An Overview of Research Focused on Assessing the Ecological Effects of PFAS at the USEPA, ORD Great Lakes Toxicology and Ecology Division*

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*Content does not necessarily reflect US Environmental Protection Agency (USEPA) position or policy.

Ecological Effects of PFAS: An Emerging Issue

- Initial emphasis on multiple human health endpoints (e.g., immune suppression, cancer, thyroid disease, etc.)

- Increasing attention on potential ecological effects
  - Detected in many ecosystems from point/nonpoint sources
  - Can be highly persistent and bioaccumulative (may biomagnify)
  - Some have significant toxicity potential
  - Large universe (100s/1000s?) of (mostly) poorly understood chemicals

- Recent activities worldwide focused on exposure to/possible ecological effects of PFAS
  - Systematic monitoring of point sources
  - Development of effects-based benchmarks/criteria

Needs for Ecological Assessment of PFAS

- Curation/systematic evaluation of existing ecotoxicological data
- Derivation of effects benchmark/criteria values for aquatic life
- Evaluation of mixture effects in sensitive taxa
- New approach methods (NAMs) for effects prediction for data-poor classes/structures
- Field studies with at risk species/populations
- Evaluation and prediction of bioaccumulation

Curation and Evaluation of PFAS Data: Open Access ECOTOX Knowledgebase

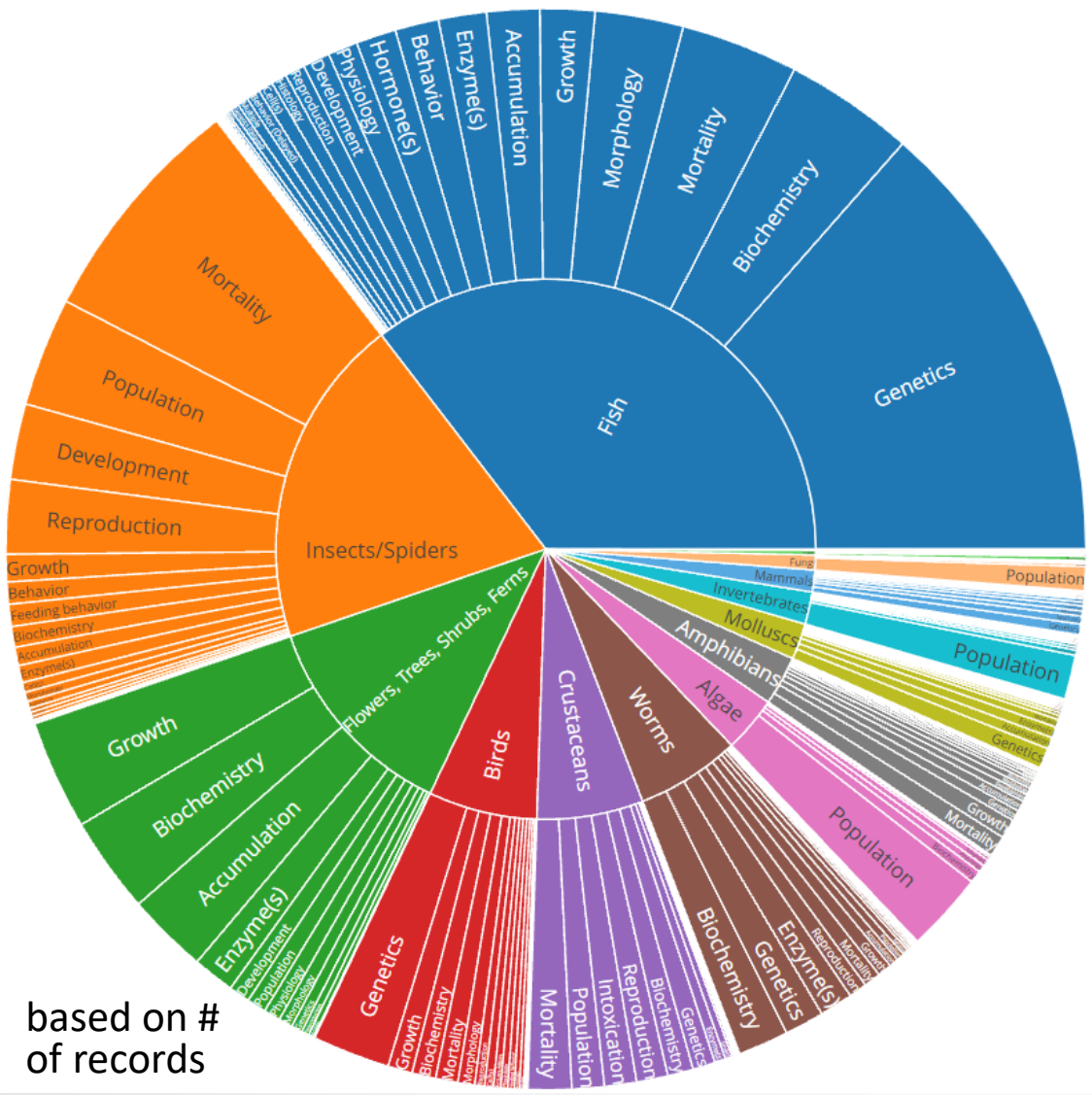
Background

- Existing data helps define
  - Sensitive and susceptible species
  - Read-across predictions
  - Benchmarks and thresholds for ecological effects
  - Data gaps
- ECOTOX maintained by Duluth lab for >30 years (50,000+ papers)

