PFAS Health Database: A Protocol for a Systematic Evidence Map of Per- and Polyfluoroalkyl Substances Exposure and Human Health Outcomes

Dr. Erik Lundqvist[1]*
[1]Karolinska Institutet, Institute of Environmental Medicine and Epidemiology, Stockholm, Sweden

Keywords
PFAS, Per- and Polyfluoroalkyl Substances, systematic evidence map, toxicology

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

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 23 PFAS of emerging and growing concern.

Search and study eligibility: We will search the electronic database PubMed for health or toxicological studies on 23 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 from the included studies and checked for accuracy by a second reviewer. The extracted 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.

1.0 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 many have been found to bioaccumulate, or build up, in humans and animals.

Best known for their original use in producing Teflon and 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. As a result, virtually all Americans tested have one or more PFAS in their bodies (Katoet al. 2011). Unfortunately, PFAS have been linked to many harmful health effects, including cancer, immune system 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). Due to increasing public concern over the harm these chemicals cause to human health, wildlife, and the environment, PFOA and PFOS have largely been phased out of production in the US; however, PFOA and PFOS may still be imported from other countries for use in manufacturing, and there are no limitations on their use or generation as waste in the production of other PFAS. 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 (Cordneret al. 2019). For example, in 2009, the U.S. Environmental Protection Agency (EPA) first issued provisional drinking water health advisories for PFOA at 400 parts per trillion (ppt) and for PFOS at 200 ppt. In 2016, after significantly more research had been completed, the EPA issued drinking water health advisories of 70 ppt for PFOA and PFOS, individually or combined (US EPA 2016b; c).

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. Both altered mammary gland development for PFOA (Maconet al. 2011; Tuckeret al. 2015; Whiteet al. 2011) and immunotoxicity for PFOS (Dongeet al. 2009; Grandjean and Budtz-Jorgensen 2013; Gurugeet al. 2006; Peden-Adamset 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; New Jersey Drinking Water Quality Institute 2017; 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; New Jersey Drinking Water Quality Institute 2017; 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 (Blumet al. 2015; Scheringeret 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.

1.2 Objectives

The goal of this systematic evidence map is to identify and organize the available health and toxicology related literature on a set of 23 PFAS of emerging and growing concern (Table 1).

Table 1. List of PFAS included in systematic evidence map

Abbreviation	Chemical Name	CASRN
PFBA	Perfluorobutanoic acid	375-22-4
PFPeA	perfluoro-n-pentanoic acid	2706-90-3

PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
PFUnA	Perfluoroundecanoic acid	2058-94-8
PFDoA	Perfluorododecanoic acid	307-55-1
PFTA	Perfluorotetradecanoic acid	376-06-7
PFTrDA	Perfluorotridecanoic acid	72629-94-8
PFBS	Perfluorobutanesulfonic acid	375-73-5
PFPeS	Perfluoropentanesulfonic acid	2706-91-4
PFHxS	Perfluorohexanesulfonic acid	355-46-4
PFHpS	Perfluoroheptanesulfonic acid	375-92-8
PFNS	Perfluorononanesulfonic acid	68259-12-1
PFDS	Perfluorodecanesulfonic acid	335-77-3
ADONA	4,8-dioxa-3H-perfluorononanoic	919005-14-4
GenX	Hexafluoropropylene Oxide (HFPO) Dimer Acid	13252-13-6
HFPO-TA	Hexafluoropropylene Oxide (HFPO) Trimer Acid	13252-14-7
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
6:2 FTSA	h,1h,2h,2h-Perfluorooctanesulfonic acid	27619-97-2
8:2 FTSA	2-(Perfluorooctyl)ethane-1-sulfonic acid	39108-34-4

2.0 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 will be registered at Prospero, Zenodo, or a similar site.

2.1 Information sources

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. Figure 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.

