior to approving novel PFAS for use.

New approaches for developing PFAS toxicity information

When it comes to determining which PFAS should be prioritized for further testing, there are too many chemicals, even in one subclass, for traditional approaches. Numerous creative and high-throughput methodologies are being developed and tested to provide valuable data on PFAS with no toxicity data.

Collaborative approaches. Problem formulation and approach must be guided by available equipment, funds, and technical staff, and important principles: 1) What biological activity and toxicology information can be generated in a *responsive time frame*? 2) Can this information be used to make public health decisions? 3) What are appropriate tools to bring to this problem (platforms, species/sex of cells used, metabolic competency of the model system, and data analysis)? 4) How do we organize, and what are the best mechanisms to report useful biological activity/toxicological information?

Developing "how" to evaluate potential health effects of new PFAS requires some thought to PFAS heterogeneity. Although subclass names have been suggested by several investigators (Buck et al. 2011; Wang Z et al. 2017; Sha et al. 2019), there is still disagreement on those groupings. In addition, half-lives and biological persistence are not predictable based on structure, and exposure routes may be complex. Given that traditional approaches to generate toxicity information are resource-intensive, new approach methodologies, which may include in vitro high-throughput toxicity screening and toxicokinetic testing, will be needed to inform further (in vivo) testing of PFAS.

One example of how agencies/institutes are collaborating to prioritize a list of PFAS needing further study is the REACT Program (Responsive Evaluation and Assessment of Chemical Toxicity). Scientists from the USEPA and the National Institute of Environmental Health Sciences (NIEHS) National Toxicology Program have joined forces to determine if read-across approaches would work. Essentially, they will use existing data for a data-rich substance (the source, e.g., PFOA or PFOS) as an anchor for a data-poor substance (the target, a novel PFAS), which is considered similar enough to the source substance to use the same data as a basis for the safety assessment. For example, the US National Toxicology Program 28-d PFAS or chronic PFOA data set (National Toxicology Program 2020c) could be used as an anchor. The goal is to group PFAS by biological activities and then use in vitro to in vivo extrapolation data and models to estimate oral equivalent exposures for PFAS. For example, multiple biological endpoints (Table 2) were chosen to generate data on 150 PFAS (Patlewicz et al. 2019), representing several structural subclasses for use in read-across.

Selecting assays shown in Table 2 based on PFOA and PFOS health effects covers a broad range of biology. However, because of the structural diversity of PFAS, biological activity of subclasses of PFAS may be missed; but this can be addressed in 2 ways. First, using transcriptomics as a screen, similar and unique pathways altered by different PFAS can be identified. Second, structure–activity relationships may predict potentially missing biological activities. As an example, Leadscope model predictions conducted at the NIEHS predicted biology that was covered in assays already chosen for evaluation, which increased confidence in the approaches chosen. Because model predictions are only as robust as data sets from which they are generated, these outputs should be used to identify assays for screening efforts and not as synonymous with toxicities induced by PFAS. Ultimately, the REACT program aims to prioritize PFAS for additional targeted testing and follow-up with in vivo studies as needed.

Molecular dynamics and protein interactions. Advances in computational tools, many developed for drug discovery, allow environmental and public health researchers to better anticipate some impacts of emerging contaminants even in the absence of substantial experimental data (Rabinowitz et al. 2008). For example, molecular docking and molecular dynamics to predict strengths of interactions between biomolecules and

TABLE 2: Fit-for purpose assays proposed in the REACT program

Endpoint of interest	Assay proposed
High-throughput transcriptomics	Metabolically competent human liver cells/MCF-7 (Tempo-Seq®)
Hepatotoxicity	2D HepaRG® cells
Developmental toxicity	Zebrafish embryo assay
Developmental neurotoxicity	Multielectrode array in neonatal cortical cells and neurite outgrowth
Immunotoxicity	Cytokine alterations in human vascular endothelial cells (BioSeek®)
Hepatic clearance	Metabolic clearance in 50 donor-pooled hepatocyte suspensions
Plasma protein binding	Serum protein binding assay using human serum
Enterohepatic recirculation	Qualyst B-CLEAR® hepatocyte transporter assay
In vitro disposition	In vitro disposition in cell lines under study

REACT = Responsive Evaluation and Assessment of Chemical Toxicity.

contaminants can be an in vitro screening tool for assessing legacy and emerging PFAS for bioaccumulation potential, to identify potential sites of toxic action (Salvalaglio et al. 2010; Ng and Hungerbuehler 2015; Cheng and Ng 2018; Li et al. 2019) and to gain insights into toxic mechanisms (Sheng et al. 2018). Relatively strong binding with particular proteins (e.g., serum albumin, liver fatty acid binding protein) has already proven useful in correlating PFAS structure with potential for bioaccumulation (Ng and Hungerbühler 2014; Cheng and Ng 2017). Tools including molecular docking and molecular dynamics can correlate relative binding affinities of emerging PFAS with these target proteins and subsequently compare with affinities of legacy chemicals with known bioaccumulation potentials, thus providing a first-tier rapid screening mechanism (Luebker et al. 2002; Cheng and Ng 2018).

The use of fluorinated substances in pharmaceutical products has led to an unexpected data source for discovery of structural features in PFAS associated with various types of bioactivity. These data were recently used to train machine learning models to predict potential bioactivity for thousands of untested PFAS (Cheng and Ng 2019). Classification approaches such as these serve as preliminary screening tools for identifying PFAS as a first step in a tiered assessment when detailed mechanistic information is not available.

Addressing mixtures. Based on their potential for complex exposure patterns, PFAS are a mixtures issue. Communities with water-monitoring programs reporting PFAS concentrations demonstrated that they are exposed to mixtures of PFAS. This mixture may be from one or more point sources releasing multiple PFAS and/or PFAS by-products into the air and water, such as a Chemours plant in North Carolina, and suggest that exposures may be substantial (McCord and Strynar 2019). However, numerous other PFAS sources are known to impact community exposure to PFAS mixtures, such as landfill leachate, biosolids recycling, and aqueous film–forming foam contamination of drinking water sources, among others (Sunderland et al. 2019; Solo-Gabriele et al. 2020). Aqueous film–forming foam and other mixtures evident in drinking water, food packaging, health and beauty products, and food-based sources are often poorly characterized (Sunderland et al. 2019; Susmann et al. 2019).

Discussions on whether PFAS may be addressed using a relative potency framework or toxic equivalency factor approach are ongoing. Substances could be grouped by bioaccumulation and persistence (toxicokinetics), function (biology), molecular initiating events, with potency factors derived from several assays, or subclass (structural similarity).

SPECIAL CONSIDERATIONS IN FUTURE STUDY DESIGNS

Future epidemiological studies

Future human studies need to characterize immune outcomes including (and not limited to) immune effects from exposure in early pregnancy and possible roles of PFAS in initiating allergic and autoimmune processes, conditions for which a dose response is hard to predict. Interactions of immune pathways with liver and lipid toxicity deserve additional consideration.

Liver and lipid studies have reasonably characterized associations between PFAS and effects and should now address why and what to do about it. Characterization of possible a priori susceptibility, such as in the obese, is important. Human and animal lipid data suggest that future experimental studies should focus on mitochondrial toxicity, alterations in bile acid metabolism, cholestasis, and resultant steatosis. These outcomes are already known to be associated with altered serum lipids, liver enzymes, and uric acid in the human population regardless of PFAS (Cohen and Fisher 2013; Sattar et al. 2014; Arguello et al. 2015; Jensen et al. 2018).

Studies of human kidney markers related to PFAS exposures illustrate the importance of understanding physiology to inform study design choices and reasonable interpretations. These substances have complex excretion mechanics that vary with dose, state of the healthy or progressively diseased kidney, as well as a potentially additional causative effect on kidney disease outcome(s). Appropriate definition of biological and mechanistic targets and more precise investigation of PFAS subclasses will better inform study designs and research questions. For example, consistent reports of disrupted cholesterol metabolism should prompt mechanistic studies evaluating effects on steroid hormones that may influence cancer, fecundity, lactation, and developmental signals seen in human population data. More attention could be given to effects of PFAS on the hypothalamic–pituitary–gonadal axis and then reconsidered based on life stages.

The history of long-chain PFAS studies indicates that collaborative team approaches featuring clinical, epidemiologic, computational modeling, and laboratory toxicological expertise are needed. Future population designs and more sensitive analytical methodologies should address replacement chemicals, typically found as mixtures; study designs must account for shorter PFAS half-lives and unpredictable PFAS detection in exposed individuals/communities. Innovative use of biomarkers in specifically designated risk subpopulations (obesity, immune) will likely be important.

Sex differences, nonmonotonic dose responses, sensitive subpopulations

Although serum-level differences exist between men and women similarly exposed to individual PFAS, sex-dependent differences in half-life have not been reported in human populations for short-chain (PFBS, PFBA) or long-chain perfluoroalkyl acids thus far (Li et al. 2017b). Perhaps the half-life differences between the sexes is similar to interindividual variability and cannot be detected above background, or studies deriving data sets used to model half-lives were not designed to detect sex differences (convenience sampling or workers were mostly male, etc.). However, sex-specific elimination half-lives are defined (Table 1) for some PFAS in rodent models.

In addition, developmental exposure studies in experimental models have consistently shown effects at lower doses than adult-only exposures and should be given priority in testing replacement chemicals. In vitro and alternative models that capture developmental susceptibility are encouraged. In summary, care should be taken in testing replacement PFAS in rodent or alternative (cell-based or zebrafish, for example) models to consider 1) the possibilities of sex-based differences in elimination half-lives, 2) dose range used (to include human relevant exposures), 3) life stage represented in the model system, and 4) variability of the response to enable the use of data generated for risk assessment.

Future experimental model studies

Experimental rodent studies have been essential in confirming PFAS health effects (liver and thyroid disease, lipid homeostasis), even when effects were not identical to those in humans; in some cases, novel targets (mammary and immune changes) were identified in animals. Future animal, cell-based, and high-throughput toxicity screening should enhance transparency in reporting to include blinded dose allocation, reporting of all data, adherence to Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al. 2010), and dose ranges that approach human relevance (adjusted to reflect the differences in elimination between species and potentially chronic exposures) so that they suitably inform systematic reviews that may be used in chemical regulation.

Model selection for health effects evaluation is critical. An appropriate model should be sensitive, be susceptible to the outcome(s) of interest (obesity, immune), and produce outcomes that will inform human health effects. Alternative research models, such as transgenic mice, zebrafish, developmental models for most affected target tissues, and diet-challenged designs in susceptible rodent strains, will strengthen our knowledge of PFAS-related health effects. Validation of fish neurobehavior models to inform mammalian, including human, developmental responses is needed.

Finally, advanced human cell-based platforms—that have been validated for relevant outcomes in humans—will facilitate concurrent screening of larger numbers of PFAS, but bioavailability of PFAS in the culture system needs to be understood because binding to media proteins or labware, the instability of some PFAS in some vehicles, and altered metabolism may exist in some cases (Gaballah et al. 2020; Liberatore et al. 2020).

Future alternative approaches

One way to determine the toxicity of the large number of PFAS compounds currently used in commerce is to develop quantitative structure–activity relationships (QSAR). Such QSAR attempt to define relationships between a PFAS compound structure with a specific biological activity or response that identifies or is a biomarker for toxicity. Few data are available for receptor binding of PFAS, mainly limited to a few PFCAs and PFSAs; and even between carboxylates and sulfonates of similar chain length substantial differences have been observed (Cheng and Ng 2017, 2018). If there are substantial differences between perfluoroalkyl carboxylic and sulfonic acids, which differ only in their acid head group, construction of successful QSAR for the large and diverse class of all PFAS will be particularly challenging. Several QSAR may be developed, each predictive of toxicity of a distinct class or subclass of PFAS, based on a unique functional moiety or other feature. Although this brings additional challenges in finding sufficient data for QSAR training and validation, big data approaches, such as the recently developed machine learning models to predict PFAS bioactivity (Cheng and Ng 2019), show promise for advancing these computational approaches at the screening level.

For example, it may be determined by affinity for receptor-specific binding and nonspecific interactions with cellular membranes that the specific toxic effect exhibits a multiphasic dose response reflecting 2 potential modes of action. In addition, the critical effect may change with levels of PFAS exposure. Add to this that people are typically exposed to PFAS mixtures, each of which may have a different affinity for a binding site and ability to impact cellular membrane fluidity, and the potential to predict PFAS toxicity becomes extremely complicated. In the foreseeable future, we may be limited to assessing PFAS toxicity using high-throughput assays designed to inform regulators as to the relative toxicity of PFAS mixtures or compounds. Such approaches are suited to the use of artificial intelligence (i.e., machine learning approaches) that integrate data from multiple sources to identify bioaccumulation potential, relevant pathways triggered, protein binding affinities, and modes of action involved in the development of individual and mixture toxicity of PFAS.

The utility of any future approach to determining PFAS toxicity must consider tissue-specific modes of action. Such an approach may rely on molecular interactions with specific binding sites on enzymes/storage/transport proteins or the nonspecific ability to alter cell membrane fluidity by which membrane-bound protein activities are altered within a particular organ/system. Regardless of the mode of action, model, and/or simulation, the predictive result should be biologically plausible and represent dose–effect responses across species.

CONCLUSION

Future research on the health effects of replacement PFAS and mechanistic studies on legacy PFAS must apply "lessons learned" such as those highlighted in the present review. There are only a handful of PFAS with enough health effects data for use in decision-making, as evidenced by state-led standard setting. There are numerous health effects reported for those PFAS tested, which sets this family of chemicals apart from many others and elevates the need for precautionary action. With hundreds of PFAS lacking health effects data, translational research teams using innovative methodologies and carefully designed studies will be critical to our state of knowledge on

PFAS-related health effects and our enhanced strategies for informing risk assessment of this large family of chemicals.

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Reproduction, Fertility and Development

The perils of poly- and perfluorinated chemicals on the reproductive health of humans, livestock, and wildlife

Mark P. Green[A], Cameron Shearer[B], Rebecca Patrick[C], Shervin Kabiri[D], Nicola Rivers[E] and Brett Nixon[F,G,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Brett Nixon
Hunter Medical Research Institute Research Program in Infertility and Reproduction, New Lambton Heights, NSW 2305, Australia
Email: brett.nixon@newcastle.edu.au

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Graeme Martin

ABSTRACT

Poly- and perfluoroalkyl substances (PFAS) are a prominent class of persistent synthetic compound. The widespread use of these substances in various industrial applications has resulted in their pervasive contamination on a global scale. It is therefore concerning that PFAS have a propensity to accumulate in bodily tissues whereupon they have been linked with a range of adverse health outcomes. Despite this, the true extent of the risk posed by PFAS to humans, domestic animals, and wildlife remains unclear. Addressing these questions requires a multidisciplinary approach, combining the fields of chemistry, biology, and policy to enable meaningful investigation and develop innovative remediation strategies. This article combines the perspectives of chemists, soil scientists, reproductive biologists, and health policy researchers, to contextualise the issue of PFAS contamination and its specific impact on reproductive health. The purpose of this article is to describe the challenges associated with remediating PFAS-contaminated soils and waters and explore the consequences of PFAS contamination on health and reproduction. Furthermore, current actions to promote planetary health and protect ecosystems are presented to instigate positive social change among the scientific community.

Keywords: emerging contaminants, human health, livestock, persistent synthetic compounds, planetary health, poly- and perfluoroalkyl substances (PFAS), remediation, reproduction, reproductive health, wildlife.

Introduction

Optimal reproductive function requires an environment that adequately supports critical processes such as gametogenesis, mating, pregnancy, and the nurturing of offspring. Humans are having an increasingly negative influence on the environment with knock on consequences for the reproductive health of numerous species (Aulsebrook *et al.* 2020). The extent to which human activity has impacted Earth's geology and ecosystems has likely crossed an epoch-scale boundary resulting in the proposal of a new epoch of geological time, the 'Anthropocene' (Zalasiewicz *et al.* 2011).

In the hope of promoting environmental stability, nine 'planetary boundaries' were defined as a framework to quantify and monitor the impact of human activity on the Earth's biophysical systems (Rockstrom *et al.* 2009). The planetary boundaries define thresholds that should not be breached to ensure sustainable human activity without causing significant harm to the ecosystems upon which humans rely for our prosperity and wellbeing (Steffen *et al.* 2015). In 2022, the threshold for novel entities (i.e. chemical pollution) was exceeded due to the large production and release of human-derived chemicals outweighing the capacity to monitor and breakdown such compounds (Persson *et al.* 2022). Of particular concern are compounds deemed to be resistant to typical methods of breakdown, causing them to be classified as 'persistent'. These persistent compounds can bioaccumulate in the tissues of humans, wildlife, and agricultural species resulting in potential long-term effects on biological function.

Poly- and perfluoroalkyl substances (PFAS) are a prominent class of synthetic chemicals that fall into the category of persistent compounds (Buck *et al.* 2011). These substances have

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been extensively used in various industrial applications since the 1950s, ranging from non-stick coatings to firefighting foams and, unfortunately, the widespread use and disposal of PFAS have resulted in their pervasive contamination on a global scale (Kurwadkar *et al.* 2022). Current evidence suggests PFAS contamination can result in elevated cholesterol, ulcerative colitis, and thyroid and kidney disease (Steenland *et al.* 2020). It is estimated that the disease burden and social costs associated with PFAS exposure in the USA could amount to between US$5.52 billion and US $62.6 billion annually (Obsekov *et al.* 2023). In response to the mounting evidence of the negative impacts of PFAS on health, PFAS contamination has recently featured at the centre of two large class action settlements in 2023, one in Australia (Australian Associated Press 2023) and one in the USA (Friedman and Giang 2023).

Despite now being ubiquitous in the environment, clear data regarding the impact of PFAS on reproduction in humans, domestic animals, and wildlife is lacking. Emerging evidence indicates that PFAS can be passed to the next generation through the placenta and lactation (Gronnestad *et al.* 2017). The effects of PFAS on reproductive function is of particular concern for species that are already at risk of extinction due to declining population numbers. The potential far-reaching consequences of PFAS contamination on reproductive health necessitates urgent action to identify and address the pervasiveness of PFAS in the environment.

Addressing the persistent nature of chemicals like PFAS requires a multidisciplinary approach, combining the fields of chemistry, biology, and policy to adequately investigate and develop innovative remediation strategies. This article combines the perspectives of chemists, soil scientists, reproductive biologists, and health policy researchers, to contextualise the issue of PFAS contamination and its impact on reproduction. The purpose of this article is to describe the challenges associated with remediating PFAS-contaminated soils and waters and explore the consequences of PFAS contamination on health and reproduction. Furthermore, current actions to promote planetary health and protect ecosystems are presented to instigate positive social change among the scientific community.

What makes some chemicals persistent?

The conversion of chemicals is a fundamental aspect of nature. Examples include the conversion of CO_2 into sugars during photosynthesis, microbial breakdown of complex organic matter into rich soils (de Vries and Wallenstein 2017), and the weathering of limestone and rock by water to create natural landmarks. Chemicals in nature are generally converted through one (or a combination) of reactions with water, sunlight, air, or microbial action.

Chemicals that are resistant to conversion through any of these means are generally persistent in nature. The Stockholm Convention (2023) maintains a list of organic chemicals that a panel of experts have deemed persistent, widespread, bioaccumulate, and harmful. Notably, all listed chemicals contain carbon to halogen bonds (C-F, C-Cl, C-Br). These bonds are very strong; sunlight does not have sufficient energy to break them (Stockholm Convention 2023), they react very slowly in water and are rare in nature, so efficient microbial processes have not yet developed to breakdown these human-made chemicals.

Poly- and perfluoroalkyl substances

Poly- and perfluoroalkyl substances are a class of synthetic chemicals which consist of a hydrophobic tail with either a fully or partially fluorinated carbon chain and a hydrophilic head group, such as a carboxylic acid or sulfonate (Buck *et al.* 2011) (Fig. 1*a*). PFAS most commonly consist of a single carbon chain with 4–10 carbons (c_4–c_{10}), with the most prevalent being perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) (Fig. 1*a*). However, more than 4000 different PFAS have been used in industry including straight chains, branched isomers, as well as production by-products and precursors (Wang *et al.* 2018).

Poly- and perfluoroalkyl substances have found a myriad of applications since the 1950s including adhesives, non-stick cooking surfaces (e.g. Teflon), water-repelling coatings (e.g. Scotch Guard), anti-corrosive coatings on metals (e.g. Zonyl FSP), anti-fogging coatings on glass and mirror, cosmetics, inks, lubricants, leather treatments, emulsifiers, wetting agents in paints, herbicides, insecticides and coatings, and as aqueous film-forming foams (AFFF) for fire-fighting foams (Kissa 2001). The extensive use and disposal of PFAS has resulted in significant release to the environment where their inherent stability has led to pervasive contamination on a global scale (Moody and Field 1999; Weiß *et al.* 2012; Hepburn *et al.* 2019; Turner *et al.* 2019) (Fig. 1*a*).

In humans, PFAS are routinely detected in blood and organs in people without industrial exposure (Hansen *et al.* 2001; Yeung *et al.* 2006; Calafat *et al.* 2007; Perez *et al.* 2013; Ye *et al.* 2018) (Fig. 1*b*). In June 2022, the US Environmental Protection Agency announced an interim health advisory, which reduced the safe drinking water level of some PFAS (i.e. PFOA and PFOS) to 0.004 ng/L for PFOA and 0.02 ng/L for PFOS (previous value was 70 ng/L for each) (United States Environmental Protection Agency (US EPA) 2022). The large decrease in the safe drinking water level of PFAS will require tremendous improvements in PFAS remediation considering the combined PFOA and PFOS concentration is currently measured at 1 ng/L or greater in the drinking water for upwards of 200 million people in the USA alone (Andrews and Naidenko 2020). Notably, no alternative to substantial remediation projects is possible considering PFAS are detected

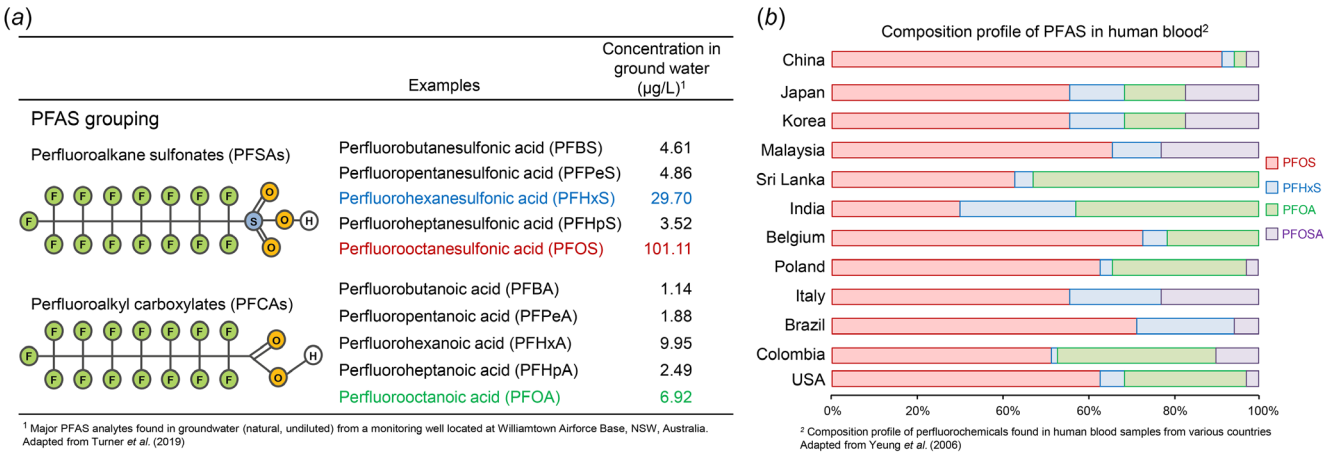

Fig. 1. Summary of common poly- and perfluoroalkyl substances (PFAS) structure, nomenclature, and representative concentrations in contaminated groundwater and compositional profile in human blood. (*a*) PFAS are a class of synthetic chemicals consisting of a hydrophobic tail with either a fully or partially fluorinated carbon chain, which are broadly grouped on the basis of their hydrophilic head group consisting of different functional group e.g. carboxylic acid or sulfonate. PFAS most commonly consist of a single carbon chain with 4–10 carbons, with two of the most prevalent being perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). The extensive use and disposal of PFAS has resulted in significant release to the environment where their inherent stability has led to pervasive contamination on a global scale, with representative levels of 10 major PFAS analytes found in groundwater (natural, undiluted) from a monitoring well located at Williamtown Airforce Base, NSW, Australia (adapted from Turner *et. al.* 2019); all of which are orders of magnitude above that considered safe drinking water levels (i.e. 0.004 ng/L for PFOA and 0.02 ng/L for PFOS; United States Environmental Protection Agency (US EPA) 2022). (*b*) A concerning consequence of this contamination is that PFAS are readily detected in human blood sampled from populations across the globe (adapted from Yeung *et al.* 2006).

in bottled water at quantities greater than the new safe limits (Chow *et al.* 2021).

Perspective for soil and water rehabilitation

The extensive application of PFAS in the last seven decades has resulted in near-ubiquitous contamination of soils, sediments, groundwater, and surface water including drinking water with several studies showing that soils contaminated with PFAS can serve as a significant long-term source of PFAS release (Strynar *et al.* 2012; Brusseau *et al.* 2020). Soil contamination occurs with the direct application of PFAS-laden AFFFs (Nickerson *et al.* 2020), via atmospheric deposition or with the deposition of PFAS-contaminated materials including biosolids, municipal sludges, and irrigation water (Bolan *et al.* 2021). Similarly, an abundance of landfilled PFAS-containing products has led to landfill leachates becoming another source of soil and groundwater contamination.

Once present in the soil matrix, PFAS can interact with different soil components before leaching down to groundwater or moving laterally to surface waters in runoff from rainfall. PFAS interactions with soil are complex, but are always related to the soil's physical and chemical properties (e.g. organic carbon content, texture (sand, silt, clay content), pH, salt and mineral contents of the soil), and PFAS chemistry (carbon-fluorine chain length, functional head group and charge) (Li *et al.* 2018; Kabiri *et al.* 2022). Critically, many PFAS, such as PFOA and PFOS, are anionic at environmental pH and can be repelled by soil or groundwater's negatively charged components such that they are relatively mobile in the soil and groundwater and can travel kilometres from the source zone (Interstate Technology and Regulatory Council (ITRC) 2023). Another major challenge related to PFAS presence in soil or water is the transformation of precursors (polyfluorinated PFAS) to perfluorinated PFAS. Many precursor chemicals bind strongly to soils, creating a stable long-term source zone that consistently releases more mobile PFAS species as they transform (Lenka *et al.* 2021). Furthermore, many of these compounds go undetected in the environment as most of them are not detectable by conventional analytical techniques (Ross *et al.* 2018).

One of the major routes of human exposure to PFAS is the consumption of PFAS-contaminated food or water (Brown *et al.* 2020) (Fig. 2). Literature-derived data has revealed elevated concentrations of PFAS being detected in certain foods, including eggs (Bao *et al.* 2019), grains (Noorlander *et al.* 2011), vegetables, fruits (Herzke *et al.* 2013; Li *et al.* 2019) milk, and meat (Guruge *et al.* 2008; Li *et al.* 2019) that are produced close to contaminated sites. Dietary survey data and toxicokinetic modelling has also estimated that seafood accounts for up to 86% of total PFOS exposure for adults (EFSA CONTAM Panel *et al.* 2020). By contrast, in infants, eggs and egg products were the most important source of chronic exposure to PFOS (up to 42%) and drinking water was the dominant source of PFOA exposure (up to 60%) (EFSA CONTAM Panel *et al.* 2020). Food contamination occurs

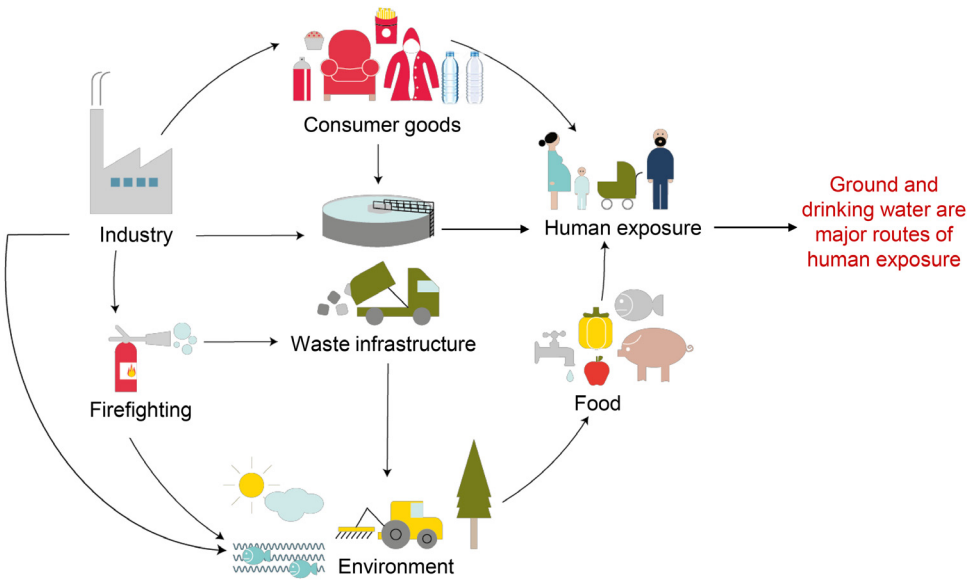

Fig. 2. Summary of human PFAS exposure pathways. Human exposure to PFAS may occur through multiple pathways including food, consumer products, food packaging, cleaning products, personal care products, household dust, and contact with a variety of other contaminated media. While dietary intake resulting from the ingestion of contaminated drinking water and/or foods grown in contaminated groundwater rank among the key exposure routes for the general population, emerging evidence indicates that plastic containers, such as those used for storage of food, drinks, personal care products, pharmaceuticals, and cleaning and industrial products, may represent a major new PFAS exposure source. Figure adapted from European Environment Agency – Emerging chemical risks in Europe – 'PFAS' (2019).

when foods are grown in contaminated soils or fished from contaminated waters, and in the former case may be further intensified by the application of contaminated water and fertiliser products (Sunderland *et al.* 2019). Therefore, widespread presence of PFAS in foods and their hazard to human health demands their removal from diverse soil and water sources. However, the varied chemistry of PFAS, complex environmental interactions, and the extremely high standard of removal (parts per trillion) required to reduce their hazard, complicate their clean-up. Practical approaches to clean-up PFAS from the environment contain the following steps (CRC CARE 2018): (1) preliminary and detailed investigation of contaminated sites; (2) development of conceptual site models to identify the source of PFAS and potential migration pathway; (3) risk assessment of the contaminated site; (4) site management and remediation technology; and (5) management of the residual contamination.

If the preliminary site investigation and conceptual site model shows the contaminated site presents an exposure risk to people or the environment, then a detailed site assessment and treatment is required (CRC CARE 2018). To date, a wide array of PFAS treatment technologies have been applied to treat contaminated soil and water. Overall, these strategies can be divided into destructive and non-destructive technologies that can be applied either *in situ* or *ex situ* to immobilise, separate, or more rarely, destroy PFAS at a site (CRC CARE 2018). The proving and scaling up of technologies for soil and water remediation remains an area of extensive research. In a field study, a soil washing technique was able to remove up to 96% of PFOS from soil (Swedish EPA 2018), while others demonstrated the remediation of 1000 tonnes of PFAS-contaminated soil by stabilising PFAS in soil using a mixed mode sorbent material (Swedish EPA 2018). Other non-destructive techniques, such as phytoremediation, and destructive techniques, such as thermal oxidation and chemical oxidation, have been applied at field scale, but with varying efficacy (Bolan *et al.* 2021). For contaminated water sources, activated carbon, ion exchange resins, and reverse osmosis have been used widely and at scale to remove PFAS (Bolan *et al.* 2021). However, there remains a considerable mismatch between the scale and breadth of PFAS contamination, and the applicability and cost-effectiveness of the tools required to resolve this pollution. For this reason, achieving large scale/throughput and cost-effectiveness with short-term measures, such as landfilling contaminated soil, will be key benchmarks for new technologies.