Overview

- ECOTOX comprehensive literature search and systematic review process completed for **>400 PFAS** (April 2018 - present)
- Ecological toxicity data from **1000 references**:
  - **159 PFAS**
  - **600 species**
  - Diversity of effects endpoints

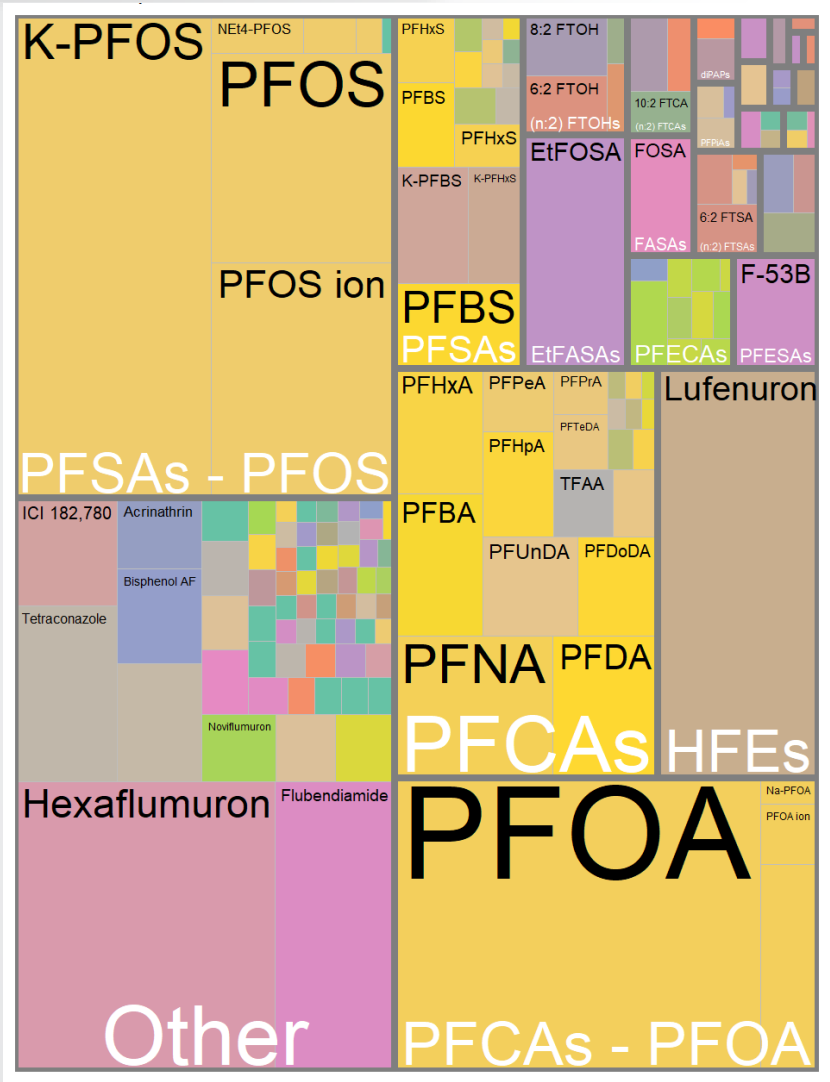
Existing PFAS Data: Taxonomic Distribution



based on # of records

	PFAS data in ECOTOX		
Group	# of Compounds	# of References	# of Records
All Species	159	1,118	29,797
Fish	115	320	10,560
Insects/Spiders	31	387	5,898
Flowers, Trees, Shrubs, Ferns	53	118	3,792
Birds	41	55	1,940
Crustaceans	43	78	1,900
Worms	32	64	1,857
Algae	46	60	997
Amphibians	24	35	879
Molluscs	23	29	643
Other Invertebrates	26	26	576
Mammals	19	18	389
Fungi	5	31	266
Reptiles	2	3	86
Miscellaneous	3	2	14

PFAS with Ecotoxicity Data
(as of September 2021 ECOTOX update)



Box size represents # of references for each chemical

DoD Tri-Services ERA Work Group

- Ecological Screening Values (ESVs) for screening-level risk assessments at US Air Force, Navy, Army, and other DOD sites (*Final Report Sept 2021*)
- Coordination with USEPA OLEM, OW, OCSPP, ORD, Regions)

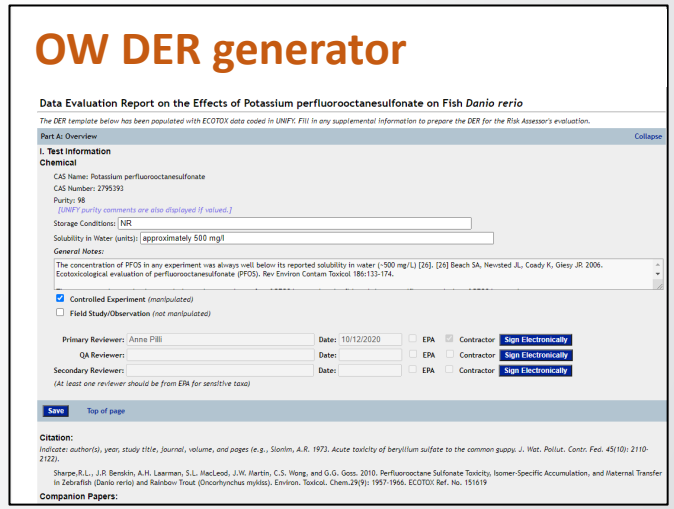
PFAS Compounds Included in the Literature Search for ESV Development

