Environment International 130 (2019) 104851

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review article

PFAS health effects database: Protocol for a systematic evidence map

Katherine E. Pelch[a,*], Anna Reade[b], Taylor A.M. Wolffe[c], Carol F. Kwiatkowski[a,d]

[a] The Endocrine Disruption Exchange, PO Box 54, Eckert, CO, USA
[b] Healthy People & Thriving Communities Program, Natural Resources Defense Council, 111 Sutter Street, Floor 21, San Francisco, CA, USA
[c] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK
[d] Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

ARTICLE INFO

Edited by Hanna Boogaard

Keywords:
PFAS
Per- and polyfluoroalkyl substances
Systematic evidence map
Toxicology

ABSTRACT

Background: *Per-* and polyfluoroalkyl substances (PFAS) confer waterproof, greaseproof, and non-stick properties when added to consumer products. They are also used for industrial purposes including in aqueous film forming foams for firefighting. PFAS are ubiquitous in the environment, are widely detected in human biomonitoring studies, and are of growing regulatory concern across federal, state, and local governments. Regulators, scientists, and citizens need to stay informed on the growing health and toxicology literature related to PFAS.
Objectives: The goal of this systematic evidence map is to identify and organize the available health and toxicology related literature on a set of 29 PFAS of emerging and growing concern.
Search and study eligibility: We will search the electronic database PubMed for health or toxicological studies on 29 PFAS of emerging concern. Eligible studies must contain primary research investigating the link between one or more of the PFAS of interest and a health effect, toxicological, or biological mechanistic endpoint.
Study appraisal and synthesis methods: Title and abstract screening and full text review will require a single reviewer for inclusion to the next level and two independent reviewers for exclusion. Study quality will not be conducted for this evidence mapping. Study characteristics will be extracted and coded from the included studies and checked for accuracy by a second reviewer. The extracted and coded information will be visualized in a publicly available, interactive database hosted on Tableau Public. Results of the evidence mapping will be published in a narrative summary.

1. Introduction

1.1. Rationale

Over the past few decades per- and polyfluoroalkyl substances (PFAS) contamination has grown into a serious global health threat. PFAS are a large class of synthetic chemicals that contain an alkyl chain with at least one fully fluorinated carbon atom. Although the class is broad, they are related in their extreme persistence in our environment and are often referred to as “forever chemicals”. PFAS are also highly mobile in the environment and some have been found to bioaccumulate, or build up, in humans and animals.

Best known for their original use in producing the fluoropolymer Teflon and the stain-resistant coating Scotchgard, these chemicals are now used in a wide range of consumer and industrial products where grease or water proofing is desired, or surfactant action is a benefit. These products include food packaging and non-stick cookware, cosmetics, waterproof and stain-proof textiles and carpet, aqueous film forming foam (AFFF) to fight Class B fires, and as part of metal plating processes.

Widespread use of PFAS has resulted in the ubiquitous presence of these chemicals in the environment including in rivers, soil, air, house dust, food and drinking water from surface water and groundwater sources. Virtually all Americans have multiple PFAS at detectable levels in the blood serum (CDC, 2018). Unfortunately, PFAS have been linked to many harmful health effects, including cancer, immune system

Abbreviations: ADME/PK/TK, absorption, distribution, metabolism, excretion, pharmacokinetic or toxicokinetic properties; AFFF, aqueous film forming foam; AI, artificial intelligence; ATSDR, Agency for Toxic Substances and Disease Registry; COI, conflict of interest; EPA, US Environmental Protection Agency; hpf, hours post-fertilization; MCL, maximum contaminant level; NJDWQI, New Jersey Drinking Water Quality Institute; NTP, National Toxicology Program; PECO, populations, exposures, comparators, and outcomes; PFAS, per- and polyfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; PPAR, peroxisome proliferator activated receptor; ppt, part per trillion; QC, quality control

* Corresponding author.
E-mail addresses: katiepelch@tedx.org (K.E. Pelch), areade@nrdc.org (A. Reade), t.wolffe@lancaster.ac.uk (T.A.M. Wolffe), carolkw@tedx.org (C.F. Kwiatkowski).

https://doi.org/10.1016/j.envint.2019.05.045
Received 19 February 2019; Received in revised form 15 May 2019; Accepted 16 May 2019
Available online 05 July 2019

dysfunction, liver damage, developmental and reproductive harm, and hormone disruption (ATSDR, 2018).

The most well-known and well-studied PFAS are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) (ATSDR, 2018). Due to increasing concern over the harm these chemicals cause to human health, wildlife, and the environment, the U.S. Environmental Protection Agency initiated the PFOA Stewardship Program in 2006 (US EPA, 2006). Through the program, the major PFAS manufacturing companies committed to phasing out PFOA, its precursor chemicals and related higher homologue chemicals from production in the US by 2015; however, PFOA and PFOS are still produced internationally and consumer products containing PFOA and related-PFAS may still be imported into the US (US EPA, n.d.) This, in combination with their extreme persistence in the environment, ensures that their legacy remains.

