Computational Toxicology and Exposure Communities of Practice

Sharing research and promoting collaboration

Thursday, December 7, 11 AM-12 PM ET

Agenda:

- Opening remarks: Sammy Hanf
  (Communications Specialist, Center for Computational Toxicology and Exposure)
- Presentation: Dan Villeneuve
  (Center for Computational Toxicology and Exposure)
- Q&A
- Closing remarks: Sammy Hanf

For more information on the CompTox CoP, visit:
epa.gov/chemical-research/computational-toxicology-communities-practice

Dan Villeneuve

Combining new approach methodologies and adverse outcome pathways for ecological risk-based screening and prioritization

This presentation will use four case studies to illustrate the complementary use of NAMs and adverse outcome pathways (AOPs) to help prioritize higher tiers of testing and support efficient ecological risk assessment.

Combining new approach methodologies and adverse outcome pathways for ecological risk-based screening and prioritization

Dan Villeneuve, United States Environmental Protection Agency, Office of Research and Development, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division, Duluth, MN, USA

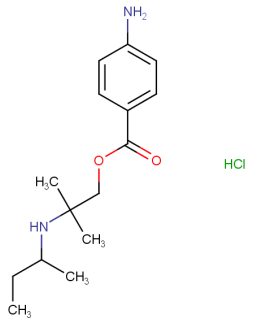
The contents of this presentation neither constitute, nor necessarily reflect US EPA policy.

Any mention of trade names or commercial products doses not constitute endorsement or recommendation for use.

Outline

- Traditional toxicity testing
- NAMs and the need for alternatives
- Blueprint for computational toxicology at US EPA
- Examples
  - ER active PFAS
  - PFAS transcriptomics
  - Eco-transcriptomics (Great Lakes)
  - Environmental mixtures

Toxicity testing

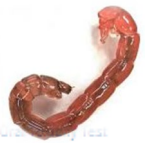


52 Guideline tests for ecotoxicity alone

>80 for human health

Majority are some variation of expose an animal, observe what happens (typically at a high level of biological organization).

23 Nov 2004	Test No. 202: Daphnia sp. Acute Immobilisation Test	OECD
04 Jul 2023	Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	OECD
21 Jan 2000	Test No. 217: Soil Microorganisms: Carbon Transformation Test	OECD
21 Jan 2000	Test No. 216: Soil Microorganisms: Nitrogen Transformation Tes	OECD
21 Jan 2000	Test No. 215: Fish, Juvenile Growth Test	OECD
21 Sept 1998	Test No. 214: Honeybees, Acut	OECD
21 Sept 1998	Test No. 213: Honeybees, Acute	OECD
21 Sept 1998	Test No. 212: Fish, Short-term Toxicity Test	OECD
04 Apr 1984	Test No. 207: Earthworm, Acute Toxicity Tes	OECD
04 Apr 1984	Test No. 206: Avian Reproduction Test	OECD
04 Apr 1984	Test No. 205: Avian Dietary Toxicity Test	OECD
04 Apr 1984	Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	OECD
26 Sept 2014	Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test	OECD
26 Sept 2014	Test No. 238: Sediment-Free Myriophyllum Spicatum Toxicity Test	OECD
26 Jul 2013	Test No. 210: Fish, Early-life Stage Toxicity Test	OECD
26 Jul 2013	Test No. 237: Honey Bee (Apis Mellifera)	OECD
26 Jul 2013	Test No. 236: Fish Embryo Acute Toxicity	OECD
02 Oct 2012	Test No. 229: Fish Short Term Reproducti	
	: Daphnia magna Reproducti	
	n Test	
	Cycle Toxicity Test Using Spike	
	bition Test (Carbon and Ammonium Oxidatio	
	OECD	
	st No. 230: 21-day Fish Assay	
	225: Sediment-Water Lumbriculus Tox	
	224: Determination of the Inhibition of the Activity of Anaerobic Bacteria	
	mary of Considerations in the Report from the OECD Expert Group on Ecotoxicology	
	OECD	
17 Aug 2006	Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test	OECD
17 Aug 2006	Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	OECD
11 Jul 2006	Test No. 221: Lemna sp. Growth Inhibition Test	OECD
04 Jul 2023	Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT)	OECD
04 Jul 2023	Test No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water	OECD
30 Jun 2022	Test No. 251: Rapid Androgen Disruption Activity Reporter (RADAR) assay	OECD
	349: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay	
	50: EASZY assay - Detection of Endocrine Active Substances, acting through estrogen , using transgenic tg(cyp19a1b:GFP) Zebrafish embryos	
	48: Xenopus Eleutheroembryonic Thyroid Assay (XETA)	
	03: Fish, Acute Toxicity Test	
	47: Bumblebee, Acute Oral Toxicity Test	OECD
09 Oct 2017	Test No. 246: Bumblebee, Acute Contact Toxicity Test	OECD
	45: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding)	
	44: Protozoan Activated Sludge Inhibition Test	OECD
29 Jul 2016	Test No. 232: Collembolan Reproduction Test in Soil	OECD
29 Jul 2016	Test No. 228: Determination of Developmental Toxicity to Dipteran Dung Flies(Scathophaga stercoraria L. (Scathophagidae), Musca autumnalis De Geer (Muscidae))	OECD
29 Jul 2016	Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil	OECD
29 Jul 2016	Test No. 223: Avian Acute Oral Toxicity Test	OECD
29 Jul 2016	Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)	OECD
29 Jul 2016	Test No. 220: Enchytraeid Reproduction Test	OECD
29 Jul 2016	Test No. 243: Lymnaea stagnalis Reproduction Test	OECD
29 Jul 2016	Test No. 242: Potamopyrgus antipodarum Reproduction Test	OECD
28 Jul 2016	Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA)	OECD



2004

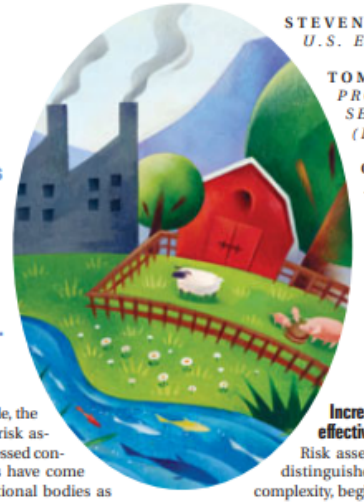
Meeting *the* Scientific Needs *of* Ecological RISK Assessment *in* a Regulatory Context

STEVEN P. BRADBURY
U.S. EPA

TOM C. J. FEIJTEL
PROCTER & GAMBLE SERVICES COMPANY NV/SA (BELGIUM)

CORNELIS J. VAN LEEUWEN
EUROPEAN COMMISSION

Three strategies could move both science and regulation forward.



During the past decade, the field of ecological risk assessment has progressed considerably. Advances have come from such international bodies as the Organisation for Economic Co-operation and Development (OECD), the World Health Organisation (WHO), the European and Mediterranean Plant Protection Organisation (EPPO), and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (*1–8*). Risk assessments have played a critical role in the development of various regulations within the European Commission (EC) as well as in other parts of the world, including the United States, Canada, and Japan (*9–17*). But scientists and regulators are faced with three significant challenges: streamlining the risk-assessment process, quantifying risks in a spatially explicit manner, and acquiring the correct kind of environmental data to enable regulatory programs to effectively focus on future environmental protection activities.

Increasing efficiency, cost-effectiveness, and focus

Risk assessment is a tiered process distinguished by levels of increasing complexity, beginning with the preliminary categorization step, followed by a refined or screening assessment, and progressing to the full, comprehensive risk assessment (*4, 18, 19*). For each tier, a minimum level of information is required. For example, OECD has established an international program—called the Screening Information Data Sets (SIDS)—for surveying high-production-volume chemicals (HPV) for potential effects. SIDS include the basic information needed to perform a preliminary assessment of a chemical's potential risk (*20*).

Applying the current risk-assessment paradigm and meeting the associated data-generation requirements, combined with the increased need to evaluate the potential effects posed by thousands of industrial chemicals, are big challenges for the chemical industry, national and international regulatory

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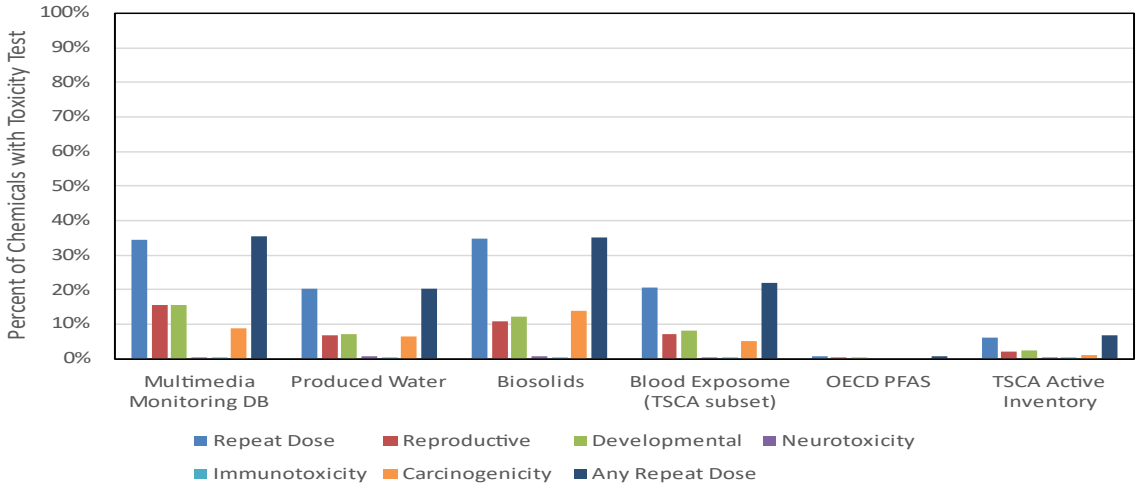
Traditional testing with defined batteries of in vivo tests

- Too many chemicals
- Too costly
- Too much time to generate and interpret
- Too many animals
- Inefficient
  - Typically only a subset of the data are used for the assessments

Bradbury SP, Feijtel TC, Van Leeuwen CJ. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol. 2004 Dec 1;38(23):463A-470A. doi: 10.1021/es040675s.



https://www.epa.gov/system/files/documents/2023-06/ETAP%20Sci%20Support%20Doc_BOSC%20Report_Draft%20Final_5_31_23_508%20tagged.pdf

Meeting *the* Scientific Needs *of* Ecological RISK Assessment *in* a Regulatory Context

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- If one assumes all chemicals on “a list” do not need to be tested, and for those that do, not all can be tested for all possible endpoints at once, then the following questions must be addressed:
  - Which chemicals should be tested *[in vivo]*?
  - And of these, which should be tested first?
  - For what endpoints [*in vivo*]?
  - Based on what rationale?