PFAS	CASRN
PFBA	375-22-4
PFHxA	307-24-4
PFOA	335-67-1
PFNA	375-95-1
PFDA	335-76-2
PFBS	375-73-5
PFHxS	355-46-4
PFOS	1763-23-1

US EPA Office of Water

- Data for PFOS and PFOA aquatic life criteria development
- Data Evaluation Record (DER) generator

OW DER generator



Deriving Benchmark/Criteria Values

- **Context:** OW/OST/HECD with support from ORD is currently developing ALC for PFOS and PFOA, but adequate data for ALCs for the broader universe of PFAS not available

- **Research question:** ***How can we develop an approach to derive/estimate aquatic life criteria (or benchmarks) for PFAS with limited information?***

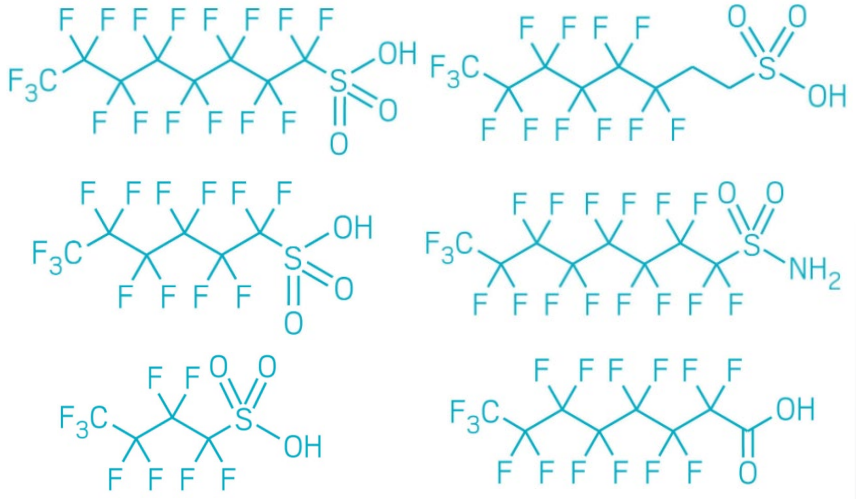
- **Research challenges:**
  - Key effects of PFAS are sub-lethal, but most chronic tests have long duration
  - Literature toxicity data highly variable; need consistent index of toxicity
  - Need to relate potency to chemical structure
  - Need to identify highly susceptible taxa (e.g., chironomids?)
  - PFAS occur as mixtures—need to predict interactive effects

Sublethal Toxicity of PFAS and PFAS Mixtures

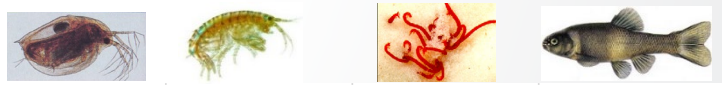
Approach

- Develop short-term (7-d) sub-lethal toxicity tests for a suite of diverse species

- Develop toxicity data for a structured set of PFAS chemicals for multiple species using sublethal endpoints



- Evaluate mixture toxicity and interactions
- Evaluate relative potency across chemicals and species to determine sensitive species and structures of particular concern



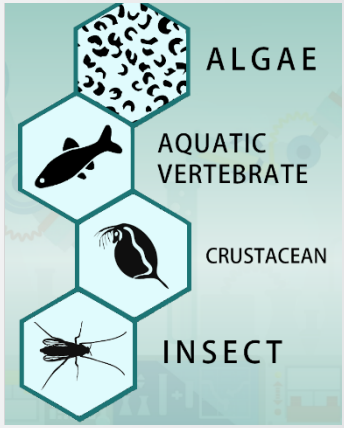
	Ceriodaphnia	*Hyalella*	*Chironomus*	*Pimephales*
PFBS				
PFBA				
PFHxS				
PFHxA				
PFOS				
PFOA				

Enhancing Mixture Prediction: SERDP Award

- **Project Title:** Measuring and predicting the aquatic toxicity of PFAS mixtures associated with AFFF (ER22-3175)
- **Research Need:** SERDP Statement of Need to address the ecological effects of PFAS mixtures associated with AFFF contamination
- **Research Approach:** Combine sublethal toxicity tests with individual PFAS and PFAS mixtures with computational chemistry techniques to develop a predictive toxicity model applicable to PFAS mixtures
- **Collaborators:** University of Delaware – Dr. Dominic Di Toro and colleagues

Applying NAMs to Ecological Assessment of PFAS: Transcriptomic Points of Departure (tPODs)