In the last two decades, the life cycle regulation of PFAS has improved, particularly with the discontinued manufacture of the most demonstrably harmful PFAS species. These regulatory changes have had the dual effect of somewhat reducing the influx of PFAS into the environment and raising the standard

of treatment of water and soil required, thereby incentivising further innovation in remediation technology. However, decades of PFAS use without regulation or monitoring mean that regardless of future inputs, the volumes of water and soil requiring clean-up are enormous and globally distributed, thus demanding a correspondingly concerted response.

Impacts of PFAS on human health

Recent changes to the US EPA health advisory concerning PFAS consumption come in the wake of the Scientific Committee on Health labelling PFAS as a potential risk for humans and the environment (Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) 2018). Such advisories reflect increasing awareness of the propensity of PFAS to accumulate in human tissues combined with mounting epidemiological evidence supporting negative associations between PFAS exposure and an array of human health conditions (Calvert *et al.* 2021). Despite endeavours to phase out the so-called long-chain PFAS, the inherent stability of these compounds has resulted in omnipresence in the global environment. Thus, as discussed later, many industrialised nations are seeking to implement additional measures to limit, detect, and eradicate PFAS contamination. Although all paths of human exposure remain to be identified, it is thought that dietary intake from food packaging or environmental contamination remains a key exposure pathway for the general population (Xing *et al.* 2023; Zhu *et al.* 2023) (Fig. 2). In this context, recent evidence suggests that plastic containers, such as those used for storage of food, drinks, personal care products, pharmaceuticals, and cleaning and industrial products, may represent a major new exposure point (Landrigan *et al.* 2023) (Fig. 2). Such contamination has been linked to the use of PFAS as lubricants during the plastic manufacturing process as well as from the practice of fluorination, whereby plastic containers are treated with fluorine gas to improve their stability and reduce their permeability. This creates the potential for several troubling scenarios, including the contamination of plastic recycling streams and unintentional leeching of PFAS into the consumer product.

Irrespective of the route of exposure, upon entering the body, PFAS bind to proteins in the blood stream and thereafter accumulate within the body's protein-rich tissues (Jensen and Leffers 2008; Perez *et al.* 2013). Consequently, PFAS are readily detectable in most bodily fluids (including urine, breast milk, blood, and seminal plasma) as well as throughout the human body and may take several years to be fully excreted (Calafat *et al.* 2007; Jian *et al.* 2018). Despite this knowledge, it has proven challenging to definitively link PFAS exposure to impacts on human health owing to factors such as variations in chemistries and potential biological activities among the different sub-classes of PFAS, the duration and degree of exposure, and potential synergistic or antagonistic effects of PFAS combinations in the body (Rand and Mabury 2017). This situation is further compounded by differences in the mechanisms and routes of PFAS exposure as well as the genetic constitution of exposed individuals, the latter of which may influence PFAS clearance rates and susceptibility to the biological effects of these chemicals.

Notwithstanding these limitations, the balance of evidence supports the potential for PFAS exposure to elicit adverse health sequelae across the life course (Kirk *et al.* 2018). This conclusion is also borne out by the findings of the C8 Health Project; a comprehensive investigation of an entire community of more than 69,000 people exposed to PFAS via consumption of contaminated drinking water (Frisbee *et al.* 2009). This study revealed probable links between PFOA exposure and six diseases: kidney and testicular cancer, thyroid disease, high cholesterol, ulcerative colitis, and pregnancy-induced hypertension. Building on this evidence, the most consistently reported metabolic consequence of PFAS exposure is dyslipidemia, with several notable studies finding links between serum PFAS and dysregulated lipid profiles, including increased low-density lipoprotein, triglycerides, and total cholesterol, in addition to diminished high-density lipoprotein (Olsen and Zobel 2007; Sakr *et al.* 2007*a*, 2007*b*; Costa *et al.* 2009; Frisbee *et al.* 2009).

Epidemiological evidence has also linked PFAS exposure to the prevalence of testicular cancer, with the International Agency for Research on Cancer (IARC) concluding PFOA is possibly carcinogenic to humans (IARC 2023) and the US EPA declaring it a likely carcinogen (United States Environmental Protection Agency (US EPA) 2021, 2022). The potential significance of these positive associations is highlighted by parallel correlations between the widespread increase in worldwide PFAS usage and the rising prevalence of testicular cancer; a pathology that has significantly increased in recent times to become the most common malignancy in young men aged 20–40 years (Skakkebaek *et al.* 2007; McIver *et al.* 2013). Although the characterisation of testicular cancer remains incomplete, there is speculation that environmental factors, as opposed to genetic factors, are a key contributor to the aetiology of this form of cancer. Further evidence of testicular dysfunction is supported by large cohort studies, which have revealed associations between PFAS exposure and several indicators of human sperm quality, including reductions in total sperm count, normal sperm morphology, and sperm motility (Joensen *et al.* 2009; Vested *et al.* 2013; Governini *et al.* 2015; Louis *et al.* 2015; Song *et al.* 2018). PFAS have also been linked a variety of additional reproductive characteristics, including direct disturbance of testicular steroidogenic cells and an attendant dysregulation of reproductive hormone profiles (Olsen *et al.* 1998; Zhang *et al.* 2014; Cui *et al.* 2020). Taken together, such correlations raise the prospect that the testis is a vulnerable organ for PFAS-induced damage.

These findings highlight the requirement for further investigation and the identification of reliable biological models that can inform health risks, allowing sensitive assessment of the spectrum of effects of PFAS exposure on humans. In this regard, there is clearly much to learn from the study of pre-clinical animal models, wherein PFAS exposure can be controlled to mitigate many of the confounders that continue to plague the study of human cohorts.

Impacts of PFAS on animals

Exposure to high levels of PFAS poses a health risk not just for humans but for domestic animals and wildlife. Understanding the abundance, distribution, and effects of PFAS on livestock and wildlife species can help identify any adverse impacts on top of other anthropogenic-created issues faced by animal species on their health, population dynamics, and habitat. Notably, PFAS exposure is another anthropogenic factor thought to drive population decline and extinction (Kannan *et al.* 2006; Ishibashi *et al.* 2008).

In terms of habitat and the exposure of wildlife to PFAS, it is well documented that the long-chain (≥8 carbons) PFAS, such as PFOA and PFOS, as well as shorter chain PFAS are transported long distances to remote environments via air (Zhou *et al.* 2022) and water currents (De Silva *et al.* 2021). Atmospheric pathways are particularly important as many PFAS are water soluble, so are highly persistent in surface and groundwater (Szabo *et al.* 2018; Zhao *et al.* 2020; Szabo *et al.* 2023), as well as oceans (Gonzalez-Gaya *et al.* 2014). Hence, transport by ocean currents is thought to be the main pathway for the global distribution of PFOS and PFOA, with PFAS commonly being redistributed from lower latitudes to the poles (Yamashita *et al.* 2008; Armitage *et al.* 2009*a*, 2009*b*; Stemmler and Lammel 2010). Loss of PFAS from the water column through settling and mixing to the deep waters of the ocean is estimated to remove approximately 25% of the total global PFOA emissions (Armitage *et al.* 2009*a*), resulting in PFAS being present and having the ability to circulate in the world's oceans for many centuries after their production ends. Despite a large percentage of PFOA and PFOS entering the sediment, concentrations in the ocean are known to vary from 57 to 200 ng/L in coastal waters around cities to 1–15 pg/L in the central Pacific Ocean (Yamashita *et al.* 2005).

Detection of PFAS in the oceans and waterways unsurprisingly has resulted in their uptake by wildlife (Letcher *et al.* 2010). PFAS in wildlife was first reported by Giesy and Kannan (2001) who demonstrated the global distribution of PFOS in fish and birds, as well as in marine and terrestrial mammals. In addition, that PFOS concentrations in animals from relatively more populated and industrialised regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were generally greater than those in animals from remote locations. Equally, temporal trends in the concentration of various PFAS are evident (Martin *et al.* 2004; Houde *et al.* 2011), which along with diet, feeding behaviour and habitat, as well as trophic order and metabolic rate all contribute to variation in PFAS profiles and concentrations in wildlife (Lein 1972; Hobson and Welch 1992; Robuck *et al.* 2020). Despite this, PFOS remains the predominant PFAS found in all wildlife species, tissues, and locations analysed around the world (Houde *et al.* 2006; Houde *et al.* 2011). It stands to reason that if PFAS are in the environment and detectable in wildlife and in humans, they will also be present in livestock, potentially affecting their health and reproduction. This troubling scenario has been confirmed whereby the migration of PFAS onto agricultural properties has been documented to result in accumulation in livestock, also likely contributing to the impact on humans through the food chain (Death *et al.* 2021; Drew *et al.* 2021; Jha *et al.* 2021; Mikkonen *et al.* 2023).

Indeed, movement of PFAS through food webs and the bioaccumulation potential of PFOA and other long-chain PFAS (≥8 carbons), especially in animals with a high body fat content, is evident and of great concern (Kelly *et al.* 2009; Ahrens 2011; Houde *et al.* 2011). Consequently, apex predators, such as killer whales, polar bears, and bald eagles, harbour PFAS concentrations higher than those measured in their diet (Smithwick *et al.* 2006; Kelly *et al.* 2009; Ahrens 2011; Lindstrom *et al.* 2011), indicating the bioaccumulation and biomagnification of PFAS in food webs (Giesy and Kannan 2001; Kannan *et al.* 2005). Species' differences in the concentration and ability to bioaccumulate specific PFAS congeners are apparent. For example, PFOS concentrations are generally lower, longer-chain perfluoroalkyl carboxylic acids (PFCA) concentrations are greater, and short-chain PFCA are more commonly detected in birds than in marine mammals (Letcher *et al.* 2010; Muir *et al.* 2019). In addition, within an animal the distribution of PFAS in body fluids and tissues is congener-specific (Gebbink and Letcher 2012), underpinned by PFAS interactions with proteins (Bangma *et al.* 2022). Increasing body burden with age and differences between sexes are also evident (Bangma *et al.* 2022), especially for egg-laying species, due to associations of PFAS with lipids and proteins in the yolk; hence, this is a major excretion route for PFAS in females (Newsted *et al.* 2007; Tartu *et al.* 2014; Lopez-Antia *et al.* 2019). The high PFAS concentration in the egg is thus likely to impact *in ovo* fetal growth and potentially long-term health of that offspring. Equally, species that consume eggs, including humans, risk ingesting highly concentrated PFAS in their diet. Further well-documented maternal PFAS transfer routes include via *in utero* gestation (Ma *et al.* 2022) and lactation (Gronnestad *et al.* 2017), as evidenced by high PFAS concentration in young and juveniles (Ishibashi *et al.* 2008). Collectively, these studies highlight how reproduction, food webs, and agricultural practices can exacerbate PFAS intake and their effects on subsequent generations.

Once PFAS is in the body, adverse effects to the genealogical, neurological, and endocrinological systems of wildlife species are reported (Dietz *et al.* 2019; Bangma *et al.* 2022), resulting in potential detrimental immunotoxicological, histopathological, and reproductive effects (Kannan *et al.* 2006; Letcher *et al.* 2010; Dietz *et al.* 2019; Bangma *et al.* 2022). This is supported by studies of laboratory animals showing associations between exposure to PFAS and adverse health and reproductive effects across generations (Kato *et al.* 2015; Chen *et al.* 2017; Haimbaugh *et al.* 2022). Attribution of these impacts to a single PFAS or the total PFAS burden is unclear, because most wildlife studies are based on association and observation, with limitations in accurate quantification and ability to measure all PFAS congeners. To comprehensively summarise the current data and our understanding of PFAS exposure effects on wildlife, novel systematic evidence maps and bibliometric analyses are being developed (Vendl *et al.* 2021). However, there is still clearly an urgent need for studies to identify PFAS exposure and impacts across wildlife species. Alongside this, the development of better non-invasive sampling methodologies is required to minimise sampling stress on wildlife. Identifying the effect of PFAS on wildlife is therefore fundamental in helping to regulate and legislate their use, not to mention how consumption of wildlife and domesticated animals by humans is a considerable exposure route (Fig. 2).

Social impacts and health policies

As documented above, there is mounting evidence that PFAS contamination has the potential to harm ecosystem health as well as human health and reproduction. In this capacity, PFAS may already be contributing to the biodiversity crisis, climate change, and degradation of fundamental ecosystem services including food and water supplies. PFAS pollution is therefore a public health issue globally, with clearly traceable physical health outcomes (as forementioned), as well as psychosocial impacts for those living in impacted areas (Banwell *et al.* 2021). There is now compelling evidence from social and psychological science that fear of and anxiety about uncertain futures posed by planetary health disasters, such as chemical pollution, is driving new mental health symptomology such as 'eco-anxiety' and 'ecological grief' (Patrick *et al.* 2023*a*, 2023*b*). For young people, anxiety about the future of the planet is causing functional and cognitive impairment at levels which are affecting their work, family, and social lives (Patrick *et al.* 2023*a*, 2023*a*). Indeed, planetary health concerns are now factoring into young peoples' reproductive choices in many countries (Schneider-Mayerson and Leong 2020). Conversely, taking action on planetary health issues can promote mental and social wellbeing and enhance environmental stewardship (Gunasiri *et al.* 2022).

An urgent and concerted global effort is needed to the respond to the complex drivers of these planetary health issues, including those arising from chemical pollution. In the Australian context, current actions to promote human health and protect ecosystems include:

- The PFAS policy solutions: The Australian PFAS National Environmental Management Plan (PFAS NEMP) 2020 provides nationally agreed guidance on the management of PFAS contamination in the environment, including prevention of the spread of contamination (HEPA 2020). It supports collaborative action on PFAS by the Commonwealth, state and territory and local governments around Australia.
- The Climate and Health Policy solutions: A coalition of leading Australian health experts and organisations, along with federal parliamentarians, have launched a Framework for a National Strategy on Climate, Health and Well-being for Australia (Climate and Health Alliance 2021). This framework for a National Strategy on Climate, Health and Well-being for Australia provides a roadmap to support the Commonwealth Government in taking a leadership role in protecting the health and wellbeing of Australian communities from climate change, and in fulfilling its international obligations under the Paris Agreement.
- The circular economy and plastics solutions: A World Health Organization circular economy approach offers an avenue to sustainable economy and good health, whilst saving the environment and its natural resources (WHO 2018). Key elements of a circular economy approach for plastics include: eliminating of problematic/unnecessary plastic packaging through redesign; reuse where relevant, reducing the need for single-use plastics; ensuring all plastic packaging is 100% reusable, recyclable, or compostable; plastic is decoupled from the consumption of finite natural resources; and plastic packaging is free of hazardous chemicals ensuring the health and safety of all involved in the supply chain (Ellen MacArthur Foundation 2022).
- Scientists and plastics leadership: The Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia has set a target of an 80% reduction in plastic waste entering the Australian environment by 2030 (CSIRO 2023). Strategies include human behaviour change and incentives, waste innovation, and developing best practice and standards.

Summary and future considerations

Gradually, institutions are moving to phase out the use of PFAS. In Australia, an action plan to voluntarily phase out all PFAS from fibre-based food packaging is underway (APCO 2023). However, the Australian Government is still working to align with the Stockholm Convention, which would subject PFAS to enhanced regulation including restriction, waste

management, and elimination (Department of Climate Change, Energy, the Environment and Water (DCCEEW) 2023). While limiting new sources of PFAS exposure is a positive step, remediating already contaminated environments will be challenging due to the complex interactions between these chemicals and the environment. As such, there is an urgent need to understand the impacts of PFAS on reproductive function in humans, domestic species, and wildlife.

Collaborative efforts are needed to minimise the adverse effects of PFAS on the environment and reproductive health. Such efforts include monitoring the effects of compounds like PFAS and designing methods to eliminate or manage their presence in the environment. Importantly, scientists have an important role to play in terms of acting as advocates and effectively educating the public and policy makers on the complexity of the issues surrounding persistent compounds like PFAS and the potential consequences to the health of all species.

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Author affiliations

[A]School of BioSciences, Faculty of Science, University of Melbourne, Parkville, Vic. 3010, Australia.

[B]Department of Chemistry, School of Physics, Chemistry and Earth Sciences, Faculty of Sciences, Engineering and Technology, University of Adelaide, SA 5005, Australia.

[C]School of Health and Social Development, Faculty of Health, Deakin University, Geelong, Vic. 3220, Australia.

[D]School of Agriculture, Food and Wine, Faculty of Sciences, Engineering and Technology, Glen Osmond, SA 5064, Australia.

[E]Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Clayton, Vic. 3168, Australia.

[F]Hunter Medical Research Institute Research Program in Infertility and Reproduction, New Lambton Heights, NSW 2305, Australia.

[G]School of Environmental and Life Sciences, College of Engineering, Science and Environment, The University of Newcastle, Callaghan, NSW 2308, Australia.

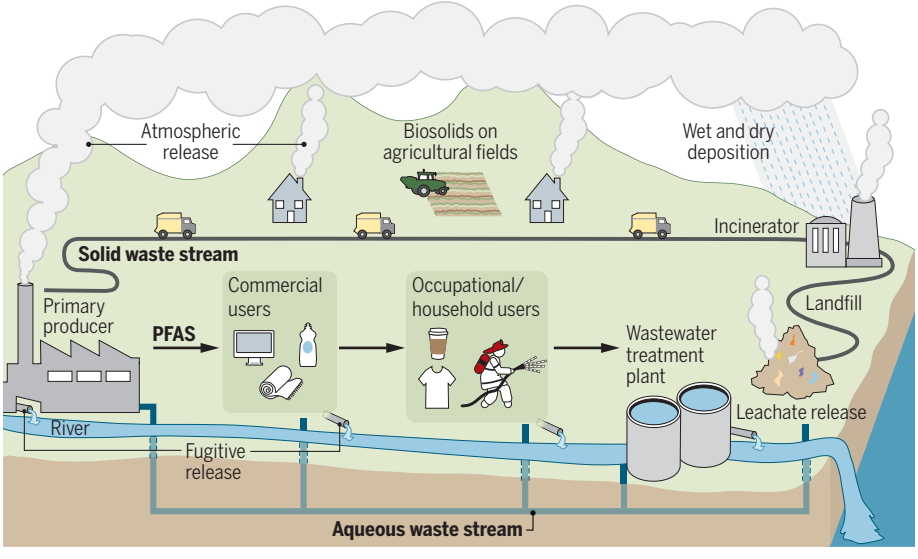
REVIEW SUMMARY

CHEMICAL POLLUTION

Per- and polyfluoroalkyl substances in the environment

Marina G. Evich†, Mary J. B. Davis†, James P. McCord†, Brad Acrey, Jill A. Awkerman, Detlef R. U. Knappe, Andrew B. Lindstrom, Thomas F. Speth, Caroline Tebes-Stevens, Mark J. Strynar, Zhanyun Wang, Eric J. Weber, W. Matthew Henderson*, John W. Washington*

BACKGROUND: Dubbed "forever chemicals" because of their innate chemical stability, per- and polyfluoroalkyl substances (PFAS) have been found to be ubiquitous environmental contaminants, present from the far Arctic reaches of the planet to urban rainwater. Although public awareness of these compounds is still relatively new, PFAS have been manufactured for more than seven decades. Over that time, industrial uses of PFAS have extended to >200 diverse applications of >1400 individual PFAS, including fast-food containers, anti-staining fabrics, and fire-suppressing foams. These numerous applications are possible and continue to expand because the rapidly broadening development and manufacture of PFAS is creating a physiochemically diverse class of thousands of unique synthetic chemicals that are related by their use of highly stable perfluorinated carbon chains. As these products flow through their life cycle from production to disposal, PFAS can be released into the environment at each step and potentially be taken up by biota, but largely migrating to the oceans and marine sediments in the long term. Bioaccumulation in both aquatic and terrestrial species has been widely observed, and while large-scale monitoring studies have been implemented, the adverse outcomes to ecological and human health, particularly of replacement PFAS, remain largely unknown. Critically, because of the sheer number of PFAS, environmental discovery and characterization studies struggle to keep pace with the development and release of next-generation compounds. The rapid expansion of PFAS, combined with their complex environmental interactions, results in a patchwork of data. Whereas the oldest legacy compounds such as perfluoroalkylcarboxylic (PFCAs) and perfluoroalkanesulfonic (PFSAs) have known health impacts, more recently developed PFAS are poorly characterized, and many PFAS even lack defined chemical structures, much less known toxicological end points.



The PFAS life cycle. PFAS product flows from primary producer to commercial user to consumers to disposal. Each step is attended by atmospheric and aqueous fugitive releases. Soils constitute a long-term environmental sink, slowly releasing PFAS to the hydrosphere and allowing uptake in biota, but the ultimate reservoir is deep marine sediment.

ADVANCES: Continued measurement of legacy and next-generation PFAS is critical to assessing their behavior in environmental matrices and improving our understanding of their fate and transport. Studies of well-characterized legacy compounds, such as PFCAs and PFSAs, aid in the elucidation of interactions between PFAS chemistries and realistic environmental heterogeneities (e.g., pH, temperature, mineral assemblages, and co-contaminants). However, the reliability of resulting predictions depends on the degree of similarity between the legacy and new compounds. Atmospheric transport has been shown to play an important role in global PFAS distribution and, after deposition, mobility within terrestrial settings decreases with increasing molecular weight, whereas bioaccumulation increases. PFAS degradation rates within anaerobic settings and within marine sediments sharply contrast those within aerobic soils, resulting in considerable variation in biotransformation potential and major terminal products in settings such as landfills, oceans, or soils. However, regardless of the degradation pathway, natural transformation of labile PFAS includes PFAS reaction products, resulting in deposition sites such as landfills serving as time-delayed sources. Thus, PFAS require more drastic, destructive remediation processes for contaminated matrices, including treatment of residuals such as granular activated carbon from drinking water remediation. Destructive thermal and nonthermal processes for PFAS are being piloted, but there is always a risk of forming yet more PFAS products by incomplete destruction.

OUTLOOK: Although great strides have been taken in recent decades in understanding the fate, mobility, toxicity, and remediation of PFAS, there are still considerable management concerns across the life cycle of these persistent chemicals. The study of emerging compounds is complicated by the confidential nature of many PFAS chemistries, manufacturing processes, industrial by-products, and applications. Furthermore, the diversity and complexity of affected media are difficult to capture in laboratory studies. Unquestionably, it remains a priority for environmental scientists to understand behavior trends of PFAS and to work collaboratively with global regulatory agencies and industry toward effective environmental exposure mitigation strategies. ■

The list of author affiliations is available in the full article online.
*Corresponding author. Email: henderson.matt@epa.gov (W.M.H.); washington.john@epa.gov (J.W.W.)
†These authors contributed equally to this work.
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REVIEW

CHEMICAL POLLUTION

Per- and polyfluoroalkyl substances in the environment

Marina G. Evich[1]†, Mary J. B. Davis[1]†, James P. McCord[2]†, Brad Acrey[1], Jill A. Awkerman[3], Detlef R. U. Knappe[4,5], Andrew B. Lindstrom[6], Thomas F. Speth[7], Caroline Tebes-Stevens[1], Mark J. Strynar[2], Zhanyun Wang[8], Eric J. Weber[1], W. Matthew Henderson[1]*, John W. Washington[1,9]*

Over the past several years, the term PFAS (per- and polyfluoroalkyl substances) has grown to be emblematic of environmental contamination, garnering public, scientific, and regulatory concern. PFAS are synthesized by two processes, direct fluorination (e.g., electrochemical fluorination) and oligomerization (e.g., fluorotelomerization). More than a megatonne of PFAS is produced yearly, and thousands of PFAS wind up in end-use products. Atmospheric and aqueous fugitive releases during manufacturing, use, and disposal have resulted in the global distribution of these compounds. Volatile PFAS facilitate long-range transport, commonly followed by complex transformation schemes to recalcitrant terminal PFAS, which do not degrade under environmental conditions and thus migrate through the environment and accumulate in biota through multiple pathways. Efforts to remediate PFAS-contaminated matrices still are in their infancy, with much current research targeting drinking water.

The ubiquitous presence of per- and polyfluoroalkyl substances (PFAS) in the environment after decades of manufacturing and consumer use (Fig. 1) has garnered global interest, with an ever-expanding inventory of >1400 individual chemicals in the Toxic Substances Control Act Inventory and >8000 unique known structures (*1*). PFAS have been incorporated in >200 use areas ranging from industrial-mining applications to food production and fire-fighting foams because of the innate chemical and thermal stability of the carbon–fluorine bond and ability to repel oil and water (*2*). As PFAS flow through commerce from primary manufacturer to commercial user to final disposal, environmental release occurs through both controlled and fugitive waste streams. The stability of many PFAS degradants fosters their ubiquity in the environment. The growing number of PFAS susceptible to partial degradation (*3*) further complicates environmental fingerprinting and remediation efforts. Whereas some PFAS transformation pathways have been well characterized, others degrade through as-yet unknown pathways, expanding the already immense PFAS inventory by untold numbers. Of the known PFAS, there is a paucity of data adequately describing potential impacts to ecosystems and their provisioning services, and few of these chemicals are well characterized by ecotoxicity studies, with the widely known perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) alone covering 21 and 39% of the ECOTOX Knowledgebase (*4*), respectively. Furthermore, with their detection in sera across the human population, coupled with epidemiological evidence of the health impacts for legacy PFAS (*5*, *6*), information on associations with human disease for emerging PFAS is needed. With global production volumes of fluoropolymers surpassing 230,000 tonnes/year (*2*) and estimated cumulative global emissions of perfluoroalkyl acids totaling ≥46,000 tonnes (*7*), scientists struggle to keep pace with manufacturing, use (Fig. 1), and subsequent release. Here, we summarize central concerns in PFAS production, persistence, environmental mobility, exposure, and remediation to inform the international community.

[1]U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), Center for Environmental Measurement and Modeling, Athens, GA 30605, USA. [2]EPA, ORD, Center for Environmental Measurement and Modeling, Durham, NC 27711, USA. [3]EPA, ORD, Center for Environmental Measurement and Modeling, Gulf Breeze, FL 32561, USA. [4]Department of Civil, Construction, and Environmental Engineering, North Carolina State University, Raleigh, NC 27695, USA. [5]Center for Human Health and the Environment, North Carolina State University, Raleigh, NC 27695, USA. [6]EPA, ORD, Center for Public Health and Environmental Assessment, Durham, NC 27711, USA. [7]EPA, ORD, Center for Environmental Solutions and Emergency Response, Cincinnati, OH 45268, USA. [8]Institute of Environmental Engineering, ETH Zürich, 8093 Zürich, Switzerland. [9]Department of Geology, University of Georgia, Athens, GA 30602, USA.
***Corresponding author. Email: henderson.matt@epa.gov (W.M.H.); washington.john@epa.gov (J.W.W.)**
†These authors contributed equally to this work.

Major PFAS groups and uses

PFAS are a class of substances within a wide universe of organofluorine compounds (*8*), as first laid out by Buck *et al.* in 2011 (*9*). In 2021, the Organisation for Economic Cooperation and Development released a revised definition of PFAS, "PFAS are fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it)" (*10*). This revised definition is more inclusive with unambiguous inclusion of PFAS such as side-chain fluorinated aromatics (Fig. 2) (*11*, *12*). By contrast, most historical work within the research community has focused on a small set of perfluoroalkyl(ether) acids and their precursors, with an emphasis on environmental and biological occurrence investigations. Whereas the persistence associated with the perfluorinated-carbon chain is a fundamental underlying concern, PFAS also have a wide range of bioaccumulation and adverse-effect concerns, governed by their varied physiochemical properties.

Although industrial reviews include general synthetic routes and major applications of some PFAS groups (*13*), inadequate public information exists for many PFAS internationally, particularly those currently in use, because of confidential business information claims and insufficient regulatory structures (*14*–*16*). Critical data gaps include PFAS identities, locations and quantities of production and processing, and final uses of products, limiting the capability to identify where environmental and human exposure occur. Here, we summarize synthetic routes, structural traits, and uses of the major PFAS groups (Figs. 1 and 2) and describe implications and knowledge gaps for future research and action.

The fluorine in PFAS is mined from fluorite (CaF_2) mineral deposits, which is digested to form hydrofluoric acid (HF) (Fig. 1). HF and other non–PFAS-based chemicals are used in either of two general synthetic techniques to produce starting materials (e.g., perfluoroalkanoyl fluorides in Fig. 2) of individual PFAS groups, namely direct fluorination (i.e., turning nonfluorinated to fluorinated substances; e.g., electrochemical fluorination) and oligomerization (i.e., converting monomers to larger molecules; e.g., fluorotelomerization). Direct fluorination is aggressive and often results in uncontrolled chemical reactions such as carbon chain shortening and rearrangement (*17*–*19*), leading to a wide range of by-products including cyclic and branched isomers. Oligomerization is less aggressive and mainly results in a homologous series of target compounds (*9*), as have been observed near fluoropolymer (*20*) and perfluoropolyether (*21*) manufacturing and processing sites. Within individual PFAS groups, the functional moieties of starting materials may further react following conventional reaction pathways to yield different PFAS (*9*); thus, depending on the complexity of synthetic routes, final products may contain a number of unreacted intermediates and degradation products (*22*, *23*). Whereas the summary below focuses on target and/or intentional PFAS, these unintentional PFAS can constitute an important part of human and environmental exposure and merit scrutiny.