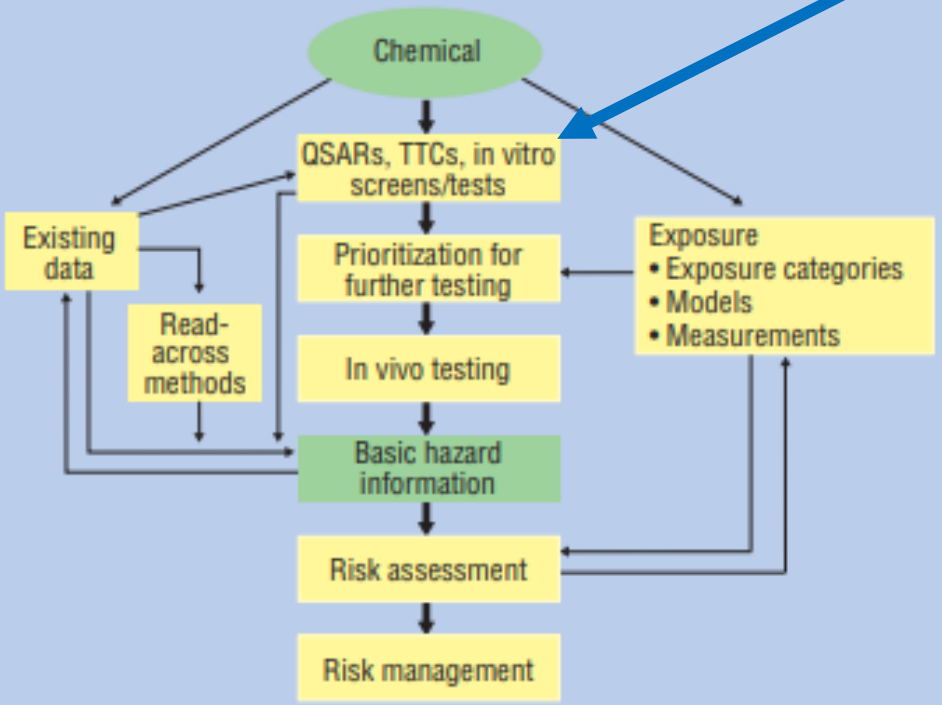
Bradbury SP, Feijtel TC, Van Leeuwen CJ. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol. 2004 Dec 1;38(23):463A-470A. doi: 10.1021/es040675s.

2004

FIGURE 1

Efficient risk assessment

Combining use and exposure information and effects information obtained from quantitative structure–activity relationships (QSARs), read-across methods, thresholds of toxicological concern (TTCs), and in vitro tests prior to in vivo testing is a more rapid, efficient, and cost-effective way to perform risk assessment of chemicals.



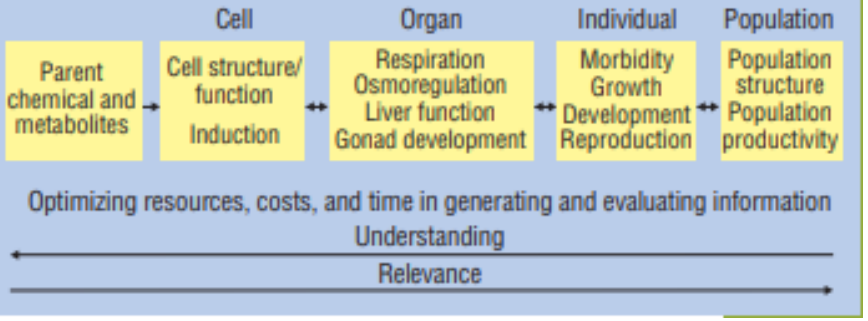
NAMs

FIGURE 2

Toxicity pathways

AOPs

Linking toxicological responses across levels of biological organization would help prioritize risk-based assessment questions and associated data and information needs.



Bradbury SP, Feijtel TC, Van Leeuwen CJ. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol. 2004 Dec 1;38(23):463A-470A. doi: 10.1021/es040675s.

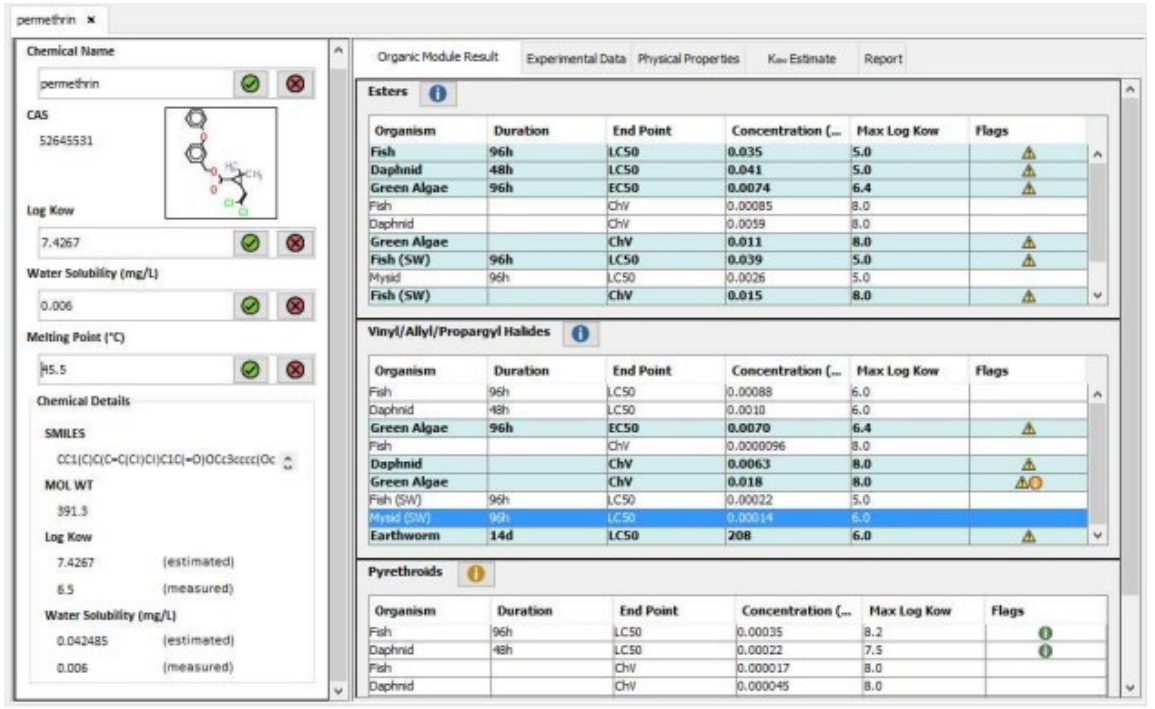
New approach methodologies

- **NAMs**: any technology, methodology, approach, that can provide information on chemical hazard and risk assessment without the use of intact [*protected life stages of vertebrate*] animals, including *in silico*, *in chemico*, *in vitro*, and *ex vivo* approaches (ECHA, 2016b; EPA, 2018d).

- ECHA (2016b). *New approach methodologies in regulatory science*. Proceedings of a scientific workshop. Helsinki: European Chemicals Agency. doi:10.2823/543644.
- EPA (2018d). Strategic plan to promote the development and implementation of alternative test methods within the TSCA program. U.S. Environmental protection agency. EPA-740-R1-8004. Available at: https://www.epa.gov/sites/default/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf.

Ecological Hazard Assessment Embraced QSARs long ago

- Quantitative structure-activity relationships (QSARs) have been used by the U.S. Environmental Protection Agency since 1981 (>40 years) to predict the aquatic toxicity of new industrial chemicals in the absence of test data.
- As of 2015, 709 QSARs had been developed for 111 organic chemical classes and integrated into ECOSAR.
- Strongest for so-called “baseline” toxicity, and a couple more specific modes of action.
- Gaps for specifically-acting chemicals: e.g., endocrine disruptors, pharmaceuticals, next generation pesticides, etc.