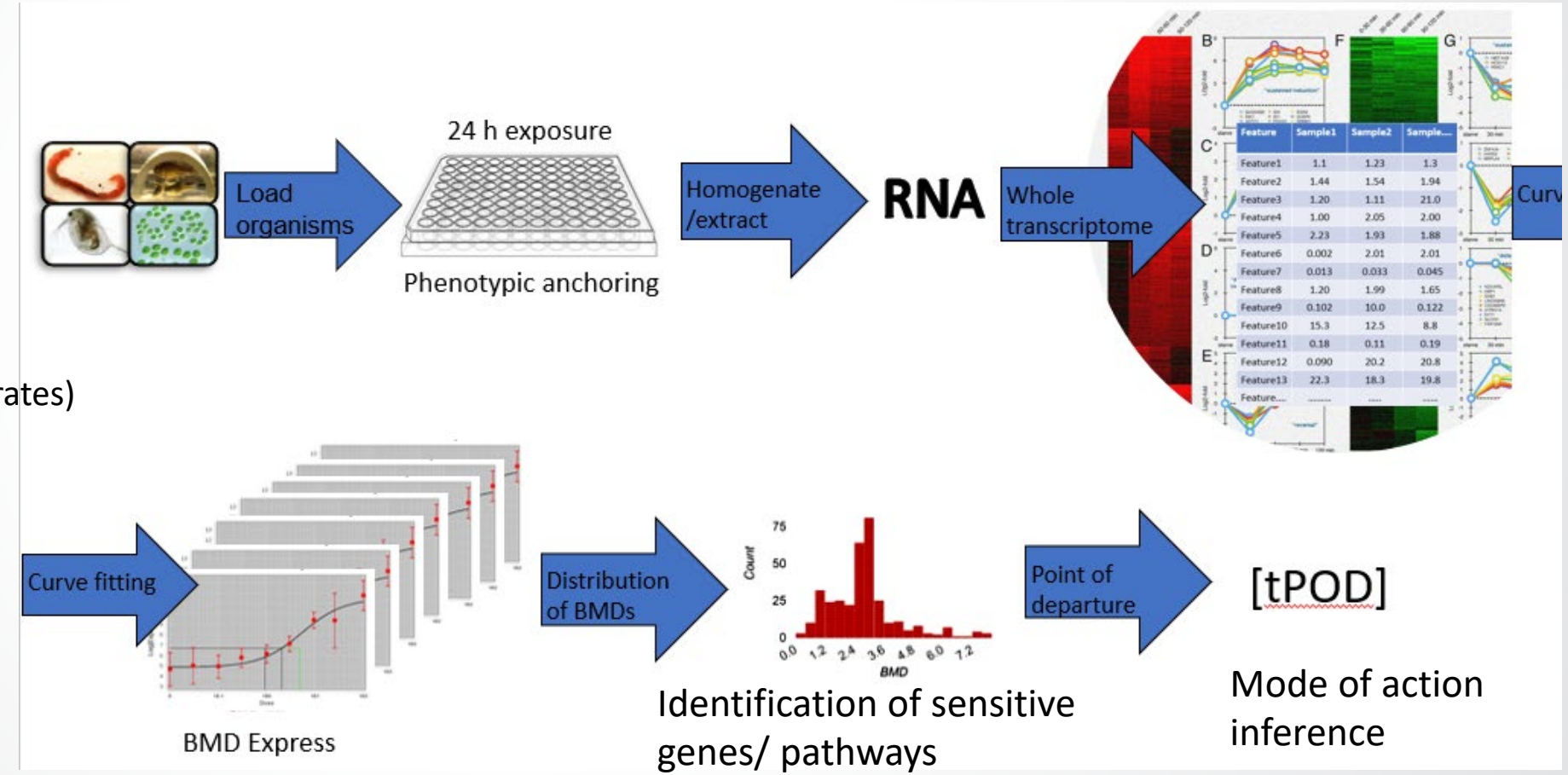
- Impossible to conduct even short-term assays with 100s of PFAS of potential concern
- EPA/ORD using rodent models to derive POD estimates of effects of data-poor PFAS based on gene expression
- Can be used for conservative effects benchmarks
- Can parallel eco-focused assays be developed to complement screening/categorization efforts for PFAS?



Approach – Eco-HTTr

(Ecological High Throughput Transcriptomics)

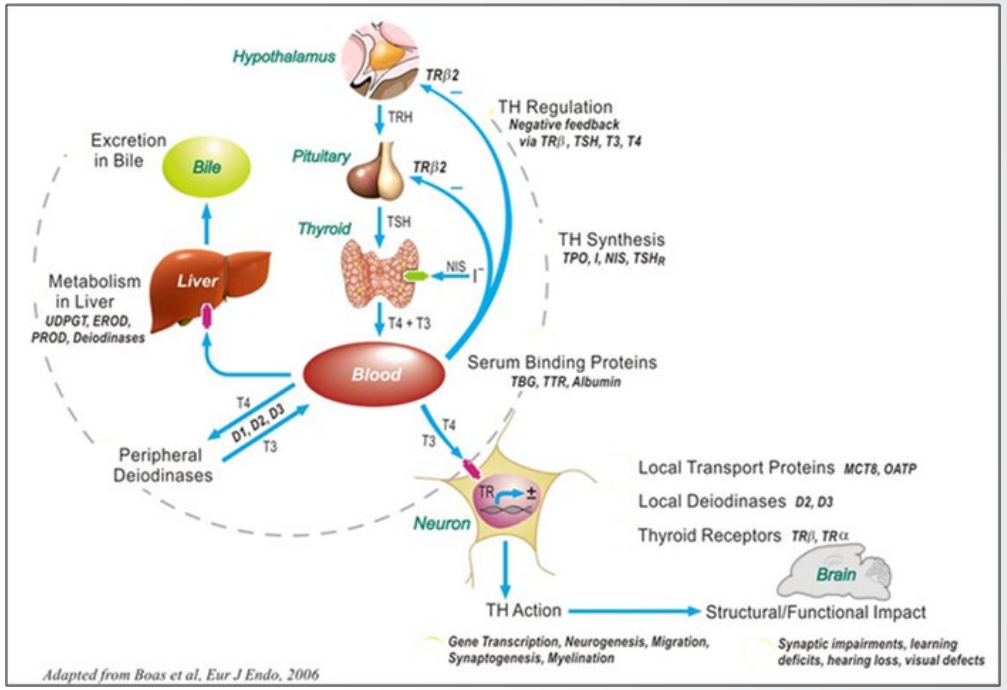
- Rapid (24 h)
- Small scale
  - 96 well plates
  - 700 µl/well
- Whole genome coverage
- Three trophic levels
  - Primary producer (algae)
  - Primary consumer (invertebrates)
  - Secondary consumer (fish)
- Assays “validated” with 10 model chemicals
- Testing ongoing with 20+ high priority PFAS