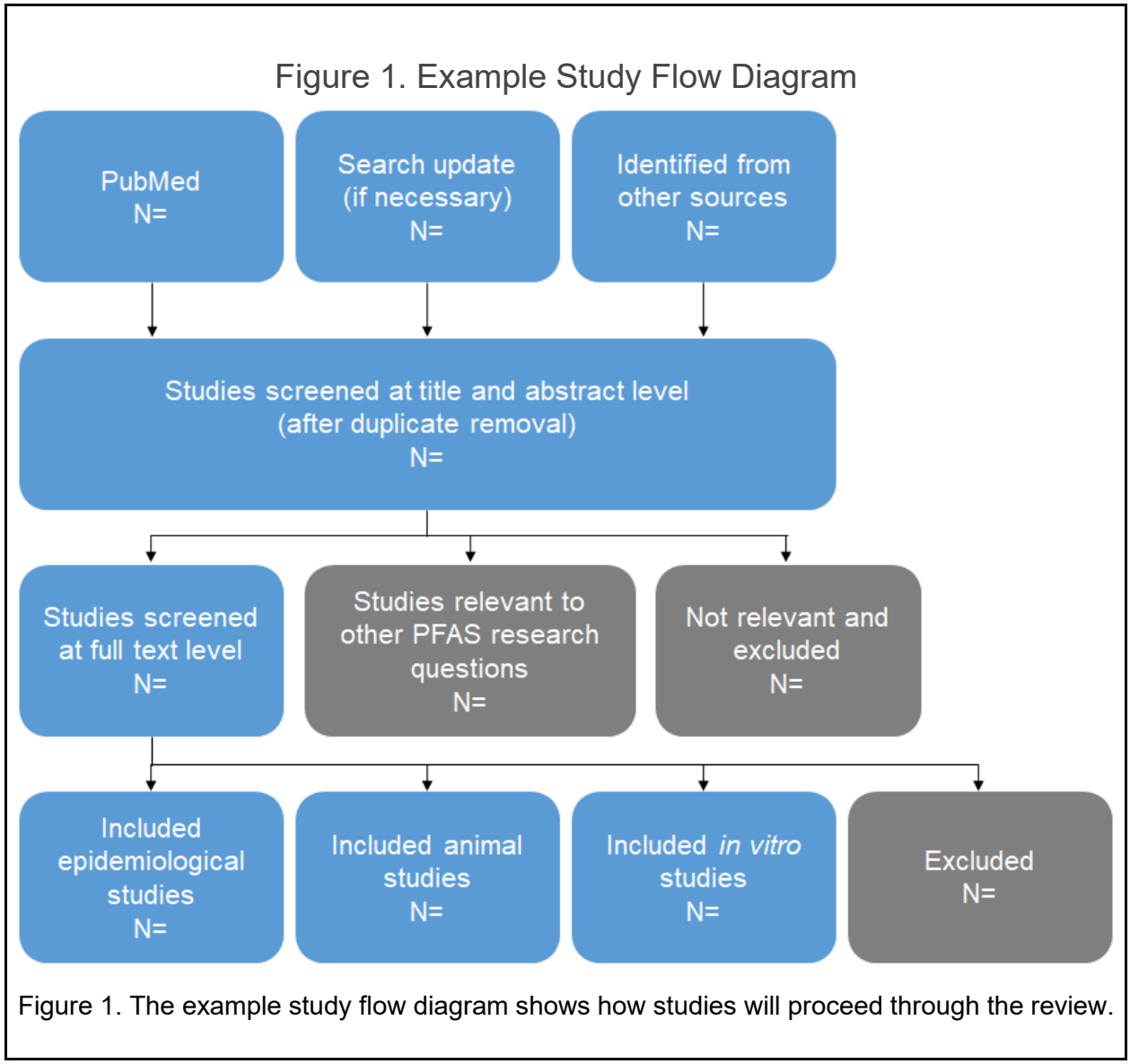

Figure 1. The example study flow diagram shows how studies will proceed through the review.

2.2 Search strategy

The Pubmed search will include names and synonyms for 23 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. Because PFOA and PFOS have been recently reviewed by US EPA (US EPA 2016a; c), ATSDR (ATSDR 2018), and NTP (NTP 2016), they were not prioritized for incorporation in this systematic evidence map.

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; b). 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.

Table 2. **P**opulations, **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.

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

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 (Figure 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 a .csv file that can be directly uploaded to Tableau Desktop Professional Edition vs 2018.3 (Tableau; Seattle, WA) for visualization.

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 and data extraction will be conducted by a single reviewer with a secondary reviewer confirming accuracy and completeness of extracted 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 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 23 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 $\sum_{PFAS}$ presented in a study will be categorized as $PFAS_{mix}$ in addition to the component PFAS.
Human study elements	<u>Study type:</u> ● Case control ● Cohort ● Cross-sectional ● Ecological/community <u>Study location:</u> ● US (list US state abbreviation) ● Non-US ● *The city, state, and/or country of study location will be captured as free text* <u>Exposure type:</u> ● General population ● Occupational <u>Study population sex:</u> ● 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 ● 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 <u>Study population sex:</u> ● Male ● Female ● Both <u>Study N:</u> ● *The study N will be collected as free text for the range of N from different experimental groups assessed throughout the study* <u>Timing of exposure:</u> ● *The timing of exposure according to study authors will be captured as free text and will also be further categorized as:* ● For rodents: ○ 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 <u>Route of exposure:</u> ● *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 <u>Exposure assessment:</u> ● *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 <u>Exposure/dose range:</u> ● Minimum reported exposure/dose ● Maximum reported exposure/dose ● Reported units of measured exposures/dose <u>Timing of assessment:</u> ● *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	<u>Cell species:</u> ● *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 <u>Cell line name:</u> ● *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 <u>Cell type:</u> ● *Example cell types are provided below. Controlled additions to this list will be allowed as needed:* ● Leukocytes ● Oocytes ● Neuronal ● Kidney ● Breast cancer ● Normal breast <u>Exposure timing:</u> ● *The range of exposure lengths used for the various experiments in a study will be recorded as free-text.* <u>Endpoint description:</u> ● *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 <u>Dose range:</u>

	• 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 Figure 2.