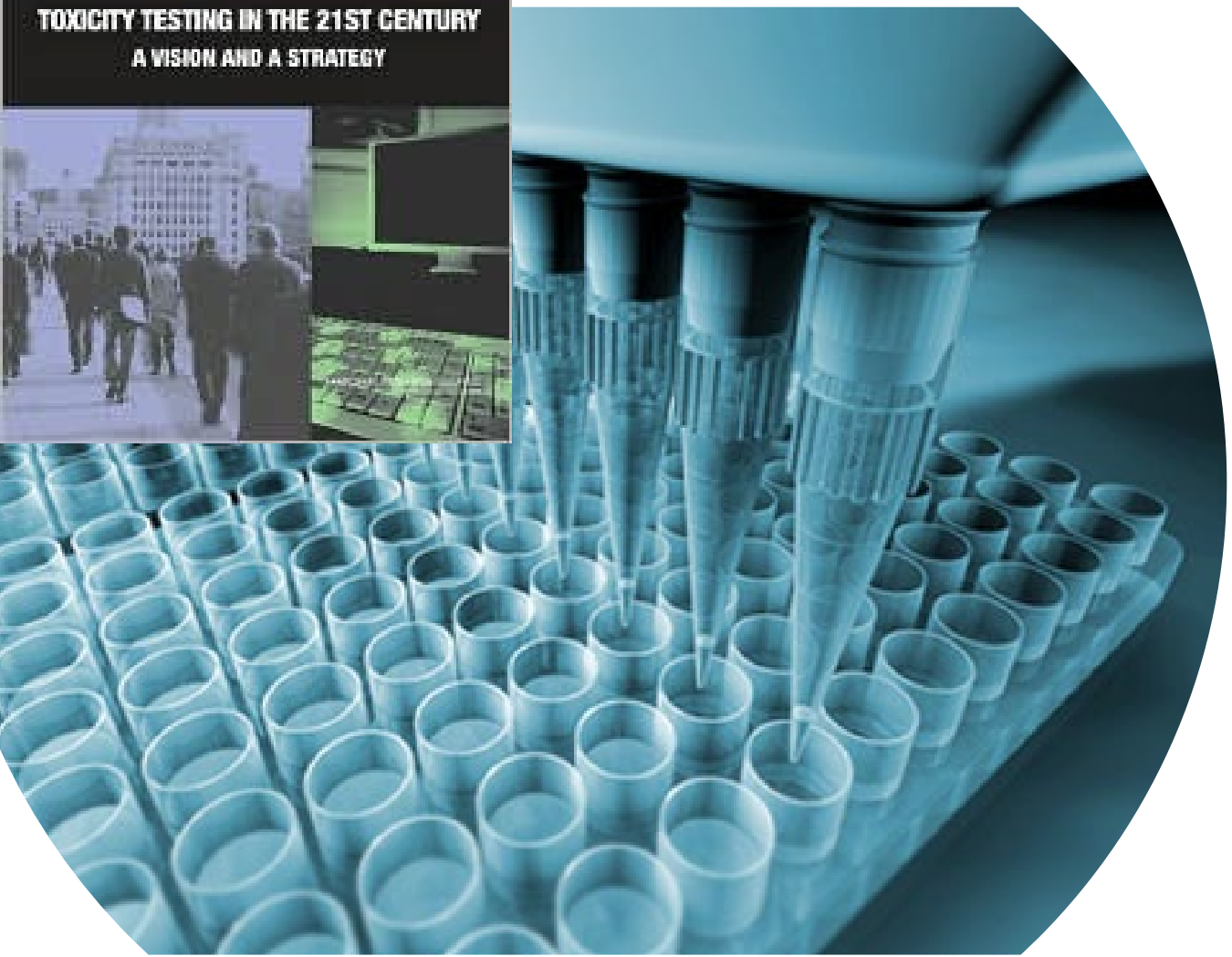


Operation Manual for the ECOlogical Structure-activity Relationship Model (ECOSAR) Class Program v. 2.2 (Feb. 2022). https://www.epa.gov/system/files/documents/2022-03/operation-manual-v.2.2_1.pdf

ECOSAR (https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model)

ToxCast™

Tox21



NRC TT21C - 2007

"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

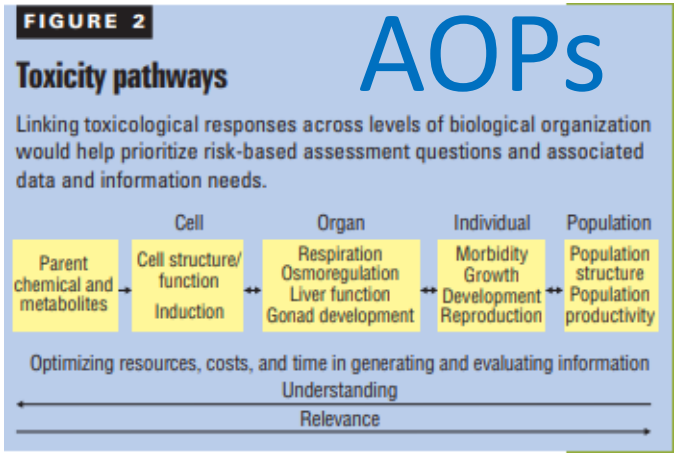
"The vision emphasizes the development of suites of predictive, high-throughput assays ….."

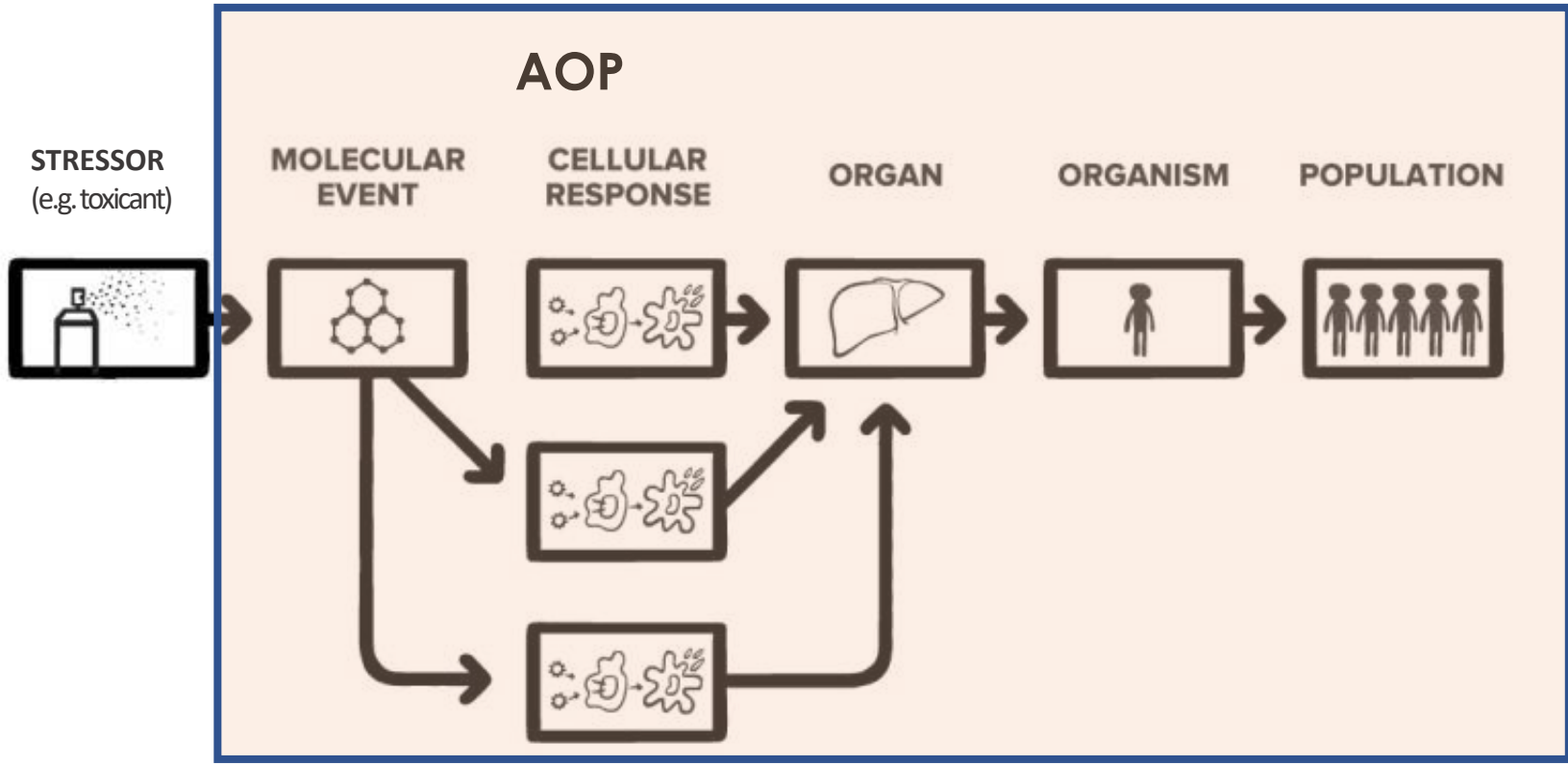
"The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves."

"Key Research Questions in Developing Knowledge to Support Pathway Testing"

- "Toxicity pathway identification – what are the key pathways whose perturbation results in toxicity?"
- "Adversity – what adverse effects are linked to specific pathway perturbations…"

2010





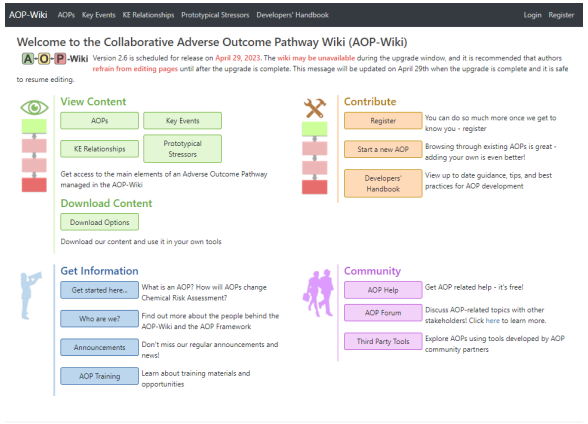
An <u>Adverse Outcome Pathway</u> (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct <u>molecular initiating event</u> and an <u>adverse outcome</u>, at a level of biological organization relevant to risk assessment.