Applying NAMs to Ecological Assessment of PFAS: Screening and Testing for Thyroid Effects

Background

- Multiple PFAS shown to impact thyroid axis
- Impacts might occur via broad range of molecular initiating events (MIEs) within axis
- Large number of PFAS and thyroid targets amenable to HTP analysis



Key Science Questions

- Which thyroid MIEs/targets are affected?
- What are the organismal consequences of this?

Approach

- Employ suite of high throughput (HTP) assays thyroid MIEs to screen 160+ PFAS
- Conduct amphibian testing with active PFAS to assess effects on early development

Applying NAMs to the Ecological Assessment of PFAS: Estrogenic Effects

Toxicology 457 (2021) 152789

Contents lists available at ScienceDirect

Toxicology

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

Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure

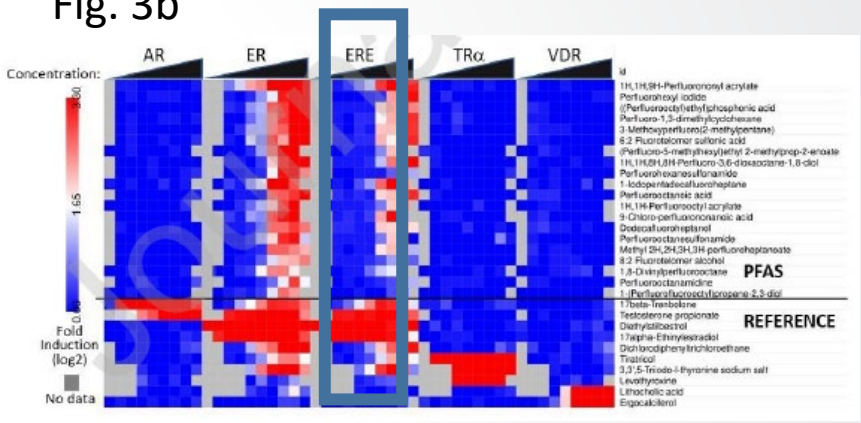
Keith A. Houck [a,*], Grace Patlewicz [a], Ann M. Richard [a], Antony J. Williams [a], Mahmoud A. Shobair [a], Marci Smeltz [a], M. Scott Clifton [a], Barbara Wetmore [a], Alex Medvedev [b], Sergei Makarov [b]

[a] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA
[b] Attagene, Inc., 7020 Kit Creek Rd, Morrisville, NC, 27560, USA

Houck et al. 2021. Toxicology. DOI: 10.1016/j.tox.2021.152789

- Screened 142 PFAS for activity against 25 human nuclear receptors (81 transcription factor activities overall)
- Detected multiple PFAS that activate the human estrogen receptor (ER)

Fig. 3b

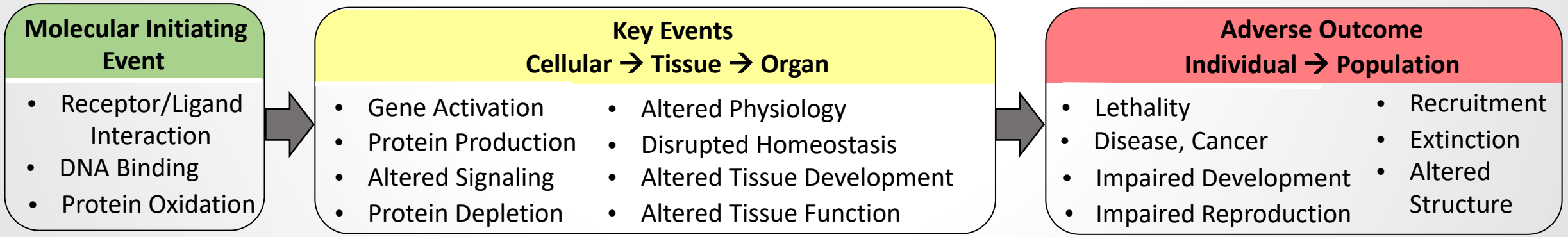


- In fish, activation of ER associated with reproductive and developmental toxicity
- Studies ongoing with 5 PFAS to examine in vivo estrogencity

Employing NAM Data for PFAS to Predict Effects: Role of Adverse Outcome Pathways

Adverse Outcome Pathways (AOPs)

