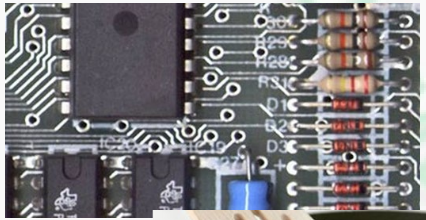
Assessing the Ecological Risks of PFAS: Challenges and Opportunities*

Gary Ankley, PhD, ORD, USEPA, Duluth, MN



*Content does not necessarily reflect EPA position or policy.

PFAS: Human Health and Ecological Concerns

- Initial emphasis on human health (e.g., immune suppression, cancer, thyroid disease, elevated cholesterol)
  - Drinking water and dietary exposures (e.g., fish consumption advisories)

- Concern has evolved to include potential ecological effects
  - Potential contamination of aquatic and terrestrial ecosystems
  - Evidence of persistence, bioaccumulation, toxicity of some PFAS

- Regulatory authorities throughout world increasing activities focused on occurrence and possible ecological risks of PFAS

Select PFAS ERA Activities Around the World

- Canada
  - National ERAs for PFOS, PFOA, >C9 PFCAs
  - FEQGs for PFOS
- Australia/New Zealand
  - Freshwater effects guidelines for PFOS, PFOA
- European Union
  - Multiple PFOS guideline values for freshwater effects
- United States
  - Development of ALC (EPA)/Screening Values (DoD) for PFOS, PFOA
  - State guidelines for aquatic/wildlife effects (MI, MN)

SETAC

Environmental Risk Assessment of PFAS

12–15 August 2019 | Durham, NC, USA

SETAC North America Focused Topic Meeting

Four-day workshop with mix of expert presentations and topic group breakouts/discussions

Topic groups: Analytical Chemistry; Exposure; Human Health Effects; Ecological Effects; Risk Characterization

Open forum discussions with tripartite participation

Peer-reviewed summary papers for each topic group

SETAC FTM Eco-Effects Expert Group

G. Ankley (USEPA)
P. Cureton (ECCC)
R. Hoke (DuPont)
M. Houde (ECCC)
A. Kumar (CSIRO)
J. Kurias (ECCC)
R. Lanno (OSU)
C. McCarthy (Jacobs)
J. Newsted (Ramboll)
C. Salice (Towson U)
B. Sample (EcoRisk)
M. Sepulveda (Purdue)
J. Steevens (USGS)
S. Valsecchi (IRSA-CNR)

Ankley et al. 2020. Assessing the Ecological Risks of PFAS: Current State-of-the Science and a Proposed Path Forward. *Environmental Toxicology and Chemistry.* https://doi:10.1002/etc.4869 (open access)

What We Do Know: PFAS Exposure

- Some PFAS are very persistent
- Widespread distribution of some in environmental matrices and biota in aquatic and terrestrial ecosystems
- Associated both with point and non-point sources, with strong evidence of global transport
- Bioaccumulation and, in certain instances, biomagnification of some PFAS occurs
- Increasing ability measure to multiple PFAS in samples with targeted and nontargeted techniques enhancing quantity and quality of monitoring data

What We Don’t Know: PFAS Exposure

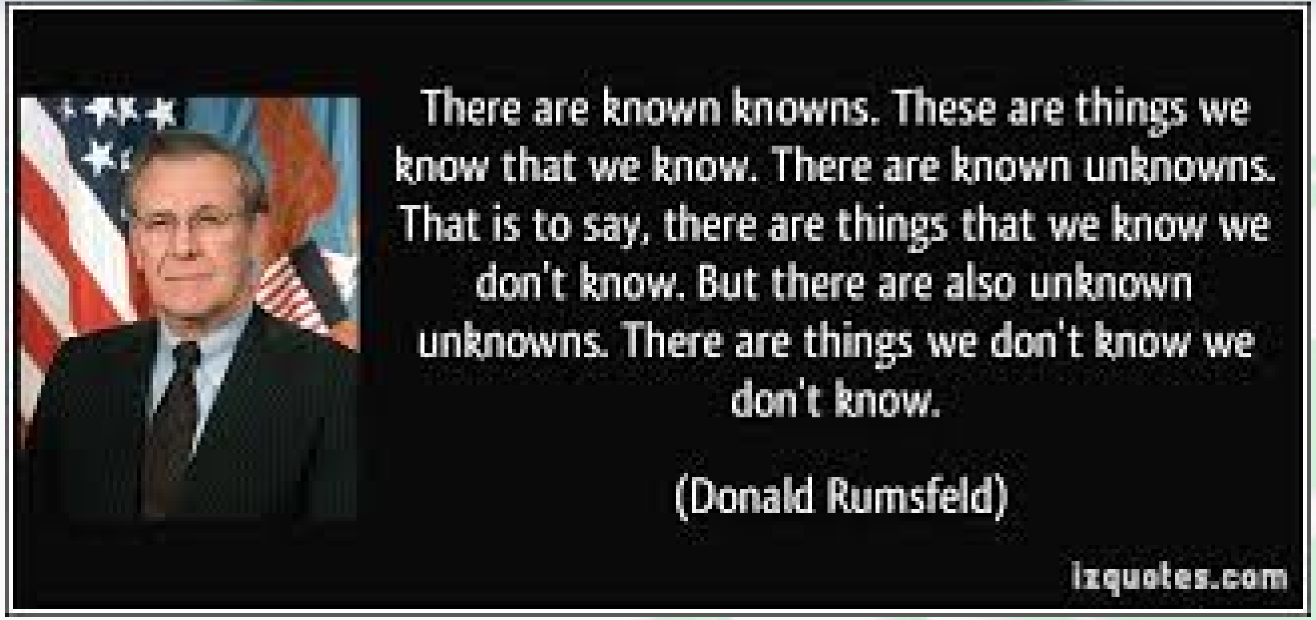
- Full suite of PFAS entering or present in the environment
  - Enhanced targeted and non-targeted analytical techniques
- Fate (of most) in different environments
  - Distribution/degradation
- Probabilistic distributions of compounds (e.g., extent of elevated contamination) in various systems
- Processes underlying bioaccumulation/biomagnification
  - Predictive models critical, but in their infancy
  - Basic mechanistic data needed (protein binding, metabolism)