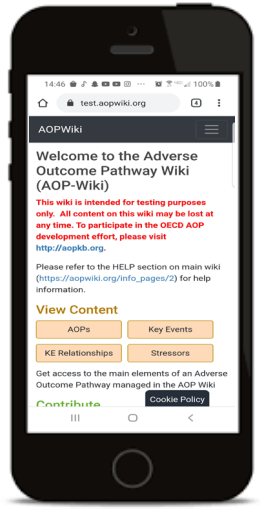
(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

2010-2014 – Formalization of AOP Framework

- **Organize and assemble the specialized scientific knowledge required to interpret results from new approach methodologies (NAMs).**

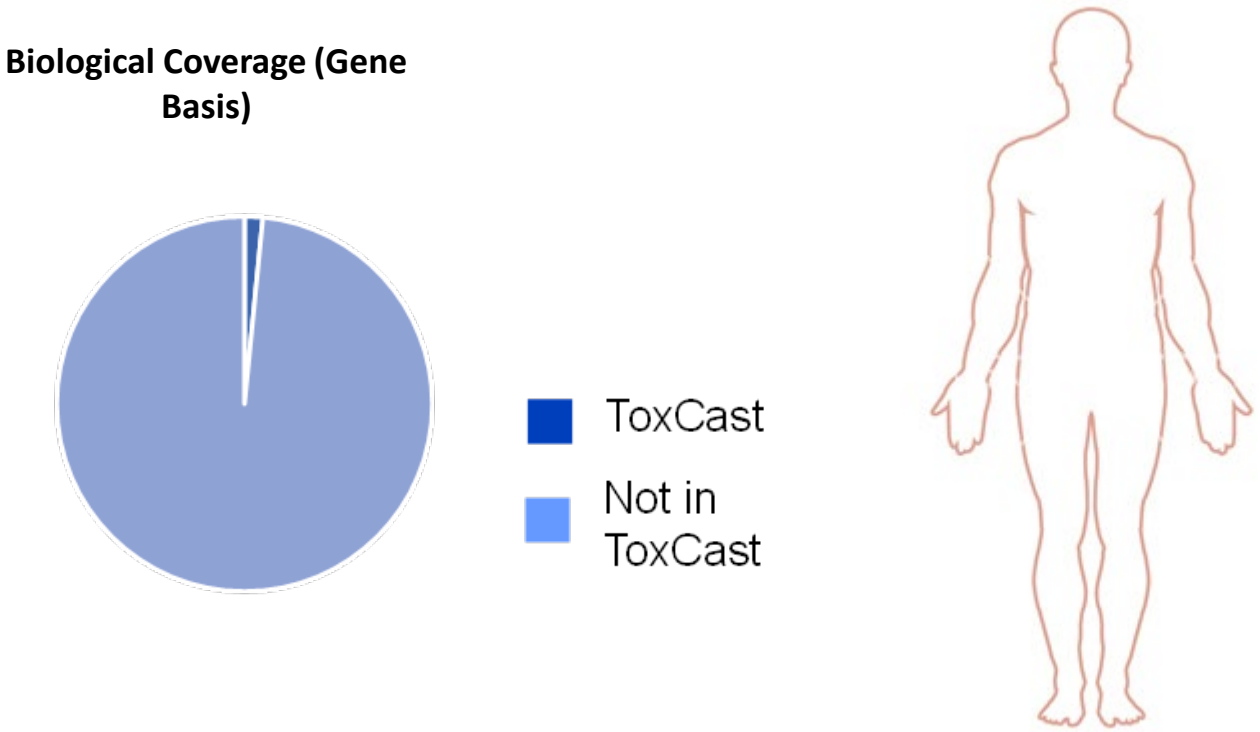
- Present it in a simple to follow graphical and narrative format
  - Supported by scientific literature and evidence
  - Searchable, globally accessible, and transparent
  - Aopwiki.org



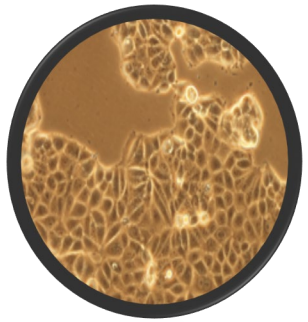


"Throughout the development and execution of ToxCast and Tox21, key limitations of the current suite of HTS assays have been identified (Tice, et al., 2013). The limitations include ***inadequate coverage of biological targets and pathways***"

Thomas et al. 2019 – The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Toxicol. Sci. *Toxicol. Sci. 169: 317-332.*



5 day rodent test



Whole human transcriptome

Principle 1
Transcriptomics is a reliable tool for detecting alterations in gene expression that result from endogenous or exogenous influences on the test organism

Principle 2
Alterations in gene expression are indicators of adverse or adaptive biological responses to stressors in an organism.

Principle 3
Transcriptomics can be employed to establish a POD from short-term, in vivo studies at a dose level below which a concerted molecular change (CMC) is not expected.

Principle 4
The use of a transcriptomic POD (set at the CMC dose level) will provide strong protection for use in risk assessment.

NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Research Report on
National Toxicology Program Approach to Genomic Dose-Response Modeling

NTP RR 5

APRIL 2018

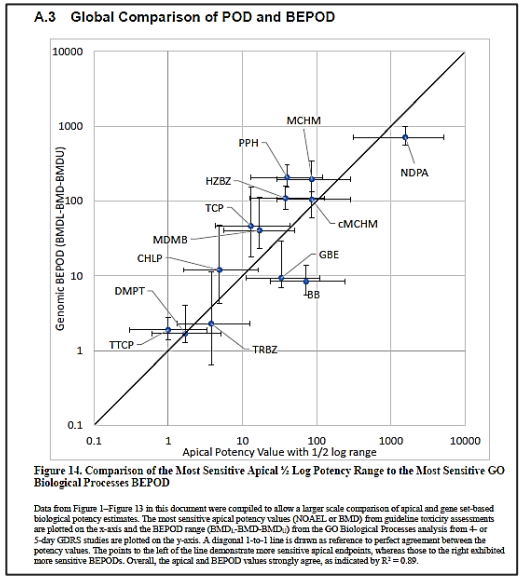
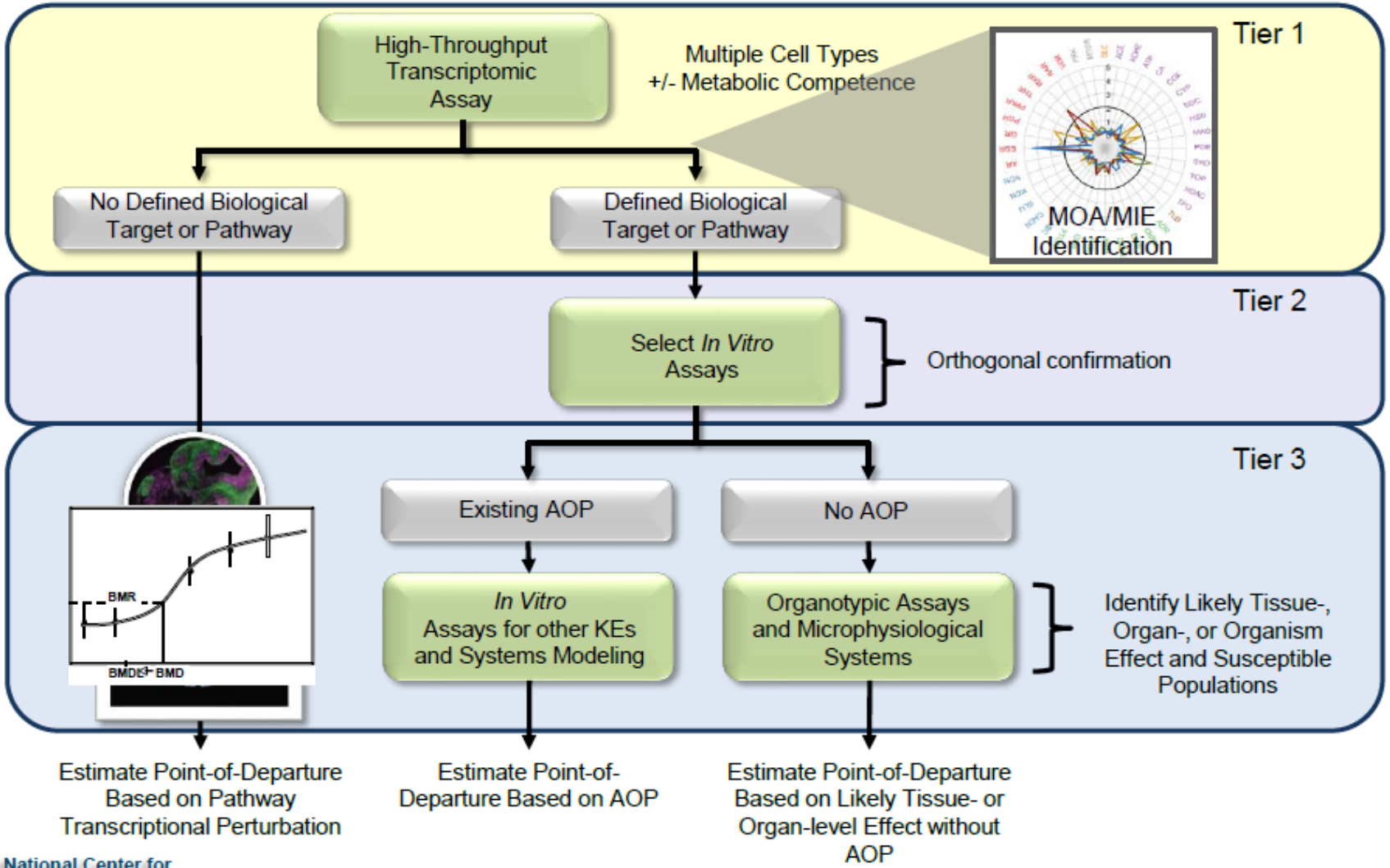


Figure 14. Comparison of the Most Sensitive Apical ½ Log Potency Range to the Most Sensitive GO Biological Processes BEPOD

Data from Figure 1–Figure 13 in this document were compiled to allow a larger scale comparison of apical and gene set-based biological potency estimates. The most sensitive apical potency values (NOAEL or BMD) from guideline toxicity assessments are plotted on the x-axis and the BEPOD range (BMD_L-BMD-BMD_U) from the GO Biological Processes analysis from 4- or 5-day GDRS studies are plotted on the y-axis. A diagonal 1-to-1 line is drawn as reference to perfect agreement between the potency values. The points to the left of the line demonstrate more sensitive apical endpoints, whereas those to the right exhibited more sensitive BEPODs. Overall, the apical and BEPOD values strongly agree, as indicated by $R^2 = 0.89$.