Integrative, organizational framework establishing causal linkages between perturbation of molecular targets and apical endpoints relevant to risk assessment and regulatory decision making



Eco-Relevant AOPs for PFAS: Ongoing Efforts

- Thyroid signaling inhibition leading to impaired metamorphosis in amphibians
- Estrogen receptor activation by weak ligands linked to developmental and reproductive impairment in fish
- Thyroid signaling inhibition leading to decreased early life-stage survival in avian species
- PPAR activation linked to developmental (early life-stage) and reproductive (adult) effects in fish

AOP 323*: PPARα Agonism Impairs Fish Reproduction

- MIE 227: Activation, PPARα

KER 2073

Decreased cholesterol, Altered HDL/LDL/VLDL

KER 2072

- KE 807: Decreased, Cholesterol

Dose and time concordance

- KE 1756: Decreased, 11-Ketotestosterone

KER 2076

Dose concordance

KER 2274

- KE 1758: Impaired, Spermatogenesis

Dose concordance

- AO: Impaired, Fertility

KE 406

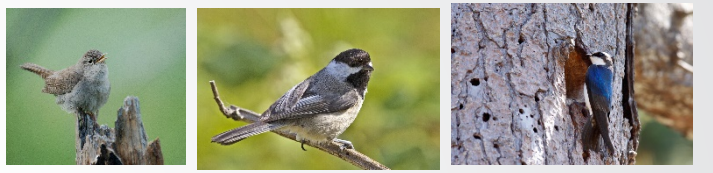
*aopwiki.org

Field Studies with at Risk Taxa

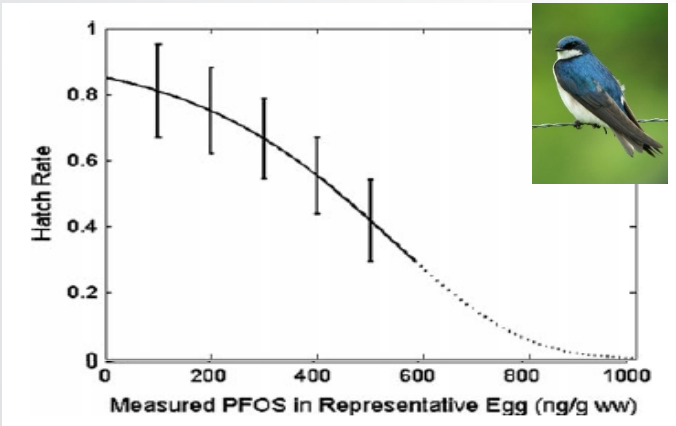
Monitoring data show elevated PFAS in variety of avian species, including some often considered at lower risk for chemical effects (e.g., songbirds)

Lab studies indicate PFAS can reduce hatching success/embryo survival in avian species and field studies suggest association between PFAS and reproductive success in tree swallows

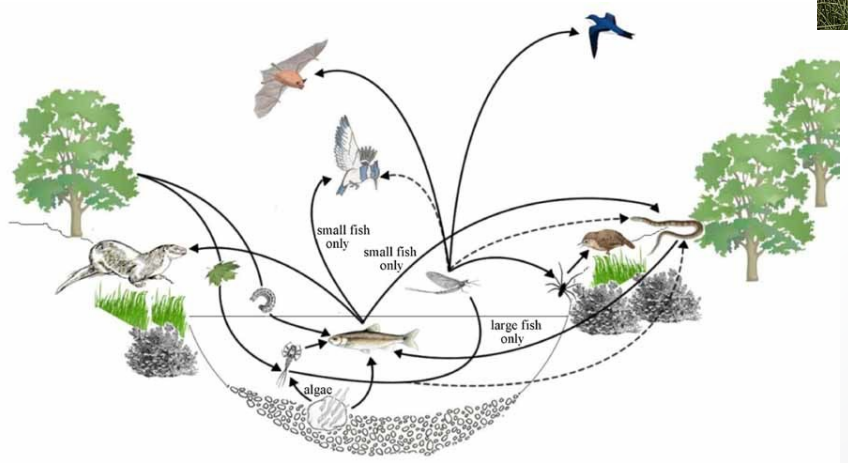
Initial nest box studies conducted at local sites to define PFAS exposure and effects in songbirds (wrens, chickadees, swallows)



Site Name	Status	N	Established
1. DANGB	contaminated	40	2021
2. Martin Rd	contaminated	50	2019
3. Rice Lk	reference	40	2020
4. Boulder Lk	reference	20	2020
5. Univ. MN	experimental	30	2020
6. Lester Park	experimental	30	Pre-2018



Custer et al. 2014. Arch Env. Contam. Tox.