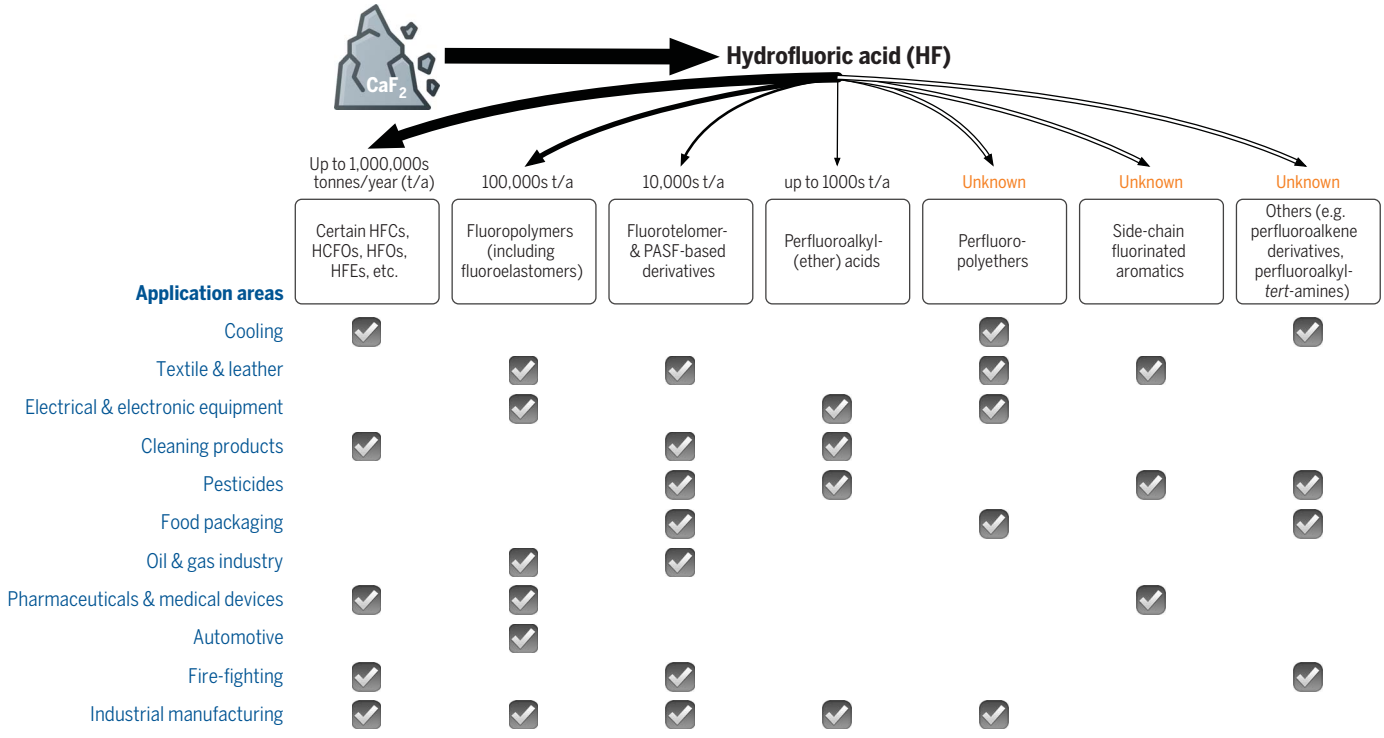


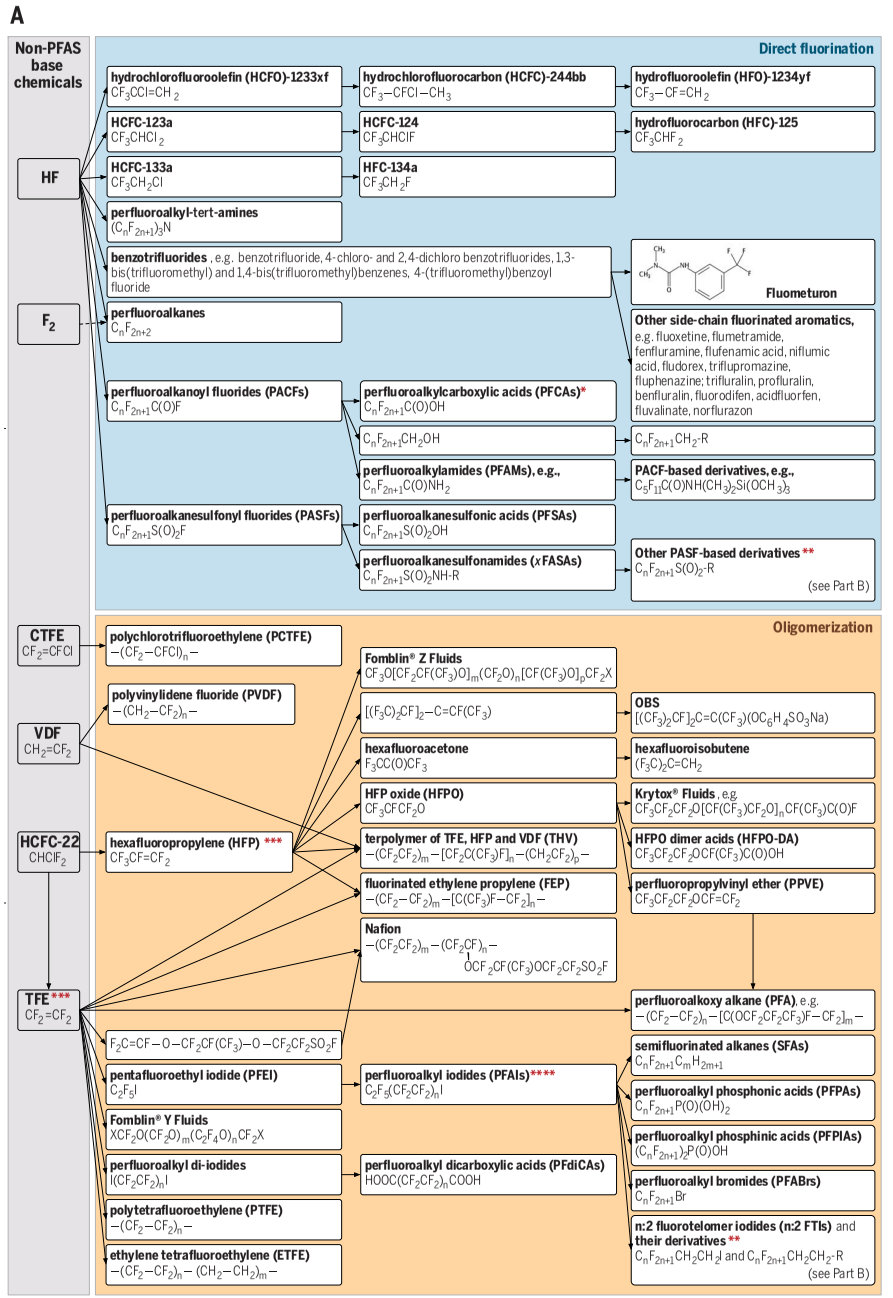
Fig. 1. Non-exhaustive summary of PFAS manufacturing, from production to consumer use. Numerous product fluxes are reasonably documented, but considerable lacunae remain. See text for details and citations. HFC, hydrofluorocarbon; HCFO, hydrochlorofluoroolefin; HFO, hydrofluoroolefin; HFE, hydrofluoroether; PASF, perfluoroalkanesulfonyl fluoride.

Major PFAS groups from direct fluorination include those hydrofluorocarbons, hydrofluoroethers, hydrochlorofluoroolefins, and hydrofluoroolefins that contain a $-CF_3$ moiety and have an overall global production of >1 megatonne/year (*24*). Including a range of low-molecular-weight and low-boiling-point compounds that are used as refrigerants, heat-transfer fluids, solvents, and foaming agents (*2*, *24*), these compounds replaced ozone-depleting chlorofluorocarbons and hydrochlorofluorocarbons. Because of their high global-warming potential, the international community has agreed to phase down and eventually eliminate hydrofluorocarbons (*25*, *26*). An ongoing industrial transition is taking place, including increasing large-scale replacement of hydrofluorocarbons with hydrofluoroethers and hydrofluoroolefins. Although they have low global-warming potentials, hydrofluoroethers and hydrofluoroolefins can ultimately degrade to highly persistent perfluoroalkylcarboxylic acids (PFCAs) such as trifluoroacetate, and a steep accumulation of trifluoroacetate in the environment is becoming increasingly evident (*27*).

Another important PFAS group resulting from direct fluorination is side-chain fluorinated aromatics (*11*, *12*), with unknown but likely considerable amounts being produced and used annually. A common starting point is the synthesis of benzotrifluorides from benzotrichlorides by reaction with HF (*8*). Addition of the $-CF_3$ moiety can reduce biological degradation, increase biological activity, and assist with membrane transport, making the parent compound longer lasting or more effective; therefore, many side-chain fluorinated aromatics are used in pharmaceutical (*12*) or agricultural (*11*) applications. These substances can also degrade to PFCAs such as trifluoroacetate.

Two other major PFAS groups produced from direct fluorination include perfluoroalkyl-tert-amines (*28*) and perfluoroalkanoyl/perfluoroalkanesulfonyl fluorides (PACF/PASFs), which are further reacted to produce PFCAs, perfluoroalkanesulfonates (PFSAs), and other derivatives (Fig. 2). Historically, hundreds of PACF/PASF–based derivatives with a wide range of perfluorocarbon-chain lengths were produced, on the order of kilotonnes/year (*15*, *29*), and used for industrial and consumer applications (*2*). Since the early 2000s, numerous long-chain (fluoroalkyl carbon number ≥6) PACF/PASF–based derivatives have been—and are being—phased out because of widespread concern, whereas shorter-chain PACF/PASF-based derivatives still are being produced and widely used, although in unknown amounts (*15*, *29*). In the environment and biota, PACF/PASF–based derivatives may degrade and partially transform into different PFCAs and/or PFSAs.

On the oligomerization side, two major PFAS groups are fluoropolymers and perfluoropolyethers. These are high-production polymers having fluorinated backbones, with fluoropolymers being produced on the scale of 100 kilotonnes/year and unknown but likely considerable amounts for perfluoropolyethers. Despite often having simple names such as polytetrafluoroethylene, substances in these two groups can be highly diverse, including both nonfunctionalized (with $-CF_3$) and functionalized termini, with different structural combinations and molar ratios of monomers (for copolymers), and from low (< 1000 Da) to very high (> 100,000 Da) molecular weight (*30*–*32*); this complexity has not been clearly communicated with a comprehensive overview of different fluoropolymers and perfluoropolyethers on the market. Depending on structure, different fluoropolymers and perfluoropolyethers can be used in a range of industrial and consumer applications (*2*); in some applications, perfluoropolyethers are used as alternatives to PACF/PASF–based derivatives. Given their variety and complexity, their subsequent bioavailability and degradability are highly variable and complex, which is generally overlooked, understudied, and/or unknown.



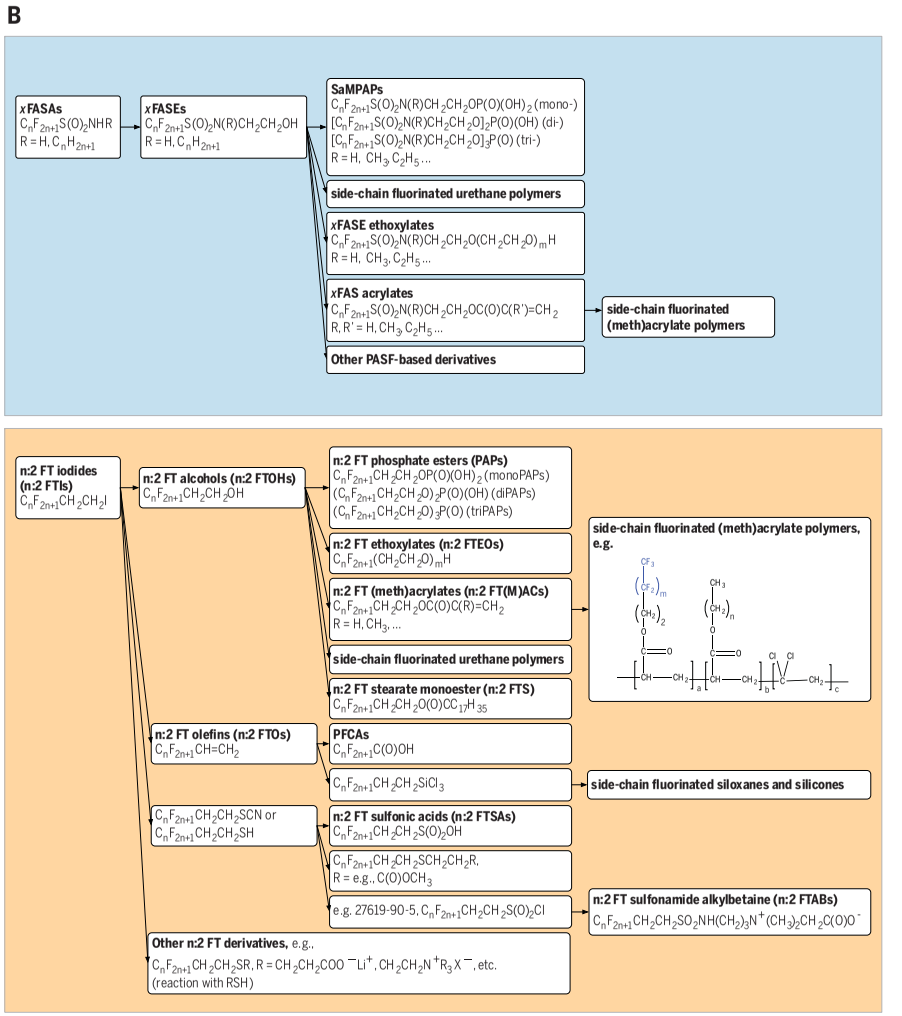


Fig. 2. Multistep multicomponent syntheses yield a complex PFAS universe. Shown are intermediate and final manufacturing products (**A**, **B**). *PFCAs have also been synthesized using other routes, e.g., from PFAIs or n:2 fluorotelomer iodides. Different synthesis routes may generate PFCAs with different perfluorocarbon chain lengths. **Additional details on known synthesis routes of PASF-based and n:2 fluorotelomer derivatives can be found in (B). ***For many compounds such as HFP and TFE, there are different synthesis routes with different starting materials, and only one of them is shown here. ****PFAIs may also be synthesized from other initial substances such as $(CF_3)_2CFI$. [Sources: for Fig. 2 (*8*), (*9*), (*29*), (*30*), (*164*).]

Three other major PFAS groups formed from oligomerization are fluorotelomers, perfluoroalkyl(ether) carboxylic and sulfonic acids, and perfluoroalkene derivatives. Fluorotelomers share many similarities to PACF/PASF–based derivatives other than perfluoroalkyl (ether) acids, including molecular structures, degradability (*9*, *23*, *29*), use applications (*2*), and manufacturing trends from a wide range of perfluorocarbon chain lengths to predominantly shorter chains. Fluorotelomers were historically produced on the order of 9 kilotonnes/year (*33*), with the current amounts produced unknown. Unknown amounts of perfluoroalkyl(ether) carboxylic and sulfonic acids are being used to replace long-chain PFCAs and PFSAs (*34*) in industrial applications such as fluoropolymer production and metal plating, respectively. Perfluoroalkene derivatives such as *p*-perfluorous nonenoxybenzene sulfonate have been produced since the 1980s; large-scale production (on the scale of kilotonnes/year) was recently initiated in China as an alternative to PFOS in firefighting and oil production (*35*). Despite having an unsaturated bond, *p*-perfluorous nonenoxybenzene sulfonate is not readily biodegradable (*36*).

Environmental stability, degradation schemes, and transformation rates

Despite typically having high stability as a group, ~20% of PFAS may undergo transformation in the environment (*3*). These labile compounds are precursors to recalcitrant, terminal transformation products such as PFCAs and PFSAs. For example, frequently detected precursors including perfluorooctane sulfonamides, fluorotelomer alcohols (FTOHs), and fluorotelomer sulfonates, have been found to contribute up to 86% of total PFAS identified in wastewater-treatment plant sludge (*37*).

Although PFAS can undergo complete degradation to inorganic components using high-energy remediation technologies, precursor transformations under environmental conditions, including processes such as hydrolysis (*38*), oxidation (*39*, *40*), reduction, decarboxylation and hydroxylation (*41*), ultimately yield stable PFAS. Despite the low vapor pressure and high water solubilities of many PFAS, some conditions (e.g., within industrial stacks) can promote partitioning to air through particulate sorption, and volatile PFAS such as FTOHs can exist in the gas phase (*42*), making atmospheric and photochemical transformation possible. In the soil-water environment, microbe-facilitated functional group biotransformation can occur aerobically (*43*, *44*) or anaerobically (*45*–*47*), and some microbes that carry out these reactions have been identified (*46*, *48*, *49*). Biotransformation of labile PFAS also can be mediated by plant-specific enzymes. For example, microbial transformation of 8:2 FTOH was substantially enhanced with the addition of soybean root exudates in solution (*50*), and perfluorooctane sulfonamide was transformed in the presence of carrot and lettuce crops, but not in their absence, in amended soils (*51*). In both studies, enhanced degradation was attributed to the organic carbon content of the soil, because the addition of carbon sources can increase microbial degradation rates through co-metabolic processes (*52*).

Several PFAS can undergo transformation, resulting in the formation of FTOHs through processes such oxidation, reduction (*53*), desulfonation (*54*), and hydrolysis (*38*, *55*–*58*) (Fig. 3A). Although some fluorotelomers evidently transform without forming intermediate FTOHs (*9*, *22*, *49*, *59*), one of the archetypal "legacy PFAS" transformation schemes involves FTOHs that are subject to (bio)transformation through numerous intermediates, leading to the formation of terminal PFCA through chain-shortening processes (Fig. 3A). The efficiency of these transformations decreases from aerobic to anoxic to anaerobic (*60*, *61*) conditions, and PFCA yields and rates of formation depend on specific precursor and transformation conditions (*9*). On average, PFOA yields from 8:2 FTOH were reported to be 25% in aerobic soils compared with <1% in anaerobic sludge (*62*). This process is initiated by the oxidation of 8:2 FTOH to yield the inferred 8:2 fluorotelomer aldehyde and then the 8:2 fluorotelomer carboxylic acid, which is reduced through the loss of F to form 7:3 unsaturated fluorotelomer acid, which can form the terminal acid perfluorohexanoic acid (*53*, *63*, *64*) (Fig. 3A). A key step in the pathway is hydroxylation in the β position and subsequent oxidation to form the 7:3 3(keto) fluorotelomer carboxylic acid, which then undergoes β-oxidation to form PFOA, as well as α-decarboxylation to form the 7:2 ketone (*53*, *63*, *64*). The ketone then is reduced to form the secondary alcohol, 1-perfluoroheptyl ethanol [also known as 7:2 (sec) FTOH], which is oxidized to form PFOA (*53*, *63*, *64*).

In a second major transformation scheme, *N*-ethyl perfluorooctane sulfonamido ethanol is proposed to oxidize to form the aldehyde and subsequently to *N*-ethyl perfluorooctane sulfonamidoacetic (Fig. 3B) (*65*, *66*). *N*-deacetylation of *N*-ethyl perfluorooctane sulfonamidoacetatic acid then leads to the formation of *N*-ethyl perfluorooctane sulfonamide followed by C-hydroxylation to form perfluorooctane sulfonamido ethanol. Oxidation of perfluorooctane sulfonamido ethanol to perfluorooctane sulfonamido acetic acid is proposed to occur through the perfluorooctane sulfonamide aldehyde. *N*-deacetylation of perfluorooctane sulfonamido acetic acid to form perfluorooctane sulfonamide is then observed. Perfluorooctane sulfonamide may also form directly from the *N*-dealkylation of *N*-ethyl perfluorooctane sulfonamide (*65*, *66*). Deamination of perfluorooctane sulfonamide to form perfluorooctane sulfinic acid is commonly followed by oxidation to form the terminal product, PFOS.

PFAS transformation under environmental conditions can be approximated using first-order kinetics (*67*). Environmental degradation of labile precursors is observed to occur in a "tree structure," with the formation of numerous intermediates along branching transformation pathways (*53*, *68*). Along each branch, the formation and disappearance of intermediates can be modeled as a sequential decay chain (*23*), with each step characterized by a pseudo first-order rate constant (*67*).

In soils and sediment, sorption can slow the observed rate of microbial transformation (*69*). With long-chain PFAS preferentially adsorbing to soil phases, molecular weight can be used as an approximate indicator of relative stability among PFAS sharing common reaction centers (*43*). To address the effects of reversible sorption, some have proposed use of a double-first-order, in-parallel model (*67*), wherein rate-limited reversible sorption is included as a first-order process.

In addition to sorption, transformation rate is dependent on a number of other environmental factors including pH, temperature, and microbial population (*70*), and these factors contribute to a wide variation of reported precursor half-lives. For example, biodegradation studies of *N*-ethyl perfluorooctane sulfonamido ethanol in sludge reported a half-life of 0.7 to 4.2 days, yet the biodegradation in marine sediments was found to proceed at much slower rates ($t_{1/2,\ 4°C}$ = 160 days and $t_{1/2,\ 25°C}$ = 44 days), which could explain reports of elevated concentrations of *N*-ethyl perfluorooctane sulfonamido ethanol in marine environments (*66*). Similarly, the anaerobic biotransformations of 6:2 and 8:2 FTOHs slowed substantially (30 and 145 days, respectively) compared with aerobic conditions (<2 and 2 to 7 days, respectively) (*62*), which can foster enhanced levels of telomer acids [e.g., 5:3 fluorotelomer carboxylic acid by hydrogenation of the 5:3 fluorotelomer unsaturated carboxylic acid (*53*)] in landfills (*71*). Therefore, PFAS that typically are intermediates in oxidizing settings may exist as terminal products under reducing conditions. For example, variations in PFAS species detected in leachate from waste collection vehicles compared with landfill leachate suggest alternative biodegradation pathways in long-term anaerobic settings such as landfills (*72*). Consequently, degradation studies conducted under controlled conditions result in considerable variation in biotransformation potential and possibly different major stable perfluorinated degradation products when extrapolating half-lives and major products from laboratory to environmental conditions.

In addition to accounting for environmental conditions (*67*), another complicating factor is that contaminants commonly exist as components in complex mixtures. One common precursor source is aqueous-film-forming foam (AFFF), formulations of which contain mixtures of PFAS, and co-contaminants such as nonfluorinated surfactants. High concentrations of organic solvents have been shown to inhibit PFOA degradation under in situ remedial chemical oxidation studies, suggesting that interactions of PFAS with other non-PFAS co-contaminants can alter PFAS transformation (*40*). Additionally, the presence of different PFAS has resulted in changing compositions of microbial communities when comparing cultures spiked with PFOA or PFOS against microbial compositions without PFAS (*46*). Considering that PFAS environmental transformation is mediated primarily by microbes, data suggest that the presence of complex mixtures could indirectly alter biodegradation and that the presence of one PFAS may affect the transformation rate of another,

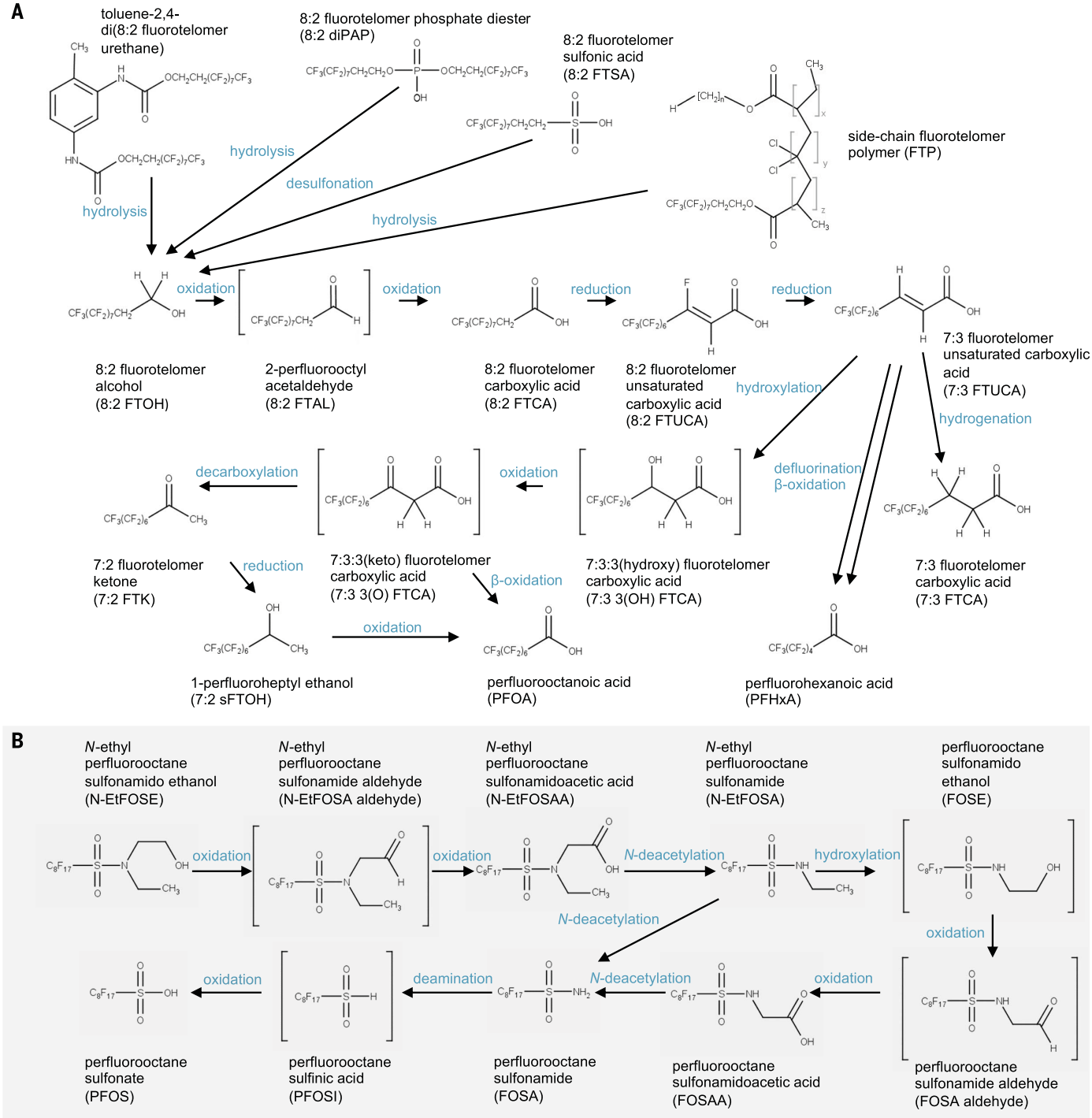


Fig. 3. Breakdown pathways of classes of PFAS. Shown are reaction schemes for 8:2 FTOH (*47*, *53*, *63*) (**A**) and *N*-EtFOSE (*65*, *66*) (**B**). Transformation products proposed by the original investigators are shown with brackets.

although transformation kinetics of PFAS mixtures has not been reported. Furthermore, these complex mixtures could have downstream implications for PFAS mobility, because co-contaminants in AFFF mixtures affect microbial toxicity and PFAS solubility, partitioning (*73*), and remediation [PFAS can be transformed during treatment of organic contaminants (*39*)].

Taken together, the complexity of real-world environmental conditions acting on primary precursors, intermediates and terminal products can result in divergence from reaction schemes and degradation rates derived under laboratory conditions. These complexities are aggravated by the many experimental challenges associated with larger PFAS such as fluoropolymers and side-chain fluorinated polymers, the structure and monomeric compositions of which often are not completely characterized (*23*, *38*, *74*). In addition, there remain uncertainties regarding the levels of impurities or synthetic by-products and life cycle emissions of these polymers, which may affect degradation rates, further necessitating nontargeted analyses in conjunction with transformation prediction simulators such as EnviPath (*75*) and the Chemical Transformation Simulator (*76*) to identify new PFAS and transformation products in the environment.

Environmental mobility and distribution

The mobility of PFAS in the environment is dictated by properties of the mobile (usually air and water) and immobile phases [e.g., natural organic matter (NOM) and mineral assemblages] as well as the PFAS species. The transformation rates discussed above affect the time available for migration. When transformation rates of short-lived intermediates exceed environmental transport rates, these intermediates can remain proximate to their precursors, a phenomenon well established for the environmental distribution of short-lived radionuclides (*77*) because of secular (radio-decay) equilibrium with long-lived parents (*78*). Further, this secular equilibrium of short-lived intermediates might contribute to the undetectable status of some inferred compounds (e.g., 2-perfluorooctyl acetaldehyde; Fig. 3). For PFAS with intermediate transformation rates (e.g., FTOHs and fluorotelomer unsaturated carboxylic acids; Fig. 3) relative to environmental transport processes, these compounds can migrate considerable distances before transformation to recalcitrant PFAS, thereby dispersing widely in the environment (*79*).

Early precursor PFAS include volatile species (FTOHs and sulfonamido ethanols; Fig. 3), the presence of which has been established globally (*80*–*82*). Atmospheric residence time governs transport distance (*83*) and depends on a variety of PFAS properties, including volatility, reactivity, molecular weight, and vapor-particulate partitioning (*82*, *84*, *85*). Atmospheric lifetimes have been reported for FTOHs of ~20 days (*86*). Consistent with these atmospheric lifetimes, air samples collected at remote oceanic locations are reported to contain several FTOH and/or perfluorosulfonamido ethanol species in both gas and particulate phases (*80*). On the basis of these and related observations, a large portion of PFAS global distribution, including that to remote regions, has been attributed to atmospheric transport (*79*, *87*). For example, in a study of soils collected from remote sites globally, all samples contained PFAS, with homolog ratios [e.g., PFOA/perfluorononanoic acid (PFNA)] consistent with atmospheric transport (*79*). These soil concentrations have been used to define global-background PFAS ranges in surface soils (means ~10 to 60 pg/g), such that surface soils rarely contain lower PFAS, and higher concentrations suggest local or regional sources (*88*). Atmospherically transported ionic PFAS also have been shown to disperse widely, perhaps as far afield as >400 km (*21*, *89*, *90*), although the form of these species, e.g., free acid, dissolved in droplets or sorbed to particulates, has not been resolved.

In terrestrial settings, PFAS transport usually occurs through aqueous advection, with migration retarded by sorption on NOM, minerals, and at fluid-fluid interfaces (particularly air-water) (*91*). Most PFAS sorption studies have been conducted with surface soils in which NOM, which is typically present at relatively high concentrations (Fig. 4) (*92*), constitutes a major substrate. Exploring surface-soil sorption mechanisms of two PFAS having sulfonate termini revealed an easily extractable fraction, as well as less reversibly sorbed fractions composed of perfluoroalkyl groups hydrophobically associating with NOM, sulfonate moieties covalently binding to NOM–OH groups forming ester linkages, and physical entrapment in NOM or minerals (*93*). Comparing the sorption of cationic, zwitterionic, and anionic PFAS showed concentration-dependent sorption for cationic and zwitterionic PFAS, pronounced sorption hysteresis for zwitterions, and major electrostatic and NOM sorption for cationic and zwitterionic PFAS (*94*).

The high NOM concentrations of surface soils typically diminish precipitously in the first several centimeters below the ground surface, where mineral surfaces come to dominate the vertically more expansive subsurface realm (Fig. 4) (*92*). Authigenic minerals typically are abundant in the subsurface, and these minerals have surface charges for electrostatic sorption. Aluminosilicate clays bear permanent negative surface charges, presenting potential sorption sites for cationic and zwitterionic PFAS. Ferric and aluminum (oxy)hydroxides bear pH-dependent, positive surface charges below their zero point of charge at a pH of ~8, so these minerals can electrostatically sorb anionic PFAS. In the vadose zone, recent studies have shown that the surfactant nature of PFAS also fosters sorption at the air-water interface, retarding PFAS migration (*91*).

To assess sorption across a wide breadth of PFAS species and complex sorption matrices, experiments have been performed on 29 PFAS in 10 soils (*95*). This study concluded that a simple distribution coefficient, K_d (soil/water concentration), effectively characterized relative distribution among PFAS. Recognizing that lower values of log K_d favor partitioning to water, thereby favoring higher environmental mobility, general patterns in these data (Fig. 4A) include the following: (i) the distribution coefficient increases logarithmically with fluoroalkyl carbon numbers >5, (ii) distribution coefficients converge to similar values among PFAS species and chain-lengths having fluorinated carbons ≤5, and (iii) for equal fluoroalkyl carbon numbers, sorption generally decreases according to zwitterions > sulfonamides > telomers > PFSAs > PFCAs > ethers. It also was observed that log K_d for anionic PFAS increased with decreasing pH, a pattern consistent with increasing positive electrostatic charge on pH-dependent surfaces of (oxy)hydroxide minerals and amorphous solids.

When precursor degradation does not complicate interpretation (*96*), relative values of log K_d are reflected in PFAS distribution patterns across the spectrum of environmental settings. Figure 4B depicts geometric mean ratios (subsoil/surface soil) of PFAS for three soil profiles after biosolids application at the ground surface (*97*); consistent with log K_d values, subsoil accumulation of PFCAs exceeds PFSAs for the common fluoroalkyl number 8, shorter chains vary little from each other, and shorter chains exceeds that of longer chains. It is noteworthy that subsoil accumulation for fluoroalkyl number >10 also varies little with chain length, perhaps reflecting facilitated transport of PFAS sorbed to colloids winnowing through the soil column (*98*).