The scientific literature on PFAS has increased exponentially in the last decade, which has resulted in a greater understanding of the potential adverse health effects associated with PFOA and PFOS exposure (Grandjean, 2018). For PFOA and PFOS this has resulted in increasingly stricter health thresholds proposed by various agencies (Cordner et al., 2019). In 2016 the EPA issued lifetime drinking water health advisories of 70 ppt for PFOA and PFOS, individually or combined (US EPA, 2016b, 2016c). Recently several states (*e.g.* MN, NH, NJ, VT, MI) have proposed drinking water regulatory or guideline levels below 70 ppt (Cordner et al., 2019; MDHHS, 2019; NHDES, 2019).

For various reasons, including uncertainties in data and biological significance, the EPA did not select the most sensitive health effects currently associated with PFOA and PFOS when generating their 2016 health advisories. There is evidence that both altered mammary gland development for PFOA (Macon et al., 2011; Tucker et al., 2015; White et al., 2011) and immunotoxicity for PFOS (Dong et al., 2009; Grandjean and Budtz-Jorgensen, 2013; Guruge et al., 2006; Peden-Adams et al., 2008) can occur at levels an order of magnitude or lower than the health effects selected by the EPA. Since the EPA issued its 2016 advisories, the National Toxicology Program (NTP) released a report concluding that both PFOA and PFOS are presumed to constitute immune hazards to humans (NTP, 2016). And most recently, the New Jersey Drinking Water Quality Institute (NJDWQI) and the Agency for Toxic Substances and Disease Registry (ATSDR) have either acknowledged or attempted to account for these more sensitive health effects in generating their proposed health standards (ATSDR, 2018; NJDWQI, 2017; NJDWQI, 2018). As a result, both NJDWQI and ATSDR have proposed significantly more protective (5–10 times lower) health thresholds for PFOA and PFOS than the EPA health advisories (ATSDR, 2018; NJDWQI, 2017; NJDWQI, 2018).

The expansion of research on PFAS has also resulted in increasing concern over the rising use of and exposure to replacements for legacy PFAS. Most legacy PFAS, including PFOA and PFOS, are “long-chain” chemicals, meaning their molecular structure contains a chain of six (for perfluoroalkyl sulfonic acids) or seven (for perfluoroalkyl carboxylic acids) or more carbon atoms. While there is less toxicity data on shorter-chain and other alternative PFAS replacing long-chain PFAS, evidence is growing quickly that indicates they collectively pose similar threats to human health and the environment; which, combined with similar concerns over the environmental fate and persistence, have led independent scientists and other professionals from around the globe to express concern about the continued and increasing production and release of PFAS (Blum et al., 2015; Scheringer et al., 2014).

Due to the health concerns related to PFAS exposure and concerns over their environmental fate and persistence, there have been various efforts at the local, state and federal level to regulate PFAS. For example, severe contamination of drinking water with both legacy and alternative PFAS in communities across the nation, has led to considerable efforts at the state-level to set enforceable drinking water maximum contaminant levels (MCLs). It is expected that efforts to regulate PFAS in drinking water (as well as in ground and surface waters, air, consumer products, *etc.*) will continue over the coming years. Staying abreast of the current PFAS health effects literature is a major barrier for setting effective regulations to protect human and environmental health. Further, as additional communities learn of their own PFAS contamination, there is a desire from citizens and citizen-led groups to know more about these chemicals and how they may impact the health of their communities.

The ATSDR Draft Toxicological Profile for Perfluoroalkyls provides estimates concerning the volume of available human and experimental animal studies through May 2016 for PFOA (Fig. 2-1; $n = 271$), PFOS (Fig. 2-2; $n = 218$) and 12 additional PFAS (Fig. 2-3; $n = 127$) (ATSDR, 2018). Though helpful, the figures provided by ATSDR do not allow the end-user much flexibility in sorting, filtering, or deeply exploring the available evidence. Additionally, ATSDR Fig. 2-3 presents the evidence for 12 PFAS of emerging interest, but it is not possible to determine how the identified studies are distributed among the chemicals, which limits its utility to state agencies proposing regulatory values for individual PFAS beyond PFOA and PFOS.

To this end, we will use systematic evidence mapping methodology to improve citizen, scientific and regulatory access to current evidence regarding the health effects associated with exposure to PFAS. Systematic evidence maps collate and characterise evidence available on a broad research topic. They distill a potentially vast, heterogeneous evidence base into a (computationally) accessible, comparable and easily updated format using transparent and reproducible methodology. Systematic evidence maps take the form of searchable databases of references and meta-data, including data extracted and coded from each individual included study. This format removes the barriers associated with manually assessing large volumes of data by affording end users a broad overview of the evidence base, allowing fast identification of emerging trends, including the presence of evidence gaps and evidence clusters (James et al., 2016). As such, systematic evidence maps do not attempt to synthesise or integrate evidence in answer to any one specific research question, but rather provide users with the means of exploring the evidence according to their own varied research interests - identifying trends which might form the basis of future syntheses or further research.

Here, we propose to create a systematic evidence map that transparently and systematically surveys the available health and toxicological evidence associated with PFAS exposure. The result will be an online, interactive, interrogable, and user-friendly database (Miake-Lye et al., 2016). Given the pace at which the evidence base appears to be growing, it would seem that now is a good time to establish a systematically and transparently created interactive database, such as the one proposed in this protocol. A database concerning the health effects of “short-chain PFAS” has been previously suggested, but to our knowledge has not yet been produced (Bowman, 2015).