What We Do Know: PFAS Hazard/Effects

- Relatively extensive toxicity data for some (PFOS, PFOA)
- Freshwater fish and some aquatic invertebrates most commonly-tested taxa
- Low acute toxicity, but chronic effects can be pronounced
- Structural aspects important determinant of toxicity (e.g., PFSA>PFCA; ↑C chain length ↑ toxicity)
- Multiple biological pathways affected by PFAS

What We Don’t Know: PFAS Hazard/Effects

- Limited data for majority of PFAS; no data for some classes
- Little data for several vertebrate taxa (herps, birds)
- Limited/no effects data for most invertebrate taxa, plants
- Knowledge concerning chronic, sublethal effects sparse
- Biological pathways affected by PFAS poorly defined
- Field studies associating PFAS exposure with effects lacking



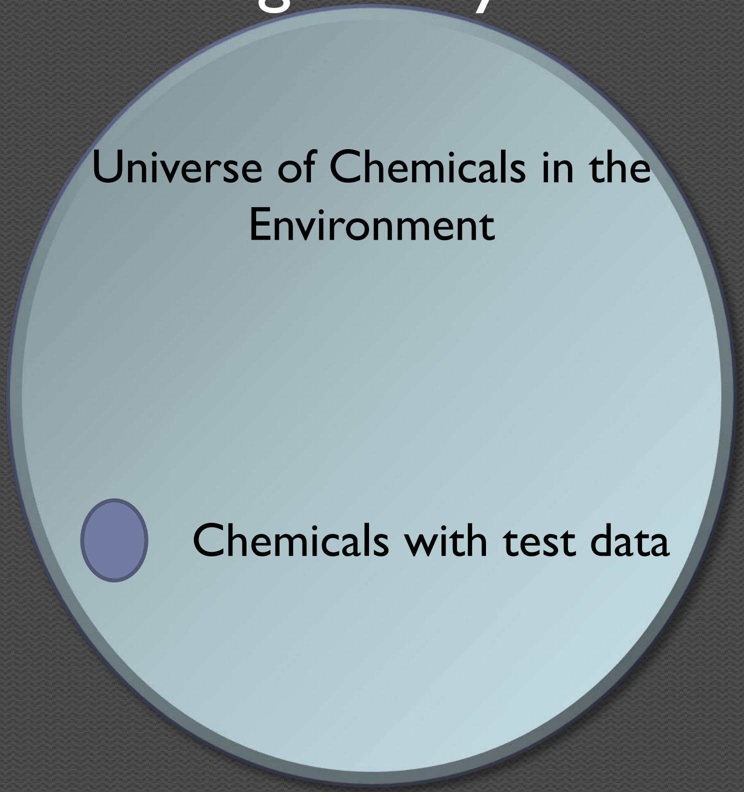
Assessing ecological hazards of PFAS: Too many known unknowns and unknown unknowns

Challenge with PFAS a microcosm of larger issue in regulatory toxicology…

The Great Chemical Unknown
[Scientific American October 28, 2010]

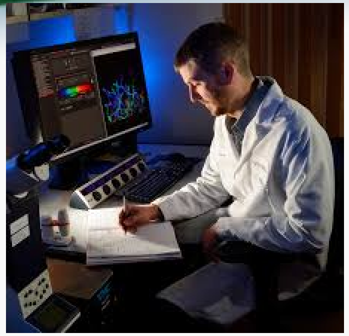
Only a tiny fraction of the compounds around us have been tested for safety

- *Chemicals used by U.S. consumers and industry: >100,000*
- *Tested in vivo: <500*



This unknown is a direct consequence of limited testing resources

A Paradigm Shift in Regulatory Toxicology



Assessing Hazard with Limited Data: New Approach Methodologies (NAMs)