Transport of PFAS into terrestrial plants occurs through a variety of pathways, with the most studied being uptake through roots. As with transport in soils, vegetative accumulation factors (VAF = $[PFAS]_{vegetation}/[PFAS]_{soil}$) are influenced by the propensity of specific PFAS to partition into water as they are transported through plants. These VAFs have revealed plant species- and tissue-specific trends (*99*–*101*). However, a recent review of VAFs across numerous species and tissues reported uniformly declining trends in total VAF with increasing fluoroalkyl number for PFCAs and PFSAs (*102*) (Fig. 4C) (*101*). VAF trends with chain length and among terminal moieties

Typical Environmental Properties				
Horizon or stratigraphic unit	Thickness (m)	NOM (% mass)	Clays (% mass)	Fe oxides (% mass)
A & E	≤ 0.5	1 - 30	0 - 25	0 - 2
B & C	0.5 - 9	0.05 - 0.5	10 - 70	2 - 10
Subsoil Regolith	0 - 50	ND - 0.05	0 - 40	0 - 5
Bedrock aquifer	0 - 150	ND - 0.05	low	variable

Sorption Substrate	CEC (meq/100 g)	AEC (meq/100 g)
NOM	200-400	low
Fe oxides	low	0-20
clays	10-100	low

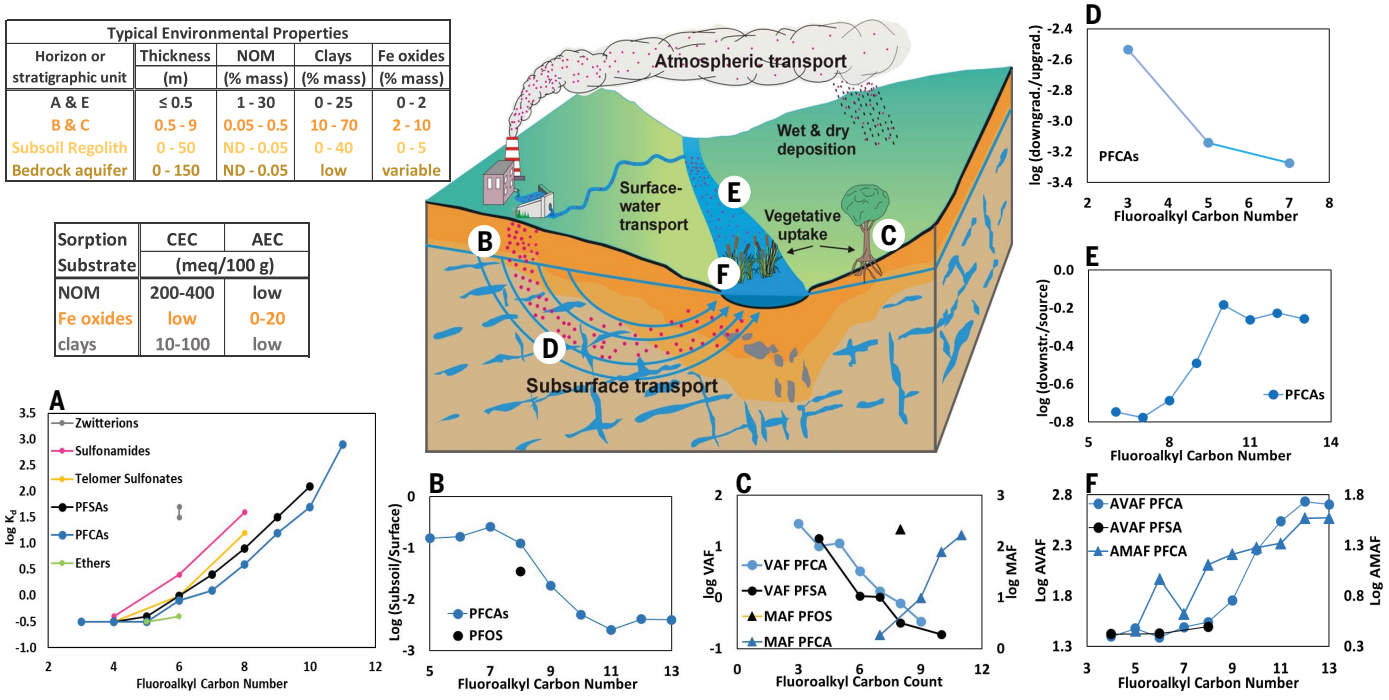


Fig. 4. PFAS partitioning in environmental media (log K_d). The environmental sorption complex varies grossly with setting, with NOM concentrated in shallow soil horizons and ferric (oxy)hydroxides commonly dominating in subsurface media (Properties). Log K_d varies as a function of fluoroalkyl number and terminal moiety [(**A**) (*95*); pH = 5.2 values depicted]. Because of this partitioning behavior, when not complicated by precursor degradation, relative mobility among PFAS commonly varies with fluoroalkyl carbon number [(**B**) (*97*), (**D**) (*105*), (**E**) (*106*)], and terrestrial vegetation accumulation diminishes with increasing fluoroalkyl number, but accumulation in terrestrial detrital feeders increases with fluoroalkyl number [(**C**) (*101*)]. In aquatic settings, vegetative and detrital-feeder accumulation both increase with fluoroalkyl number [(**F**) (*107*)]. CEC/AEC, cation-exchange capacity/anion-exchange capacity.

suggest that chemical properties of PFAS also exert a strong influence over plant uptake. Reports of plant uptake of emerging PFAS compounds are limited, but studies examining the concentration of chloroether sulfonic acids (F-53B, a replacement for PFOS in electroplating industry) suggest similar variation with chain length (*103*).

In contrast to the VAF patterns, which are largely governed by relative PFAS aqueous-sorbed partitioning, soil macroinvertebrates feeding directly on long-chain-rich vegetative detritus and NOM tend to express trends opposite to that for VAFs. For example, macroinvertebrate accumulation factors (MAF = $[PFAS]_{macroinvertebrate}/[PFAS]_{soil}$) reported for earthworms (*Eisenia andrei*) in biosolid-amended soil have trends of increasing MAF with fluoroalkyl number (Fig. 4C) (*104*).

After percolating through the vadose zone, relative PFAS mobility patterns have been reported in groundwater plumes. For example, PFAS concentrations were reported for wells in a groundwater plume flowing from a landfill, to an observation well, and then to water-supply well (*105*). Given travel times exceeding 24 years for flow from the landfill to the water-supply well, several PFCA homologs fell to undetectable levels, but perfluorobutanoic acid, perfluorohexanoic acid, and PFOA exhibited a pattern of lower downgradient/upgradient ratios (specifically, downgradient well 1/upgradient well OW1f03) with increasing PFCA chain length (Fig. 4D).

In a riverine setting, sediments downstream of a carpet industry have been reported to retain higher ratios of long-chain homologs than short (downstream site 5/upstream source site 4; Fig. 4E) (*106*), consistent with preferential sorption of the longer homologs (perhaps affected by precursor transformation as well). In turn, this pattern also is expressed at the base aquatic autotrophic level; for example, aquatic vegetative-leaf accumulation (AVAF = $[PFAS]_{vegetation}/[PFAS]_{water}$; Fig. 4F) was relatively higher for long-chain compounds (*107*). Mirroring these AVAF trends, aquatic macroinvertebrate accumulation factors (AMAF = $[PFAS]_{macroinvertebrate}/[PFAS]_{sediment}$; Fig. 4F) for blackworms (*Lumbriculus variegatus*) increases with fluoroalkyl number as well (*107*).

Environmental exposure

Widespread global persistence of PFAS has resulted in detectable concentrations of the compounds in the blood of almost the entire human population (*6*). Human health effects from exposure to PFAS have been studied extensively, identifying possible carcinogenic, reproductive, endocrine, neurotoxic, dyslipidemic, and immunotoxic effects (*6*, *108*, *109*). However, with animal models reflecting similar postulated mechanisms of action, the potential toxicity of these compounds for wildlife cannot be dismissed (*110*). For humans, direct exposure through manufactured products can be managed more expediently than indirect exposure to accumulated sources in aquatic ecosystems. PFAS exposures through food chains are more difficult to resolve, and dietary exposure through drinking water and contaminated food sources (e.g., seafood and other animal products) are among the greatest exposure sources for ecosystems and human populations alike (*109*, *111*). Here, we review the consequences of PFAS persistence in the environment and the resulting bioaccumulation in biota, present ecotoxicological details in the context of environmental distribution and exposure potential, and discuss the ecological effects of PFAS mixtures (*112*).

Estimation of environmental exposure to PFAS is hindered by the sheer number of functionally diverse PFAS and is further complicated by their presence as complex mixtures. A fundamental understanding of ecotoxicology requires comprehensive knowledge of all PFAS species to which target organisms have been exposed. Although pragmatic limitations have fostered studies reporting

summary characterizations such as Total Organic Fluorine and Total Oxidizable Precursor assays as proxies for more informative chemical-specific studies (*113*–*116*), more exhaustive approaches providing identification of individual compounds within PFAS mixtures remains the more informative strategy (*117*, *118*). Ideally, such characterizations would include details regarding branched- versus linear-chain homologs, homolog ratios, isomer comparisons, and forensics with high-resolution mass spectrometry. In addition to pinpointing potential point sources, these methods can distinguish between receptor contact with precursor compounds and their terminal products.

An accurate assessment of PFAS risk must consider exposure to precursor compounds because these compounds transform and are thus important for characterizing environmental PFAS mixtures (*119*, *120*). PFAS precursors are susceptible to in vivo metabolic conversion to terminal acids or sulfonamides after exposure, as well as transformation during (or subsequent to) atmospheric or oceanic transport (see previous sections). For example, whereas PFSAs were the most abundant PFAS in both sediment and water at sites contaminated with AFFF (*114*), aquatic invertebrates exposed to AFFF displayed elevated concentrations of PFCAs as well as the 6:2 fluorotelomer sulfonate (*114*, *115*). Given the common detection of precursors, environmental-organismal uptake and distribution models should include both parent and degradant PFAS to best describe patterns of exposure and influence on biomagnification, especially considering the rapidly expanding incorporation of new, shorter-chain PFAS that tend to be detected less frequently in biota (*121*).

Key to understanding distribution of PFAS in biota are the specific interactions between PFAS and biological molecules. Although the bioaccumulation of some persistent organic pollutants is often related to lipid partition coefficients, PFAS are not exclusively associated with lipids (*120*). Bioaccumulation modeling suggests that both protein interactions and lipid partitioning are important parameters for accurately assessing PFAS (*122*, *123*), although predicting biomacromolecule interactions has proven difficult because of their physiochemical properties. PFAS do not behave like neutral, hydrophobic organic contaminants and instead are hypothesized to involve both phospholipids and proteinaceous tissues due in part to their anionic nature (*123*). Cooperative binding models have further correlated (and predicted) protein associations, relying on traditional measures of hydrophobicity and its effect on biomacromolecule interactions (*124*). Therefore, both membrane-water partitioning and protein-water coefficients could be informative bioaccumulation indicators (i.e., bioconcentration factors, bioaccumulation factors, and trophic magnification factors), and coupled with hepatic- and renal-clearance mechanisms across taxa are all vital in understanding PFAS persistence in organisms. Nevertheless, the specific physiochemical differences, such as chain length, result in different distribution of PFAS in biological tissues (*125*).

Ecotoxicological study of PFAS is further complicated by diversity of the PFAS class. Bioaccumulation factors for terrestrial vegetation are greater for PFCAs than for PFSAs, with shorter-chain perfluoroalkyl acids bioaccumulating to a greater degree than longer-chain ones, largely driven by variation in PFAS solubility (*126*), followed by uptake and translocation into tissues (Fig. 4C) (*100*, *101*). Conversely, potential perfluoroalkyl acid bioaccumulation in other fauna is greatest in long-chain compounds (*120*), with clear trends of bioaccumulation increasing with chain length (Figs. 4, C and F, and 5) (*121*). Long-chain PFAS concentrations tend to increase with trophic level in aquatic food webs, consistent with biomagnification processes (*127*). However, transformation of precursors in exposure media and biota can confound interpretation of high concentrations of some PFAS (e.g., PFOS) as biomagnification without explicit identification of trophic magnification (*128*).

Biomagnification in predators is related to trophic level, food-chain length, and capacity to metabolize PFAS precursors (*125*). Seabirds, marine mammals, and terrestrial species show the greatest magnification factors compared with exclusively aquatic food webs, in which organisms with gills eliminate perfluoroalkyl acids more efficiently (*120*). Effects in predators, also frequently seen in humans, seem to be largely cytotoxic, immunological, reproductive, or carcinogenic (*125*). Exposure models for aquatic food webs at AFFF-contaminated sites found benthic invertebrate consumers to be the avian dietary guild at highest exposure risk (*114*). At higher trophic levels, PFSAs (e.g., PFOS) bioaccumulate at greater rates than PFCAs (e.g., PFOA) of the same chain length (Fig. 5) (*114*, *129*) and tend to be more toxic (*4*).

Estuarine, marine, and freshwater environments have demonstrated trophic magnification of long-chain PFAS (Fig. 5) (*130*, *131*). Discrepancies in the relative concentrations of PFAS in fish compared with benthic invertebrates appear largely dependent on the compounds' functional group and exposure routes, with elevated PFAS concentrations often linked to site-specific sources and/or benthic prey (*131*–*133*). Solubilized (i.e., waterborne) rather than dietary exposure was linked to reduced amphipod survival and reproduction (*133*), but higher trophic-level organisms are exposed primarily through ingestion (*109*). Counterintuitively, exposure to low concentrations of PFAS can exacerbate bioconcentration, motivating biologically based, physiological models exploring this phenomenon (*127*). Overall, evidence suggests that the ultimate global reservoirs of PFAS are oceans and marine sediments (*134*), emphasizing the importance of elucidating consequences of PFAS contamination in these ecosystems (*135*).

Ecological implications of PFAS exposure to aquatic and terrestrial organisms highlight the need to assess and incorporate new-approach methodologies that prioritize real-world hazard of organismal exposure and subsequent risk. Mechanism-based studies and in silico approaches are beginning to fill data gaps pinpointing the cellular and molecular pathways resulting in toxicity (*136*, *137*). Elimination half-life has been identified as an end point relevant to bioaccumulation and effects (*4*). In addition to prioritizing chemical selection based on environmental fingerprinting, cross-taxa and sensitive-taxa toxicity testing research should focus on in silico model development that can determine tissue distribution, molecular perturbations, and trophic-level accumulation. As the scale of assessment expands, so does the need for the continued development of adverse-outcome-pathway models to facilitate translation of exposure concentration/dose to organismal-effect end points for the projection of population-level consequences, including multigenerational effects. For instance, unexposed progeny of fish exposed to PFOA and PFOS had lower survival rates, reduced growth, and thyroid-related effects as revealed by histology (*138*). Similarly, lipid metabolism (*139*) and behavioral end points (*140*) were affected in subsequent generations of other species.

Although data are available on potentially common mechanisms of action and toxicity between species (e.g., lipid metabolism, modification of cell membrane integrity, protein binding, and nuclear receptor activation), the large number of PFAS underscores the need to augment conventional in vivo testing with in vitro and in silico approaches (*4*). Using these approaches, a number of moderate- and long-chain PFAS have been shown to elicit varying degrees of oxidative stress and modify the antioxidant defense systems of invertebrates, induce neurotoxic and reprotoxic effects across species, and reside in organisms longer than or comparable to any known class of anthropogenic contaminants (*120*). PFAS toxicity, bioaccumulation, and persistence generally are increasingly problematic with increasing chain length.

Remediation

Treatment and remediation of PFAS-affected media is especially challenging because the chemistry of PFAS renders them unaffected by most traditional treatment technologies (*141*). Given the strength of the carbon–fluorine

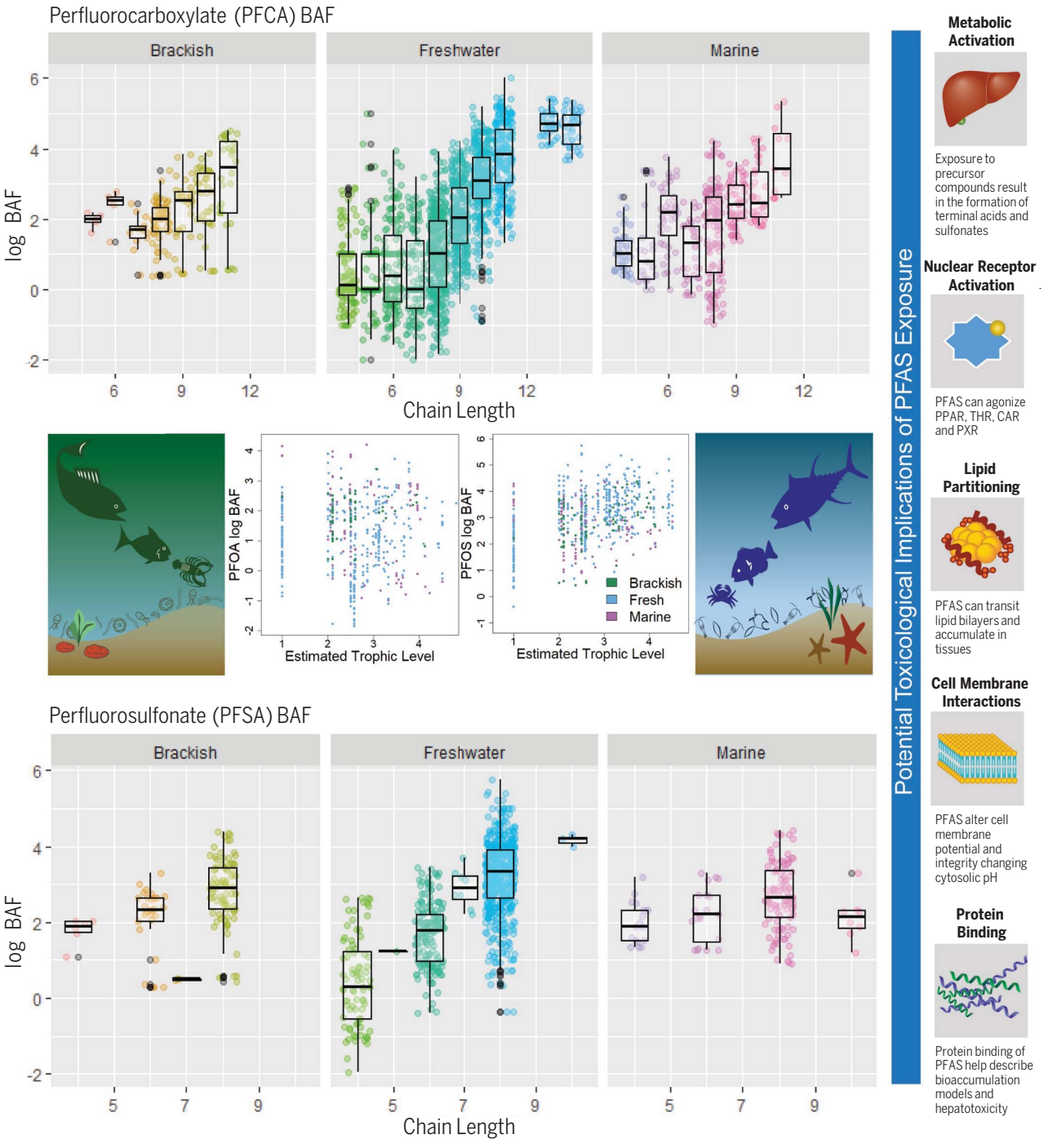


Fig. 5. Trophic transfer and environmental exposures. Bioaccumulation factors (BAFs) in aquatic food webs are greater for long-chain perfluorocarboxylates (top panel) and perfluorosulfonates (bottom panel) than for short-chain perfluoroalkylcarboxylates. Higher trophic-level organisms demonstrate greater bioaccumulation of PFOS than PFOA (center panel); trophic-level accumulation was estimated for data with a single-prey classification method (FishBase) and standardized bioaccumulation factor by wet weight of organism. Multiple toxicological implications (right panel) reflect the diversity of PFAS physicochemical properties and have been linked to both functional group and fluoroalkyl carbon chain length. Data were originally compiled by Burkhard (*127*).

bond, complete mineralization is difficult, with fluorinated products of incomplete destruction remaining a concern (*142*, *143*). Many existing treatment technologies are only capable of concentrating PFAS (*144*), and concentrated treatment residuals can result in the reintroduction of PFAS into the environment (Fig. 6). For example, treatment of drinking water can reduce human exposure at the site of treatment while also acting as a PFAS source where residuals are generated, reinforcing the need for a preventative and holistic approach (*145*). Therefore, treatment and remediation approaches for contaminated media should be considered in terms of a total management approach influenced by the primary source(s), the affected media, and the ultimate method of destruction or long-term storage of PFAS.

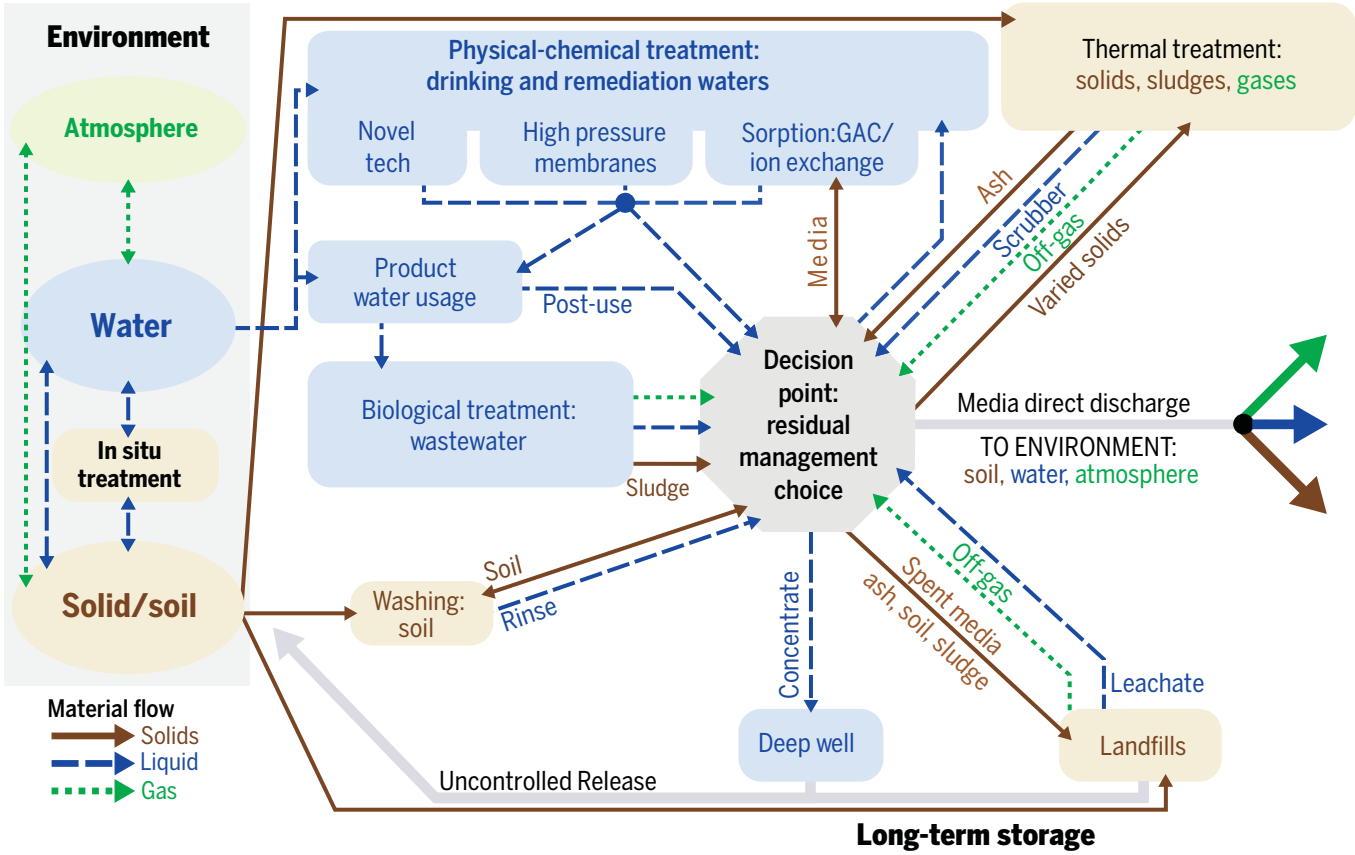


Fig. 6. Site management options for media streams containing PFAS. Brown, blue, and green indicate solid/semisolid, water/liquid, and air/gas phases, respectively. PFAS, including precursors and products of incomplete destruction, cycle through the management options based on treatment and operational choices. Without informed management choices, the persistence of PFAS results in rereleases into the environment. Only complete mineralization, with HF control, offers a permanent solution for breaking the treatment cycle.

PFAS-affected drinking water often is the primary route of human exposure (*146*), and treatment techniques for aqueous media are the most well established, although performance and cost for the removal of some short-chain PFAS can be particularly challenging. Management can occur at primary sources (i.e., treatment of industrial wastewater effluent), at the secondary concentration source (e.g., drinking water treatment plants or landfill leachate), or in diffuse environmental media (e.g., groundwater). Treatment of diffuse media can involve ex situ "pump-and-treat" approaches to adjoin groundwater to aqueous treatment technologies. The most established treatments for water are sorption to granular activated carbon (GAC) or ion-exchange stationary phases (*141*). Powdered sorbents can be used; however, particle-separation technology is needed to physically recover the spent sorbent (e.g., conventional treatment, microfiltration, or ultrafiltration).

Removal performance of sorbents differs among targeted PFAS, concentrations, background water quality, and sorbent properties among other parameters (*141*, *147*, *148*). Another concentrative approach is the use of high-pressure membrane systems such as reverse osmosis or nanofiltration. The residual stream for sorbent technologies are the spent media or a regenerate stream for regenerable ion-exchange media, whereas high-pressure membranes yield an enriched retentate. Both residual streams need to be processed further (Fig. 6). GAC typically is reactivated and single-use resins typically are incinerated, but little is known regarding PFAS fate in full-scale facilities. Likewise, studies evaluating treatment options for PFAS-laden reverse-osmosis membrane concentrate or ion-exchange regenerant are in their infancy (*149*). Other, less-used techniques include membrane distillation, electrodialysis reversal, flotation, electrocoagulation, and evaporation. The niche applications of these technologies are because of their performance, cost, and lack of process familiarity.

Environmental media such as soils can be diffusely contaminated through wet/dry deposition; land application of PFAS-enriched materials such as biosolids, wastewater, or leachate; usage of PFAS-containing products such as AFFFs and pesticides or uncontrolled release through unlined landfills or spills. Soil contamination is a threat to nearby water sources because of downward and lateral migration of PFAS into receiving water bodies (Fig. 4). In some cases, the large volume of soil that is affected makes ex situ removal and destruction a considerable logistics problem. Another approach to site management is in situ modification to enhance mobility of PFAS for pump-and-treat application or to stabilize PFAS migration using GAC or other sorbents (e.g., clays) to limit impacts (*150*). Although this can be an effective short-term site-management technique, it is not a permanent solution, and likely will not retain all PFAS species effectively (*148*, *150*, *151*). In situ treatment of PFAS in aquifers requires different techniques, such as permeable reactive barriers or addition of powdered activated carbon – of which, none have shown the ability to control PFAS plumes in the long term (*150*).

The terminal destination of PFAS wastes is of primary concern for the life cycle management of these compounds. Currently, two commercially viable long-term storage approaches are landfilling affected media or underground injection of contaminated water (*145*). Such sequestration is a temporary solution. Because

most PFAS do not naturally degrade to nonfluorinated chemical species, these long-term sinks are time-delayed sources. For example, landfills are recognized PFAS sources through PFAS-enriched landfill gas and liquid leachates (*71*). The only permanent solution to PFAS is the destructive remineralization of the underlying fluorine, whether directly acting on contaminated media or from treatment of residual streams of other treatment techniques, such as spent sorbents or regenerant solutions.

Thermal treatment is a destructive approach that can achieve PFAS mineralization. Incineration by itself has been shown to at least partially destroy even highly fluorinated wastes (*143*), and advanced thermal oxidation can be used on solid, liquid, and gas samples to convert PFAS to constituent gases with an acid-scrubber cleanup (*152*). Ideally, this process yields HF, NO_x, SO_x, and CO_2 gases that are handled by traditional air pollution control technologies. However, thermal treatment requires substantial temperatures (>700°C) for a sufficient period to convert PFAS into HF and nonfluorinated products, with more highly fluorinated species requiring more time and higher temperature (*153*, *154*). Catalytic oxidation at lower temperatures (e.g., 400°C) has been demonstrated for some PFAS (*155*). Thermal processes, however, have not been demonstrated at scale, where inefficiencies can reduce performance. Atmospheric emission of products of incomplete destruction or the air pollution control technologies associated with thermal treatment processes, including the regeneration of spent GAC, can become additional PFAS sources. Capture or destruction of these products in the exhaust of thermal processes also is an area of active research, although forefront technologies are like those applied for other media, namely scrubbers, activated-carbon adsorption, and thermal oxidation.

Other destructive treatments for aqueous streams include electrochemical degradation, sonolysis, nonthermal plasma, advanced oxidation (e.g., sulfate radicals) and reduction (solvated electrons), biodegradation (Feammox), zero-valent iron, hydrothermal, and supercritical water oxidation (*149*, *156*). Although many of these technologies have shown the ability to destroy select PFAS, none have demonstrated long-term performance approaching mineralization at full scale with natural and industrial water matrices for a wide assortment of PFAS. Also, the energy costs of many of these technologies limit their sustainability and desirability, and the formation of harmful by-products (e.g., bromate, perchlorate) remains a concern (*144*). The lack of widespread testing and limited field usage has led to a reluctance in using these technologies because additional management of the waste or residual streams will be needed. These unknowns, among others, further demonstrate the need to minimize use of PFAS and find a total waste-management approach in which complete destruction of PFAS is ensured.

Conclusions

The pool of new PFAS, for which physical, chemical, and toxicological data remain undetermined, is expanding rapidly and now includes untold numbers of compounds having widely varying chemical structures, volatilities, and solubilities, as well as uncertain potential exposure consequences. Early studies on structurally similar PFAS suggest that behavioral trends gleaned from legacy PFAS studies can be useful as a basis to predict fate, toxicity, and remediation strategies for emerging compounds. Recently, an internationally authored paper called for PFAS to be managed as a class based upon widespread use in commerce, shared inclusion of strongly bonded perfluorocarbon moiety, and the resulting environmental persistence of common terminal products (*157*).

Current international reporting practices used to document PFAS synthesis, production volumes, and potential releases vary among countries and are not always tailored to provide the knowledge necessary to adequately track and understand the movement of these compounds in the environment. These efforts typically serve as a critical first step in developing knowledge to be used in future assessment and potential regulation of PFAS. In the United States, expansion of the Toxic Release Inventory will include ~172 long-chain PFAS starting in 2021, providing limited but valuable information in the form of sources, compositions, and quantities released for these compounds. However, under regulatory frameworks around the world, information on many PFAS is protected as confidential business information and will not be disclosed publicly (*16*), thereby necessitating substantial continued discovery and forensic identification efforts around the world. Other PFAS, such as many of those classified as chemical substances of unknown or variable composition, by-products, or biological materials and polymers, may be too complex to fully characterize and can challenge scientific investigation.

There is an ongoing need to advance responsive PFAS science, particularly regarding investigating environmental sources and sinks, toxicity, and remediation technologies, but evidence suggests that preventative upstream actions are critical to facilitating the transition to safer alternatives and minimizing the impact of PFAS on human health and the environment. Examples of these upstream actions include the EPA's Stewardship Program (*158*), the Amendment to the Polymer Exemption Rule removing side-chain fluorotelomer polymers from the Exemption Rule (*159*), the Significant New Use Rule removing an exemption for a set of PFAS used as coatings (*160*), the recently announced Comprehensive National Strategy to confront PFAS pollution (*161*), and a ban on PFAS in food contact paper in Denmark (*162*). Regardless of the regulatory approach implemented, collaborative efforts among scientists, industrial producers, and policy makers will remain key in finding effective and timely solutions (*163*).