1.2. Objectives

The objectives of this systematic evidence map are to:

1. Identify and organize the available scientific research on the physiological health effects of a set of 29 PFAS (see Table 1), individually or combined, as measured in human, animal, or *ex vivo/in vitro* models.
2. Present the literature in a user-friendly, online, interactive database that will connect end-users directly to referenced primary studies.
3. Identify data gaps and research needs, and publish a narrative summary of the systematic map.

The protocol described here, serves to document decisions made *a priori* regarding the conduct of the systematic evidence mapping.

Table 1
List of PFAS included in systematic evidence map

Abbreviation	Chemical name	CASRN
PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
PFBS	Perfluorobutanesulfonic acid	375-73-5
PFHxS	Perfluorohexanesulfonic acid	355-46-4
PFUnA	Perfluoroundecanoic acid	2058-94-8
PFDoA	Perfluorododecanoic acid	307-55-1
NEtFOSAA	2-(N-ethyl-perfluorooctane sulfanamido) acetic acid	2991-50-6
NMeFOSAA	2-(N-Methyl-perfluorooctane sulfanamido) acetic acid	2355-31-9
GenX	Hexafluoropropylene Oxide (HFPO) Dimer Acid	13252-13-6
PFTA	Perfluorotetradecanoic acid	376-06-7
PFTrDA	Perfluorotridecanoic acid	72629-94-8
ADONA	4,8-dioxa-3H-perfluorononanoic	919005-14-4
6:2 Cl-PFESA	6:2 chlorinated polyfluorinated ether sulfonic acid	73606-19-6
8:2 Cl-PFESA	8:2 chlorinated polyfluorinated ether sulfonic acid	83329-89-9
PFBA	Perfluorobutanoic acid	375-22-4
PFPeA	Perfluoro-n-pentanoic acid	2706-90-3
Nafion BP2	Nafion Byproduct 2	749836-20-2
PFO4DA	Perfluoro-3,5,7,9-tetraoxadecanoic acid	39492-90-5
PFO5DoDA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	39492-91-6
Hydro-Eve	2,2,3,3-Tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy) propanoic acid	773804-62-9
6:2 FTSA	h,1h,2h,2h-Perfluorooctanesulfonic acid	27619-97-2
8:2 FTSA	2-(Perfluorooctyl)ethane-1-sulfonic acid	39108-34-4
PFPeS	Perfluoropentanesulfonic acid	2706-91-4
PFHpS	Perfluoroheptanesulfonic acid	375-92-8
PFNS	Perfluorononanesulfonic acid	68259-12-1
PFDS	Perfluorodecanesulfonic acid	335-77-3
HFPO-TA	Hexafluoropropylene Oxide (HFPO) Trimer Acid	13252-14-7

2. Methods

This protocol has been prepared in accordance with the ENVINT PRISMA-SM-P report (available at (Elsevier, 2017)) and based on guidance from the Collaboration for Environmental Evidence (Collaboration for Environmental Evidence, 2018). The protocol has been registered at Zenodo (Pelch et al., 2019).

2.1. Information sources

PFAS (Table 1) were prioritized for inclusion in this systematic evidence map due to their inclusion in the ATSDR Draft Toxicological Profile for Perfluoroalkyls (ATSDR, 2018), their presence in US EPA Method 537.1 (Shoemaker and Tettenhorst, 2018), because they were reported to be detected in blood in the GenX exposure study (NC State Center for Human Health and the Environment, 2018a; NC State Center for Human Health and the Environment, 2018b), or because they were suggested to be of interest by members of the NGO community (personal communication). Because PFOA and PFOS have been recently reviewed by US EPA (US EPA, 2016a, 2016c), ATSDR (ATSDR, 2018), and NTP (NTP, 2016), they were not prioritized for incorporation in this systematic evidence map.

The peer-reviewed published literature will be identified by searching PubMed electronic database with no date or language restrictions. If a search update is needed, the PubMed search will be repeated but limited to studies published since the date of the last search using the "date-create" field in the PubMed Advanced Search Builder. The number of studies retrieved from searching will be tracked in a study flow diagram (*e.g.* Fig. 1), which will also track how the studies progress through the review. Any studies identified from sources other than PubMed (*e.g.* identified by hand searching included studies or relevant reviews) will be marked as "Identified from other sources" on the study flow diagram.

2.2. Search strategy

The Pubmed search will include names and synonyms for 29 PFAS of emerging interest. Specific search terms can be found in Appendix 1. There will be no search limitations based on health outcome or other aspects of study design or conduct. Furthermore, the search will be conducted without limit on publication year or language.

Search terms were identified for the PFAS of interest by searching the CASRN for each chemical, the common abbreviation, and full chemical names, which have been identified as synonyms for the chemical in PubChem. The search logic for GenX and PFBS are adapted from the recent EPA GenX and PFBS Draft Toxicity Assessments (US EPA, 2018a, 2018b). The search logic for PFAS in general has been adapted from the search logic used in the NTP monograph (NTP, 2016). When possible, the search will also include CASRN and relevant search terms for associated salts (see Table 2).

2.3. Eligibility criteria

Study eligibility is based on the PECO statement provided in Table 2.

To be included in this systematic evidence map, studies must contain primary research investigating the link between one or more of the PFAS of interest and a health effect, toxicological, or biological mechanistic endpoint. Epidemiological, animal, and *in vitro* and mechanistic evidence will be included. Studies that do not contain health, toxicological, or mechanistic information on the PFAS of interest will be excluded at the title and abstract level and will not be further data extracted.