Johnson KJ, Auerbach SS, Stevens T, Barton-Maclaren TS, Costa E, Currie RA, Dalmas Wilk D, Haq S, Rager JE, Reardon AJF, Wehmas L, Williams A, O'Brien J, Yauk C, LaRocca JL, Pettit S. A Transformative Vision for an Omics-Based Regulatory Chemical Testing Paradigm. Toxicol Sci. 2022 Sep 27:kfac097. doi: 10.1093/toxsci/kfac097.

Blueprint of Computational Toxicology at US EPA



Broad screening in simplified biological systems, QSARs, read-across

Greater pathway specificity

Greater biological complexity/realism as needed

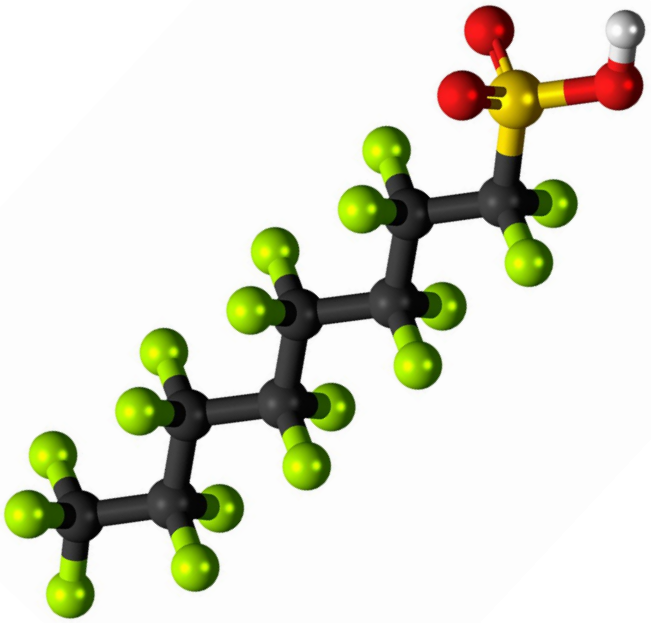
National Center for Computational Toxicology

Thomas et al. 2019 – The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Toxicol. Sci. 169: 317-332.

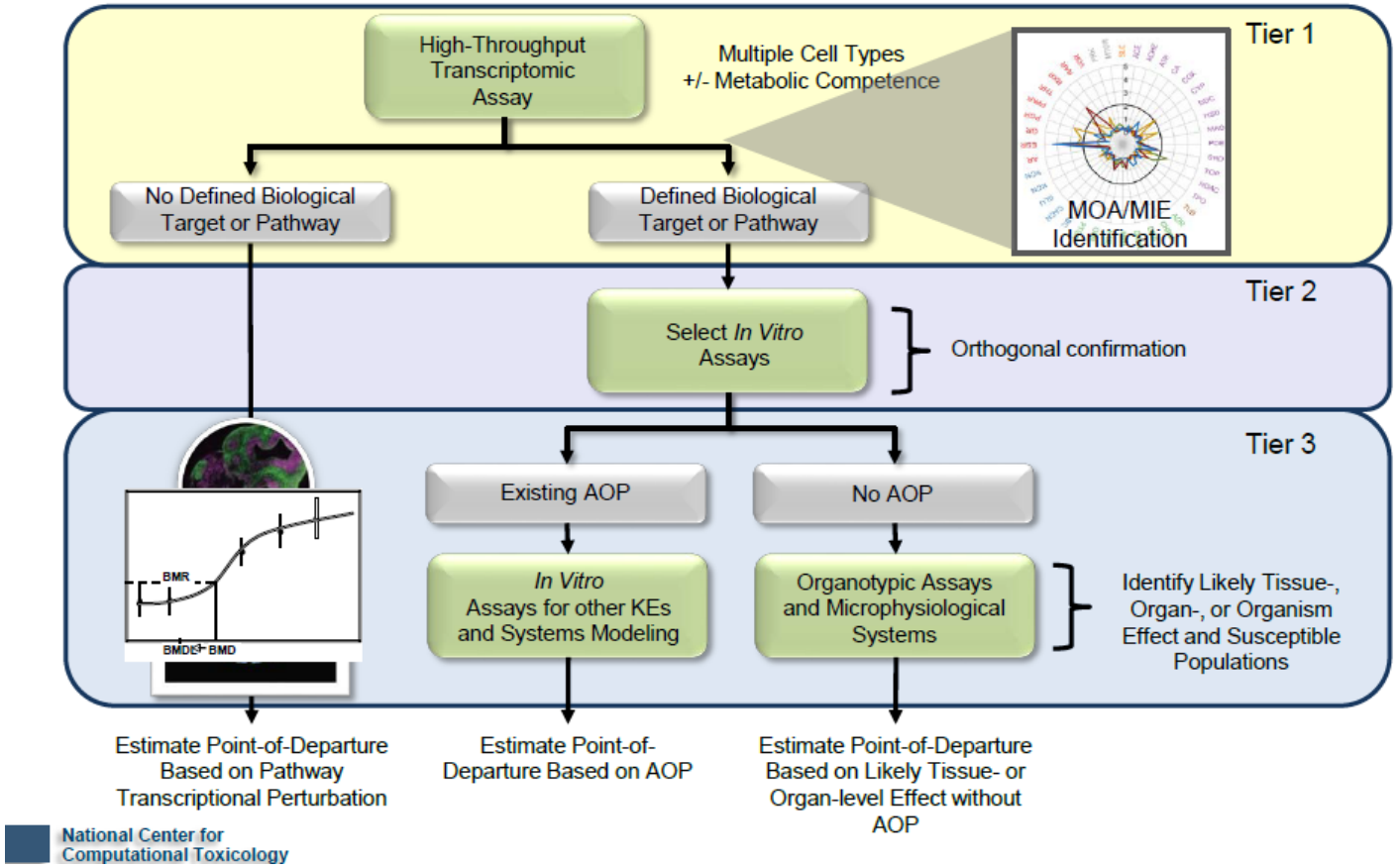
Applying NAMs and AOPs for Chemical Prioritization and Endpoint Selection

Per- and polyfluoroalkyl substances (PFAS)

- Broad chemical group of concern due to environmental persistence, exposure and accumulation in humans and wildlife, and potential toxicity (thousands of structures)
- Feb 7, 2023 the European Chemicals Agency (ECHA) posted proposal to restrict around 10,000 PFAS (https://echa.europa.eu/-/echa-publishes-pfas-restriction-proposal)
- While certain PFAS (e.g., PFOS, PFOA) have been heavily studied, exposure, bioaccumulation, and effects data are lacking for the vast majority of PFAS



Hazard Screening



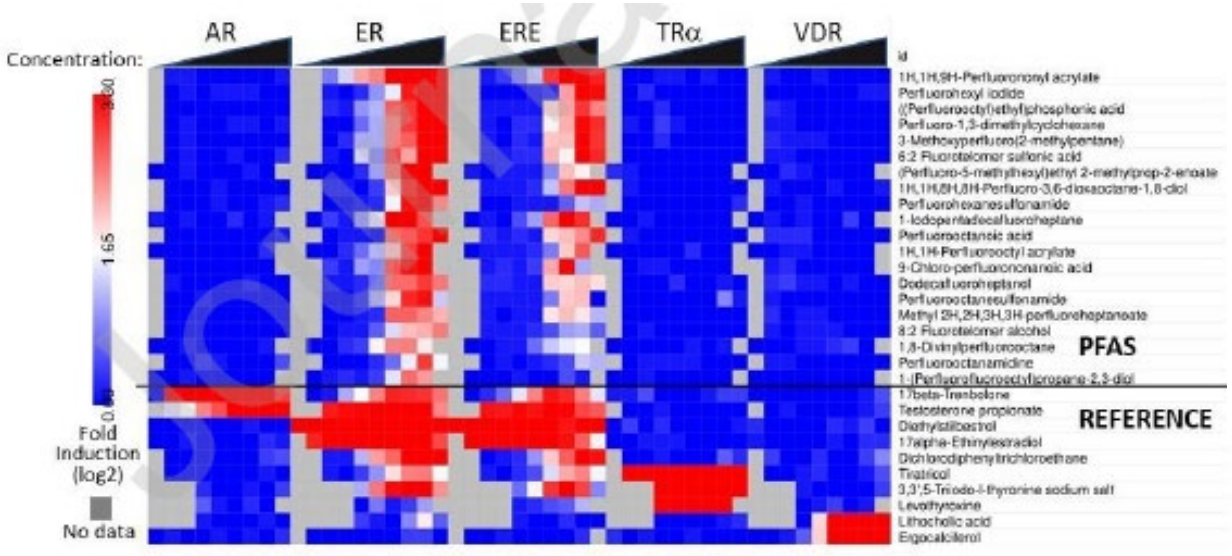
Library of ≈ 150 PFAS selected for HTS based on structural diversity, Agency interest, ability to procure and properties for testing

Houck et. al., screened for ability to interact with human nuclear receptors using a multi-factorial assay

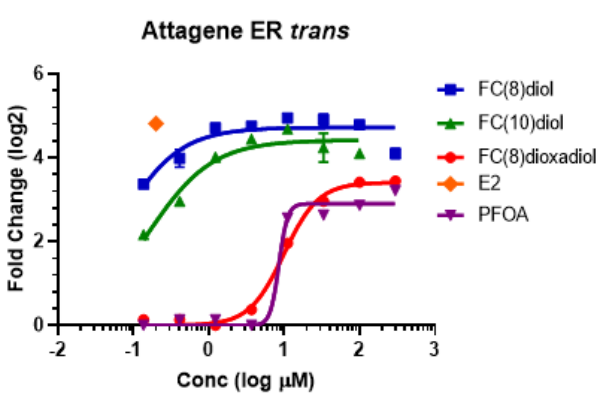
Thomas et al. 2019 – The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Toxicol. Sci. 169: 317-332.