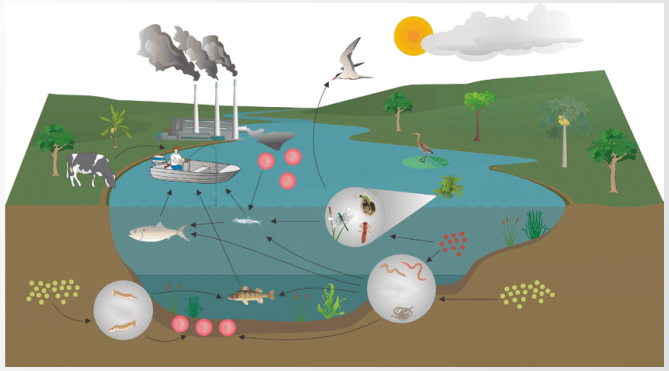


PFAS Effects in Songbirds: SERDP Award

- **Project Title:** Food Web Exposure and Consequent Effects of PFAS on Birds (ER22-3202)
- **Research Need:** SERDP Statement of Need for assessment of effects of PFAS on avian species in aquatic and terrestrial food webs
- **Research Approach:** Conduct controlled and semi-controlled field studies; develop integrated approach based on bioaccumulation, population, and AOP models to predict exposure and effects of PFAS in diverse field settings and species
- **Collaborators:** Dr. Christine Custer and colleagues, USGS; Major Ryan Blazevic and colleagues, Duluth Air National Guard

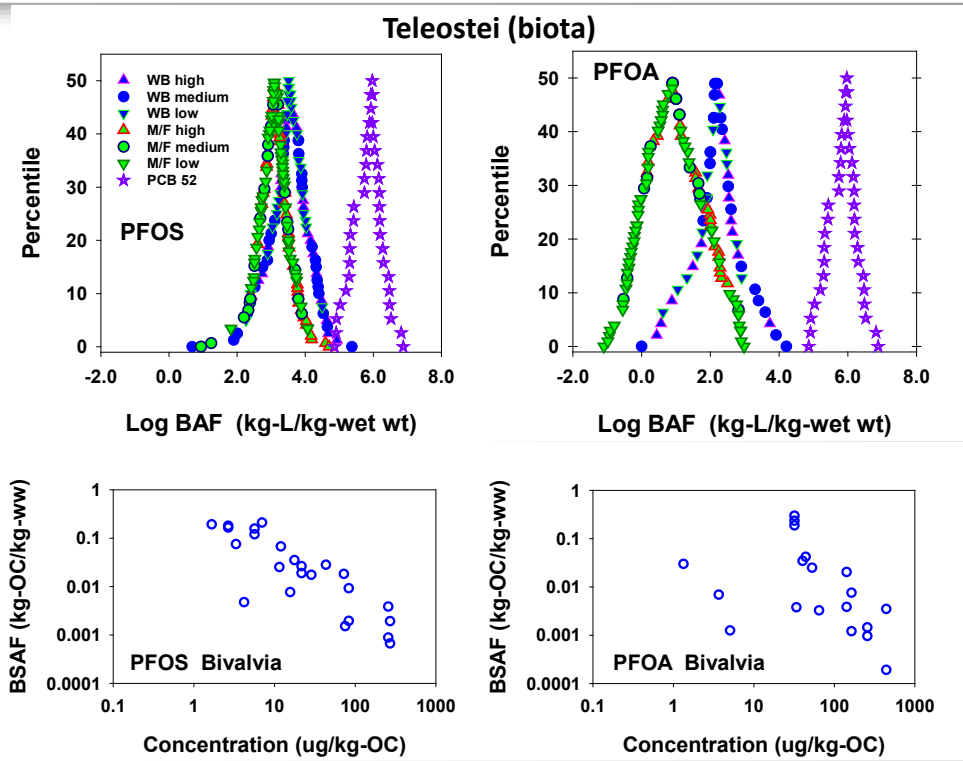
PFAS Bioaccumulation: A Key Uncertainty for Exposure Assessment

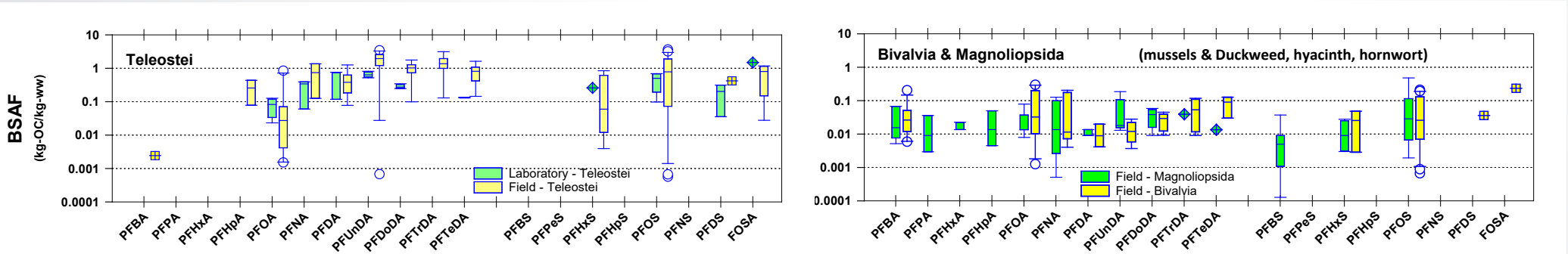
- Eco and human health issue (OW ALC; State Fish Consumption Advisories)
- Some bioaccumulate and a few also biomagnify in food webs (e.g., PFOS)
- Processes controlling bioaccumulation uncertain
- Widely-used lipid-based models (e.g., for PCBs) not appropriate
- Empirical relationships best current option to predict bioaccumulation