Studies that investigate aspects of PFAS other than health outcomes will be tagged and categorized as to the nature of the evidence and may be made available upon request or as a downloadable list on the TEDX website (www.tedx.org). This includes studies on environmental detection, environmental fate and transport, biomonitoring, detection in wildlife, reports on the absorption, distribution, metabolism, excretion, pharmacokinetic or toxicokinetic properties (ADME/PK/TK), *in silico* and read across analyses, reviews, and systematic reviews of the PFAS of interest. Though they will be tagged and collated, studies that lack health outcome endpoints will not proceed past title and abstract screening.

Given that this is a systematic evidence map rather than a systematic review, efforts will be made to include non-English language studies if essential information (*i.e.* chemicals tested and health outcomes assessed) can be obtained from the title and abstract. Non-English studies will be denoted with square brackets on the title. Conference abstracts, presentations, posters, and theses/dissertations will not be included in this systematic evidence map.

2.4. Data management

2.4.1. Management of literature updates and study flow diagram

A study flow diagram will be maintained that describes the number of studies evaluated in each step of the review (Fig. 1). Any search updates or modifications to the protocol will also be noted as amendments to the registered protocol.

Literature search results will be imported to EndNote X6. Duplicate records will be identified using EndNote's "Find Duplicates" feature based on title and author fields. All records will receive a unique identification number upon import to EndNote X6 that will be maintained throughout the review. Records will then be exported and uploaded to DistillerSR (Evidence Partners; Ottawa, Ontario, Canada). Customized forms in DistillerSR will be used to manually screen studies at the title and abstract level and to extract study details from full-text documents. Extracted information will be exported from DistillerSR to one of three .csv files that can be directly uploaded to Tableau Desktop Professional Edition *vs* 2018.3 (Tableau; Seattle, WA) for visualization.

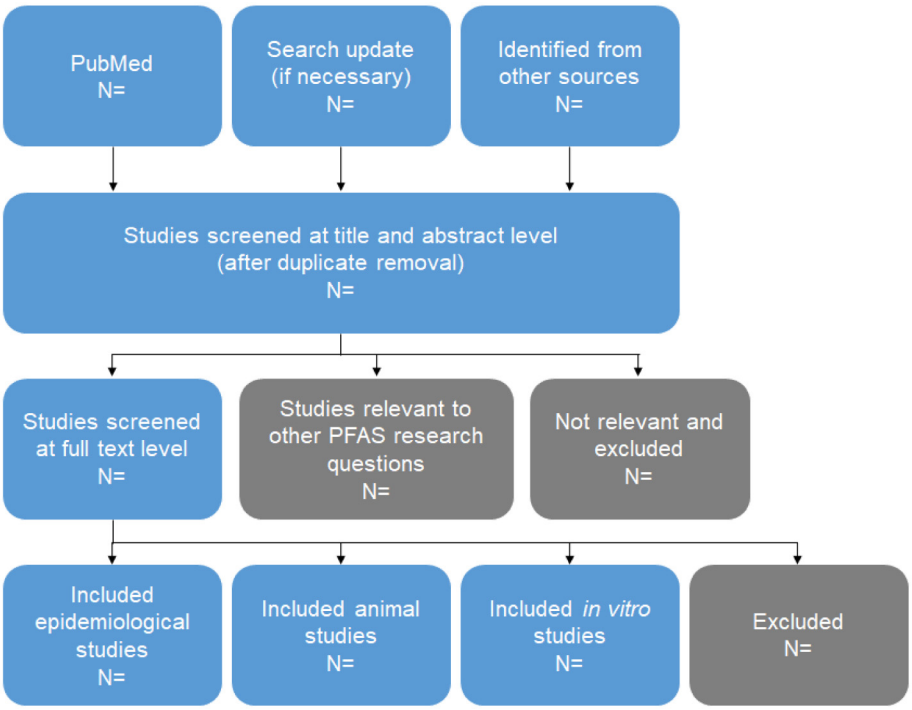

Fig. 1. Example study flow diagram
The example study flow diagram shows how studies will proceed through the review.

Table 2
Populations, **E**xposures, **C**omparators, and **O**utcomes (PECO) Statement.

PECO element	Evidence
Populations	Any human, animal (whole organism including experimental and observational studies), or *ex vivo/in vitro* models utilizing organs, tissues, cell lines, or cellular components (*e.g.* cell-free receptor binding assays).
Exposures	Exposure to at least one of the PFAS or the associated salts listed in Table 1 (*e.g.* perfluorobutane sulfonic acid (PFBS; CASRN 375-73-5) and potassium perfluorobutane sulfonate (K + PFBS; CASRN 29420-49-3)). Exposures may include, for example: biomarkers of exposure, modeling of potential exposures, and/or administered exposures. Mixtures of PFAS will also be included and listed as $PFAS_{mix}$. There are no limitations on the timing, route, level, or determination of estimated exposure.
Comparators	Humans, animals, organs, tissues, cell lines, or cellular components exposed to a lower level of a PFAS than the more highly exposed subjects or treatment groups, or vehicle-only treatment.
Outcomes	Any health outcome or type of biological response measured in the exposed population.