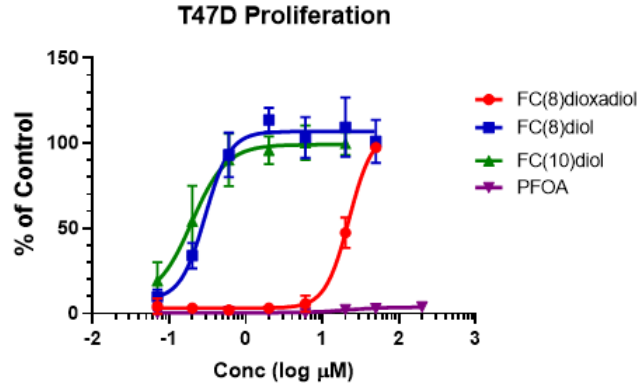
Patlewicz et al. A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing. Environ Health Perspect. 2019 doi: 10.1289/EHP4555.

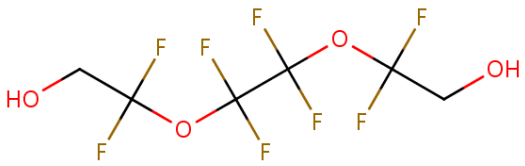
Estrogenic PFAS



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



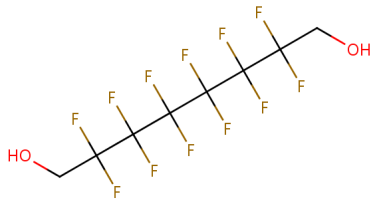




FC8-DOD

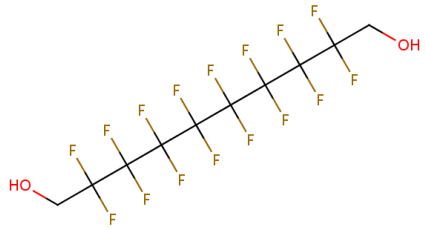
1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-diol (DTXSID70381090)

- Out of 142 PFAS screened, 40 showed interaction with ERα or EREs
- Among those, three were identified as particularly potent and efficacious



FC8-diol

1H,1H,8H,8H-Perfluorooctane-1,8-diol (DTXSID70381090)



FC10-diol

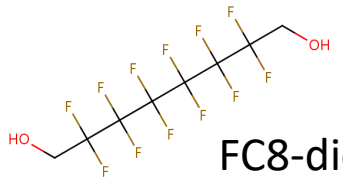
1H,1H,10H,10H-Perfluorodecane-1,10-diol (DTXSID50369896)

In vivo confirmation

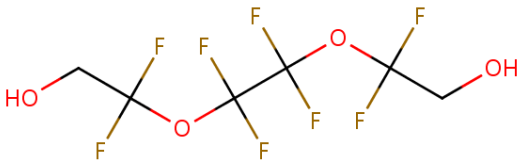
- Five *in vivo* experiments
  - Four ER-active PFAS of varying potency
    - FC8-diol
    - FC10-diol
    - FC8-DOD
    - PFOA
  - One ER-negative PFAS
    - HFPO-DA (GenX)
- Adult male fathead minnows exposed to PFAS for 96 h
  - Included E2 positive control
- Gene expression (QPCR)
  - Four orthogonal ER-regulated genes
    - Two – expected up-regulation
    - Two – expected down-regulation

Results:

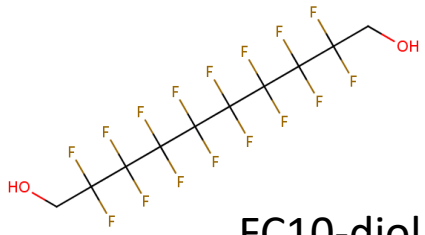
PFAS identified as ER agonists in human cells do elicit estrogenic responses in fish, in vivo.



