Assessing the Ecological Effects of PFAS: Current Knowledge, Existing Uncertainties, and a Path Forward*

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ENVIRONMENTAL RISK ASSESSMENT OF PFAS
SETAC North America Focused Topic Meeting

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PFAS Ecological Effects: Four Key Questions

1. What do we currently know regarding PFAS ecotoxicity to assess ecological risk(s)?
2. What do we need to know regarding PFAS ecotoxicity to assess ecological risk(s)? What are key data gaps?
3. Are there emerging methods that might help address data gaps and uncertainties?
4. What are appropriate approaches for assessing the ecological effects of PFAS mixtures?

Answering the Questions: Basic Approach

- Nine platform presentations (Day 2)
  - General ERA needs and specific activities from different regions
  - Overviews of existing data for different PFAS by taxonomic group
  - Status of "traditional" and emerging approaches for testing
- Facilitated breakout group discussions (Days 3&4)
  - Core experts group (coauthors of this talk)
  - Approximately 80-90 "observers"
- Final plenary/exchange with all other workgroups

PFAS ERA Challenges and Needs

- Large number (1000s) of structurally-diverse chemicals, the majority of which have little (or no) fate/exposure/effects data
- Retrospective *and* prospective approaches required
  - Alternatives "challenge" (new products onboarding continually)
- "Hot" spots (e.g., airbases) and broader/non-point source issues (e.g., atmospheric transport, ocean circulation)
- Pressing needs
  - Effect-based benchmark values for "triggers" and clean up
  - Sensitive/susceptible species (and endpoints) for testing/monitoring
  - Prediction of bioaccumulation potential (human and eco issue)

Environment Canada: One Example of ERA Activities for PFAS

REGULATORY ACTIVITIES

- From 2006 – 2012, PFOS, PFOA & LC PFCAs, their salts and precursors were concluded as harmful to the environment and added to Schedule 1 – List of Toxic Substances
- Risk management activities are ongoing
- Attention in Canada has now shifted to SC PFCAs/PFSAs (C4 – C7) and LC PFSAs (C9 – C20)
- To help inform regulatory activities in Canada, 1400 publically available papers were analyzed (early 1990s to 2019) to identify ecological data gaps and assessment challenges for SC PFCAs/PFSAs and LC PFSAs *(see Poster (Tuesday), J. Kurias)*

GUIDELINE ACTIVITIES

- Ecological Guidelines available for PFOS (surface water, fish health, wildlife diet-mammalian and avian, soil for agricultural, residential, parkland, commercial and industrial)
- Commissioned tests to fill data gaps (rainbow trout, soil invertebrates)
- ECCC Biological Test Methods - valuable resource to conduct high quality standardized testing/reporting; data can be used for Guidelines and risk assessment
- PFOS levels in Canadian surface water and fish are below environmental quality guidelines to protect aquatic life and fish health
- PFOS levels in fish in some drainage basins are above the environmental quality guidelines to protect mammals and birds that may consume fish
- Ecological Guidelines underway for PFOA

MONITORING/RESEARCH ACTIVITIES

- Monitoring of PFAS in biotic and abiotic matrices from Great Lakes and Canadian Arctic
- Research on:
  - metabolism/transformation
  - bioaccumulation, biomagnification in aquatic food webs
  - acute and chronic effects
  - multi-generational effects
- Non-targeted screening of new PFAS

LC = long-chain (C9-C20): SC = short-chain (C4-C7)

What do we currently know regarding PFAS ecotoxicity to assess ecological risk(s)?