The three .csv files will represent the three evidence streams: human, animal, and *in vitro*. The .csv files will also be submitted as supplemental files to the journal with the final report.

The systematic evidence map will be hosted on TEDX's public profile on Tableau Public, which is available at https://public.tableau.com/profile/the.endocrine.disruption.exchange#!/. A link to the visualization will also be found on the TEDX website along with additional systematic evidence map details including links to the published and registered protocols.

2.5. Selection and data collection processes

Title and abstract screening will be performed in DistillerSR by senior researchers (KEP, AR, TW), none of which have authored peer reviewed articles that would be relevant for inclusion in this systematic evidence map. DistillerSR's artificial intelligence (AI) text mining functionality may be utilized to prioritize studies for title and abstract screening. Title and abstract screening and full text review will require a single reviewer for inclusion to the next level and two independent reviewers for exclusion. Discrepant screening results will be resolved by discussion. Likewise, full text review, data extraction, and coding will be conducted by a single reviewer with a secondary reviewer confirming the accuracy and completeness of extracted and coded data using DistillerSR's quality control (QC) feature. We will attempt to contact study authors *via* email if it is unclear which PFAS was investigated (*e.g.* missing CASRN or structure, or ambiguous chemical name). Other missing information will be flagged as missing, but study authors will not be contacted. Prior to commencing the search, DistillerSR forms will be piloted by KEP, AR, and TW on a small set of studies to ensure ease and accuracy of data extraction and export for visualization in Tableau.

2.6. Data coding strategy

Data extraction will be conducted on full-text studies using structured forms in DistillerSR. The following information will be collected from all included studies: authors, journal, reference information, year of publication, which evidence streams were investigated (human, animal, or *in vitro)*, conflict of interest statement (COI), funding statement, acknowledgements statement, chemicals evaluated, and the health outcome category (see Table 3). Data specific to each evidence stream will also be collected as outlined in Table 3. All data will be captured at the study level rather than at the level of each individual endpoint. In other words, for each study, data extractors will be instructed to select all responses that apply to each question.

Table 3
Data coding and recording.

Data category	Data captured
Bibliographic information	● authors ● year of publication ● journal ● title ● reference information ● study URL ● COI statement ● authors' acknowledgments statement ● funding source
Evidence stream	*Evidence stream is defined by the type of subject or population being exposed to the chemical.* ● Human epidemiological studies ● Animal (including experimental and observational whole animal studies) ● *In vitro* (includes mechanistic studies in humans and other species, *ex vivo*, and cell free)
Health effects studied	*Health outcomes will be tagged as follows (these headings were derived from the MedLinePlus ontology, which is available with definitions from the Unified Medical Language Systems Database (US NLM (United States National Library of Medicine), 2016):* ● Blood, heart, and circulation ● Bones, joints, and muscles ● Brain and nerves ● Cancers ● Digestive system ● Ear, nose, and throat ● Endocrine system ● Eyes and vision ● Female reproductive system ● Genetics/birth defects ● Immune system ● Injuries and wounds ● Kidneys and urinary system ● Lungs & Breathing ● Male reproductive system ● Mental health and behavior ● Metabolic problems ● Mouth and teeth ● Mortality ● Pregnancy and reproduction ● Sexual health issues ● Skin, hair, and nails
Chemicals studied	Data will be collected on the 29 PFAS listed in Table 1. If PFAS other than those listed in Table 1 are studied in included studies, they will be permanently added to the list of options so that they might be tracked for any future updates or expansions to this systematic evidence map. Mixtures of PFAS or Σ_{PFAS} presented in a study will be categorized as $PFAS_{mix}$ in addition to the component PFAS.
Human study elements	Study type: ● Case control ● Cohort ● Cross-sectional ● Ecological/community Study location: ● US (list US state abbreviation) ● Non-US ● *The city, state, and/or country of study location will be captured as free text* Exposure type: ● General population ● Known or suspected point source pollution ● Occupational Study population sex: ● Male ● Female ● Both Study N: ● *The study N will be collected as free text for the total number of study participants (e.g. all cases and controls)* Timing of exposure assessment: ● *The timing of exposure according to study authors will be captured as free text and will also be further categorized as:* ● Preconception ● Pregnancy ● birth-1 years of age ● > 1–3 years of age ● > 3–12 years of age ● > 12–20 years of age ● > 20 years of age Exposure assessment: ● *The exposure assessment method as described by the study authors will be captured as free text and will also be further categorized as follows, with controlled additions allowed as needed:* ● Adipose tissue ● Amniotic fluid ● Breast milk ● Cord blood ● Distance to source ● Drinking water concentration ● Hair ● Nails ● Serum ● Urine ● Whole blood Exposure level: ● Minimum reported exposure ● Maximum reported exposure ● Reported units of measured exposures Timing of outcome assessment: ● *The timing of outcome assessment according to study authors will be captured as free text and will also be further categorized as:* ● Pregnancy ● Birth −1 years of age ● > 1–3 years of age ● > 3–12 years of age ● > 12–20 years of age ● > 20 years of age
Animal study elements	Animal subjects: ● Species - species will be categorized as follows, with controlled additions allowed as needed: ○ Daphnia ○ Monkey ○ Mouse ○ Rat ○ Frog ○ Fish ● Strain - will be captured as free text Study population sex: ● Male ● Female ● Both Study N: ● *The study N will be collected as free text for the range of N from different experimental groups assessed throughout the study* Timing of exposure: ● *The timing of exposure according to study authors will be captured as free text and will also be further categorized as:* ● For rodents:

(*continued on next page*)

Table 3 (*continued*)

Data category	Data captured
	○ Gestational ○ Postnatal (for rodents postnatal day (PND)0-PND14) ○ Developmental (gestational + postnatal) ○ Juvenile (for rodents PND15–40) ○ Adult (for rodents PND41 +) ● For zebrafish: ○ Embryonic (hpf 0–72) ○ Larval (hpf 72–30 days) ○ Adult (> 30 days) ● For other model systems: ○ Will develop as needed with expert consultation Route of exposure: ● *The exposure assessment method as described by the study authors will be categorized as follows, with controlled additions allowed as needed:* ● Inhalation ● Intraperitoneal injection ● Embryonic injection (*e.g.* zebrafish, xenopus) ● Subcutaneous: injection ● Subcutaneous: mini osmotic pump ● Subcutaneous: silastic capsule ● Oral: drinking water ● Oral: gavage ● Oral: feed/diet/treat ● In treatment water (*e.g.* zebrafish, xenopus) ● Dermal ● Ocular Exposure assessment: ● *When relevant (i.e. observational animal studies), the exposure assessment method as described by the study authors will be categorized as follows, with controlled additions allowed as needed:* ● Adipose tissue ● Amniotic fluid ● Breast milk ● Cord blood ● Feces ● Hair ● Nails ● Serum ● Urine ● Whole blood ● Whole organism Exposure/dose range: ● Minimum reported exposure/dose ● Maximum reported exposure/dose ● Reported units of measured exposures/dose Timing of assessment: ● *The timing of outcome assessment according to study authors will be captured as free text and will also be further categorized as:* ● For rodents: ○ Gestational ○ Postnatal (for rodents PND0-PND14) ○ Juvenile (for rodents PND15–40) ○ Adult (for rodents PND41 +) ● For zebrafish: ○ Embryonic (hpf 0–72) ○ Larval (hpf > 72–30 days) ○ Adult (> 30 days +) ● For other model systems: ○ Will develop as needed with expert consultation
In vitro study elements	Cell species: ● *Cell species will be categorized as follows, with controlled additions allowed as needed:* ● Chicken ● *E. coli* ● Frog ● Guinea pig ● Hampster ● Human ● Mouse ● Rabbit ● Rat ● Yeast ● Zebrafish Cell line name: ● *Example cell line names are provided below. Controlled additions to this list will be allowed as needed:* ● 3T3L-1 ● BG-1 ● CHO ● COS-7 ● DT40 ● GH3 ● H295R ● HeLa ● HepaRG ● HepG2 ● Ishikawa ● MCF-7 ● MDA-kb2 ● NIH3T3 ● PC3 ● PZFH ● U2OS ● ZLF Cell type: ● *Example cell types are provided below. Controlled additions to this list will be allowed as needed:* ● Leukocytes ● Oocytes ● Neuronal ● Kidney ● Breast cancer ● Normal breast Exposure timing: ● *The range of exposure lengths used for the various experiments in a study will be recorded as free-text.* Endpoint description: ● *In vitro endpoints will be broadly categorized. Examples of broad categories are provided below. Controlled additions to this list will be allowed as needed:* ● Estrogen related ● Androgen related ● Thyroid related ● Glucocorticoid related ● Peroxisome proliferator activated receptor (PPAR) related ● Cell and molecular dysfunction (*e.g.* oxidative stress) Dose range: ● Minimum reported dose ● Maximum reported dose ● Reported units of measured exposures

2.7. Data mapping method

Studies will be collated by evidence stream, PFAS studied, and health outcome. The systematic evidence map will be hosted on TEDX's public profile on Tableau Public, which is available at https://public.tableau.com/profile/the.endocrine.disruption.exchange#!/. An example of how the data will be presented in shown in Fig. 2.

The display in Tableau Public will be an interactive evidence map that contains an evidence map as shown in Fig. 2, a list of all included studies, and a filter to limit the display based on evidence stream. In the freely available, online interactive display, it will be possible to filter the data to only see the studies for selected evidence streams, health

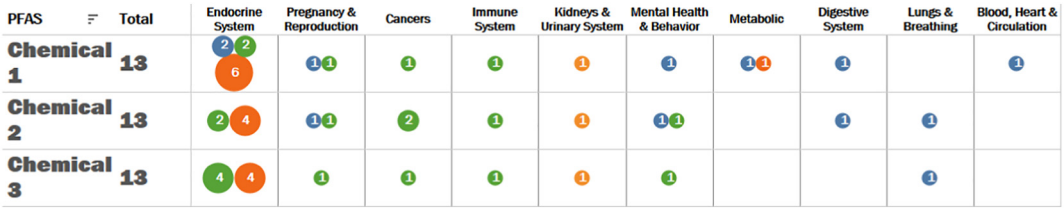

Fig. 2. Example evidence mapping
The example evidence mapping shows one aspect of how the data is expected to be presented in Tableau Public. In this example the different colored circles represent the three different evidence streams (human, animal, *in vitro*). The size of and number in each circle represents the number of studies for that specific chemical and health outcome category in that evidence stream. The rows are each different PFAS chemicals and the columns are different health outcome categories. A list of included studies is presented in another panel of the interactive figure not shown here.

outcome categories or chemicals. Users will be able to easily identify papers of interest by clicking on one of the colored circles to see a list of only those papers evaluating that specific PFAS and health outcome category. Users will be able to find additional study details (*e.g.* timing of exposure and outcome assessment, conflict of interest statement, *etc.*) and read the abstract by hovering over the name of the study in the study list. Further, clicking on a study of interest will take the user directly to the PubMed entry (or the entry on the publisher's page if the paper is not in PubMed).