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Contamination of groundwater with per- and polyfluoroalkyl substances (PFAS) from legacy landfills in an urban re-development precinct☆

Emily Hepburn [a, *], Casey Madden [a], Drew Szabo [b], Timothy L. Coggan [b], Bradley Clarke [b], Matthew Currell [a]

[a] School of Engineering, RMIT University, Melbourne, Australia
[b] Centre for Environmental Sustainability and Remediation (EnSuRE), School of Science, RMIT University, GPO Box 2476, Melbourne, Victoria, 3001, Australia

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ABSTRACT

The extent of per- and polyfluoroalkyl substances (PFAS) in groundwater surrounding legacy landfills is currently poorly constrained. Seventeen PFAS were analysed in groundwater surrounding legacy landfills in a major Australian urban re-development precinct. Sampling locations (n = 13) included sites installed directly in waste material and down-gradient from landfills, some of which exhibited evidence of leachate contamination including elevated concentrations of ammonia-N (≤106 mg/L), bicarbonate (≤1,740 mg/L) and dissolved methane (≤10.4 mg/L). Between one and fourteen PFAS were detected at all sites and PFOS, PFHxS, PFOA and PFBS were detected in all samples. The sum of detected PFAS ($\sum_{14}$PFAS) varied from 26 ng/L at an ambient background site to 5,200 ng/L near a potential industrial point-source. PFHxS had the highest median concentration (34 ng/L; range: 2.6–280 ng/L) followed by PFOS (26 ng/L; range: 1.3–4,800 ng/L), PFHxA (19 ng/L; range: <LOQ – 46 ng/L) and PFOA (12 ng/L; range: 1.7–74 ng/L). Positive correlations between $\sum_{14}$PFAS, PFOA and other perfluoroalkyl carboxylic acids (PFCAs) (e.g. PFHxA) with typical leachate indicators including ammonia-N and bicarbonate were observed. In contrast, no such correlations were found with perfluoroalkyl sulfonic acids (PFSAs) (e.g., PFOS and PFHxS). In addition, a strong positive linear correlation ($R^2 = 0.69$) was found between the proportion of PFOA in the sum of detected perfluorinated alkylated acids (PFOA/$\sum$PFAA) and ammonia-N concentrations in groundwater. This is consistent with previous research showing relatively high PFOA/$\sum$PFAA in municipal landfill leachates, and more conservative behaviour (e.g. less sorption and reactivity) of PFCAs during subsurface transport compared to PFSAs. PFOA/$\sum$PFAA in groundwater may therefore be a useful indicator of municipal landfill-derived PFAA. One site with significantly elevated PFOS and PFHxS concentrations (4,800 and 280 ng/L, respectively) appears to be affected by point-source industrial contamination, as landfill leachate indicators were absent.

1. Introduction

Understanding the sources, fate and transport of per- and polyfluoroalkyl substances (PFAS) in groundwater in urban areas to date has been poorly characterised and as such, is increasingly important to scientists and regulators worldwide (Xiao et al., 2012; Liu et al., 2017). In the last decade, PFAS have been demonstrated to be omnipresent in water, air, food, wildlife and humans due to their resistance to typical environmental degradation processes (Giesy and Kannan, 2001; Kim and Kannan, 2007; Wang et al., 2017; Xiao, 2017). Furthermore, PFAS can have negative impacts on exposed organisms (including humans) and are therefore a potential environmental and public health risk (Prevedouros et al., 2006; Eschauzier et al., 2013; DeWitt, 2015; US EPA, 2016; Barzen-Hanson et al., 2017; Hamid et al., 2018). Currently, there is a lack of data on the source, fate and ecological impact of PFAS in urban groundwater systems, particularly in the Australian context where increasing urban re-development of former industrial land for residential purposes threatens to further expose humans and environments to these substances. Determining the levels of PFAS

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* Corresponding author. GPO Box 2467, Melbourne, 3001, Australia.
E-mail address: emily.hepburn@rmit.edu.au (E. Hepburn).

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contamination in groundwater in such environments, and understanding processes governing their fate and transport is therefore vital to developing contaminant management and remediation strategies which protect human and ecological health in these settings. Occupational exposure pathways can include dermal contact and inhalation of volatile PFAS from shallow contaminated groundwater by intrusive workers during re-development activities (e.g. laying building foundations and/or de-watering operations). Non-occupational exposure pathways can include consumption of contaminated drinking water supplies and local fish/aquatic organisms, and potentially produce from local gardens if groundwater is used for irrigation. Where exposure risks are low, data collection and interpretation remain beneficial for improved understanding of PFAS releases in urban settings.

PFAS are a diverse family of fluorinated synthetic chemicals used as surfactants and polymers for a wide variety of industrial and commercial applications since the 1950s (Prevedouros et al., 2006; Paul et al., 2009). The most common applications include textile protection (Scotchgard™), surface coating for cooking implements (Teflon™), food contact paper (Begley et al., 2008), and Aqueous Film Forming Foams (AFFFs) (Rao and Baker, 1994; Buck et al., 2011). Aside from evidence that major manufacturers were aware of harmful health effects for decades (Grandjean, 2018), the broad thinking within the scientific community and among the general public was that PFAS were inert and non-toxic and were therefore widely used with little consideration of environmental dispersal or ecological impact (Giesy and Kannan, 2001, 2002). It was not until 2001 that the extent of PFAS contamination at the global-scale was first demonstrated for perfluorooctanesulfonate (PFOS; $C_8F_{17}SO_3H$) (Giesy and Kannan, 2001). Since then, PFAS have been detected in almost every wildlife sample measured (Giesy et al., 2010), ubiquitously in humans throughout the world (Toms et al., 2009), and within most environments, including pristine locations (Lindstrom et al., 2011).

Many PFAS contain a hydrophilic functional group, such as carboxylates and sulfonates, and both a hydrophobic and lipophilic fluorinated chain, varying in carbon-chain length. The anionic properties of certain PFAS such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) allow them to be water soluble while also sorbing onto soil and sediment at higher chain lengths, allowing wide environmental mobility. The most well-known and studied PFAS are PFOS and PFOA (perfluorooctanoate; $C_8F_{15}O_2^-$). The unique high-energy carbon-fluorine bond renders these compounds resistant to hydrolysis, photolysis, microbial degradation and metabolism in animals (Lindstrom et al., 2011). The US EPA (2016) estimates that the half-life of PFOS and PFOA in water are >41 and > 92 years respectively, and it is possible that these compounds may never actually degrade under natural environmental conditions (Blum et al., 2015). In recognition of the threat posed to public health and the environment, PFOS was added to the list of United Nations Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009, ratified by Australia. Based on a comprehensive analysis of information available in the public domain, the Organisation for Economic Co-operation and Development (OECD, 2018) has released a new list of over 4700 PFAS on the global market, including several new groups of PFAS, suggesting that a large proportion are not currently monitored nor quantified in environmental samples.

Initial research on the environmental fate of PFAS has largely focussed on defence and aviation facilities throughout the world where the historically widespread use of AFFF has resulted in highly contaminated soil and groundwater (Moody and Field, 2000; McGuire et al., 2014; Anderson et al., 2016; Braunig et al., 2017). Aside from point-source contamination associated with AFFFs, other sources such as wastewater discharge, landfill leachates (Benskin et al., 2012; Fuertes et al., 2017), manufacturing releases/spillages directly to ground or to atmosphere followed by deposition and migration through the vadose zone (Davis et al., 2007; Oliaei et al., 2013) and urban runoff and precipitation (Xiao et al., 2012) are also potentially ubiquitous inputs of PFAS into the global environment (Prevedouros et al., 2006; Murakami et al., 2009a). To date these sources and their interactions with groundwater systems remain generally poorly characterised (Murakami et al., 2009b; Eschauzier et al., 2013), highlighting the significant knowledge gap in the global literature with respect to PFAS in groundwater from these sources. Urban groundwater may be utilised for irrigation (or in some instances potable water supply), and may discharge to receiving waters such as wetlands, streams and bays, thus creating possible pathways and receptors for PFAS contamination. Additional sources of PFAS in groundwater include stormwater or wastewater leakage from sewers (Murakami et al., 2009a; Murakami et al., 2009b). Delineating these different sources of PFAS in groundwater and understanding the factors governing their fate in the subsurface is critical to effective contaminant management, and for limiting environmental and human health risks. Delineation may also contribute to source identification as, in some regions, multiple sources may contribute to a single, co-mingled release of PFAS to the sub-surface.

Most urban areas contain an unknown but potentially significant number of legacy landfills, many of which were constructed with little or no leachate control systems, and which may therefore contaminate local aquifers. The composition of leachate, including concentrations of PFAS, can vary substantially depending on the types of wastes accepted – e.g. municipal/domestic waste vs. Industrial or construction waste (Gallen et al., 2017), however to date the extent of PFAS contamination of groundwater surrounding such landfills is poorly constrained. International studies have identified the sum of detected PFAS ($\sum$PFAS) in raw and treated landfill leachate in the range 1,378–292,000 ng/L (Benskin et al., 2012; Yan et al., 2015; Fuertes et al., 2017); bearing in mind that only a limited sub-set of total possible PFAS were analysed. The types and concentrations of PFAS in landfill leachate have also been studied and are dependent on the type and age of the waste, regulations on the manufacture and importation of PFAS, historical and current landfill management practices, geochemical conditions (e.g. water salinity, organic carbon and pH), biodegradation (e.g. methanogenesis) within the landfill, and seasonal meteorological parameters (Benskin et al., 2012; Yan et al., 2015; Gallen et al., 2017; Hamid et al., 2018).

Eschauzier et al. (2013) reported total concentrations of PFCAs and PFSAs, together known as perfluorinated alkylated acids (PFAA), in groundwater impacted by landfill leachate of up to 4,400 ng/L, with elevated concentrations of certain PFCAs such as PFOA corresponding with other typical indicators of leachate impact (such as ammonium and methane). Relatively lower concentrations of other PFSAs (PFOS and PFHxS) in leachate impacted groundwater have been hypothesised to be related to a greater propensity for sorption of these compounds and/or lower levels of occurrence in landfill waste (Eschauzier et al., 2013; Hamid et al., 2018). Therefore, where specific PFAA, such as PFOA are found to correlate with other typical leachate indicators in groundwater, they may prove useful as relatively conservative tracers of landfill vs. non-landfill derived contamination. Examination of ratios of PFAA (e.g. PFOA/$\sum$PFAA) in addition to absolute concentrations can assist by controlling for the effect of overall contamination source strength – a factor that is typically variable in complex urban areas due to different degrees of source dilution and mixing in the aquifer, and age/composition of the landfill waste. No studies have as yet examined the use of such ratios (specifically PFOA/$\sum$PFAA) in leachate-impacted groundwater, as far as we are aware.

The Fishermans Bend urban re-development precinct in Melbourne, Australia encompasses 240 ha of former industrial land currently undergoing progressive re-zoning into residential land (Bolton et al., 2013). The region contains several legacy landfills, which accepted municipal and/or industrial waste during the 20th Century. It is hypothesised that these legacy landfills may have acted as sources of PFAS to the region's shallow groundwater and may represent ongoing sources. The aims of this study were therefore to: 1) determine concentrations of a range of PFAS in groundwater surrounding legacy landfills at Fishermans Bend, 2) investigate differences in the proportions of different PFAS in groundwater impacted by different contamination sources, and 3) investigate any relationships between PFAA and conventional indicators of landfill leachate contamination of groundwater. Many urban re-development projects worldwide occur in regions of similar land-use history to Fishermans Bend and include managed or unregulated legacy landfills. We hypothesise that groundwater impacted by landfills that accepted municipal waste (containing elevated levels of leachate indicators such as ammonia and methane, related to breakdown of putrescible organic waste) likely contain different proportions of particular PFAS such as PFOA in comparison to landfills which accepted industrial, construction and/or demolition waste, or other industrial point sources (e.g. Gallen et al., 2017). Further, we hypothesise that a greater proportion of PFCAs such as PFOA will be observed in plumes of groundwater contamination related to such municipal landfills, due to their more conservative behaviour in the aquifer away from the original source. Understanding the degree to which these landfills act as sources of PFAS to groundwater, and the typical concentrations and proportions of different PFAS arising from these landfills, will assist in the development of more targeted contaminant management and remediation efforts. Indices such as ratios of certain PFAA and/or correlations with other landfill-derived contaminants may help identify and differentiate PFAS contamination sources in urban areas of complex land-use history and groundwater contamination.

2. Materials and methods

2.1. Study area characteristics

Fishermans Bend is located approximately 1 km southwest of the Central Business District of Melbourne, Australia. It is located near the mouth of the Yarra River, on Quaternary river-delta sediments (Holdgate and Norvick, 2017). The shallow subsurface is typically underlain by artificial fill up to approximately 5 m thick (Neilson, 1992). The uppermost natural sediment consists of the Port Melbourne Sand (PMS) which acts as an unconfined aquifer with a shallow water table (Neilson, 1992) containing fresh (91–2,971 mg/L total dissolved solids), oxic (0.01–5.72 mg/L dissolved oxygen) groundwater (Hepburn et al., 2018). Groundwater predominantly flows towards a sewer in the south east of the area (Fig. 1a and b). There are seven known legacy landfills that accepted a range of waste types during the 1930s–1990s across the area (Fig. 1a). Two of these landfills accepted municipal waste and have thus been the subject of environmental audits, which provide some limited information about the degree of soil and groundwater contamination (Lane Consulting, 1999; SKM, 1999). However, as is the case with many legacy landfills, there is little information available for the five remaining legacy landfills concerning the type of wastes accepted, operational periods or effects on surrounding groundwater. The information that is available typically consists of observations and aerial photographs within desktop reviews (e.g. Golder Associates, 2012; AECOM, 2015) which identify historical unregulated filling within former sand quarries prominent in the centre of the study area. It is assumed that none of the landfills were equipped with modern engineering controls such as liners, drainage layers and leachate or gas collection systems, which might serve to limit interaction between leachate and groundwater.

Table 1 shows concentrations of typical landfill leachate contaminants in groundwater at the sampled sites. These data were used to categorise sites into three groups: (1) Showing indications of impact from landfill leachate (sample codes LI) (2) No indication of landfill impact (sample codes NI), and (3) a background site (B) (Table 1). The quantitative criteria used to distinguish landfill impacted sites from non-impacted sites were the minimum concentrations reported in Kjeldsen et al. (2002) for landfill leachate, as follows: 15 mg/L for ammonia-N (in older landfills); 610 mg/L for bicarbonate, 30 mg/L for total organic carbon, 50 mg/L for potassium, and 150 mg/L for chloride. Where at least three mean concentrations of these indicators were detected above the criteria at a given site, the site was deemed to be landfill impacted. Within the landfill impacted group, sites LI1-W, LI2-W and LI6-W were drilled directly in waste material and contain measurable dissolved methane consistent with landfills in the methanogenic phase (Table 1). Sites LI3, LI4, LI5, LI7 and LI8 are located down-hydraulic gradient (along the groundwater flow-path) from landfills and also show some evidence of landfill leachate impact, including relatively high concentrations of ammonia, bicarbonate, total organic carbon, potassium and chloride (Table 1). Despite indicator concentrations at sites LI5, LI7 and LI8 typically at or slightly below the criteria, these sites were deemed to be landfill impacted due to their location within former landfill cells (Fig. 1a and b), and the presence of construction/demolition waste in the fill above the screened interval. The remaining sampled sites (NI1, NI2, NI3 and NI4) are also located down-hydraulic gradient from landfills but show no indication of any landfill-leachate related contamination (e.g. dissolved methane was not detected, and ammonia was present at low levels – Table 1). The background site (B) is located up-gradient from any known landfills and appears to have experienced minimal contamination. Information including bore depths, screened intervals and lithology are presented in Table S1 (supplementary material). All sites included in this study are screened within the Fill/Port Melbourne Sand aquifer (see cross-section presented in Fig. 1b).

2.2. Sample collection

Groundwater samples were collected from thirteen shallow monitoring bores (which we term 'sites' throughout the rest of the paper) (Fig. 1a) using a low flow pump with dedicated low-density poly ethylene (LDPE) tubing, into 250 mL polypropylene bottles. Prior to sample collection, standing water level was measured using a Solinst™ interface probe and field parameters were monitored in purged water in accordance with Standard No. 5667-11 (ISO, 2009). At sites that were installed directly within landfill waste it was not possible to measure the field parameters due to potential interference/cross-contamination (see Fig. S1; supplementary material). At these sites, the standing water level was monitored until stabilisation (i.e. no change in level) to ensure sampled water represented that recharged to the site from the aquifer during pumping. All sampling equipment was cleaned following use at each site using ultrapure water (>18Ω, Milli-Q, Millipore) only, as detergents were considered a possible source of PFAS. The sampling methodology was adjusted at site B as the bore contained an insufficient volume of water to use the pump and as such, a stainless-steel bailer was used. Upon return to the laboratory, samples had ~1 g of sodium azide (NaN_3) added as a preservative and were stored at 4 °C prior to analysis.

Sites previously sampled for leachate indicators were sampled

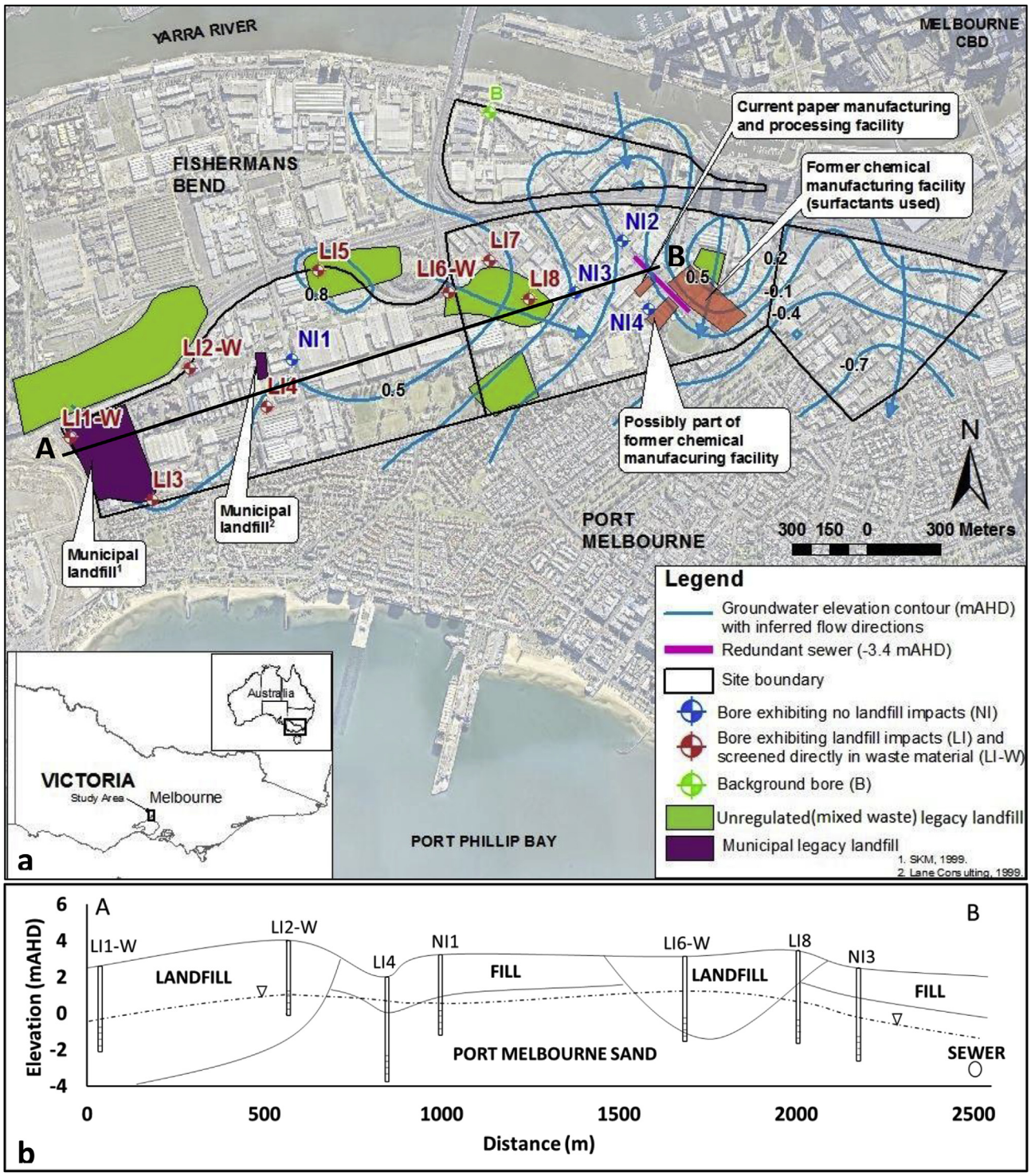

Fig. 1. a: Historical industries of interest across Fishermans Bend, including location of redundant sewer line and sampled sites; mAHD = metres above the Australian Height Datum; b: Cross-section A-B showing the major geological units of relevance.

in accordance with Standard No. 5667-11 (ISO, 2009). Samples for alkalinity and major ions were collected in 250 mL plastic bottles. Samples for total organic carbon (TOC) and dissolved methane were collected in 40 mL vials which were fully filled to ensure no headspace gas remained. All samples were stored at 4 °C prior to analysis by Australian Laboratory Services via PC Titrator (alkalinity), dual column gas chromatography with flame ionisation detector (dissolved methane), and inductively coupled plasma mass spectrometry (cations). TOC was analysed by TOC Analyser following APHA 5310B methods, and anions were analysed by Discrete Analyser, following APHA 4500 methods (2017).

2.3. Standards and reagents

Analytical standards (perfluorobutanoic acid, PFBA; perfluoropentanoic acid, PFPeA; perfluorohexanoic acid, PFHxA; perfluoroheptanoic acid, PFHpA; perfluorooctanoic acid, PFOA; perfluorononanoic acid, PFNA; perfluorodecanoic acid, PFDA;

Table 1
Landfill leachate indicator concentrations for sampled sites.

Site ID	Mean concentration in groundwater (mg/L) (range included in brackets) from previous sampling[a]					
	NH_3–N	HCO_3^-	CH_4	TOC	K	Cl
LI1-W	99 (92–106)	1660 (1600–1740)	3.88	50 (40–58)	174 (162–197)	853 (815–889)
LI2-W	69 (55–78)	1453 (1430–1470)	10.40	40 (37–42)	42 (41–44)	320 (307–331)
LI6-W	5.6 (4.1–7.2)	1090 (1020–1150)	8.32 (6.54–10.10)	17 (16–18)	51 (46–55)	160 (138–171)
LI3	8.0 (5.3–15)	922 (822–1110)	0.04	42 (35–46)	51 (35–74)	338 (203–479)
LI4	24 (17–27)	909 (894–941)	0.14	18 (14–21)	28 (26–31)	164 (142–183)
LI5	1.6 (0.8–2.2)	900 (852–936)	0.19	11	43 (41–44)	119 (101–140)
LI7	5.4 (4.9–6.2)	738 (699–832)	0.05 (0.04–0.05)	14 (13–16)	24 (23–24)	123 (113–129)
LI8	4.2 (3.2–4.7)	591 (544–616)	0.01 (0.01–0.02)	11 (10–12)	35 (34–36)	68 (51–79)
NI1	2.9 (2.7–3.4)	414 (406–428)	0.02	10	16 (15–17)	46 (38–59)
NI2	2.1 (1.8–2.5)	149 (125–173)	0.17	6 (1–9)	26 (25–27)	591 (398–771)
NI3	0.13 (0.01–0.31)	101 (88–114)	<0.01	15 (6–23)	7 (4–8)	16 (13–22)
NI4	0.49 (0.04–0.95)	176 (90–256)	0.01 (0.01–0.01)	3 (2–5)	7 (0.5–12)	26 (12–36)
B	0.01 (0.01–0.02)	91 (85–96)	–	4 (3–4)	4 (3–4)	14 (11–16)

[a] Hepburn, unpublished data, and AECOM, 2016.

perfluoroundecanoic acid, PFUnDA; perfluorododecanoic acid, PFDoDA; perfluorobutane sulfonate, PFBS; perfluoropentane sulfonate, PFPeS; perfluorohexane sulfonate, PFHxS; perfluoroheptane sulfonate, PFHpS; perfluorooctane sulfonate, PFOS; perfluorodecane sulfonate, PFDS; 6:2 fluorotelomer sulfonate, 6:2 FTS; and 8:2 fluorotelomer sulfonate, 8:2 FTS) and isotopically labelled analogues (PFHxA$^{13}C_2$, PFOA$^{13}C_8$, PFDA$^{13}C_2$, PFDoDA$^{13}C_2$, PFBS$^{13}C_2$, PFHxS$^{13}C_3$, PFOS$^{13}C_4$, PFOS$^{13}C_8$ and 6:2 FTS$^{13}C_2$) were purchased from Wellington Laboratories (Ontario, Canada) as solutions of 50 μg/mL in methanol. The solvents methanol (LC-MS grade, Honeywell, USA) and ultrapure water (Merck Millipore, Australia) were tested for PFAS contamination over the duration of the study prior to use. Ammonium hydroxide solution (28% in H_2O, ≥ 99.99%), sodium acetate, glacial acetic acid and ammonium acetate (≥99.99%) were purchased from Sigma-Aldrich (Australia).

2.4. Sample extraction and analysis

Each 250 mL sample was filtered using glass fibre filters (1.2 μm, Millipore, Ireland) pre-rinsed with ultrapure water and then spiked with 5 ng of isotopically labelled ^{13}C PFAS standards (Table S3; supplementary material) prior to solid-phase extraction (SPE). Contact time with the glass was minimised, and as the filters will sorb <15% of PFAA (Chandramouli et al., 2015; Szabo et al., 2018) the analyte recovery was not expected to be significantly reduced. Weak anion exchange cartridges (Oasis WAX, 6 CC, 150 mg, 30 μm, Waters, Australia) were pre-conditioned with 4 mL 0.1% (v/v) ammonium hydroxide in methanol, 4 mL methanol, and 4 mL ultrapure water. Water samples were loaded at ~1 mL/min and cartridges washed with 4 mL of pH 4 buffer (sodium acetate/acetic acid) then dried under vacuum for 10 min before elution into 15 mL polypropylene centrifuge tubes using 2 mL MeOH that had been used to rinse the sample bottle, then 4 mL of 0.1% (v/v) ammonium hydroxide in methanol. Eluents were evaporated to dryness under a gentle stream of nitrogen at 40 °C and reconstituted to 500 μL in 50/50 methanol/ultrapure water before analysis.

Analysis was performed on an Agilent Technologies 1290 infinity II liquid chromatograph (LC) coupled with an Agilent technologies 6495B tandem mass spectrometer (MS/MS) in negative electrospray ionisation mode (ESI) (MS/MS parameters listed in Table S2; supplementary material). Separation was achieved on a Zorbax eclipse plus RRHD C18 column (3.0 × 50 mm, 1.8 μm, Agilent Technologies, USA). Gradient elution using 5 mM ammonium acetate in ultrapure water (A) and methanol (B) at 400 μL min^{-1} was used and the first 1.5 min was diverted to waste (t_0 = 10% B; $t_{0.5}$ = 10% B; $t_{2.5}$ = 55% B; t_9 = 90% $t_{9.5}$ = 100% B; $t_{11.5}$ = 100% B; $t_{11.6}$ = 10% B; t_{14} = 10% B). A delay column (Zorbax Eclipse Plus C18 RRHD, 4.6 × 50 mm, 3.5 μm, Agilent Technologies, USA) was installed between the solvent mixer and injector module to delay instrument contamination. Injector needle wash and seat back flush lines were replaced with peek tubing and stainless-steel solvent filters. A dynamic multiple reaction monitoring (dMRM) method was created based on optimised transitions, collision energies and retention time for all compounds and can be found in Table S3. The two most abundant m/z transitions were used for qualitative identification of each compound except for PFBA and PFPeA where only one transition was available. The m/z transition with the highest intensity was used for quantitation. For PFAS with branched and linear isomers the combined peak area was quantified and reported as sum branched and linear of that compound. Linear calibration curves with 8 levels ($r^2 > 0.99$) in 50/50 methanol/ultrapure water and containing 5 ng/mL of surrogate PFAS to match sample extracts were derived.

2.5. Quality assurance/quality control (QA/QC)

Two of each of the following QA/QCs were performed: field reagent blank (FRB), method blank (MB), laboratory control sample (LCS) and two sites were selected by random number generator to be sampled and analysed in triplicate. Method blanks for all PFAS fell below the LOD except for PFBA (0.8 ng/L) and PFHxA (0.3 ng/L). The field blank contained no detectable concentrations for 14 of the 17 analysed PFAS, however it did contain minor concentrations (≤1.2 ng/L) of PFOS, PFBA and PFHxA – these concentrations were below half of the lowest detected concentrations in the samples (within the background site 'B'). The LOQ values for these compounds were subsequently adjusted to at least three times the concentration of the field blank, or the lowest calibration level, whichever was higher. LCS recoveries of 20 ng/L for target analytes were within recovery limits (70–130%) (Shoemaker et al., 2008) with the exception of PFDS (53% and 91% for the two samples). Each analyte was adjusted according to internal standards which produced adequate recoveries (Table S4; supp. material). Overall the analytical dataset and QA/QC results are considered to provide an acceptable degree of confidence in the data for the purposes of the study.

The limit of detection (LOD) was defined by the lowest calibration point with a signal to noise ratio (S/N, 10:1). The limit of quantification (LOQ) was defined as three times the concentration of the method blank for each compound. Method blanks involved the addition of ultrapure water to pre-rinsed polypropylene sample bottles and spiking with 10 ng internal standard. One method blank sample was extracted with each batch of 10 samples. In the case that no detectable contamination is present, the LOD is used as the

Table 2
PFAS concentrations in Fishermans Bend groundwater (concentrations in ng/L; average of duplicate sample analysis).

Sample code	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUDA	∑PFCA	PFBS	PFPeS	PFHxS	PFHpS	PFOS	∑PFSA	6:2 FTS	∑PFAS
LI1-W	<0.2	<0.2	46	<0.2	56	<0.2	<0.2	<0.2	100	14	<0.2	34	1	20	69	3.2	180
LI2-W	39	<0.2	<0.2	<0.2	74	<0.2	<0.2	5.3	120	8.9	6.8	34	4.4	71	130	<0.2	240
LI3	49	15	29	22	61	<0.2	<0.2	<0.2	180	16	8.8	35	<0.2	4.5	64	<0.2	240
LI4	<0.2	<0.2	17	<0.2	73	<0.2	<0.2	<0.2	90	10	3.5	14	<0.2	44	72	<0.2	160
LI5	5.1	<0.2	6.0	<0.2	5.1	<0.2	<0.2	<0.2	16	4.2	2.1	9.3	<0.2	33	49	<0.2	65
LI6-W	13	<0.2	20	<0.2	12	<0.2	<0.2	<0.2	45	12	6.2	28	<0.2	24	70	<0.2	120
LI7	11	<0.2	12	<0.2	6.0	8.6	<0.2	<0.2	38	7.3	3.7	16	<0.2	16	43	<0.2	81
LI8	9.1	<0.2	<0.2	<0.2	7.5	<0.2	<0.2	<0.2	17	9.0	6.4	45	<0.2	26	86	<0.2	100
NI1	8.8	14	13	4.8	2.1	<0.2	<0.2	<0.2	43	2.1	<0.2	3.6	<0.2	1.3	7	<0.2	49
NI2	17	13	34	12	12	0.76	<0.2	<0.2	89	31	16	96	3.9	75	220	10.0	320
NI3	11	12	19	<0.2	7.7	0.69	<0.2	<0.2	50	24	15	170	7.1	250	470	<0.2	520
NI4	24	6.3	29	3.8	18	0.73	1.3	<0.2	83	8.5	5.1	280	5.3	4800	5100	<0.2	5200
B	3.3	3.0	2.4	<0.2	1.7	0.67	2.2	<0.2	13	2.0	<0.2	2.6	<0.2	7.7	12	<0.2	26
LOD	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		0.2	0.2	0.2	0.2	0.2		0.2	
LOQ	1.7	0.2	0.7	0.2	0.2	0.2	0.2	0.2		0.2	0.2	0.2	0.2	1.8		0.2	
Detection frequency (%)	85	46	85	31	100	39	15	8		100	77	100	39	100		15	
Minimum	<0.2	<0.2	<0.2	<0.2	1.7	<0.2	<0.2	<0.2	13	2.0	<0.2	2.6	<0.2	1.3	7	<0.2	26
Maximum	49	15	46	22	74	8.6	2.2	5.3	180	31	16	280	7.1	4800	5100	10.0	5200
Median	11	<0.2	19	<0.2	12	<0.2	<0.2	<0.2	50	9.0	6.3	34	<0.2	26	70	<0.2	160

<0.2 Below limit of quantification (LOQ).