- Tools that provide basis for predictive assessment of chemicals with limited information/capacity for in vivo testing
  - Curated biological effects knowledgebases; computational and bioinformatic methods; in vitro assays (incl. HTT); short-term in vivo tests (incl. ‘omics endpoints)
- Current plausible applications to PFAS ERAs
  - Prioritization (bioactivity); categorization (biological similarities); guiding assay selection (sensitive species, endpoints)

Effectively Employing NAMs: Role of AOP Framework

- Depict causal response linkages across biological levels of organization
- Developed specifically to support use of NAM data for effects prediction in risk assessment
- Provides framework to assemble and communicate knowledge
- Multiple ongoing efforts supporting eco-AOP development for PFAS
  - Fish, amphibians, birds, invertebrates

NAMs for PFAS ERAs: Biological Knowledgebases

- ECOTOX Knowledgebase (cfpub.epa.gov/ecotox)
  - Open access tool curated for 30 y by EPA with data from 50,000+ references
  - Quarterly updates of PFAS data (to date, 134 structures from >650 references)
  - Recent upgrades enhance data extraction/display/comparison
  - Standardized format amenable to formal systematic review
  - Uses: identifying available data; generating SSDs; "read-across" analyses
- AOP Wiki (aop.wiki.org)
  - Interactive open access system with 250+ AOPs, several applicable to PFAS toxicity in fish, amphibians, birds and mammalian wildlife
    - Effects on thyroid system: swim bladder inflation (AOP 155); amphibian metamorphosis (AOP 190)

NAMs for PFAS ERAs: Computational & Bioinformatic Tools

- Predicting bioactivity based on structure
  - Cheng and Ng (2019)* employed HTT data and machine-learning algorithms to build QSARs to predict bioactivity of 3400+ PFAS
  - Data primarily from in mammalian assay systems with different protein targets
  - Are bioactivity predictions relevant to nonmammalian species?
  - Defining the biological domain of activity predictions
  - Structural/functional conservation of protein targets provides a basis for predicting potential cross-species susceptibility to PFAS
  - LaLone et al. (2018)** used novel tool to explore basis of extrapolation of HTT results across species using NCBI protein sequence data

* Environ. Sci. Technol. 53:13970-13980
** Environ. Sci. Technol. 52:13960-13971

NAMs for PFAS ERAs: In vitro Bioactivity Assays

- Attagene assay system (Houck et al. in review*)
  - Multiplexed platform for ≈70 biological pathways, many with nuclear receptors as transcription factors
  - Assay conducted with ≈130 PFAS representing different structural classes
  - Notable pathways affected: thyroid, estrogen, retinoid, PPAR signaling
  - Use(s): prioritization; biological categorization; test design; informing AOPs
  - Mammalian-based system
- Ecotox FACTORIAL Assay (Medvedev et al. 2020**)
  - Multiplexed platform similar to Attagene enabling simultaneous assessment of ER, AR, TR, PPARɣ from multiple taxa (mammals, fish, frogs, birds, reptiles)

* houck.keith@epa.gov

**Environ. Sci. Technol. doi.org/10.1021/acs.est.0c03375

NAMs for PFAS ERAs: Short Term In vivo Tests

- High throughput multi-endpoint assays (Padilla et al.*)
  - Embryonic zebrafish (<6 hpf) 120 h exposures in 96-well format with ≈130 PFAS representing different structural classes
  - Multiple apical endpoints (survival, dysmorphology)
  - Use(s): baseline toxicity data; biological categorization; SARs, informing AOPs
- High throughput transcriptomic assays (Villeneuve et al.**)
  - Embryonic fathead minnow (<12 hpf) 24 h exposures in 96-well format with 20 high priority PFAS, most with little tox data
  - Emphasis on dose-dependent transcriptomic responses
  - Use(s): Point-of-departure estimates for "default" benchmarking; informing AOPs

* padilla.stephanie@epa.gov
**villeneuve.dan@epa.gov

Summary

- PFAS present credible risks to various ecological systems and services
- Traditional approaches for exposure/effects assessments valid but require "tailoring" to properties of PFAS (e.g., bioaccumulation models; assays with susceptible species/endpoints)
- Greatest current challenge is lack of data needed to conduct ERAs for majority of PFAS
- Innovative predictive approaches needed to prioritize, categorize and guide PFAS testing
- Integration of NAMs with traditional assessment methods offer exciting opportunities for ERAs with PFAS *and* wider chemical universe

Acknowledgements

- Contributors to the SETAC PFAS FTM
  - Eco Paper coauthors
  - Breakout group discussants
  - Meeting organizers/facilitators
  - Sponsors
- USEPA/ORD PFAS Scientists
  - Research: C. Elonen, K. Houck, S. Padilla, D. Villeneuve
  - Reviewers: J. Conley, A. Gillespie, D. Hoff, C. LaLone, B. Rodan

For More Information

- Some of the research discussed in this presentation is part of EPA's overall efforts to rapidly expand the scientific foundation for understanding and managing risk from PFAS.

- For more information on EPA's efforts to address PFAS, please visit the following websites
  - EPA PFAS Action Plan - https://www.epa.gov/pfas/epas-pfas-action-plan
  - EPA PFAS Research - https://www.epa.gov/chemical-research/research-and-polyfluoroalkyl-substances-pfas