2.8. Study quality assessment

Study quality will not be assessed in this systematic evidence map.

2.9. Synthesis of results

Results of this systematic evidence map will be summarized narratively and prepared as a manuscript for peer review. We anticipate discussing the overall results of the literature search (to be described in the study flow diagram, Fig. 1) and providing an analysis of the trends in PFAS publications by year. A list/lists of studies that investigate aspects of PFAS other than health outcomes (*i.e.* environmental detection, environmental fate and transport, biomonitoring, detection in wildlife, reports on the ADME/PK/TK, *in silico* and read across analyses, reviews, and systematic reviews) for the 29 PFAS of interest may be made available upon request or as a downloadable list on the TEDX website (www.tedx.org). The human evidence will be discussed in terms of chemicals evaluated to-date, the frequency of use of different study types and locations of the studies, the frequency of use and timing of various exposure assessments, the ranges of reported exposures and the different health outcomes evaluated to-date. The animal evidence will be discussed similarly but separately for observational studies and experimental studies, and will include a discussion on the chemicals studied to-date, the frequency of study of different species, and different experimental aspects including the timing, route, and level of exposure and health outcomes evaluated. The *in vitro* evidence will be discussed in terms of the chemicals and exposure levels studied to-date, the cell or model systems used, and different types of questions addressed by the *in vitro* studies.

Financial support

Financial support for this work was provided by the Arkansas Community Foundation, Winslow Foundation, Marisla Foundation and Tides Foundation. TAMW is financially supported by the Centre for Global Eco-Innovation (a programme funded by the European Regional Development Agency) and Yordas Group, a consultancy based at Lancaster University. AR is employed by the Natural Resources Defense Council, a nonprofit environmental advocacy group.

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Author contributions

KEP and CFK conceived the protocol. KEP, AR, TAMW scoped the project and wrote the first draft. KEP, AR, TAMW, CFK reviewed and revised the protocol for submission and in response to reviewer requests.

Acknowledgements

Thank you to Paul Whaley for helpful conversations about systematic evidence maps, to Michele Dedeo for helpful conversations regarding search terminology, to Michelle Angrish for helpful conversations about health outcome ontologies, and Keshia Rose from Tableau Service Corps for guidance on creating the interactive web browser.

Declaration of Competing Financial Interests

The authors declare they have no actual or potential competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.05.045.

References

ATSDR (Agency for Toxic Substances and Disease Registry), 2018. Toxicological Profile for Perfluoroalkyls (Draft for Public Comment). Department of Health and Human Services, Public Health Service. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237, Accessed date: 2 November 2018.

Blum, A., Balan, S.A., Scheringer, M., Trier, X., Goldenman, G., Cousins, I.T., Diamond, M., Fletcher, T., Higgins, C., Lindeman, A.E., Peaslee, G., de Voogt, P., Wang, Z., Weber, R., 2015. The Madrid statement on poly- and perfluoroalkyl substances (PFASs). Environ. Health Perspect. 123, A107–A111.

Bowman, J.S., 2015. Fluorotechnology is critical to modern life: the FluoroCouncil counterpoint to the Madrid statement. Environ. Health Perspect. 123, A112–A113.

CDC (Centers for Disease Control and Prevention), 2018. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, March 2018. https://www.cdc.gov/exposurereport/pdf/fourthreport_updatedtables_volume1_mar2018.pdf, Accessed date: 20 January 2019.

Collaboration for Environmental Evidence, 2018. Guidelines and Standards for Evidence Synthesis in Environmental Management. www.environmentalevidence.org/information-for-authors, Accessed date: 9 January 2019.

Cordner, A., De La Rosa, V.Y., Schaider, L.A., Rudel, R.A., Richter, L., Brown, P., 2019. Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors. J. Expo. Sci. Environ. Epidemiol. 29, 157–171.

Dong, G.H., Zhang, Y.H., Zheng, L., Liu, W., Jin, Y.H., He, Q.C., 2009. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. Arch. Toxicol. 83, 805–815.

Elsevier, 2017. Guidance notes for authors of systematic reviews, systematic maps and other related manuscripts. Elsevierhttps://www.elsevier.com/journals/environment-international/0160-4120/guidance-notes, Accessed date: 9 January 2019.