LOQ. Samples with a weak S/N (<3:1) were considered below the detection limit. Samples with S/N between 3:1 and 10:1 are considered to be qualitatively detected, however below the limit of quantification. In statistical analyses, these values were set as one half of the limit of quantification.

2.6. Statistical analysis

Analysis of Variance (ANOVA) was used to analyse the significance of differences in PFAS concentrations among different groups of samples. The assumption of normality was assessed using the Shapiro-Wilk test (performed on the standardised residuals); where the assumption failed the data were log-transformed and the residuals re-tested. The assumption of homogeneity of variance was assessed using the Levene Statistic; where the assumption failed the Welch test was used to re-test the data. Only those sites where these two assumptions were met were included in the ANOVA. Pearson correlation coefficients were used to identify statistically significant relationships between PFAS and landfill leachate indicators (see Table 3). PFAS In all statistical analyses, censored data was substituted with one half of the detection limit or quantification limit (Mikkonen et al., 2018). All analyses were completed using the statistical package SPSS (IBM SPSS Version 23.0).

3. Results and discussion

3.1. Concentrations and geographic distribution of PFAS in groundwater

PFAS were detected in all groundwater samples (n = 13) and the sum of detected PFAS ($\sum_{14}$PFAS) ranged from 26 to 5,200 ng/L (Table 2, Fig. 2). PFOS, PFHxS, PFOA and PFBS were detected at all locations. PFHxS had the highest median concentration (34 ng/L; range 2.6–280 ng/L) followed by PFOS (median: 26 ng/L; range: 1.3–4,800 ng/L), PFHxA (median: 19 ng/L; range: <LOQ – 46 ng/L) and PFOA (median: 12 ng/L; range: 2–74 ng/L). The precursor 6:2 FTS was only detected at two sites, and PFDoDA, PFDS and the precursor 8:2 FTS were below the LOQ at all locations; as such, these compounds will not be discussed further.

The site sampled in the northern part of the study area (Site B) contained the lowest $\sum_{14}$PFAS (26 ng/L), with a maximum concentration of 3.3 ng/L for any individual PFAS (PFBA). As this site is not impacted by landfill leachate (see Table 1 and Section 2.1), these results indicate that the site is a reasonable representation of ambient (anthropogenic) background groundwater condition in the study area. The most likely sources of PFAS to groundwater at this site are urban runoff and precipitation, with some potential surface water infiltration from the adjacent Yarra River.

The proportions of different PFAS in groundwater varied considerably across the dataset; PFOS comprised the highest proportion of $\sum_{14}$PFAS at five sites, followed by PFOA (n = 4) and PFHxS (n = 3) (Fig. 2; Table S5; supp. material). Sites dominated by PFOA (LI1-W, LI2-W, LI3, LI4) are located in the western part of the study area and are screened within or immediately down-hydraulic gradient from three legacy landfills, two of which are known to have accepted domestic (municipal) waste between the 1930s and 1990s (Fig. 2). These sites had $\sum_{14}$PFAS between 160 and 240 ng/L and all contained similar concentrations (range: 56–74 ng/L) and

Table 3
Correlation between certain PFAA and selected landfill indicators.

	n^	NH_3–N	K	HCO_3	TOC	CH_4
PFBA	13	0.001	0.01	0.02	0.15	0.07
PFBA	*10*	*0.01*	*0.01*	*0.06*	*0.26*	*0.10*
PFHxA	13	0.09	0.31[b]	0.02	0.10	0.02
PFHxA	*10*	*0.25*	*0.65*[a]	*0.30*	*0.37*	*0.001*
PFHpA	13	0.07	0.01	0.05	0.06	0.14
PFHpA	*10*	*0.05*	*0.001*	*0.006*	*0.14*	*0.11*
PFOA	13	0.50[a]	0.20	0.45[b]	0.59[a]	0.15
PFOA	*10*	*0.47*[b]	*0.16*	*0.46*[b]	*0.59*[a]	*0.11*
PFBS	13	0.001	0.01	0.02	0.003	0.01
PFBS	*10*	*0.21*	*0.38*	*0.48*[b]	*0.52*[b]	*0.05*
PFPeS	13	0.07	0.08	0.12	0.03	0.01
PFPeS	*10*	*0.01*	*0.02*	*0.07*	*0.07*	*0.08*
PFHxS	13	0.04	0.07	0.22	0.13	0.05
PFHxS	*10*	*0.17*	*0.22*	*0.32*	*0.36*	*0.12*
PFOS	13	0.03	0.06	0.11	0.09	0.03
PFOS	*10*	*0.23*	*0.002*	*0.31*	*0.05*	*0.36*
∑PFAA	13	0.03	0.05	0.11	0.08	0.03
∑PFAA	*10*	*0.36*	*0.17*	*0.54*[b]	*0.68*[a]	*0.20*

^ = half the value of the LOQ used where data points < LOQ.
Italicised text = scenario with omitted sites likely impacted by industrial point sources (NI2, NI3 and NI4).
[a] Significant at the 0.01 level.
[b] Significant at the 0.05 level.

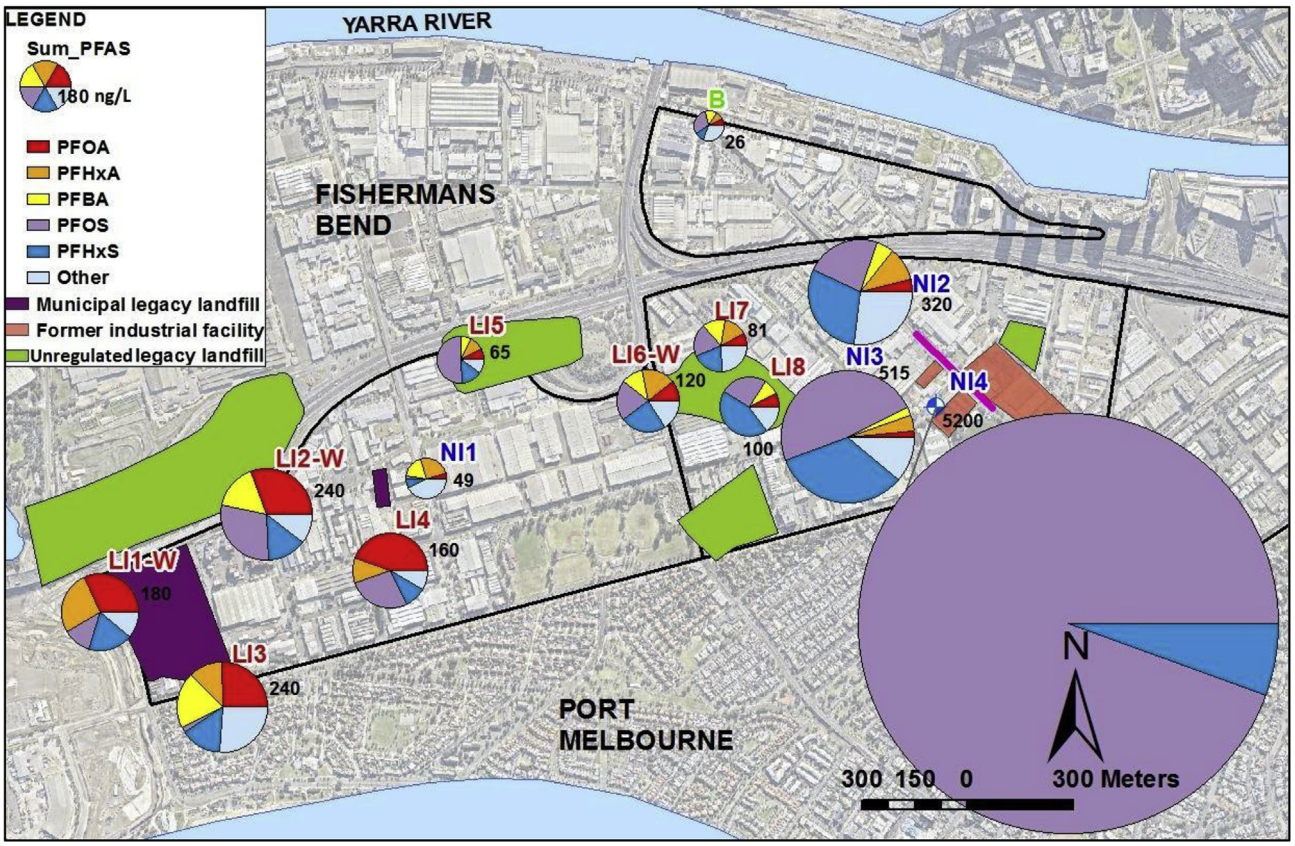

Fig. 2. Sum of detected PFAS concentrations ($\sum_{14}$PFAS) and proportions of major PFAS in the total detected for the 13 sampled sites across Fishermans Bend.

proportions (25%–45%) of PFOA (Table 2; Table S5). These data are consistent with previous studies of municipal landfill leachate and/or leachate impacted groundwater, which have shown comparable proportions of PFOA/$\sum$PFAA (e.g., 29%–42%, Yan et al., 2015; Fuertes et al., 2017). A one-way ANOVA revealed significant differences between these four sites (i.e. LI1-W, LI2-W, LI3 and LI4) and the remaining sampled sites across the study area for (log-transformed) PFOA and PFOA/$\sum$PFAA ($P \leq 0.005$). Sites LI2-W and LI3 also contained relatively high concentrations of PFBA (39 and 49 ng/L respectively; Table 2) whereas this compound was below detection at sites LI1-W and LI4) possibly reflecting different waste types and/or ages in the different municipal landfills.

In contrast, sites dominated by PFOS and PFHxS included three sites unimpacted by landfill leachate (NI2, NI3 and NI4), located in the eastern part of the study area (i.e. away from any known municipal landfills). In addition, there were four sites dominated by PFOS and PFHxS in the centre of the study area which show impacts from landfill leachate (LI5, LI6-W, LI7 and LI8) (Fig. 2); however, the legacy landfills located near these sites are not known to have accepted municipal waste. Based on field observations and aerial photographs, these landfills appear more likely to have been unregulated dumping grounds within former quarries, where construction, demolition and/or general industrial waste was deposited. Such waste does not typically generate leachate components such as ammonia and methane, which are derived from the breakdown of organic, putrescible wastes (Kjeldsen et al., 2002). However, the presence of ammonia and elevated methane (mean = 8.32 mg/L) in the groundwater at site LI6-W indicates that while these landfills did not officially accept municipal waste, there was likely some disposal of putrescible material, in addition to the disposal of industrial, construction and demolition waste. Our data suggest (see section 3.2 below) that such waste is associated with different types of PFAS (e.g. lower proportions of PFCAs) compared to the municipal landfills. However, it is acknowledged that various environmental factors such as fate and transport and precursor transformation could contribute to the observed PFOS and PFHxS dominance in sites proximal to industrial, construction and demolition waste.

A notable outlier in terms of PFOS concentrations occurred at NI4 (4,800 ng/L; comprising 93% of the total mass of detected PFAS at the site), which exceeded the median PFOS concentration (26 ng/L) by more than two orders of magnitude. The concentration of PFHxS (280 ng/L) at this site was also nearly one order of magnitude higher than the overall median. NI3, the closest sampled site to NI4, exhibited the second highest PFOS concentration (230 ng/L), PFHxS concentration (170 ng/L) and $\sum$PFSA concentration (470 ng/L), while the next closest sampled site (NI2) exhibited the third highest PFOS concentration (75 ng/L), PFHxS concentration (96 ng/L) and $\sum$PFSA concentration (220 ng/L) (Table 2). Due to their geographic proximity, these data indicate a possible common source of perfluoroalkyl sulfonic acids (PFSAs) at NI2, NI3 and NI4, which is probably unrelated to municipal landfill leachate (further evidence is discussed in section 3.2 below). A one-way ANOVA revealed these three sites (NI2, NI3 and NI4) exhibit significantly different concentrations compared to the remaining sites for PFHxS ($p = 0.002$), PFBS ($p = 0.022$) and PFPeS ($p = 0.012$). Concentrations and proportions of PFOA (7.5–18 ng/L and 0.3–3.8%, respectively) were substantially lower at these sites compared to the four sites in the western part of the study area near legacy municipal landfills. NI4, the site with the highest PFOS and PFHxS concentrations is located within 70 m of a current paper manufacturing/processing facility and within 80 m of a former chemical manufacturing facility operational from 1896 to 2013 which historically produced cleaning and oleo products (Fig. 1a; URS, 2014), each known to contain

fluorinated surfactants (Kissa, 2001). The elevated PFOS, PFHxS and other PFSAs may therefore be a result of an industrial point-source, such as a chemical storage area or historical chemical spillage, resulting in a localised plume.

3.2. Relationships between PFAS and other landfill leachate indicators

Other typical indicators of landfill leachate impacting groundwater quality include elevated concentrations of ammonia/ammonium, bicarbonate, potassium, total organic carbon and dissolved methane (Eschauzier et al., 2013). These parameters show clear correspondence with legacy landfill locations known to have accepted municipal wastes (i.e., putrescible organic waste as well as other household domestic waste) in the study area (Table 1). Correlation coefficients for PFAS concentrations and these landfill indicators are shown in Table 3. PFCAs, particularly PFOA, are typically associated with municipal landfill leachate (Eschauzier et al., 2013; Gallen et al., 2017; Hamid et al., 2018). PFCAs in the study area showed positive correlations with landfill leachate indicators, for example PFOA with ammonia-N ($R^2 = 0.50$, $p = 0.009$), total organic carbon ($R^2 = 0.59$, $p = 0.002$) and bicarbonate ($R^2 = 0.45$, $p = 0.012$), and PFHxA with ammonia-N ($R^2 = 0.39$, $p = 0.029$) and potassium ($R^2 = 0.37$, $p = 0.035$). Omitting sites suspected to be impacted with sources of PFAS other than landfill leachate (i.e. NI2, NI3 and NI4; see section 3.1 above) allows for a broader assessment of the correspondence between PFCAs and leachate indicators, without confounding influences from anomalous point sources. In this scenario, the correlation coefficients remained similar for PFOA and typically increased for ∑PFAA; for example, R^2 values for ∑PFAA with bicarbonate, total organic carbon and ammonia-N increased to 0.54 ($p = 0.016$), 0.68 ($p = 0.003$) and 0.36 ($p = 0.066$), respectively. In addition, the correlation coefficients typically increased for PFBS in this scenario; for example, R^2 values increased for bicarbonate ($R^2 = 0.48$, $p < 0.05$) and total organic carbon ($R^2 = 0.52$, $p < 0.05$) suggesting the presence of some PFBS in leachate-impacted groundwater.

PFOA/∑PFAA also exhibited a strong positive correlation with (log-transformed) ammonia-N concentrations ($R^2 = 0.69$), while moderate positive correlations with (log-transformed) bicarbonate and total organic carbon were also observed ($R^2 = 0.54$ and 0.51, respectively) (Fig. 3 and Fig. 4). This is consistent with PFOA constituting a significant proportion of landfill-leachate derived PFAA and behaving relatively conservatively during sub-surface transport (Eschauzier et al., 2013). Examination of ratios of PFAA, in addition to absolute concentrations, allows for an assessment of the degree of correspondence of these compounds to the other known leachate indicators, independent of the original magnitude of PFAA source(s) in the landfills, and allows assessment of the degree of modification/change of these PFAA (relative to other PFAA) during transport in the aquifer. Relatively strong correlations between these ratios (as opposed to ratios of PFSAs to ∑PFAA) is consistent with the hypothesis that these particular compounds behave relatively conservatively in typical aquifer environments (Eschauzier et al., 2013; Hamid et al., 2018). PFOA/∑PFAA may therefore serve as a potentially useful indicator of municipal landfill, as opposed to non-landfill (or industrial/construction waste landfill) derived PFAS in areas with complex land-use history and multiple potential sources.

There was no significant positive correlation between PFOS or PFHxS (the PFAA with the highest median and maximum values) and the typical landfill leachate indicators (Table 3). Previous studies have generally found PFOS and other PFSAs in relatively smaller albeit still significant proportions (Yan et al., 2015; Gallen et al., 2017) in landfill leachate or leachate impacted groundwater compared to PFOA and other PFCAs. The lack of correlation suggests that these compounds may relate to other contamination sources. As discussed above, this appears to include a point source of industrial contamination impacting three of the sites (NI2, NI3 and NI4). A further four sites with relatively high concentrations of PFHxS and PFOS (LI5, LI6-W, LI7 and LI8), but relatively low concentrations of $\sum_{14}$PFAS (65–120 ng/L) and concentrations of typical landfill leachate indicators (e.g. ammonia) occur in proximity to suspected unregulated landfills, which likely accepted construction, demolition and/or general industrial waste (as opposed to municipal waste). Landfills accepting such wastes have been shown to typically contain higher concentrations of PFOS, PFHxS and other PFSAs, and lower concentrations of PFOA and other PFCAs relative to municipal solid waste landfills (Eggen et al., 2010; Gallen et al., 2016; Hamid et al., 2018). Such landfills typically do not generate high levels of ammonia, methane or other typical municipal landfill leachate indicators, due to low putrescible organic waste fractions (Kjeldsen et al., 2002).

Additional potential PFAS sources in the region include urban stormwater runoff and precipitation; Murakami et al., (2009a & 2009b) and Xiao et al. (2012) found significant PFAS concentrations in street runoff, which likely recharges groundwater in the study area. However, the generally low or non-detect PFAS concentrations in the background site (B) indicate that if runoff and/or precipitation were acting as significant sources across the precinct, they must be occurring in localised areas only. The low observed PFAS concentrations at the background site therefore indicate that runoff and/or precipitation are unlikely to be significant PFAS sources in this region, although they may be minor contributors of certain PFAS (Loewen et al., 2005; Scott et al., 2006; Cai et al., 2012). Sewer leakage is another possible source; however, this is considered unlikely as sewers are generally deeper than the water table and drain groundwater rather than leaking to it (e.g., Fig. 1a and b).

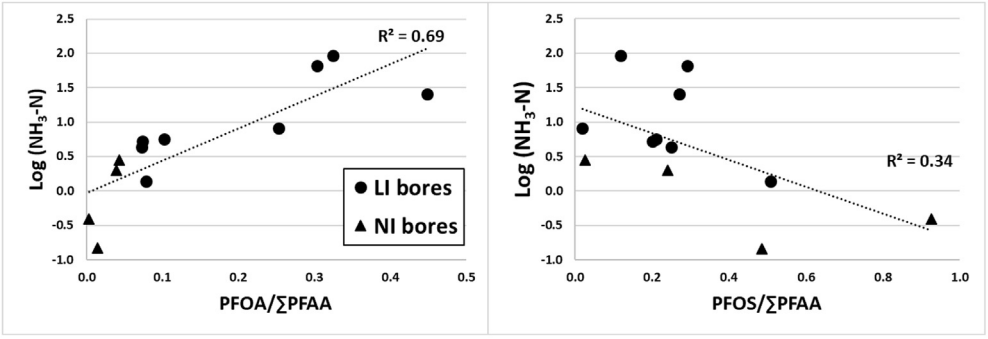

Fig. 3. Relationship between two commonly detected PFAA (as a proportion of the sum of detected PFAA) and concentrations of ammonia-N (averages over 2 to 5 sampling rounds per site) which is an indicator of the degree of legacy landfill impact on groundwater quality.

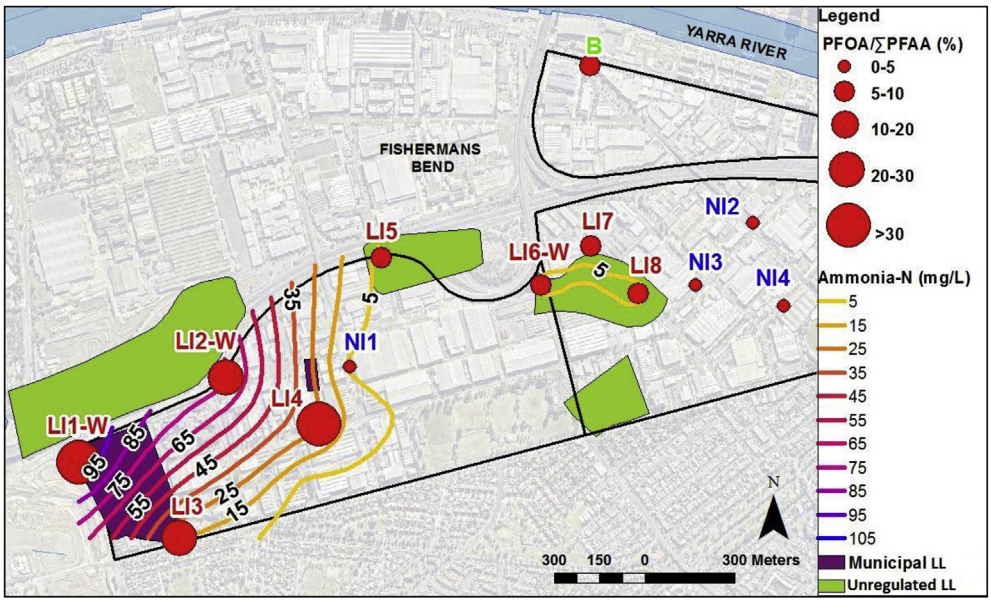

Fig. 4. PFOA/∑PFAA and ammonia-N concentrations in groundwater for the 13 sampled sites across Fishermans Bend (LL = Legacy Landfill).

Previous sampling has also not identified common indicators of sewer leakage (e.g. bacterial contamination; Hepburn, unpublished data).

It is acknowledged that many factors can influence PFAS fate and transport in groundwater and that the detail required to characterise all possible controls in the study area is largely unavailable. However, all sampled sites were screened within a relatively uniform hydrogeological horizon (see cross-section presented in Fig. 1b) which has been well characterised (Neilson, 1992; Leonard, 2006). This unit contains relatively minor organic carbon and fresh, oxic groundwater (section 2.1) recharged by precipitation (Hepburn et al., 2018). As such, significant sorption/degradation of PFAS due to water-aquifer interaction are considered unlikely, or at least, unlikely to be occurring at highly different rates across the study area.

3.3. New framework for identifying legacy landfill PFAS impacts in groundwater

A new framework for identifying PFAS impacts in groundwater surrounding legacy landfills is presented in Fig. 5. The framework may be used by practitioners, regulators and academic researchers to isolate landfill-related PFAS impacts to groundwater in settings where multiple PFAS sources may exist, such as in urban redevelopment areas. The framework consists of a flowchart which systematically outlines which landfill indicators to analyse, followed by the use of PFOA/∑PFAA to determine the likelihood of various sources being attributable to the observed PFAS impacts (e.g. groundwater impacted by fluoropolymer manufacturing sites might be expected to contain elevated PFOA/∑PFAA but would not typically contain other elevated landfill indicators). The framework also encourages the use of historical site knowledge, where available.

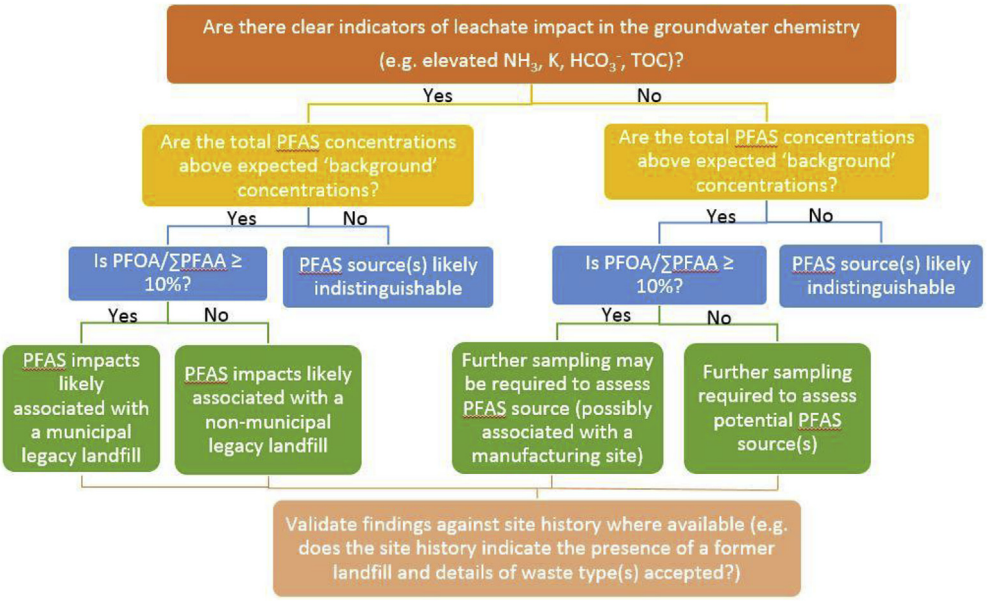

Fig. 5. New framework for assessing legacy landfill PFAS impacts to groundwater.

3.4. Comparison to australian and international PFAS concentrations in landfill leachate

Hamid et al. (2018) recently reviewed and compiled PFAS data from a range of landfill types reported worldwide (Hamid et al., 2018 Fig. 2 and Table 1). In general, PFASs in groundwater from this study, including the sites screened in legacy landfill waste, were far below those reported in raw and treated leachate for operating and recently closed landfills (e.g. PFOA $\leq$ 214,000 ng/L; PFBA $\leq$ 9,270 ng/L; PFHxA $\leq$ 25,000 ng/L). This can be attributed to the long period of time since closure of the landfills in the study area (e.g. 1990 or earlier), which has likely resulted in a large proportion of readily leachable PFAS in the waste material having already been removed by groundwater. The ranges of concentrations in this study are similar to those reported in leachate-impacted groundwater (as opposed to raw leachate) by Eschauzier et al. (2013) (74–4,400 ng/L $\sum$PFAA – see Table 4 for comparison to mean concentrations). Further field studies utilising the approach taken in this paper to assess PFAS concentrations in leachate-impacted groundwater in other regions is vital for 1) evaluating the effectiveness of this approach, and 2) developing a better understanding of the risks posed by PFAS-containing waste to human health and the environment surrounding legacy landfills.

Concentrations of C4 and C8 chemistries (PFBA, PFBS, PFOA and PFOS) were relatively low in the groundwater in this study compared to typically reported landfill leachate (Table 4). PFBA and PFBS have been used as replacements for PFOS and PFOA in recent times (Buck et al., 2012) driven by the deliberate phasing out of PFOS and PFOA via voluntary agreements between their primary manufacturers and the US EPA (US EPA, 2006; 2009). For this reason, PFBA and PFBS are likely to be seen in higher concentrations in relatively new landfills. The relative lack of significant concentrations of these replacement compounds in our study is somewhat expected given the landfill operational periods (1930s–1990s); although these compounds were still detected at nearly all sampled sites.

In comparison to raw and treated landfill leachate, groundwater down-gradient from legacy landfills is subject to a significant degree of dilution with regional groundwater, and possibly, attenuation processes such as sorption and/or degradation of precursor PFAS (given the long timeframe of waste disposal and industrial activity in the region). As discussed above, the relatively high concentrations of PFOA in comparison to PFSAs near the (legacy) municipal landfills, and the positive correlations between PFCAs (but not PFSAs) and typical leachate indicators are consistent with a lesser degree of sorption of the former during subsurface transport. No significant negative correlation between groundwater TOC and PFAS was observed in the data (in fact a moderate positive correlation was observed between PFOA/$\sum$PFAA and TOC, likely because TOC is associated with landfill leachate), nor was any significant correlation with pH observed, which may otherwise indicate a strong control exerted by sorption behaviour on the observed concentrations (Higgins and Luthy, 2006). The relative persistence of PFOA in such settings may also relate to the degradation of various PFAA precursors to PFOA after disposal to landfill (Hamid et al., 2018).

3.5. Comparison to reported literature values of PFAS concentrations and ratios by source

The concentrations of selected PFAS and key ratios (including PFOA/$\sum$PFAA) from this study are further compared to those in

Table 4
Summary of reported literature values of PFAS concentrations and ratios by source and region.

Region	n	Mean concentrations (ng/L)					Key ratios (mean)		Source
		PFOA	PFOS	PFBA	PFBS	$\sum$PFAA	PFOA/$\sum$PFAA	PFOA/PFOS	
AFFF-impacted groundwater									
Europe	3	29	481	12	30	867	0.03	0.1	Wagner et al. (2013)
	–	12,000	26,000	1,300	1,100	77,350	0.16	0.5	Woodard et al. (2017)
Australia	13	200	2,600	200	500	7,170	0.03	0.1	Braunig et al., 2017
USA	24	33,596	34,796	16,346	28,729	329,704	0.11	2.7	Houtz et al. (2013)
	10	36,110	32,000	–	–	–	[a]	1.9	Moody et al. (2003)
Manufacturing-impacted groundwater									
Asia	37	22,384	5.6	1,564	2.4	26,052	0.65	1705	Liu et al. (2016)
	4	1,422	0.4	1,544	375	3,340	[a]	2942	Wang et al. (2016)
	17	156	6.3	21	3	254	0.58	95	Lu et al. (2018)
	10	335	35	12	108	806	0.42	11	Wei et al. (2018)
Recycled Wastewater (partially treated)-impacted groundwater									
Europe	31	1	1	<1	<1	40	0.10	1.1	Boiteux et al. (2012)
	164	3	4	–	<0.3	–	[a]	0.8	Loos et al. (2010)
Australia	28	2.2	11	6.1	4.4	37	0.09	0.4	Szabo et al. (2018)
Background									
Africa	12	0.2	0.8	0.2	0.2	1.8	0.08	0.3	Kaboré et al. (2018)
Asia	102	4.8	4.4	2.4	10	42	0.12	2.2	Wei et al. (2018)
Australia	1	1.7	7.7	3.3	2	26	0.07	0.2	This study
Landfill leachate-impacted groundwater									
Australia (municipal waste)	4	66	35	44	12	204	0.33	4.8	This study
Australia (mixed waste)[b]	4	8	25	10	8	92	0.08	0.3	This study
Netherlands (mixed waste)[c]	4	559	–	393	39	1,259	0.24	–	Eschauzier et al. (2013)
Raw landfill leachate – operating landfills									
Australia (municipal waste)	12	520	300	–	–	3,466	[a]	–	Gallen et al. (2017)
China (municipal waste)	5	49,246	2,716	3,518	15,236	80,220	0.30	9.4	Yan et al. (2015)
Canada (mixed waste)[d]	3	210	80	70	28	2547	0.08	2.6	Benskin et al. (2012)
Raw landfill leachate – closed landfills									
Australia (municipal waste)	7	390	180	–	–	2,219	[a]	–	Gallen et al. (2017)
USA (municipal waste)	6	663	109	748	567	3,889	0.20	6.5	Huset et al. (2011)

[a] Ratio only calculated where the total number of PFAA compounds analysed were comparable to this study.
[b] Mixed waste (inferred construction, demolition and/or general industrial waste; likely some additional putrescible material).
[c] Mixed waste (household and construction).
[d] Mixed waste (soils/sand, municipal, construction and demolition); "-" = not analysed.

groundwater impacted by AFFF, manufacturing and recycled wastewater as well as background concentrations and raw leachate in Table 4. The concentrations of PFAA (including PFOA and PFOS) are significantly lower in leachate-impacted groundwater compared to groundwater impacted by AFFF and manufacturing sites, likely due to higher starting concentrations of the source at these sites, compared to landfills. In comparison, concentrations of PFAA are significantly higher in leachate-impacted groundwater compared to groundwater impacted by wastewater, the concentrations of which are similar to background concentrations.