FC8-diol

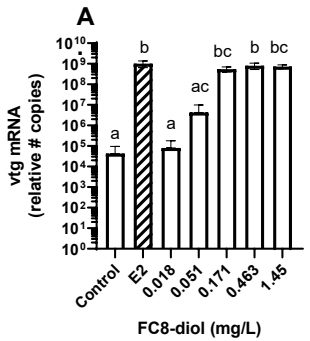


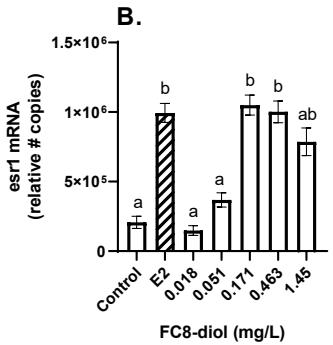
FC8-DOD

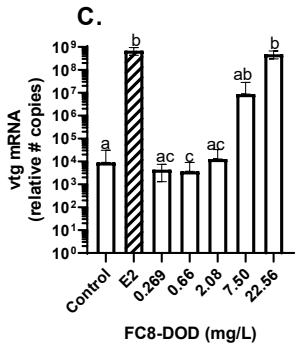


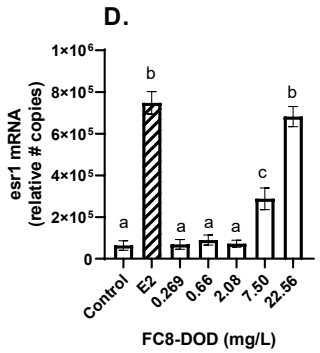
FC10-diol

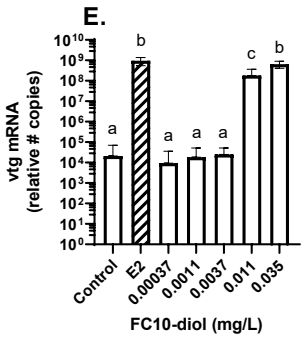
Upregulation expected

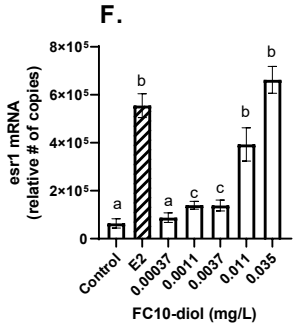




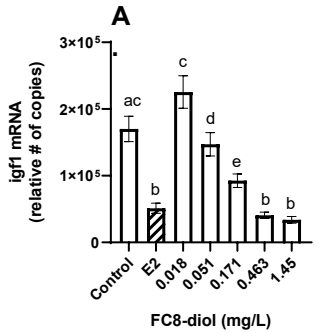


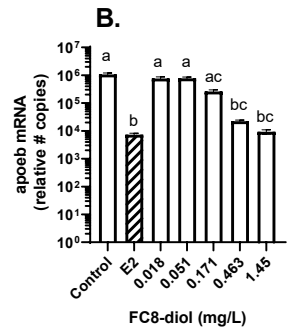


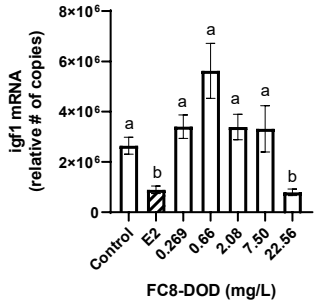


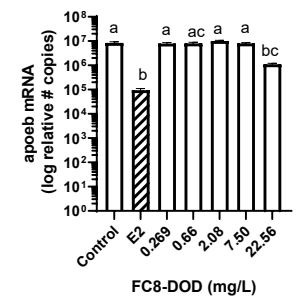


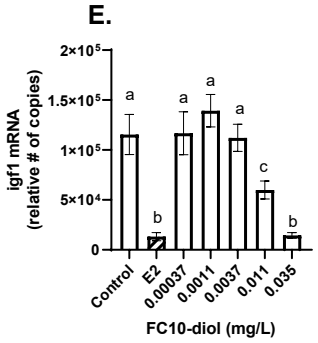
Downregulation expected

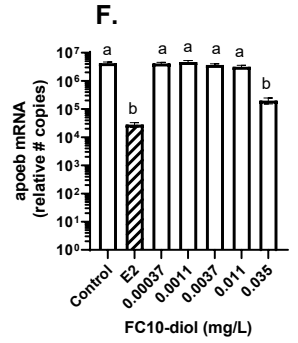










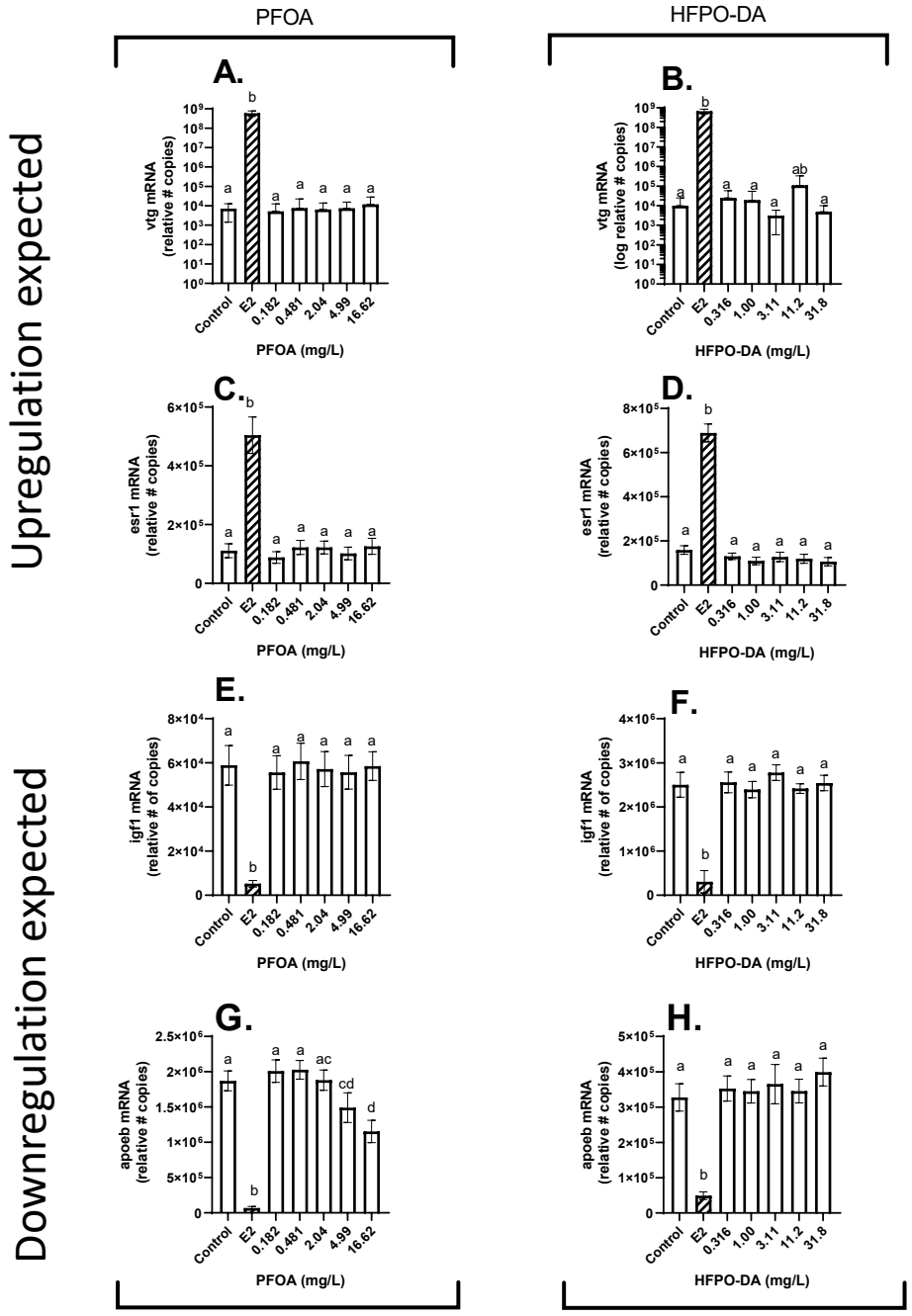


Villeneuve DL, et. al. *Verification of In Vivo Estrogenic Activity for Four Per- and Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists via New Approach Methodologies.* Environ Sci Technol. 2023 Mar 7;57(9):3794-3803. doi: 10.1021/acs.est.2c09315.

Results:

Weakly estrogenic PFAS (PFOA) caused a weak response in vivo, only impacting expression of 1 of 4 of the ER-regulated genes

Non estrogenic PFAS did not elicit ER-dependent gene expression *in vivo*



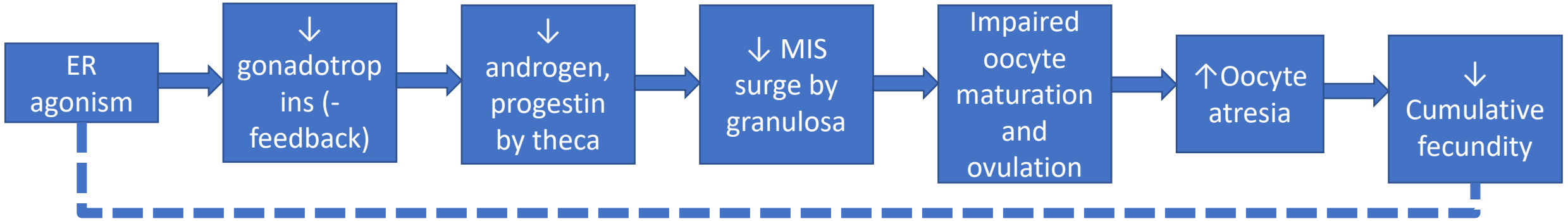
Villeneuve DL, et. al. *Verification of In Vivo Estrogenic Activity for Four Per- and Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists via New Approach Methodologies.* Environ Sci Technol. 2023 Mar 7;57(9):3794-3803. doi: 10.1021/acs.est.2c09315.

AOP: 445

Title

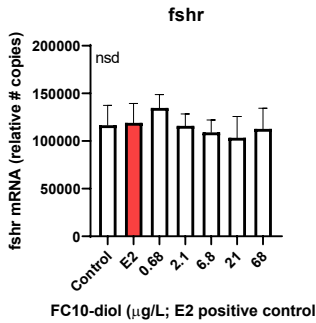
https://aopwiki.org/aops/445

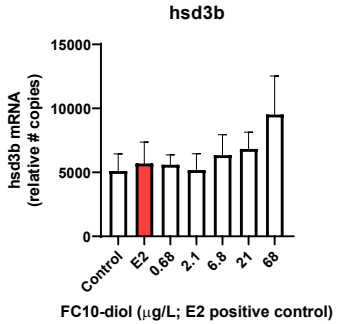
Estrogen Receptor Alpha Agonism leads to Impaired Reproduction

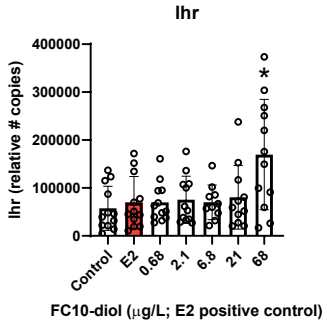


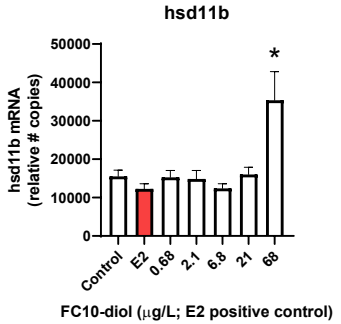
Most potent of the estrogenic PFAS (FC10-diol) tested in a 21 d reproduction test