Figure 2. Example evidence mapping

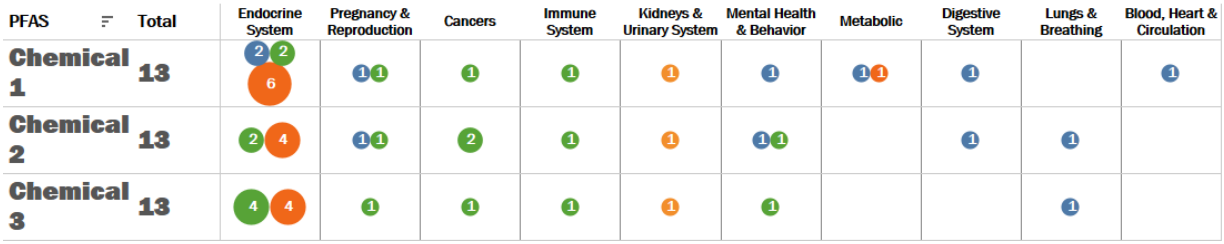

Figure 2. The example evidence mapping shows one aspect of how the data is expected to be presented in Tableau Public. In this example the different colored circles represent the three different evidence streams (human, animal, *in vitro*). The size of and number in each circle represents the number 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.

The display in Tableau Public will be an interactive evidence map that contains an evidence map as shown in Figure 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 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, Figure 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 23 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.

3.0 Financial support

Financial support for this work was provided by the Arkansas Community Foundation, Winslow Foundation, Marisla Foundation and Tides Foundation. TAMW's 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.

4.0 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.

5.0 Acknowledgements

Thank you to Paul Whaley for helpful conversations about systematic evidence maps, to Michele Dedo 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.

6.0 Declaration of Competing Financial Interests

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

7.0 References

ATSDR. Toxicological Profile for Perfluoroalkyls (Draft for Public Comment). in: Department of Health and Human Services P.H.S., ed; 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. The Madrid Statement on Poly- and Perfluoroalkyl Substances (PFASs). Environmental health perspectives 2015;123:A107-111

Collaboration for Environmental Evidence. Guidelines and Standards for Evidence synthesis in Environmental Management. 2018

Cordner, A.; De La Rosa, V.Y.; Schaider, L.A.; Rudel, R.A.; Richter, L.; Brown, P. Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors. Journal of exposure science & environmental epidemiology 2019;29:157-171

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

Elsevier. Guidance notes for authors of systematic reviews, systematic maps and other related manuscripts. Elsevier; 2017

Grandjean, P. Delayed discovery, dissemination, and decisions on intervention in environmental health: a case study on immunotoxicity of perfluorinated alkylate substances. Environmental health : a global access science source 2018;17:62

Grandjean, P.; Budtz-Jorgensen, E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environmental health : a global access science source 2013;12:35

Guruge, K.S.; Yeung, L.W.; Yamanaka, N.; Miyazaki, S.; Lam, P.K.; Giesy, J.P.; Jones, P.D.; Yamashita, N. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicological sciences : an official journal of the Society of Toxicology 2006;89:93-107

Kato, K.; Wong, L.Y.; Jia, L.T.; Kuklenyik, Z.; Calafat, A.M. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. Environmental science & technology 2011;45:8037-8045

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. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. Toxicological sciences : an official journal of the Society of Toxicology 2011;122:134-145

New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendation for Perfluorooctanoic Acid in Drinking Water. 2017

New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendation for Perfluorooctane Sulfonate in Drinking Water. 2018

NTP. Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). in: Translation O.o.H.A.a., ed. Research Triangle Park, NC: National Toxicology Program; 2016

Peden-Adams, M.M.; Keller, J.M.; Eudaly, J.G.; Berger, J.; Gilkeson, G.S.; Keil, D.E. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicological sciences : an official journal of the Society of Toxicology 2008;104:144-154

Scheringer, M.; Trier, X.; Cousins, I.T.; de Voogt, P.; Fletcher, T.; Wang, Z.; Webster, T.F. Helsingor statement on poly- and perfluorinated alkyl substances (PFASs). Chemosphere 2014;114:337-339

Tucker, D.K.; Macon, M.B.; Strynar, M.J.; Dagnino, S.; Andersen, E.; Fenton, S.E. The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reproductive toxicology 2015;54:26-36

US EPA. Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). in: Water O.o., ed. Washington, D.C.; 2016a

US EPA. Drinking Water Health Advisory for perfluorooctanesulfonate (PFOS). in: Water O.o., ed. Washington DC; 2016b

US EPA. Drinking Water Health Advisory for perfluorooctanoic acid (PFOA). in: Water O.o., ed. Washington DC; 2016c

US EPA. 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). in: Water O.o., ed. Washington DC; 2018a

US EPA. Toxicity Assessment: Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3). in: Water O.o., ed. Washington DC; 2018b

US NLM. Unified Medical Language System (UMLS). Bethesda, MD: United States National Library of Medicine; 2016

White, S.S.; Stanko, J.P.; Kato, K.; Calafat, A.M.; Hines, E.P.; Fenton, S.E. Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environmental health perspectives 2011;119:1070-1076