Overall, there is evidence that leachate-impacted groundwater (our study) has distinctly higher ratios of PFOA/$\sum$PFAA (0.33) and PFOA/PFOS (4.8) compared to groundwater impacted by AFFF and wastewater (PFOA/$\sum$PFAA range: 0.03–0.16; PFOA/PFOS range = 0.1–2.7), indicating that higher PFOS concentrations are generally associated with these sources. In comparison, leachate-impacted groundwater has similar ratios to raw landfill leachate reported in Huset et al., 2011) (PFOA/PFOS = 6.5 and PFOA/$\sum$PFAA = 0.20) and in Yan et al. (2015) (PFOA/PFOS = 9.4 and PFOA/$\sum$PFAA = 0.30). Importantly, much higher proportions of PFOA are observed in the municipal leachate-impacted groundwater (this study) compared to the mixed-waste leachate-impacted groundwater (also this study). The relative prevalence of PFOA compared to the other PFAA in municipal leachate impacted groundwater is consistent with the findings of Hamid et al. (2018) (discussed above).

Aside from at leachate-impacted sites, the only other type of site where significantly higher PFOA concentrations (both absolute and as a proportion of total PFAA) are observed is at PFAS manufacturing sites (e.g. Liu et al., 2016). The data for these sites are somewhat limited and may be biased towards whichever PFAS were manufactured in largest quantities at such sites.

4. Conclusions

To date, the extent of per- and polyfluoroalkyl substances (PFAS) in groundwater surrounding legacy landfills is poorly constrained, highlighting the significant knowledge gap in the global literature with respect to PFAS in groundwater from this source. We determined concentrations of a range of PFAS in groundwater in an urban re-development area with multiple legacy landfills and a long history of industrial activity. Total PFAS concentrations ranged from 26 to 5,200 ng/L. Sites within or immediately down-gradient from legacy landfills that accepted municipal waste from the 1930s–1990s, contained $\sum_{14}$PFAS (between 160 and 240 ng/L) that were significantly lower than active or recently closed landfills, but which were consistent with other studies of groundwater impacted by landfill leachate. These sites were dominated by PFOA (25–45% of the sum of detected PFAS), which is consistent with other studies reporting relatively high proportions of PFOA in municipal landfill-related PFAS. A strong positive correlation between PFOA/$\sum$PFAA and ammonia-N concentrations, and correlation coefficients between PFCAs and other leachate indicators are consistent with these compounds being sourced from landfill leachate and behaving relatively conservatively during subsurface transport. PFOA/$\sum$PFAA could therefore potentially be used as a tracer of PFAS derived from (municipal) landfill leachate as distinct from other sources (such as industrial point sources or construction/demolition waste landfills) in areas of complex land-use history such as Fishermans Bend. A new framework for isolating landfill-related PFAS impacts to groundwater in such settings has been presented as a potentially replicable approach for analogous precincts where groundwater contamination is widespread. Comparison of PFOA/$\sum$PFAA from this study to AFFF, manufacturing and wastewater sources indicate promise in the use of the ratio as a standalone diagnostic tool for PFAS source identification, potentially independent of the presence or absence of the more traditional landfill leachate indicators such as ammonia and methane.

High $\sum_{14}$PFAS (320–5,200 ng/L) also occurred at a subset of sites that were dominated by PFSAs, particularly PFOS and PHFxS. These sites showed no evidence of typical landfill leachate impact (such as elevated ammonia-N) and were not located near any known former landfills. It is therefore likely that the anomalously high concentrations relate to an industrial point-source.

To our knowledge this is one of the first studies to report PFAS concentrations in groundwater impacted by contamination from a range of legacy landfill types and other diffuse and localised inputs (i.e. an area of complex, mixed land-use history including former industrial facilities and multiple landfill sites which accepted different waste types over a long period). The observed concentration ranges and proportions of different PFAS, which are attributed to different sources here, may be broadly representative of PFAS contamination in such regions. The data reported here may have wider significance for environmental regulation of urban re-development projects worldwide, as many such projects are located in similar settings, with long histories of industrial activity and both municipal and unregulated landfilling. Future research involving larger sample sizes from sites worldwide is needed to verify the effectiveness of PFOA/$\sum$PFAA as an indicator of PFAS derived from municipal landfill leachate compared to conventional indicators.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.02.018.

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An investigation into per- and polyfluoroalkyl substances (PFAS) in nineteen Australian wastewater treatment plants (WWTPs)

Timothy L. Coggan [a,*], Damien Moodie [a], Adam Kolobaric [a], Drew Szabo [a], Jeff Shimeta [a], Nicholas D. Crosbie [b,c], Elliot Lee [d], Milena Fernandes [e,f], Bradley O. Clarke [a]

[a] Centre for Environmental Sustainability and Remediation, School of Science, RMIT University, GPO Box 2476, Melbourne, Vic., 3001, Australia
[b] Applied Research, Melbourne Water Corporation, Docklands, VIC, 3001, Australia
[c] Faculty of Engineering, University of New South Wales, NSW, 2052, Australia
[d] Water Corporation, Leederville, Western Australia, 6007, Australia
[e] SA Water, GPO Box 1751, Adelaide SA, 5001, Australia
[f] College of Science and Engineering, Flinders University, Adelaide, Australia

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ABSTRACT

Quantifying the emissions of per- and polyfluoroalkyl substances (PFAS) from Australian wastewater treatment plants (WWTP) is of high importance due to potential impacts on receiving aquatic ecosystems. The new Australian PFAS National Environmental Management Plan recommends 0.23 ng L^{-1} of PFOS as the guideline value for 99% species protection for aquatic systems. In this study, 21 PFAS from four classes were measured in WWTP solid and aqueous samples from 19 Australian WWTPs. The mean $\sum_{21}$PFAS was 110 ng L^{-1} (median: 80 ng L^{-1}; range: 9.3–520 ng L^{-1}) in aqueous samples and 34 ng g^{-1} dw (median: 12 ng g^{-1} dw; range: 2.0–130 ng g^{-1} dw) in WWTP solids. Similar to WWTPs worldwide, perfluorocarboxylic acids were generally higher in effluent, compared to influent. Partitioning to solids within WWTPs increased with increasing fluoroalkyl chain length from 0.05 to 1.22 log units. Many PFAS were highly correlated, and PCA analysis showed strong associations between two groups: odd chained PFCAs, PFHxA and PFSAs; and 6:2 FTS with daily inflow volume and the proportion of trade waste accepted by WWTPs (as % of typical dry inflow). The compounds PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA increased significantly between influent and final effluent. The compounds 6:2 FTS and 8:2 FTS were quantified and F–53B detected and reported in Australian WWTP matrices. The compound 6:2 FTS was an important contributor to PFAS emissions in the studied Australian WWTPs, supporting the need for future research on its sources (including precursor degradation), environmental fate and impact in Australian aquatic environments receiving WWTP effluent.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made pollutants that pose an emerging risk to the water sector, challenging established practices such as recycling and environmental discharges. They are omnipresent in water, air, food, wildlife, and humans, are resistant to typical environmental degradation processes, and can have negative impacts on exposed organisms [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. Most PFAS are recalcitrant through conventional water treatment processes and, therefore, wastewater effluents can contain PFAS that has originated from domestic and industrial sources [11, 12]. Understanding the sources of PFAS to the environment is of high importance in Australia due to the recently recommended perfluorooctane sulfonic acid (PFOS) guideline value for 99% species protection of 0.23 ng L^{-1} in aquatic ecosystems in the PFAS National Environmental Management Plan [13].

The unique and useful chemical and physical properties of PFAS have resulted in many commercial applications, such as stain-resistant coatings, water-resistant fabrics, metal plating paints, pesticides, fluoropolymers, greaseproof paper, and aqueous film-forming foams (AFFF) used in firefighting, amongst others [3, 4, 7, 9]. Although PFAS are a broad class of compounds comprising over 4700 known PFAS [10], many studies have focused on a small number of perfluoroalkyl acids (PFAAs), specifically the perfluorocarboxylic acids (PFCAs; $CF_3(CF_2)_nCOOH$) and perfluorosulfonic acids (PFSAs; $CF_3(CF_2)_nS(=O)OH$) [9]. Despite being

* Corresponding author.
E-mail address: timothy.coggan@rmit.edu.au (T.L. Coggan).

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manufactured since the 1950s, it wasn't until 2001 that the extent of PFAS global contamination was first demonstrated for perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3H$) and perfluorooctanoic acid (PFOA; $C_7F_{15}COOH$) [1]. Since then, PFAS have been detected in almost every wildlife sample measured [14], ubiquitously in humans throughout the world [15], and in most environmental compartments, including pristine locations [7].

The perfluoroalkyl substances contain at least one fully fluorinated alkyl chain bonded to a functional group, whereas polyfluoroalkyl substances contain a partially fluorinated alkyl chain with a range of functional groups. In general, the sorption potential of PFAS is determined by functional group, chemical structure, and fluorinated chain length; however, for many newer PFAS, this information is not yet available. In environmental aquatic systems, the different partitioning behavior will typically result in short-chain compounds (PFCAs: $\leq$ C6, and PFSAs: $\leq$ C5) partitioning to the aqueous phase and long-chain compounds adsorbed to the solid compartments [16, 17]. Furthermore, some PFAS (viz. fluorotelomer alcohols, phosphate esters, etc.) are precursor compounds and will transform in the environment, forming many intermediate transformation products with PFAAs such as PFOA as terminal products [18].

The growing understanding of the risks of many legacy PFAS has led to the phase-out of production of PFOS (and related compounds) and PFOA in North America (in 2000 and 2002, respectively) and an increased use of less problematic alternative compounds (such as short-chain and fluorotelomer based chemistries) [4]. An example of two PFOS alternatives used as mist suppressants in metal plating are 6:2 fluorotelomer sulfonate (6:2 FTS) and the chlorinated perfluoroether sulfonate F–53B [4, 19, 20]. In some regions, 6:2 FTS is not used as a PFOS substitute in metal plating as it cannot match the low surface tension of PFOS and approximately three to ten times the quantity is required [21]. However, 6:2 FTS has found further uses as a PFOS substitute in AFFF, oil production and primarily occurs as an intermediate degradant of complex fluorotelomer-based substances [4]. In initial testing by Dupont scientists, 6:2 FTS was found to show low risk to aquatic ecosystems making it a desirable substitute, however, studies on the environmental fate and effects were still needed [22]. Alternatively, F-53 (6:2 PFESA) then the chlorine substituted F–53B (6:2 Cl-PFESA), have been used almost exclusively in China since the 1970s with little PFOS ever used in metal plating [19]. As investigations into the fate and toxicity of F–53B progresses, it is now becoming apparent that it shows similar recalcitrance, toxicity and physiochemical properties to PFOS and is becoming widely distributed in the environment making it a less desirable substitute for PFOS [20, 23, 24, 25].

Wastewater treatment plants (WWTPs) can act as a conduit for many recalcitrant anthropogenic compounds, such as PFAS, to the environment through effluent discharges and the land application of biosolids [26]. PFAS have been detected in WWTP influent, effluent and solids worldwide [11]. Similar to other environmental compartments, hydrophobic partitioning in WWTPs is the dominant sorption mechanism, which results in long-chain PFAAs partitioning to WWTP solid matrices [27, 28, 29, 30]. Typical wastewater treatment processes are unable to remove PFAS from the final effluent. In some studies, concentrations of compounds such as perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids (PFSA) have increased from influent to final effluent [11, 27, 31]. The increase of PFAAs has been attributed to the degradation of the PFAS precursor compounds [32, 33], fluorotelomer sulfonoates (FTS) and fluorotelomer alcohols (FOTH), that have been shown to transform to stable PFAAs in WWTP sludge [34, 35].

The awareness of PFAS environmental contamination associated with AFFF application on government military sites, and evidence of widespread distribution in the Australian environment [36, 37, 38, 39, 40], have led to the development of the Australian PFAS National Environmental Management Plan (NEMP) [13]. Within the NEMP, the recommended freshwater and marine guideline values (water concentrations) for 99% species protection are 0.23 and 1900 ng L^{-1} PFOS and PFOA, respectively (HEPA 2018). As a result, there is strong interest from water industry professionals and regulators to understand the quantities of PFAS released into the environment through treated effluent, and the potential impact these emissions may have upon Australian aquatic environments.

Initial studies on PFAS emissions in Australian WWTPs have focused on the removal efficiency in two reclaimed water plants (18 PFAS measured ranging from 1.1 to 38.6 ng L^{-1}) [41] and one WWTP (8 PFAS measured ranging from 3 to 82 ng L^{-1}) [42]. An Australian-wide study measuring nine PFAS in WWTP effluent (range from n. d. to 240 ng L^{-1}) and biosolids, sampled in 2016, estimated that Australian WWTPs have discharged an estimated 33 kg PFOS and 67 kg PFOA, annually [37]. More recently, PFAS levels in influent over a four year period at two large Australian WWTPs (mean $\sum_{11}$PFAS levels 57 $\pm$ 3.3–94 $\pm$ 17 ng L^{-1} at WWTP A; and 31 $\pm$ 6.1–142 $\pm$ 73 ng L^{-1} at WWTP B) were determined to have: 1) no significant difference in daily PFAS mass load between weekdays and weekends (composite samples over 7 consecutive days), 2) very few significant seasonal differences of $\sum_{11}$PFAS (with most significant differences linked to a pulse release of PFOS at both WWTPs), and, 3) only one significantly different annual mean mass load in WWTP B over the entire four year period (linked to the same PFOS pulse event of October 2017) [43].

Australian WWTPs represent a unique case as there is no reported PFAS manufacture and low rates of PFAS are imported for direct use in industries such as car manufacture, chrome plating, leather treatment, medical imaging, firefighting and in goods already impregnated (carpets, furniture, etc.) or in products containing PFAS as impurities [13, 44]. Furthermore, unlike many parts of the world, in Australian cities, sewer systems are closed, with separate stormwater sewers and low infiltration rates, this means rainfall has limited effect on influent PFAS composition as opposed to pulse events from industrial effluent discharge. It is, however, becoming apparent that many PFAS, including PFOS and PFOA, are present in Australian WWTP effluents and are being discharged to the aquatic environment.

The aims of this study were to measure the mass loading of PFAS (including PFAAs, FTSs, and F–53B) within solid and liquid matrices from 19 Australian WWTPs of varying size, capacity, localities and treatment types. Samples were taken from various stages within the treatment train from a range of WWTPs to determine the trends in the mass flux and partitioning of PFAS within the sampled WWTPs. Finally, the data were compared to recent work estimating the Australian annual PFAS discharge, providing important data for ongoing assessments of the potential impact of PFAS on aquatic environments.

2. Materials and methods

2.1. Sampling

Field sampling kits including field blanks were prepared at RMIT University laboratories and shipped overnight to each WWTP. Three replicate aqueous and solid samples were collected from each of nineteen Australian WWTPs throughout 2017 (Table 1). Aqueous samples (influent, primary effluent, secondary effluent, final effluent, recycled water) consisting of either triplicate sub-samples from a single 24 h composite or three replicate grab samples were collected in 250 mL polypropylene bottles pre-rinsed with ultrapure water, methanol, and site water. Solid samples (primary sludge, secondary sludge, lagoon sludge, and one lagoon sludge dredge pile) were collected in 50 mL polypropylene centrifuge tubes. On receipt, samples were sterilized (aqueous samples with sodium azide ~1g L^{-1} and solid samples with 2 % w/w sodium azide solution) and refrigerated until extraction.

2.2. Chemicals and standards

The compounds quantified in this study were the perfluorocarboxylic acids (PFCAs): PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA,

Table 1
Wastewater treatment plant specifics and sample locations for replicate influent (IN), primary effluent (1E), secondary effluent (2E), final effluent (FE) and recycled water (RW). Lagoon sludge (LS) was collected from WWTPs-8, 10 and 16; primary (1S) and secondary (2S) sludge were collected from WWTPs-3, 4, 9, 17 and 18.

WWTP code	Treatment description	Month sample	WWTP type	Inflow (ML/d)	TW (%)
WWTP-1	screen (IN), IDEA (2E), balancing pond (FE)	AUG	AS	6	<10%
WWTP-2	screen (IN), SBR, filtration, UV disinfection (FE)	APRIL	AS	13	<10%
WWTP-3	screen (IN), primary sedimentation (1E), aeration, secondary sedimentation (FE) - FE excess sludge and centrifuge supernatant to DAFT, then DAFT supernatant to aeration tanks	AUG	AS	127	<5%
WWTP-4	screen (IN), primary sedimentation (1E), activated sludge reactors, clarifier (2E), stabilisation lagoons (FE), dissolved air floatation and filtration (RW-1), chlorination (RW-F) - secondary sludge and centrifuge supernatant to activated sludge reactors	AUG	AS/LAG	167	<20%
WWTP-5	screen (IN), bioselector, SBR (2E), balancing dam (FE) - Excess aeration sludge to processing, sludge supernatant to influent	DEC	AS	9.8	<5%
WWTP-6	screen (IN), bioselector, oxidation ditches (1E), clarifiers (FE), tertiary filters, UV disinfection (RW) - Excess secondary sludge to aerated storage tanks, and centrifuge supernatant to influent	SEPT	AS	4.9	<5%
WWTP-7	screen (IN), bioselector, oxidation ditches (1E), clarifiers (FE)	OCT	AS	2.7	<5%
WWTP-8	screen (IN), aeration pond, maturation pond (FE)	DEC	LAG	1.59	<5%
WWTP-9	screen (IN), primary sedimentation (1E), aeration (2E), balancing dam, media filtration, ozone, UV disinfection, chlorination (FE)	SEPT	AS	330	<20%
WWTP-10	screen (IN), aeration pond (1E), maturation pond (FE)	OCT	LAG	1.9	<10%
WWTP-11	screen (IN), bioselector, oxidation ditches (1E), clarifiers (FE) - centrifuge supernatant to bioselector	NOV	AS	10.2	<5%
WWTP-12	(IN), screen, bioselector, SBR with alum addition (1E), balancing dam (FE), tertiary filters, chlorine disinfection (RW) - excess secondary sludge to digesters, digester and centrifuge supernatant to influent	SEPT	AS	3.2	<5%
WWTP-13	screen (IN), bioselector, oxidation ditches (1E), clarifiers (FE) - Excess secondary sludge to DAFT, DAFT and centrifuge supernatant to bioselector	NOV	AS	5.5	<5%
WWTP-14	(IN), screen, bioselector, SBR with alum addition, balancing dam (FE), tertiary filters, chlorine disinfection (RW) - excess secondary sludge to aerated storage tanks, and centrifuge supernatant to influent	DEC	AS	1.5	<5%
WWTP-15	screen (IN), Imhoff tank, primary pond, secondary ponds (2E), alum dosing, polishing pond (FE), UV disinfection (RW), chlorination	NOV	LAG	1.5	<5%
WWTP-16	(IN), screen, Anaerobic ponds (1E,1E), facultative ponds, maturation ponds (FE)	AUG	LAG	3.7	<5%
WWTP-17	screen (IN), primary sedimentation (1E), aeration, secondary sedimentation (FE) - excess secondary sludge and centrifuge supernatant to DAFT, then DAFT supernatant to primary sedimentation	SEPT	AS	59	<10%
WWTP-18	screen (IN), primary sedimentation (1E), SBR (2E), balancing dam (FE) - centrifuge supernatant and excess SBR sludge to DAFT, then DAFT supernatant to Primary sedimentation tanks	AUG	AS	143	<10%
WWTP-19	(IN) anaerobic ponds, aerobic ponds, clarifiers (2E, 2E), maturation ponds (FE), polishing pond (RW-1), UV disinfection, chlorine disinfection (RW-F)	SEPT	AS/LAG	498	<30%

WWTP treatment trains were broadly classified as activated sludge (AS) and lagoon based (LAG). TW refers to the proportion of trade waste (TW) of typical dry inflow received at the sampled WWTPs. Trade waste flows were calculated from metered flows at industrial sites, industry models or estimates of commercial discharges. The acronyms IDEA (intermittently decanted extended aeration), SBR (sequencing batch reactors) and DAFT (dissolved air floatation thickeners) refer to treatment process employed within the WWTPs.

PFUdA, PFDoA, PFTrA & PFTeA; the perfluorosulfonic acids (PFSAs): PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFDS; the fluorotelomer sulfonates 6:2 FTS, 8:2 FTS, and the chlorinated perfluoroether sulfonic acids (components of the commercial product F–53B): 6:2 Cl-PFESA (F–53B) and the F–53B impurity 8:2 Cl-PFESA (full compound details and MS/MS transitions listed in Table S1). These compounds were selected as PFCAs and PFSAs have previously been demonstrated to be present in Australian WWTPs and need further baseline data [37, 41, 42]. The FTSs were selected as 6:2 FTS has been demonstrated as present in AFFF formulations impacting WWTPs [33], used as a PFOS replacement [4] and there is little current published Australian data on FTSs. Furthermore, the F–53B components are an emerging contaminant in China due to substitution for PFOS in chrome plating [20]. As Australian is part of the Asia Pacific region, and Cl-PFESAs have been detected in WWTPs in China [19, 24] it was included in this study to determine if there is an emerging risk in Australia.

Analytical standards and isotopically labeled analogues of PFAS were purchased from Wellington Laboratories (Ontario, Canada) as solutions of 50 μg mL^{-1} in methanol. Stock solutions of 100 ng mL^{-1} for native PFAS and 100 ng mL^{-1} for surrogate PFAS were prepared gravimetrically in methanol for spiking.

The solvents methanol (LC-MS grade, Honeywell, USA and LiChrosolv hypergrade, Merck Millipore, Australia) and ultrapure water (pH 8, Merck Millipore, Australia) were tested for PFAS contamination prior to use. Ammonium hydroxide solution (28% in H_2O, ≥ 99.99%), sodium acetate, glacial acetic acid and ammonium acetate (≥99.99%) were purchased from Sigma-Aldrich (Australia). The dispersive solid-phase extraction sorbents (d-SPE), sorbents C18, and primary secondary amine (PSA) were purchased in bulk from Agilent Technologies (USA).

2.3. Aqueous sample extraction

Aqueous samples were extracted using similar methods outlined in Szabo, Coggan [40], Hepburn, Madden [45] and Coggan, Anumol [46]. Briefly, samples were filtered using 1 μm glass fibre filters (Merck Millipore, Australia), spiked with 5 ng of isotopically labelled PFAS, followed by solid-phase extraction (SPE) using Oasis weak anion exchange (6 mL, 150 mg WAX) cartridges with 15 mL polypropylene centrifuge

vials used as collection vessels. Cartridges were conditioned sequentially with 4 mL 0.1% (v/v) ammonium hydroxide in methanol, 4 mL methanol, and 4 mL ultrapure water. The entire sample was passed through the cartridge under vacuum at approximately one drop per second, then washed with 4 mL of a pH 4 buffer (sodium acetate/acetic acid) and dried under vacuum for 10 min. SPE cartridges were eluted using 2 mL of methanol that was used to rinse the sample bottle, followed by 4 mL of 0.1% (v/v) ammonium hydroxide in methanol. Extracts were evaporated to 500 μL under a gentle stream of nitrogen (at 25 °C) and reconstituted to 1 mL in methanol and transferred to a polypropylene chromatography vial with polyethylene lid for analysis.

2.4. Solid sample extraction

Freeze-dried sludge samples (0.5–1 g) were spiked with 25 ng of isotopically labelled PFAS before adding 4.65 mL of 10 mM NaOH in methanol. Samples were sonicated for 30 min and shaken overnight for 12 h. Extracts were neutralized with 100 μL of glacial acetic acid and cooled on ice. Five mL of extract was then transferred to a 15 mL polypropylene (PP) tube before adding 100 mg of C18 and 50 mg primary secondary amine (PSA) to remove interfering compounds. Extracts were agitated for approximately 1 min and centrifuged (10,000 rpm, 10 °C, 10 min), with this process repeated twice. Finally, extracts were filtered using a 0.45 μm PES syringe filter (pre-rinsed with LC-MS grade methanol) into a propylene chromatography vial with polyethylene lid for analysis.

2.5. Instrumental analysis

The analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 6495B mass spectrometer coupled with an Agilent 1290 II Infinity liquid chromatograph optimised for PFAS analysis. Twenty-one PFAS compounds were quantified using isotope dilution. A surrogate compound for each PFAS was set as a mass-labeled compound from a similar class and/or close elution time. For compounds where two or more transition ions were present, the transition with the highest response was set as the quantifier, with others set as qualifier ions. The branched plus linear isomers of PFPeS, PFHxS, PFHpS, and PFOS were quantified using linear-only calibration standards and reported as a combined branched plus linear concentration.

The twenty-one PFAS quantified in the analytical method are listed in the supplementary information (Table S1). The method employed dynamic multiple reaction monitoring (dMRM) and a 2 μL injection, in negative ESI mode. MS parameters were: gas temperature 250 °C, gas flow 11 L min^{-1}, Nebulizer 25 psi, sheath gas temp 375 °C, sheath gas flow 11 L min^{-1}, capillary voltage 2500 V, high pressure ion funnel RF 90 V and low pressure ion funnel RF 60 V. Separation was achieved using a Zorbax eclipse plus RRHD C18 column (3.0 × 50 mm, 1.8 μm, Agilent Technologies, USA) with a guard column attached. Gradient elution with the solvents 5 mM ammonium acetate in ultrapure water (A) and methanol (B) at 400 μL min^{-1} was performed, and the first 1.5 min was diverted to waste (t_0 = 10% B; $t_{0.5}$ = 10% B; $t_{2.5}$ = 55% B; t_9 = 90% B; $t_{9.5}$ = 100% B; $t_{11.5}$ = 100% B; $t_{11.6}$ = 10% B; t_{14} = 10% B). A delay column (Zorbax Eclipse Plus C18 RRHD, 4.6 × 50 mm, 3.5 μm, Agilent Technologies, USA) was installed between the solvent mixer and injector module to delay instrument PFAS contamination.

2.6. Quality control

Linear calibration curves were prepared by gravimetric dilution of a mixed PFAS standard solution (100 ng mL^{-1} in methanol) with methanol to 9 levels with $r^2 > 0.99$. Limit of quantification (LOQ) was set as the lowest calibration point multiplied by four and ranged from 0.1 to 0.6 ng L^{-1} depending on the compound type and gravimetric dilution. Limit of detection (LOD) was set as the instrument detection limit (IDL), varied on a compound-by-compound basis, and ranged from 0.01 to 0.1 ng L^{-1}. IDL and instrument variability was determined using similar methods to Coggan, Anumol [46] on the same instrument and using the same instrument configuration.

A field blank was prepared with every kit, transferred to a clean bottle on-site and then extracted concurrently with samples. Field blanks were extracted within the same batches as samples and matched with corresponding WWTPs. Only one compound (PFBA) was detected above LOD in field blanks from treatment plants WWTP-7 and WWTP-17; due to this, PFBA results for these two treatment plants were set as < LOD.

Aqueous samples were extracted in batches containing two method blanks and a laboratory control sample (LCS). Laboratory control samples consisted of ultrapure water spiked with a native PFAS mixture containing all measured compounds at a mass of 5 ng, 1 ng or 0.25 ng. Mean recovery of all compounds in LCS samples ranged from 80 to 120% with s.d. < 15%, except for PFDS (72%, s.d. 13%), 8:2 Cl-PFESA (73%, s.d. 7%), PFTrA (70%, s.d. 6%) and PFTeA (76%, s.d. 6%) (Table S2). Solid LCS samples consisted of acid-washed sand spiked with 10 ng of PFAS and extracted alongside batches of 12 samples. Mean recovery of all compounds in LCS samples ranged from 80 to 120% with s.d. < 15%, except for 6:2 FTS (61% s.d. 8%). Method blanks returned less than the limit of detection (<LOD) for all batches. The use of ultrapure water and acid-washed sand as laboratory control samples may not adequately represent WWTP matrices (and the associated interferences) and present some uncertainty with analytical results. However, similar methods have been successfully employed in WWTP matrices in other studies [32, 46, 47] and overall we considered the QA/QC results provided an acceptable assurance of the quality of the data set for this study.

2.7. Data processing

Quantitation was carried out using MassHunter QQQ quantitative analysis software (version 08.00, Agilent Technologies, USA). Descriptive statistics were computed using pooled data from all samples.

Statistical analysis was carried out using R [48]. Data visualizations were also produced in R [48] using the packages reshape2 [49] and ggplot2 [50].

To compare distribution coefficients to those previously published in Eriksson, Haglund [32] and Sun, Zhang [51], similar estimation methods were employed. This calculation method is only an approximation as it assumed that the concentration of PFAS in solids and liquids at the sampled location were in equilibrium and does not consider the differences in effluent and sludge retention times. Distribution coefficients (log K_d) were calculated for the compounds PFHxA, PFHxS, 6:2 FTS, PFOA, PFNA, PFOS, and PFDA. Distribution coefficients were only calculated for these compounds at sample locations where both aqueous and solid samples were above the limit of quantitation.

Due to non-normal distributions, data for 11 compounds were first log_{10}-transformed. Pearson correlation coefficients were computed using the transformed, pooled, influent and pooled final effluent data. Linear mixed-effects analysis was performed on the transformed data using the R package lme4 [52]. P-values were obtained by likelihood ratio tests of the full model with treatment stage (influent and final effluent) included against the model without the effect in question. PCA analysis was performed on the untransformed data for the 11 compounds plus percentage trade waste and daily inflow using the correlation matrix (standardised) and visualised in R using the package factoextra [53] and ggplot2 [50].

3. Results and discussion

3.1. PFAS in WWTP matrices

Twenty-one PFAS from four classes (PFCA, PFSA, FTS, Cl-PFESA) were measured in aqueous (n = 201) and solid (n = 51) samples from the 19 Australian WWTPs. PFAS were detected in all samples from all matrices. The summary statistics are presented in Table 2 and the data is further provided in the Supplementary Information, Table S3, and Table S4.

Table 2
Summary statistics for pooled aqueous (n = 201, triplicates from 67 individual locations within 19 WWTPs) and pooled solid (n = 51, triplicates from 5 primary and secondary sludge locations, 6 lagoon sludges and a lagoon dredge pile) samples. The sum of branched plus linear isomers was reported for PFPeS, PFHxS, PFHpS, and PFOS.