Grandjean, P., 2018. Delayed discovery, dissemination, and decisions on intervention in

environmental health: a case study on immunotoxicity of perfluorinated alkylate substances. Environ. Health 17, 62.
Grandjean, P., Budtz-Jorgensen, E., 2013. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environ. Health 12, 35.
Guruge, K.S., Yeung, L.W., Yamanaka, N., Miyazaki, S., Lam, P.K., Giesy, J.P., Jones, P.D., Yamashita, N., 2006. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol. Sci. 89, 93–107.
James, K.L., Randall, N.P., Haddaway, N.R., 2016. A methodology for systematic mapping in environmental sciences. Environ. Evid. 5.
Macon, M.B., Villanueva, L.R., Tatum-Gibbs, K., Zehr, R.D., Strynar, M.J., Stanko, J.P., White, S.S., Helfant, L., Fenton, S.E., 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. Toxicol. Sci. 122, 134–145.
MDHHS (Michigan Department of Health and Human Services). Public Health Drinking Water Screening Levels for PFAS https://www.michigan.gov/documents/pfasresponse/MDHHS_Public_Health_Drinking_Water_Screening_Levels_for_PFAS_651683_7.pdf (accessed 10 April 2019). Division of Environmental Health 2019.
Miake-Lye, I.M., Hempel, S., Shanman, R., Shekelle, P.G., 2016. What is an evidence map? A systematic review of published evidence maps and their definitions, methods, and products. Syst. Rev. 5, 28.
NC State Center for Human Health and the Environment. The GenX Exposure Study. https://chhe.research.ncsu.edu/the-genx-exposure-study/ (accessed 22 January 2019). North Carolina State University; 2018a.
NC State Center for Human Health and the Environment, 2018b. GenX Exposure Study: PFAS Blood Sample Results. https://chhe.research.ncsu.edu/wordpress/wp-content/uploads/2018/11/Community-event-BLOOD-slides.pdf, Accessed date: 22 January 2019.
NHDES (New Hampshire Department of Enviornmental Services), 2019. Summary report on the New Hampshire Department of Environmental Services development of maximum contaminant levels and ambient groundwater quality standards for perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS). https://www.des.nh.gov/organization/commissioner/pip/publications/documents/r-wd-19-01.pdf, Accessed date: 4 April 2019.
NJDWQI (New Jersey Drinking Water Quality Institute), 2018. Maximum Contaminant Level Recommendation for Perfluorooctane Sulfonate in Drinking Water. https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-summary.pdf, Accessed date: 26 October 2018.
NJDWQI, 2017. Maximum Contaminant Level Recommendation for Perfluorooctanoic Acid in Drinking Water. https://www.nj.gov/dep/watersupply/pdf/pfoa-recommend.pdf, Accessed date: 26 October 2018.
NTP (National Toxicology Program). Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). Office of Health Assessment and Translation. Research Triangle Park, NC: National Toxicology Program; https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf (accessed 5 November 2018). 2016.
Peden-Adams, M.M., Keller, J.M., Eudaly, J.G., Berger, J., Gilkeson, G.S., Keil, D.E., 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicol. Sci. 104, 144–154.
Pelch, K.E.; Reade, A.; Wolffe, T.; Kwiatkowski, C.F. PFAS Health Database: A Protocol for a Systematic Evidence Map. https://zenodo.org/record/2832470#.XNt2Lo5Ki70 (accessed 14 May 2019). Zenodo; 2019.
Scheringer, M., Trier, X., Cousins, I.T., de Voogt, P., Fletcher, T., Wang, Z., Webster, T.F., 2014. Helsingor statement on poly- and perfluorinated alkyl substances (PFASs). Chemosphere 114, 337–339.
Shoemaker, J.A., Tettenhorst, D.R., 2018. In: Development O.o.R.a (Ed.), Method 537.1: Determination of Selected per- and Polyflrorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Cincinnati, OH.
Tucker, D.K., Macon, M.B., Strynar, M.J., Dagnino, S., Andersen, E., Fenton, S.E., 2015. The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reprod. Toxicol. 54, 26–36.
US EPA, 2006. PFOA Stewardship Program. Risk Management for Per- and Polyfluoroalkyl Substances (PFASs) under TSCA. United States Environmental Protection Agency. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfass#tab-3, Accessed date: 27 April 2019.
US EPA (United States Environmental Protection Agency), 2016a. Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). Office of Water, Washington, D.C.. https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final_508.pdf, Accessed date: 20 January 2019.
US EPA, 2016b. Drinking Water Health Advisory for Perfluorooctanesulfonate (PFOS). Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final-plain.pdf, Accessed date: 8 January 2019.
US EPA, 2016c. Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final-plain.pdf, Accessed date: 8 January 2019.
US EPA, 2018a. Toxicity Assessment: Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2018-11/documents/genx_public_comment_draft_toxicity_assessment_nov2018-508.pdf, Accessed date: 10 January 2019.
US EPA, 2018b. Toxicity Assessment: Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3). Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2018-11/documents/pfbs_public_comment_draft_toxicity_assessment_nov2018-508.pdf, Accessed date: 10 January 2019.
US EPA. Basic Information on PFAS. PFOA, PFOS and Other PFASs: United States Environmental Protection Agency https://www.epa.gov/pfas/basic-information-pfas (accessed 27 April 2019).
US NLM (United States National Library of Medicine). Unified Medical Language System (UMLS). Bethesda, MD: United States National Library of Medicine; https://www.nlm.nih.gov/research/umls/ (accessed 10 January 2019). 2016.
White, S.S., Stanko, J.P., Kato, K., Calafat, A.M., Hines, E.P., Fenton, S.E., 2011. Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environ. Health Perspect. 119, 1070–1076.