- Most effects testing to date has been with a limited number of model fish and to a lesser extent invertebrate species

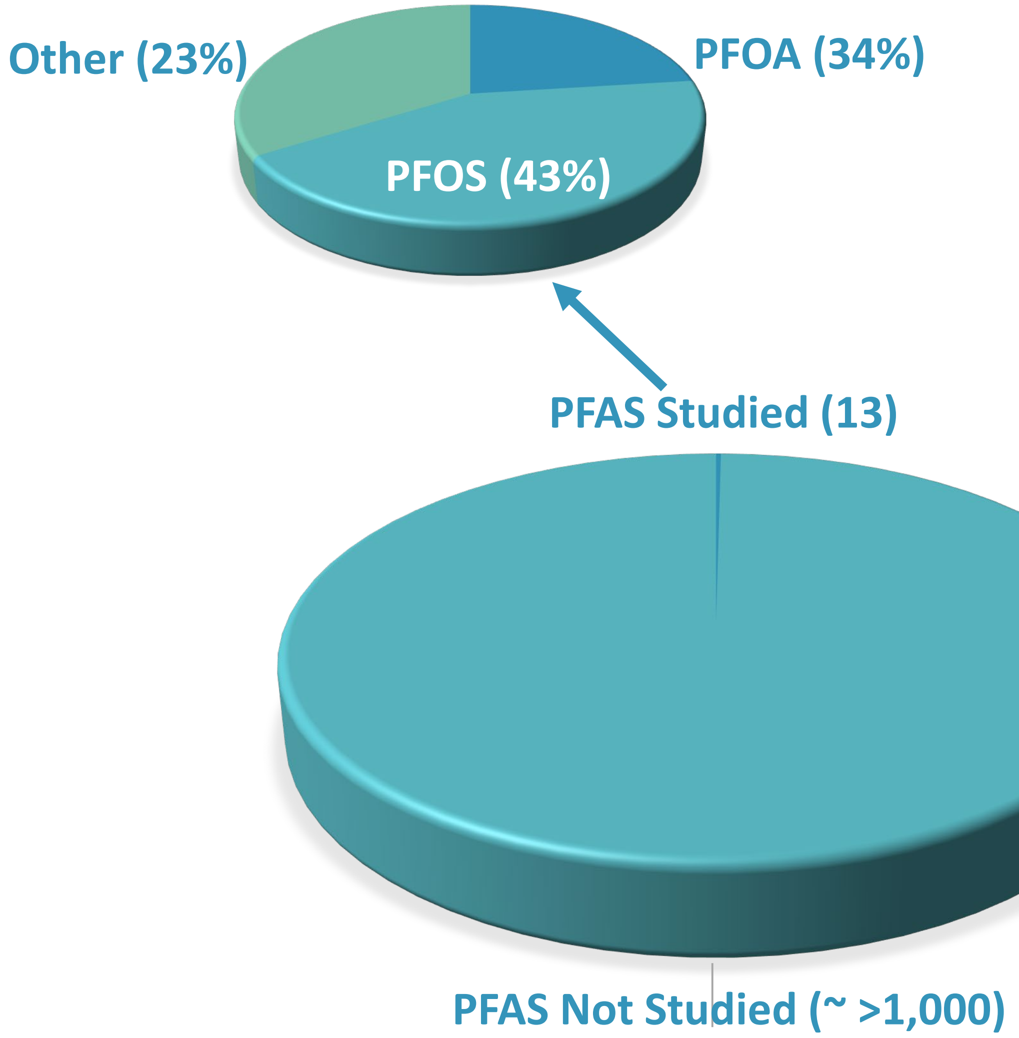
- Existing data for only a relatively few, high-visibility PFAS

- Emphasis on acute as opposed to chronic effects

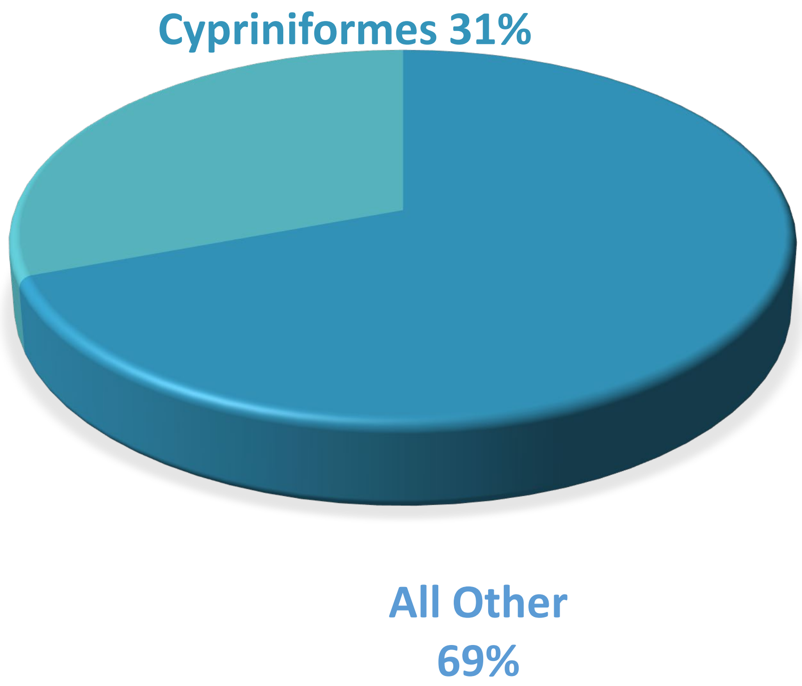
- Significant amount of existing lab-based toxicity data for PFAS has QA issues

