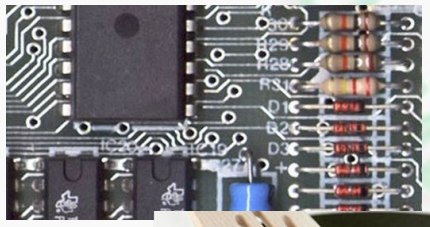
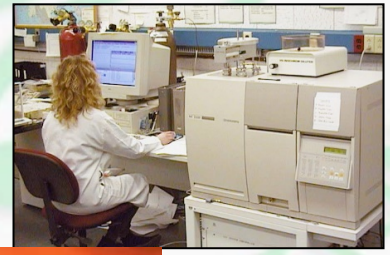
Assessing the Ecological Risks of PFAS: Challenges and Opportunities*

Gerald Ankley, USEPA/ORD, Duluth, MN







*Content does not necessarily reflect EPA position or policy.

PFAS: Human Health and Ecological Concerns

- Early (and ongoing) emphasis on human health (e.g., immune suppression, cancer, thyroid disease, elevated cholesterol)
  - Drinking water and (more recently) dietary exposures

- Increasing attention for potential ecological effects
  - Detected in media/biota in many ecosystems from point/nonpoint sources
  - Some highly persistent and potentially bioaccumulative (may biomagnify)
  - Exposure concerns encompass large universe of chemicals (100s/1000s?)

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

Environmental Risk Assessment of PFAS

12–15 August 2019 | Durham, NC, USA

SETAC North America Focused Topic Meeting

Four-day workshop with +400 attendees and mix of topic-oriented presentations breakouts/discussions, with peer-reviewed reports prepared by each topic group:

Johnson et al. Estimating environmental hazards and risk from exposure to PFAS: Outcome of a SETAC focused topic meeting

De Silva et al. Exposure pathways for humans and wildlife: Synthesis of current knowledge and key gaps in understanding

Ankley et al. Assessing the ecological risks of PFAS: Current state-of-the science and a proposed path forward

Fenton et al. PFAS toxicity and human health review: Current state of knowledge and strategies for informing future research

Mikkonen et al. Suggestions for improving the characterization of risk from exposures to PFAS

Environmental Toxicology and Chemistry, March 2021, Highlighted Issue on PFAS

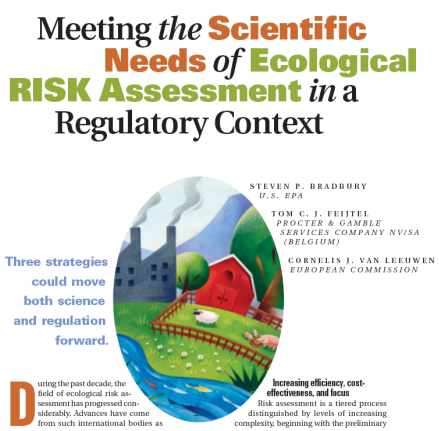
Ecological Effects of PFAS: Knowledge and Needs

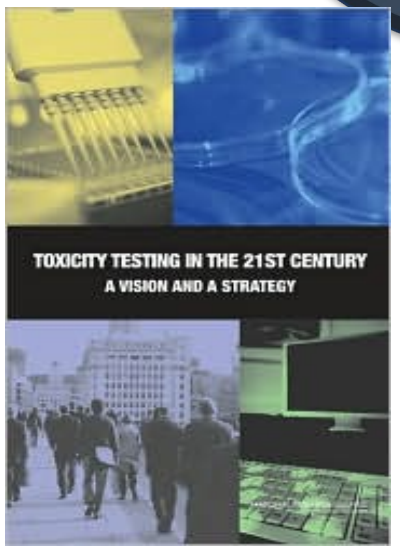
- Significant amount of (mostly) acute toxicity data in some taxa (fish, aquatic invertebrates) for few PFAS (PFOA, PFOS), but…
- Little or no information from many perspectives
  - Majority of PFAS/PFAS classes; most degradates/metabolites
  - Several important taxa (birds, amphibians, reptiles, marine mammals, terrestrial invertebrates, plants)
  - Diversity of chronic endpoints
  - Uncertainties as to mixture effects
- **Critical requirements**
  - **Generate some baseline toxicity data for diverse PFAS structures**
  - **Implement approaches to address data gaps for majority of PFAS**

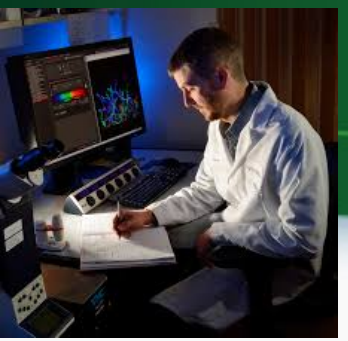
PFAS Just Latest "Visible" Group of Data-Poor Chemicals