PFAS Bioaccumulation: Mining Existing Data

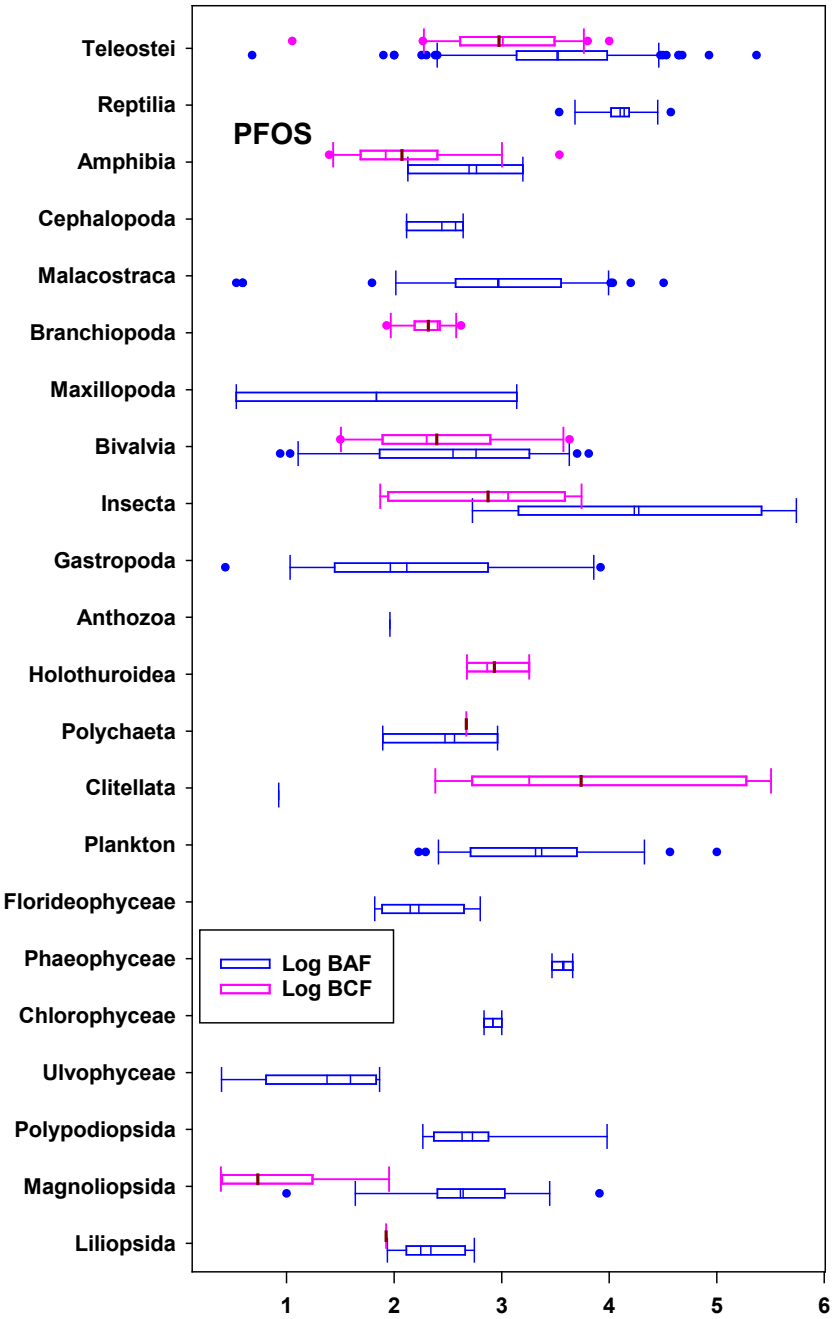
- Approach
  - Literature searching
    - ECOTOX for exhaustive literature search
    - Identify papers with bioaccumulation information using Swift Review software
  - Extract environmental and tissue PFAS data from identified papers
    - Endpoints: bioconcentration and bioaccumulation factors (BCFs, BAFs)
    - Compute bioaccumulation metrics from reported data





PFAS Bioaccumulation

- Initial Analysis*
  - Many measurements for carboxylic (PFOA) and sulfonic acids (PFOS), especially in fish
  - BCF/BAF values for some PFAS comparable to lower-chlorinated PCBs
  - Gaps and Limitations
    - Broader range of taxa needed, including marine species
    - Little/no data for most PFAS, i.e., fluorotelomers, ethers, alcohols, amides, phosphates
  - In Progress
    - Evaluation of published biota-sediment accumulation factors (BSAF), biomagnification factors (BMF) and trophic magnification factors (TMF) for PFAS
    - Support OW for human health/aquatic life criteria derivation



*Burkhard, LP. 2021. Evaluation of published bioconcentration factor (BCF) and bioaccumulation factor (BAF) data for per- and poly-fluoroalkyl substances across aquatic species. Environ. Toxicol. Chem. 40: 1530-1543.

PFAS Eco Research Contributors

- ECOTOX curation/analysis (Hoff, Olker et al.)
- ALC derivation and mixture testing (Mount, Kadlec, Erickson et al.)
- Screening for thyroid activity/effects (Degitz, Hornung, Haselman et al.)
- Eco HTP transcriptomics (Villeneuve, Flynn, LaLone et al.)
- Development of AOPs (Olker, Villeneuve, Ankley et al.)
- Exposure and effects in avian species (Etterson, Burkhard, Ankley et al.)
- Bioaccumulation (Burkhard, Kolanczyk, LaLone et al.)
- Instrumental analytical chemistry (Blackwell, Backe, Burkhard et al.)