Summary of PFAS/Fish Toxicity Studies*

PFAS Studied



Fishes Studied



*From >150 total studies reviewed

Summary of PFAS/Aquatic Invertebrate Tox Studies

				Crustacea					Mollusca		Insecta		Worms	
		Ciliate protozoa	*Rotifera*	*Copepoda Isopoda Mysida*	*Decapoda (Shrimp, Crab)*	*Amphipoda*	*Water Flea*	*Echinodermata*	*Bivalvia*	*Gastopoda*	*Midge*	*Damselfly*	*Platyhelminthe*	*Annelida*
		2 (1)	*9 (1)*	*9 (1)*	*5 (1)*	*2 (1)*	*9 (1)*	*3 (1)*	*9 (3)*	*2*	*3 (2)*	*1*	*1*	*2*
PFAS														
4	*PFBS*	X							X		X			
6	*PFHxS*								X					
8	*PFOS*	X	X	X	X	X	X	X	X	X	X	X	X	X
8	*PFECHS*						X							
8	*PSOF*							X						
10	*PFDS*								X					
PFCA														
1	*TFA*		X											
2	*PFPrA*		X				X							
3	*PFBA*		X				X							
4	*PFPeA*		X				X		X					
5	*PFHxA*		X				X		X					
6	*PFHpA*						X		X					
7	*PFOA*	X	X	X	X		X	X	X	X	X		X	X
8	*PFNA*	X					X		X		X			
9	*PFDA*	X					X		X		X			
10	*PFUnDA*						X		X		X			
10	*PFDdA*	X												
11	*PFDoDA*						X				X			

Standard test specie: *Tetrahymena thermophila, Brachionus calyciflorus, Tigriopus japonicus, Americamysis bahia, Hyalella azteca, Daphnia magna, Paracentrotus lividus, Mytilus galloprovincialis, Crassostrea gigas, Crassostrea virginica, Chironomus tentans, Chironomus riparius*

Evaluation of Existing Data: Notable QA Issues

1. Lack of verification of exposure concentrations (esp. prominent)
2. Analytical methods
   - Lack of standards
   - Method availability
3. Background contamination – low levels of PFAS detected in control treatments
4. Inconsistent measurement and reporting of environmental test conditions, test chamber composition, carrier solvents

What do we need to know regarding PFAS ecotoxicity to assess ecological risk(s)? What are key data gaps?

- Broader representation of potentially sensitive phyla (incl. plants, microbes)
- More chronic test data/sublethal endpoints
- Mechanistic basis for cross-species extrapolation of effects
- Better understanding of processes controlling bioaccumulation
- Integrated approach to prioritize PFAS for in-depth assessment/testing

Basis of a Framework for Prioritizing PFAS for Ecological Testing and Assessment

- Production volume and use
- Environmental occurrence - parents, degradates
- Possibility of bioaccumulation
- Potential for effects (in vivo toxicity, in vitro bioactivity)
- Unique physio-chemical attributes (e.g., volatility)

Are there emerging methods that might help address data gaps and uncertainties?