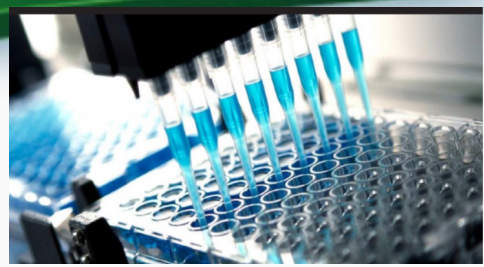
- Very limited toxicity characterization for most chemicals in commerce

- Lack of data has fueled a conceptual shift in field of regulatory toxicology









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

- Pathway-based tools that provide basis for predictive assessment of chemicals with limited information/capacity for in vivo testing
  - Biological effects knowledgebases; computational/bioinformatic methods; in vitro assays (incl. HTT); short-term in vivo tests (incl. ‘omics endpoints)
- Initial emphasis on use in human health assessment but increasingly considered in ecological assessments
- Current NAM applications to chemical hazard assessment
  - Prioritization; Categorization; Informing testing (e.g., taxa, endpoints); Estimating effects benchmarks

Role of AOP Framework in Employing NAM Data

- 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
- Ongoing international efforts focused on AOP collation, harmonization, review and regulatory implementation (OECD)

Pathway-based approaches for assessing ecological hazards/risks of PFAS: Illustrative examples of ongoing research/applications

Biological Effects Knowledgebases

Computational & Bioinformatic Approaches

In vitro HTT Assays

Short-term In vivo Assays

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
  - Recent upgrades greatly enhance data extraction/display/comparison
  - Ongoing quarterly updates of PFAS data (so far 134 structures, >650 references)
  - Uses: Informing testing; Estimating benchmarks (e.g., SSDs, “read-across”)
- AOP Wiki (aop.wiki.org)
  - Interactive open access system with 300+ AOPs, several applicable to PFAS toxicity in fish, amphibians, birds and mammalian wildlife (e.g., thyroid effects)
  - Uses: Categorization; Informing testing

NAMs for PFAS ERAs: Computational & Bioinformatic Tools

- Predicting bioactivity based on structure
  - Cheng and Ng (2019)* used HTT (e.g., ToxCast) data with machine-learning algorithms to build QSARs to predict bioactivity of 3400+ PFAS
  - Data primarily from in mammalian assay systems with different protein targets
  - Uses: Prioritization; Categorization
- Are predictions from mammalian systems relevant to nonmammalian species?
  - Structural/functional conservation of protein targets provides a basis for predicting potential cross-species susceptibility to PFAS
  - LaLone et al. (2018)** used SeqAPASS 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 mammalian nuclear receptors as transcription factors
  - Assay conducted with ≈120 PFAS representing different structural classes
  - Notable pathways affected: thyroid, estrogen, retinoid, PPAR signaling
  - Uses: Prioritization; Categorization; Informing testing (with AOPs)
- 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)

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

NAMs for PFAS ERAs: Short Term In vivo Tests

- High throughput multi-endpoint assays*
  - Embryonic zebrafish (<6 hpf) 120 h exposures in 96-well format with ≈120 PFAS representing different structural classes
  - Multiple apical endpoints (survival, dysmorphology)
  - Uses: Prioritization; Categorization; Estimating benchmarks
- High throughput transcriptomic assays**
  - Embryonic fathead minnow (<12 hpf) 24 h exposures in 96-well format with 20 high priority PFAS, most with little tox data
  - Dose-dependent transcriptomic responses for point-of-departure (POD) analyses
  - Uses: Estimating benchmarks (POD); Guiding testing (with AOPs)

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Eco-Relevant AOPs for PFAS: Some Ongoing Efforts

- PPAR activation linked to developmental (early life-stage) and reproductive (adult) effects in fish (olker.jennifer@epa.gov; nacci.diane@epa.gov)
- Thyroid signaling inhibition leading to impaired metamorphosis in amphibians (degitz.sigmund@epa.gov)
- Estrogen receptor activation by weak ligands linked to developmental and reproductive impairment in fish (villeneuve.dan@epa.gov)
- Thyroid signaling inhibition leading to decreased early life-stage survival in avian species (etterson.matthew@epa.gov)

Summary

- PFAS present plausible risks to ecological systems and services
- “Traditional” exposure/effects approaches valid for ERAs
- Greatest current challenge is lack of data needed to conduct credible ERAs for majority of PFAS
- NAMs can serve as basis for predictive approaches to prioritize, categorize and inform PFAS evaluation/testing
- Integration of NAMs with traditional methods offer substantive inroads to conducting ERAs for PFAS

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