	Aqueous samples (ng L^{-1})						Solid samples (ng g^{-1} dw)					
	Median	Mean	s.d.	min	Max	Detect (%)	Median	Mean	s.d.	min	max	Detect (%)
PFBA	5.8	13	33	<LOQ	370	100%	<LOD	0.45	0.91	<LOD	4.1	29%
PFPeA	5.3	8.3	8.8	<LOD	47	96%	<LOD	<LOQ		<LOD	5.2	20%
PFHxA	16	21	17	1.4	92	100%	0.92	1.9	2.8	<LOD	13	82%
PFHpA	5.0	6.1	5.1	<LOD	34	100%	<LOQ	0.30	0.66	<LOD	4.1	54%
PFOA	11	19	19	1.0	91	100%	<LOQ	2.6	4.4	<LOD	25	84%
PFNA	0.60	0.92	1.1	<LOD	6.6	97%	<LOQ	0.20	0.29	<LOD	1.1	50%
PFDA	1.3	2.3	2.9	<LOD	18	98%	0.60	5.1	7.7	<LOD	26	84%
PFUdA	<LOD	<LOQ		<LOD	<LOQ	12%	<LOQ	<LOQ		<LOD	1.2	54%
PFDoA	<LOD	0.47	0.55	<LOD	4.2	49%	0.48	3.8	5.9	<LOD	20	94%
PFTrA	<LOD	<LOQ		<LOD	<LOQ	19%	<LOQ	0.32	0.48	<LOD	1.8	70%
PFTeA	<LOD	0.27	0.19	<LOD	2.0	25%	<LOQ	0.69	1.1	<LOD	4.6	90%
PFBS	2.5	4.0	4.9	<LOD	33	98%	<LOD	0.83	2.0	<LOD	9.3	44%
PFPeS	<LOQ	1.9	4.1	<LOD	27	77%	<LOD	<LOQ		<LOD	2.3	14%
PFHxS	3.1	13	31	<LOD	200	95%	<LOQ	1.1	2.8	<LOD	17	50%
PFHpS	<LOQ	0.86	1.7	<LOD	11	76%	<LOD	0.29	0.67	<LOD	3.3	26%
PFOS	7.2	15	24	<LOD	140	99%	4.7	14	24	<LOD	90	94%
PFDS	<LOD	0.21	0.13	<LOD	1.1	23%	<LOD	0.78	2.1	<LOD	9.8	42%
6:2 FTS	2.4	7.3	12	<LOD	61	99%	<LOD	0.26	0.69	<LOD	2.7	26%
8:2 FTS	<LOQ	0.53	1.1	<LOD	9.2	82%	<LOD	0.73	1.6	<LOD	6.9	42%
6:2 Cl-PFESA	<LOD	<LOQ		<LOD	<LOQ	4%	<LOD	<LOQ		<LOD	<LOQ	16%
8:2 Cl-PFESA	<LOD	<LOD		<LOD	<LOD	0%	<LOD	<LOQ		<LOD	<LOQ	8%
$\sum_{21}$PFAS	80	110		9.3	520		12	34		2.0	130	

3.1.1. *Aqueous matrices*

The mean $\sum_{21}$PFAS in aqueous samples was 110 ng L^{-1} (median: 80 ng L^{-1}; range: 9.3–520 ng L^{-1}) (Table 2). The highest concentration measured in aqueous matrices for any compound was 370 ng L^{-1} for PFBA in final effluent at WWTP-12. PFBA has been used as a short-chain PFAS substitute for some PFCAs [54]. The high concentration of PFBA in final effluent and distribution within the sampled WWTPs may reflect current PFBA use.

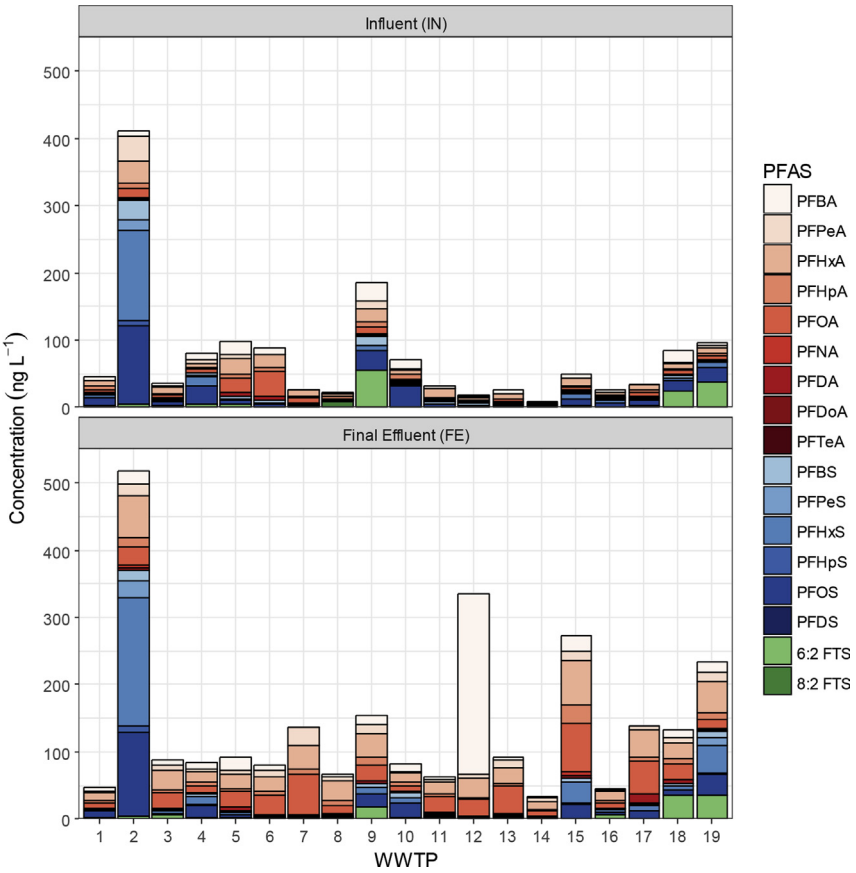

Fig. 1. Mean PFAS concentration (n = 3 replicates) in 19 WWTPs from influent (top panel) and final effluent (bottom panel) sampling points. PFUdA, PFTrA, 6:2 Cl-PFESA and 8:2 Cl-PFESA are not plotted as all values were <LOQ.

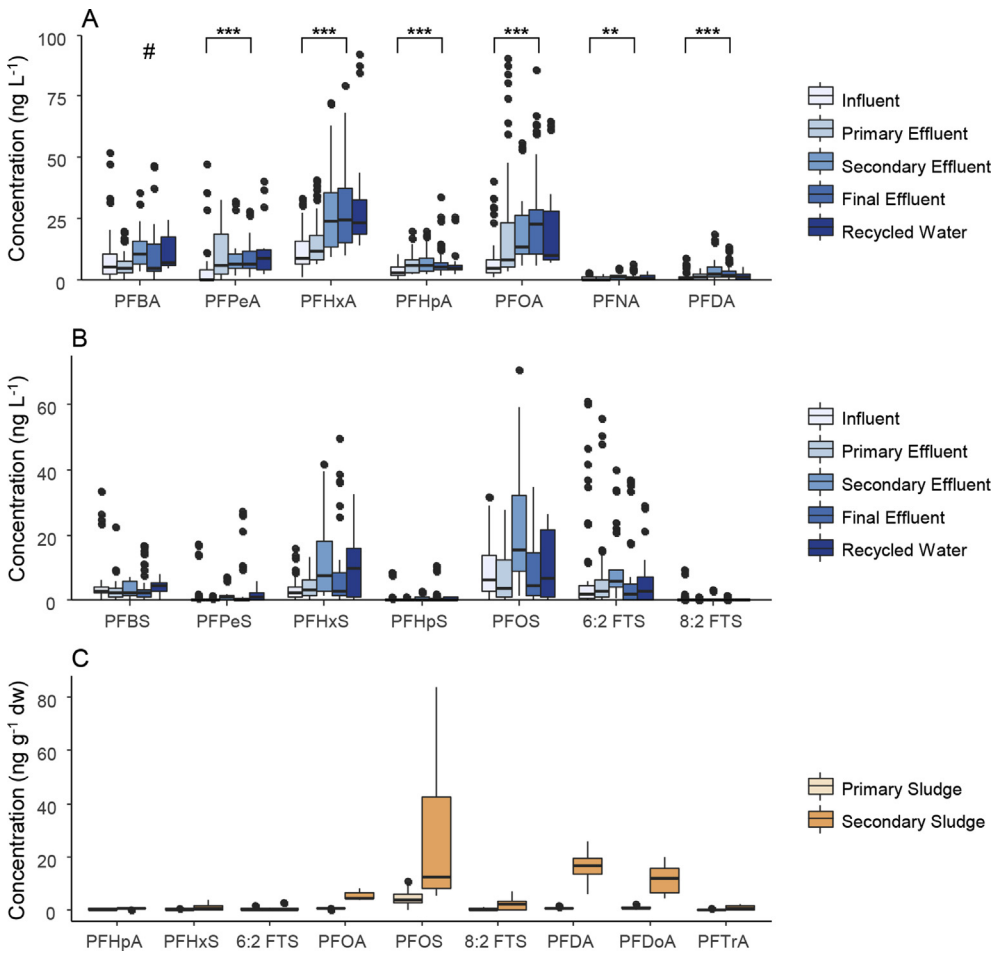

Fig. 2. Boxplots of pooled data from 19 WWTPs for A) PFCAs (perfluorocarboxylic acids); B) PFSAs (perfluorosulfonates) and FTSs (fluorotelomer sulfonates) in aqueous samples; C) selected PFAS in solid samples. Aqueous sample locations were influent (n = 57), primary effluent (n = 39), secondary effluent (n = 24), final effluent (n = 57) and recycled water (n = 24). Solid sample locations were primary sludge (n = 15) and secondary sludge (n = 15). # indicates concentration outside y-axis range for PFBA. Asterisk (*) indicates significant difference (<0.01 = **; <0.001 = ***) between influent and final effluent concentrations when tested using linear mixed effects analysis.

Eleven of the 21 compounds were detected in >90% of samples (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, 6:2 FTS), and these were used in the subsequent statistical analysis of influent and final effluent. Mean concentrations in aqueous samples followed the trend: PFHxA > PFOA > PFOS > PFHxS > PFBA > PFPeA>6:2 FTS > PFHpA > PFBS > PFDA > PFPeS > PFNA > PFHpS>8:2 FTS > PFTeA > PFDS. Mean concentrations of PFAS in aqueous samples were similar to concentrations previously reported for Australian WWTP aqueous samples [37, 41, 43]. To date concentrations of PFBA and 6:2 FTS have not been widely reported in Australian WWTPs. PFBA and 6:2 FTS have been used as C8 substitutes and are end-stage and intermediate metabolites (respectively) of many PFAS, their prevalence in the studied WWTPs may be an indicator of changing PFAS use trends in Australia.

WWTP-2 had the highest $\sum_{21}$PFAS in influent and final effluent, with concentrations of 410 and 520 ng L^{-1}, respectively. Major contributors to $\sum_{21}$PFAS loading at WWTP-2 were PFHxS (influent 130 ng L^{-1}, effluent 190 ng L^{-1}) and PFOS (influent 120 ng L^{-1}, effluent 130 ng L^{-1}) (Fig. 1). The WWTP operator reported that approximately 45% of the inflow at WWTP-2 is attributed to baseflows, and largely a result of groundwater infiltration. Furthermore, WWTP-2 is within a highly industrialised catchment which may be causing elevated $\sum_{21}$PFAS levels in both groundwater and influent. Final effluent from WWTP-2 is mixed with reverse osmosis (RO) reject water. The RO process is an effective long-chain PFAA treatment, removing them from effluent then partitioning them to the RO reject water [41]. Therefore, the re-introduction of RO reject water at this WWTP is likely contributing to the elevated PFAS concentrations observed in the final effluent.

3.1.2. Solid matrices

PFAS were detected in all WWTP solid samples, and the mean $\sum_{21}$PFAS in solid samples was 34 ng g^{-1} dw (median: 12 ng g^{-1} dw; range: 2.0–130 ng g^{-1} dw) (Table 2). Mean concentrations of PFAS in solids followed the trend PFOS > PFDA > PFDoA > PFOA > PFHxA > PFHxS > PFBS > PFDS>8:2 FTS > PFTeA > PFBA > PFHpA > PFHpS>6:2 FTS. The compounds PFOS, PFDoA, and PFTeA were detected in >90% of samples, while the compounds PFOA, PFDA, PFHxA, and PFTrA were detected in 70–90% of solid samples. Six of the seven compounds with detection frequencies above 70% had a carbon chain length of eight or higher. The increased partitioning of PFAS to the solid phase within WWTPs has been associated with increasing fluoroalkyl chain length [11, 51, 55]. The calculated mean partitioning coefficients from this study reflected this trend, increasing with increased fluoroalkyl chain length, and were higher in PFSAs compared to PFCAs of similar carbon chain length; except for 6:2 FTS which displayed the lowest mean partitioning coefficient, being primarily partitioned to the aqueous phase (Table S5).

The highest mean concentration found in WWTP solids was for PFOS (mean: 14; median: 4.7; range < LOD - 90 ng g^{-1} dw). The lagoon-based treatment plant sludge and AS primary sludge displayed low PFAS concentrations compared to AS secondary sludge. The process of concentrating and recycling AS secondary sludge through the treatment process, combined with the aeration/agitation provided, likely facilitates increased secondary sludge PFAS concentrations.

3.1.3. Detection of the PFOS alternatives 6:2 FTS and F–53B

6:2 FTS was detected in 99% of aqueous samples (mean 7.3 ng L^{-1}) and 26% of solid samples (mean 0.26 ng g^{-1} dw), At three of the larger

WWTPs (-9, 330 ML day^{-1}; -18, 143 ML day^{-1}; and -19, 498 ML day^{-1}), elevated total PFAS loading was a result of increased 6:2 FTS in influent (56, 23 and 38 ng L^{-1}, respectively). 6:2 FTS has been employed in aqueous film-forming firefighting foam (AFFF) in mixtures with fluoroalkylthioamido sulfonates [56], used as a PFOS replacement in metal plating applications [57] and is a transformation intermediate in the degradation of more complex fluorotelomer-based compounds [4]. High concentrations of 6:2 FTS in WWTP effluents have been associated with AFFF use in catchments in the USA [33]. It is possible that elevated levels of 6:2 FTS observed may be associated with AFFF use or PFOS substitution in metal plating, however, it more likely indicates the presence of a range of not yet measured precursor compounds with 6:2 FTS as an intermediate degradation product.

The compound 6:2 Cl-PFESA was only detected in 4% of aqueous samples and 16% of solid samples between LOD and LOQ (Table 2). The compound 8:2 Cl-PFESA was not detected above LOD in any aqueous sample, and in 8% of solid samples between LOD and LOQ. These compounds have been demonstrated as the major components of the commercial product F–53B after purification, with a reported 6:2 Cl-PFESA content of 77.6% and 8:2 Cl-PFESA comprising an unreported percentage of the remaining fraction [19]. F–53B is used as a PFOS alternative for mist suppression in metal plating applications used in China that has recently been detected in Chinese WWTPs and the environment [19, 24, 25]. In Australia between 2006 and 2007, 99% of the directly imported PFOS was for use as a mist suppressant in metal plating which is listed as an approved, essential use [58]. The Australian metal plating industry has no need to switch to alternatives like F–53B as PFOS is still approved for use. The low F–53B concentrations detected in this study may be a result of contamination of products sourced from markets that utilise F–53B.

3.1.4. Distribution within WWTPs

PFAS concentrations generally increased in both aqueous and solid matrices through the wastewater treatment process (Fig. 2). The mean concentration of $\sum_{21}$PFAS increased as wastewater treatment progressed from influent, to primary effluent, secondary effluent, final effluent and recycled water (76, 89, 140, 140 and 120 ng L^{-1}, respectively). PFCA concentrations in aqueous samples also increased from influent to final effluent, with levels persisting in recycled water, whilst PFSA concentrations within treatment plants varied. In influent, PFOS had the highest mean concentration (17 ng L^{-1}) (Table S4). PFOA had the highest mean concentration in primary effluent (23 ng L^{-1}), displaying an increase from mean influent concentration (7.9 ng L^{-1}). PFHxA had the highest mean concentration from pooled aqueous samples in secondary effluent, final effluent, and recycled water; increasing in concentration from influent to primary, secondary and final effluent and recycled water (11, 16, 28, 28 and 32 ng L^{-1}, respectively).

Due to the delay of transmission of PFAS (caused by hydraulic retention time) within a wastewater treatment plant, the comparison of influent and effluent over the same 24-hour period may not be directly applicable. There was, however, a large variation in all PFAS concentrations between and within treatment plants from influent to final effluent. In 16 of the 19 WWTPs, $\sum_{21}$PFAS concentrations in final effluent were greater than influent at the same WWTP, which is consistent with trends in WWTPs worldwide [11]. At WWTPs-5, 6 and 9, $\sum_{21}$PFAS concentration was greater in influent than final effluent and largely due to PFSA and FTS concentrations.

Linear mixed-effects analysis of pooled influent and effluent data confirmed that some PFAS concentrations increased between influent and final effluent (Fig. 2). Between influent and final effluent, the compounds PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA (all of which are PFCAs) increased significantly. A number of transformation pathways with stable PFCA endproducts are known [18], this may explain some of the increase in PFCAs from influent to final effluent.

Microbial degradation of the compounds 6:2 PAP and 6:2 diPAP using WWTP aerobic microbes has been shown to produce 6:2 FTOH, which was then degraded further to PFHxA [59]. Furthermore, degradation of the compound 6:2 FTOH in activated sludge has been demonstrated to produce the corresponding 5:3 acid, which is then degraded further to PFHxA [34]. Little transformation of 6:2 FTOH to PFPeA was observed as the intermediary product 5:2s FTOH was likely volatilized before biotransformation could occur. This may explain the high concentrations of PFHxA compared to PFPeA (whose precursor is partitioned to the gas phase) observed in this study. PFOA has been observed as a microbial transformation product of 8:2 diPAP in soil [60] and in gilthead bream [61]. Furthermore, both PFOA and PFHxA have displayed net positive increases from influent to effluent, associated with diPAP and unknown PFAS precursor degradation in WWTP influent and sludge in three Swedish WWTPs [32]. It is likely that similar precursor transformation processes are occurring within our studied WWTPs, contributing to increased PFCA concentrations as treatment progresses.

The concentration of PFOS, PFDA, and PFDoA was higher in sludge, compared to other PFAS (Table S4). The median concentration of PFOS, PFDA, and PFDoA increased between primary and secondary sludge from 3,8 – 12, <LOQ – 17 and <LOQ – 14 ng g^{-1} dw, respectively (Fig. 2, Table S4). This increase between primary and secondary sludge was also reflected in the calculated distribution coefficients (Table S5); where coefficients increased between primary and secondary locations by 0.17–1.22 log units for PFOS and 0.37 to 1.34 log units for PFDA.

3.2. Trends, correlations, and transformation

Pearson correlation coefficients were positive for all PFAS measured in influent (Figure S1). In influent, positive, strong ($r > 0.70$) and significant ($p < 0.05$) correlations were displayed between compounds within the same compound class PFCAs (PFHxA-PFOA, PFHxA-PFNA, PFHxA-PFDA, and PFOA-PFDA) and between PFPeA-6:2 FTS and PFPeA-PFHxS.

In final effluent, there were significant, positive, strong correlations between PFPeA-PFHxA, PFPeA-PFOA, PFHxA-PFOA, PFHxA-PFNA, PFHpA-PFHxA, PFHpA-PFNA, PFHpA-PFHxS, PFHpA-PFOS, and PFHxS-PFOS. There was only one significant negative correlation between 6:2 FTS-PFOA ($r = 0.3$). There were no significant correlations for the following: 1) PFBA and all other compounds; 2) 6:2 FTS and 5 of the eleven compounds; 3) PFBS-PFOA and PFBS-PFDA; 4) PFHxS-PFPeA and PFHxS-PFOA; 5) PFOS-PFPeA, PFOS-PFOA, and PFOS-PFDA. PFCAs and PFSAs were not strongly correlated in final effluent. This implies the distribution of PFCAs and PFSAs are WWTP specific and vary in final effluent independently of each other.

There were four principal components in the influent data, and five principal components in the final effluent data with eigenvalues above 1 (Fig. 3). For the influent data, the first four components explained 93.2% of the variation (47.8, 20.2, 15.9 and 7.32%, respectively). In component 1, the PFSAs (PFBS, PFHxS, PFOS), odd chained PFCAs (PFPeA, PFHpA, and PFNA), and PFHxA displayed strong associations and accounted for a large proportion of the variation within the data. Short-odd chain PFCAs (<C8) and PFHxA have been associated as impurities, degradants and metabolites of the short-chain fluorochemistries used to replace PFOA [4, 18, 62] Furthermore, in Australia, PFOS is still employed in approved uses and there are no current restrictions on PFHxS or PFBS [13]. The strong associations of these compounds and their contribution to the observed variation in influent data may reflect Australian PFAS usage trends and PFAS loading within specific WWTP catchments. Principal component 2 showed strong associations between WWTP daily inflow, percentage trade waste, and 6:2 FTS. This strong association was largely a result of the larger WWTPs accepting a higher proportion of trade waste, however, it shows the importance of 6:2 FTS as a possible trade waste indicator in these Australian WWTPs. In component 3, PFOA and PFDA were highly associated and in component 4, PFBA was the main contributor to the variation observed. This may indicate that these three compounds behave independently of each of in respect to PFAS loading in influent.

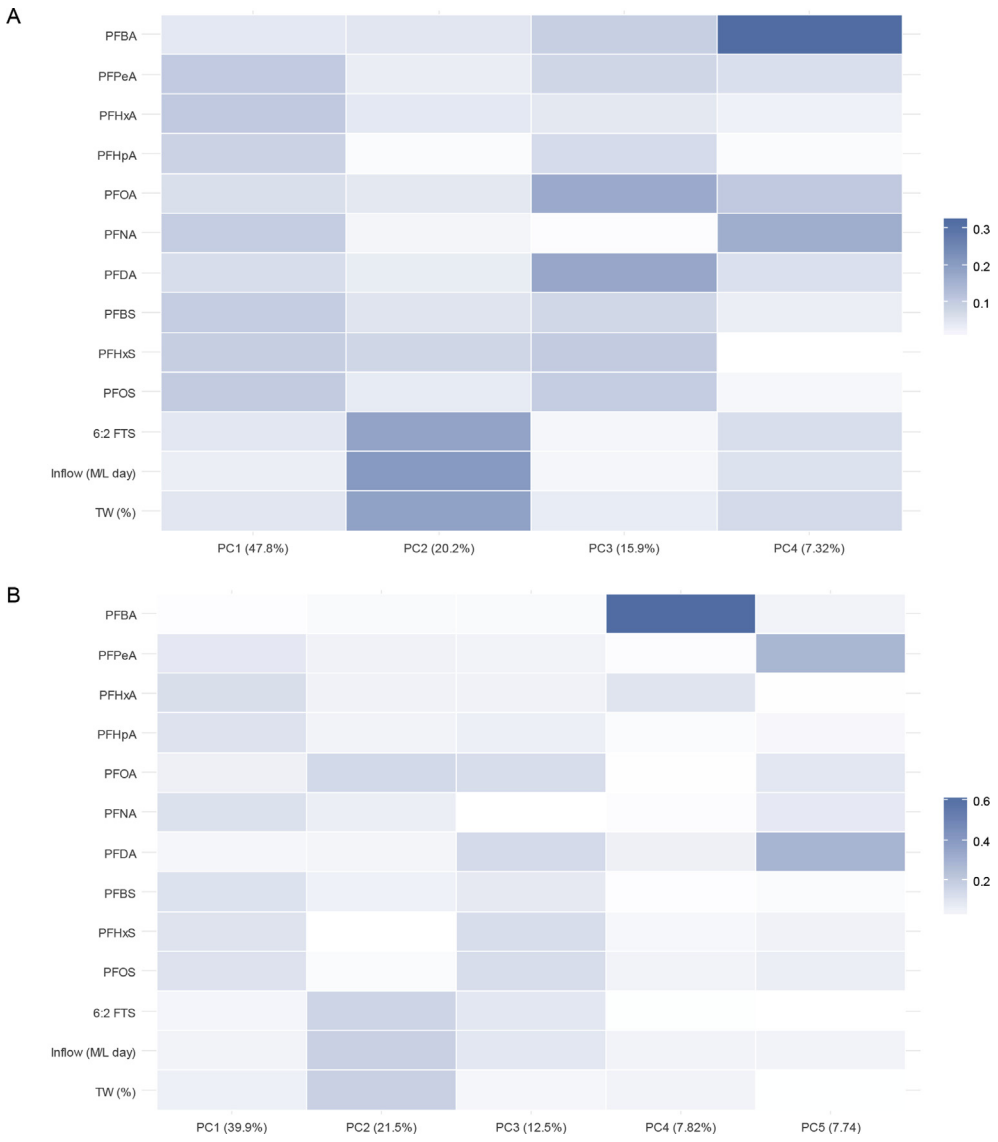

Fig. 3. Heatmap of PCA results for principal components computed using the correlation matrix (scaled) and including average daily inflow and proportion of trade waste in inflow. Components with eigenvalues above 1 from influent (A) and final effluent (B) are displayed.

For the final effluent data, the five principal components accounted for 89.5% of the variation (39.9, 21.5, 12.5, 7.82 and 7.74%, respectively). In component 1 of the final effluent, there were strong associations between PFSAs (PFBS, PFHxS, PFOS), odd chained PFCAs (PFPeA, PFHpA, and PFNA), and PFHxA. In principal component 2, there was again a strong association between daily inflow volume, percentage trade waste, and 6:2 FTS (an intermediate degradant from C6 based precursors), with the addition of PFOA. There are many demonstrated transformation pathways with PFOA as the terminal end-product [63], and the significant increase of PFOA from influent to final effluent may reflect this. The strong association of PFOA with 6:2 FTS (which showed no significant change between influent and final effluent), inflow and percentage trade waste may be a result of degradation of PFOA precursors (likely as impurities from the C6 manufacture process) associated with trade waste that are not yet measured in influent at these WWTPs. In component 3, there were strong associations between PFOS, PFHxS, PFOA, and PFDA, all of which have been used extensively in the past [4]. In principle component 4, PFBA was the main contributor to variation, and behaved independently of the other PFCAs, reflecting the trend seen in influent. The compounds PFPeA and PFDA were strongly associated, accounting for a small amount of the variation in component 5.

3.3. Environmental discharge in final effluent

Calculation of the estimated annual discharge at a WWTP from a single sampling campaign contains a high uncertainty due to daily and seasonal variation [64]. In Australian WWTPs, temporal variation of PFAS in influent [43] and effluent [37] has been shown to be low; with observed temporal variation in influent being more likely from pulse release as opposed to seasonal factors [43]. It is, however, useful to estimate annual discharge to compare to similar Australian studies. Daily discharge rates from the 19 WWTPs in this study varied greatly, were similar to previous Australian studies, similar to studies worldwide and were influenced primarily by daily inflow (and as an extension WWTP size; Table S6).

PFOS and PFOA concentrations were similar to those measured in 2014 from a study of nine PFAS in effluent from in 14 Australian WWTPs [37]. In their study, they estimated a national $\sum_9$PFAS discharge from Australian WWTPs as 175 kg per year in Australian WWTP effluent [37]. Assuming the same annual discharge volume of 3013 GL and using mean annual discharge rates from the 19 WWTPs in this study, we calculated an estimated discharge of $\sum_{21}$PFAS of 339 kg. When compared, their study and our study produce similar yearly mass discharged for PFOA,

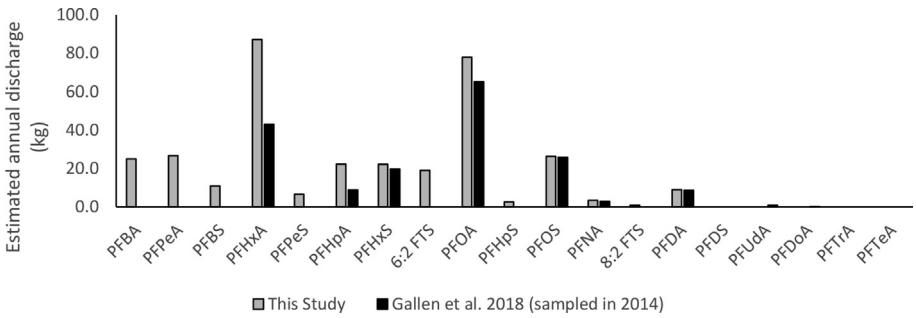

Fig. 4. Comparison of estimated annual discharge (kg) of PFAS from Australian WWTPs in this study and from Gallen, Eaglesham [37].

PFNA, PFDA, PFHxS and PFOS (Fig. 4). In our study the estimated yearly mass discharged was higher for PFHpA (8.8 vs 22 kg annually) and PFHxA (43 vs 87 kg annually). This difference may be a result of changing PFAS use patterns or bias introduced through the WWTPs selected and sampling design each study.

The annual mass discharge of three compounds not measured in [37]: PFBA, PFPeA and 6:2 FTS (25, 27 and 19 kg annually), were similar to that of PFOS calculated for both studies (26 vs 26 kg annually). If this is the case for three compounds, and as there are now over 4700 listed PFAS in the environment [10], it is likely both studies have underestimated the total PFAS emissions from Australian WWTPs.

4. Conclusions

Twenty-one PFAS from four classes (PFCAs, PFSA, FTS, F–53B) were measured in aqueous and solid samples from the 19 Australian WWTPs. PFAS was detected in every sample analysed. Many PFAS were highly correlated, suggesting similar sources of PFSAs and PFCAs and independent behavior of these compound classes within WWTPs. Statistical analyses showed an increase of PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA between influent and final effluent. When compared to Australian WWTP PFAS emission data measured in 2014, the estimated annual discharge for the newly reported compounds PFBA, PFPeA and 6:2 FTS (25, 27 and 19 kg annually) were similar to PFOS (26 kg annually). This demonstrated that it is likely both studies have significantly underestimated the total PFAS emissions from Australian WWTPs and future work is required to determine the risk profile of PFAS present and total PFAS loading at Australian WWTPs.

The compounds 6:2 FTS and 8:2 FTS quantified, and F–53B components 6:2 Cl-PFESA and 8:2 Cl-PFESA were detected in Australian WWTPs. 6:2 FTS was strongly associated with the proportion of trade waste in influent, was partitioned to the aqueous phase, had a similar estimated Australia-wide annual mass discharged in effluent to PFOS, and did not significantly decrease between influent and final effluent. Although the ecological risk of 6:2 FTS is considered low, there are many unknowns regarding the environmental fate and effects and its presence likely indicates the degradation of currently employed short-chain fluorochemistries. In Australia, the presence of 6:2 FTS may be an emerging concern in Australian WWTPs and aqueous environments receiving WWTP effluent.

Declarations

Author contribution statement

Timothy Coggan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Damien Moodie: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Adam Kolobaric, Drew Szabo: Performed the experiments.

Jeff Shimeta, Nicholas Crosbie, Elliot Lee, Milena Fernandes: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Bradley Clarke: Conceived and designed the experiments.

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Competing interest statement

The authors declare the following conflict of interests: Nicholas Crosbie is an employee of Melbourne Water who provided samples and funding. Elliot Lee is an employee of Water Corporation who provided samples and funding. Milena Fernandes is an employee of SA Water who provided samples and funding. Timothy Coggan, Damien Moodie and Drew Szabo have scholarship support from Melbourne Water through Water Research Australia. Bradley Clarke receives some research funding from Melbourne Water. Jeff Shimeta and Bradley Clarke are PhD supervisors for Timothy Coggan, Drew Szabo and Damien Moodie. Adam Kolobaric declares no conflicts of interest.

Additional information

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PFAS Contamination Class Action

From the 1970s to at least 2004, the Australian Defence Force (ADF) regularly conducted firefighting drills using a type of firefighting foam known as Aqueous Film Forming Foam. This foam consists of toxic chemicals known as 'PFAS'.

The PFAS Contamination Class Action alleged that the Department of Defence negligently allowed toxic chemicals known as 'PFAS' to escape from defence bases and contaminate local environments. These contaminants negatively impacted properties, land values and the livelihoods of surrounding communities.

In May 2023, Shine Lawyers reached an agreement with the Department of Defence, successfully achieving justice for the residents in Bullsbrook, Townsville, Darwin, Richmond, Wagga Wagga, Wodonga and Edinburgh that were affected by PFAS contamination.

On 25 August 2023, the Federal Court approved the $132.7 million settlement and distribution scheme which determined how compensation is distributed to

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Frequently Asked Questions

What is PFAS?

Is it possible to claim for personal injury?

Who is the claim against?

What is the difference between becoming a 'Represented Class Member' and a 'Registered Unrepresented Class Member'?

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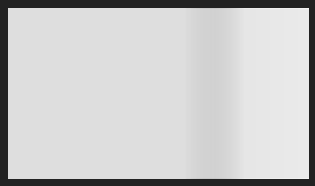

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Shine Lawyers acknowledges the Traditional Custodians of the Country throughout Australia and their connections to land, waterways and community. We pay our respects to Aboriginal and Torres Strait Islander cultures; and to Elders past, present and emerging.

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