- Curated, open-source databases (e.g., ECOTOX)

- In vitro and short-term in vivo assays with endpoints indicative of perturbation of specific mechanism/pathways

- Bioinformatic integration tools for quantitative prediction of bioactivity and cross-species extrapolation(e.g., QSARs, SeqAPASS)

- Adverse outcome pathway (AOP) framework for data assembly and “translation”

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Identifying Biological Activity of PFAS: High Throughput Screening

- 150 different PFAS subjected to HTS, building on USEPA ToxCast™ effort

- Attagene platform, featuring around 90 different pathways

- Initial results highlight handful of commonly observed bioactivities associated with diverse PFAS

Table 1 Human Nuclear Receptors Included in *trans*- Factorial-1 Assay

#	Abbreviation	Name	Nomenclature
1	M-06	*Internal control endpoint*	n/a
2	FXR	Farnesoid X receptor	NR1H4
3	AR	Androgen receptor	NR3C4
4	RARγ	Retinoic acid receptor-γ	NR1B3
5	GAL4	Yeast GAL4, negative control	GAL4
6	RXRα	Retinoid X receptor-α	NR2B1
7	GR	Glucocorticoid receptor	NR3C1
8	RARβ	Retinoic acid receptor-β	NR1B2
9	RARα	Retinoic acid receptor-α	NR1B1
10	PPARγ	Peroxisome proliferator-activated receptor-γ	NR1C2
11	ERRγ	Estrogen-related receptor-γ	NR3B3
12	RORα	RAR-related orphan receptor-α	NR1F1
13	ERα	Estrogen receptor-α	NR3A1
14	LXRα	Liver X receptor-α	NR1H3
15	ERRα	Estrogen-related receptor-α	NR3B1
16	M-19	*Internal control endpoint*	n/a
17	M-32	*Internal control endpoint*	n/a
18	PXR	Pregnane X receptor	NR1I2
19	TRα	Thyroid hormone receptor-α	NR1A1
20	LXRβ	Liver X receptor-β	NR1H2
21	CAR	Constitutive androstane receptor	NR1I3
22	PPARα	Peroxisome proliferator-activated receptor-α	NR1C1
23	RORγ	RAR-related orphan receptor-γ	NR1F3
24	RXRβ	Retinoid X receptor-β	NR2B2
25	HNF4α	Hepatocyte nuclear factor-4-α	NR2A1
26	M-61	*Internal control endpoint*	n/a
27	NURR1	Nuclear receptor related 1	NR4A2
28	VDR	Vitamin D receptor	NR1I1
29	PPARδ	Peroxisome proliferator-activated receptor-δ	NR1C3

What are appropriate approaches for assessing the ecological effects of PFAS mixtures?

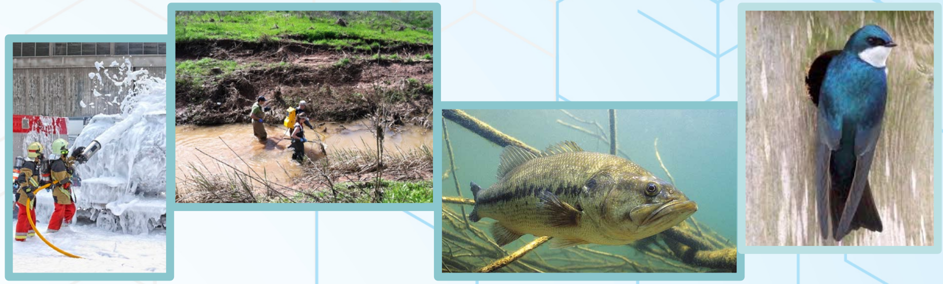
- Many PFAS enter the environment as multi-component formulations (e.g., AFFF) and virtually all field exposures involve complex mixtures

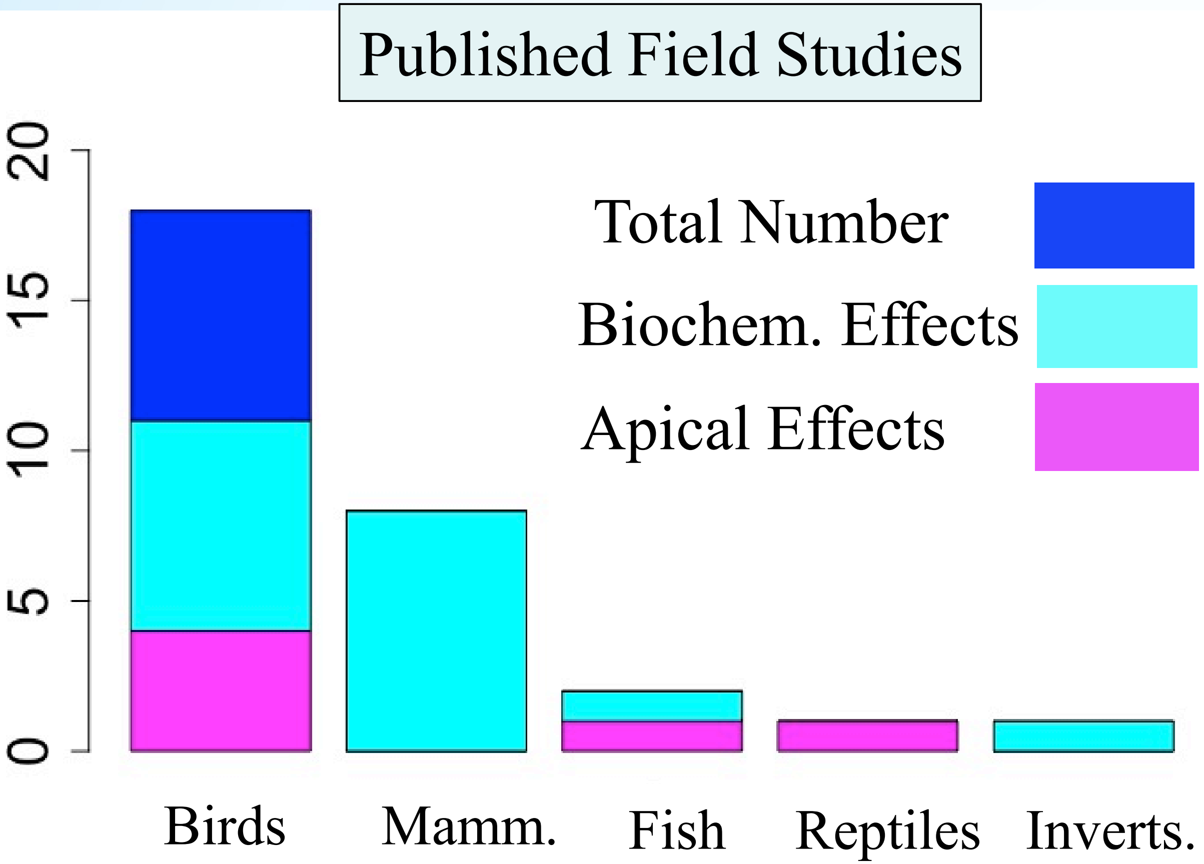
- Lack of knowledge of formulation composition and validated analytical methods for multiple PFAS in environmental matrices problematic

- Absence of toxicity/bioactivity data for many PFAS present in mixtures limits utility of predictive models (e.g., TEF-based approach)

- Combined analytical/biological approaches needed for addressing risks of complex PFAS mixtures in prospective and retrospective (field) studies

PFAS Mixtures and Field Effects





- PFAS exposure related to source
  - PFOS often dominant (but most frequently measured as well)
  - A mixture of long-chain PFAS for Arctic species (e.g., C11-C13 PFCA)
- Many studies point to biochemical effects:
  - Immunologic
  - Oxidative stress
  - Other sub-organismal
- Fewer demonstrations of effects on survival, growth, reproduction

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Next Steps and Acknowledgements

- Journal article: "*Assessing the Ecological Risks of PFAS: Current Sate-of-the-Science and Proposed Path Forward*" targeted for submission in early 2020

- Thanks for the contributions from the **entire** eco-effects breakout group of observers/participants, the PFAS FTM Steering Committee, meeting sponsors, and especially the SETAC support staff (Tamar Schlekat, Greg Schiefer, Nikki Mayo, Terresa Murdoch, Sabine Barrett, Dusty Kennedy)