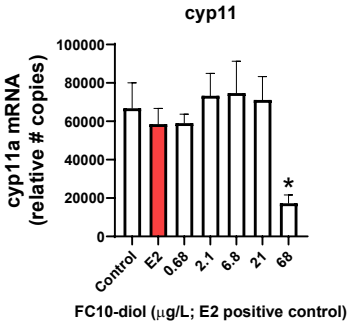
↓
androgen, progestin by theca

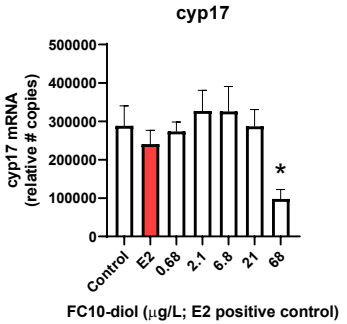


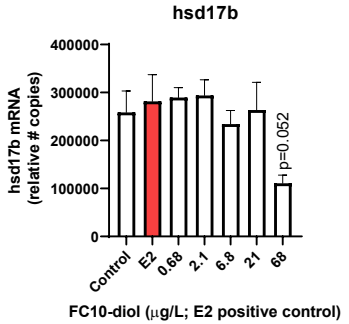


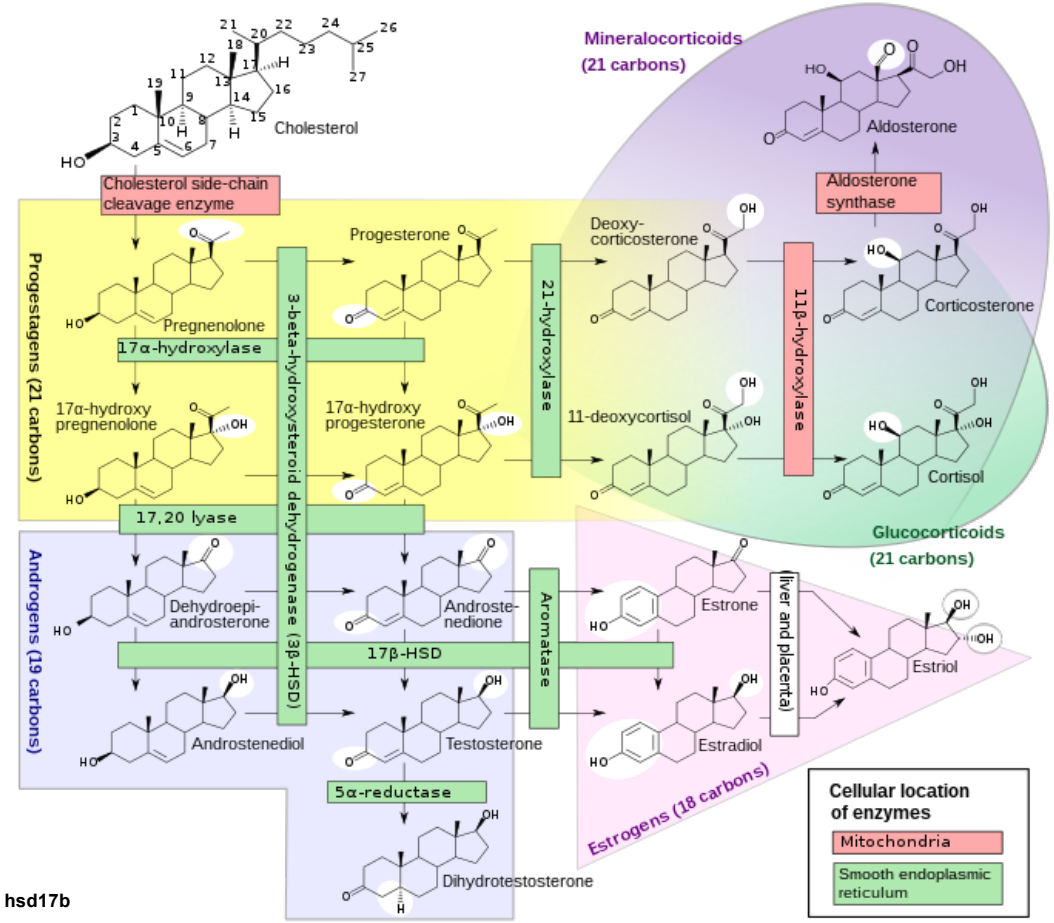










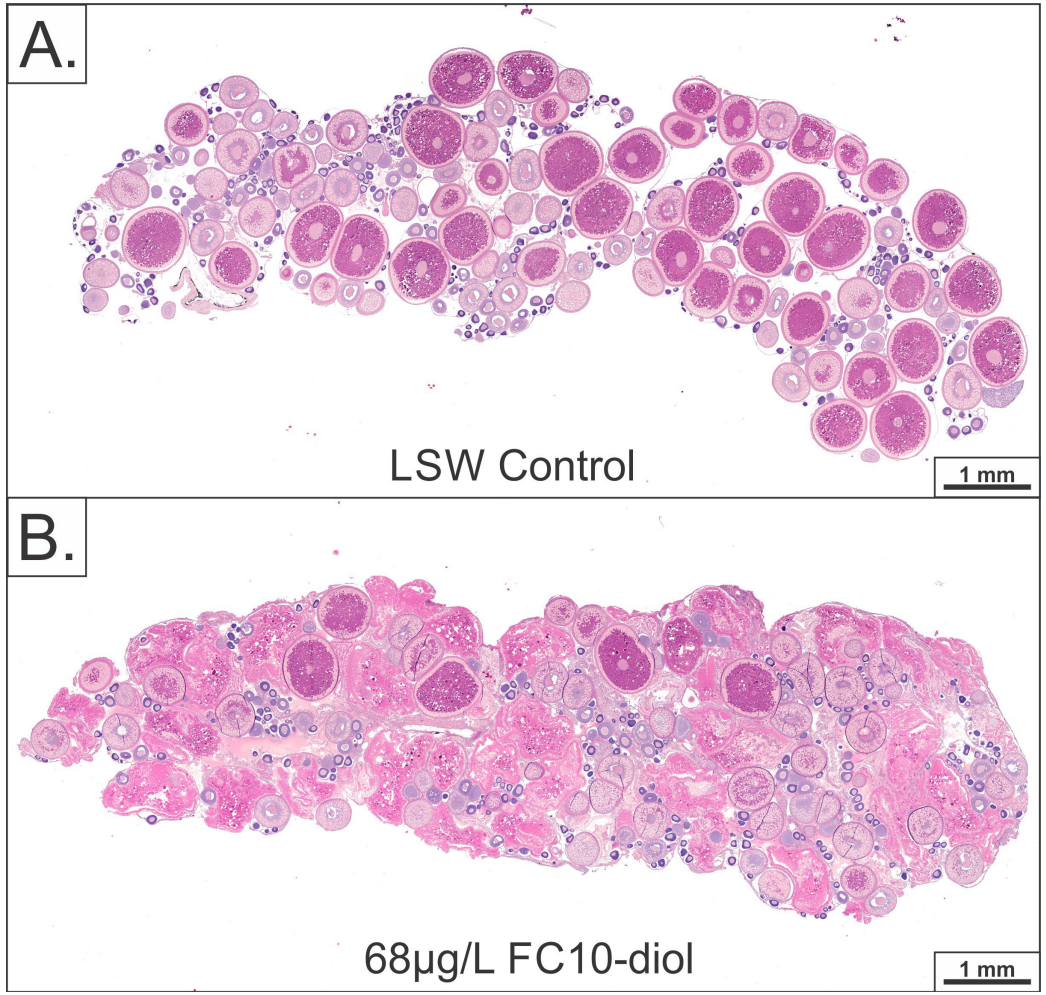


Pathway to androgen and estrogen production down-regulated

Consistent with hypothesized feedback

↑Oocyte atresia

Ovary Histology



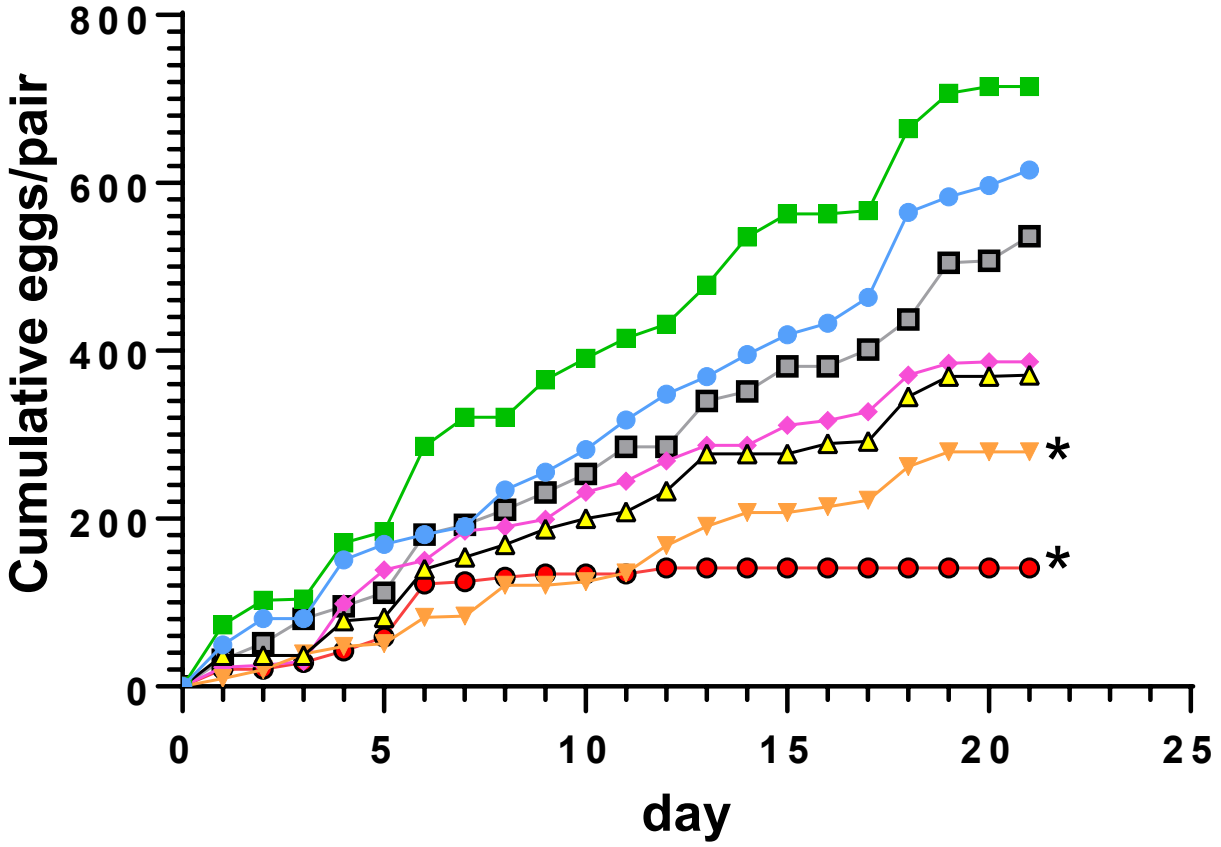
Significant increase in incidence and severity of oocyte atresia in 68 µg/L treatment.

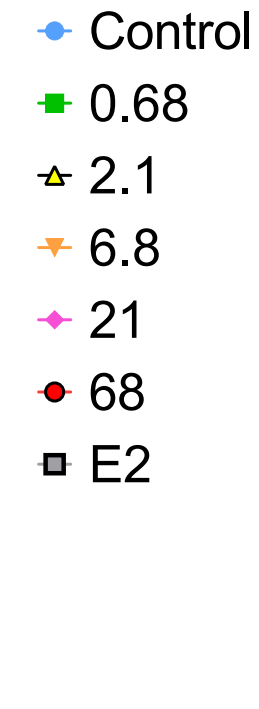
Significant increase in the incidence and severity of interstitial and intravascular proteinaceous fluid

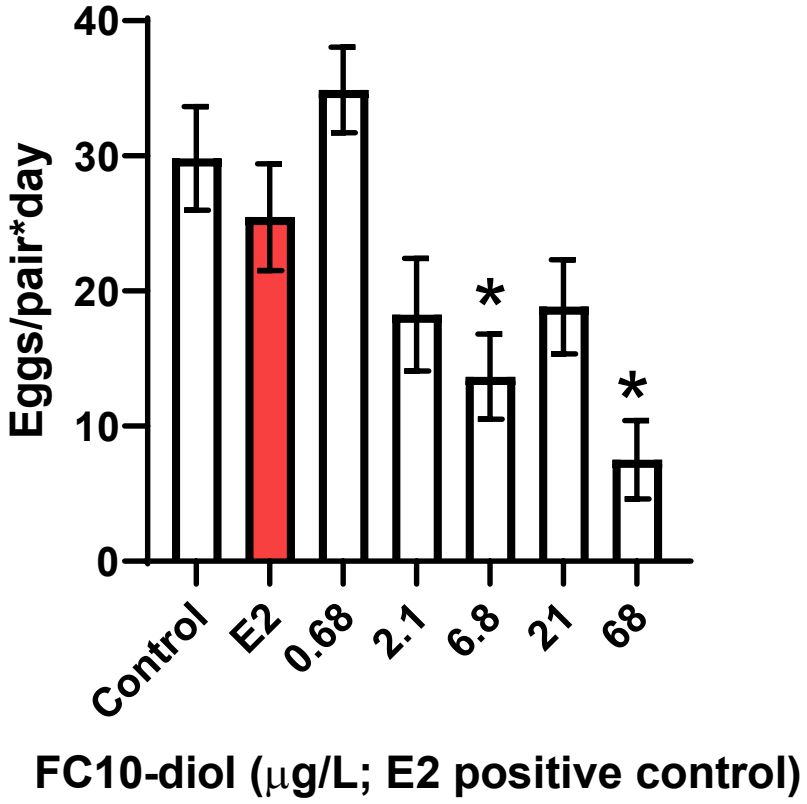
Broadly consistent hypothesized KE of increased oocyte atresia

↓ Cumulative fecundity

Cumulative fecundity was significantly reduced for pairs exposed to either 6.8 or 68 µg FC10-diol/L.







Ecological Relevance

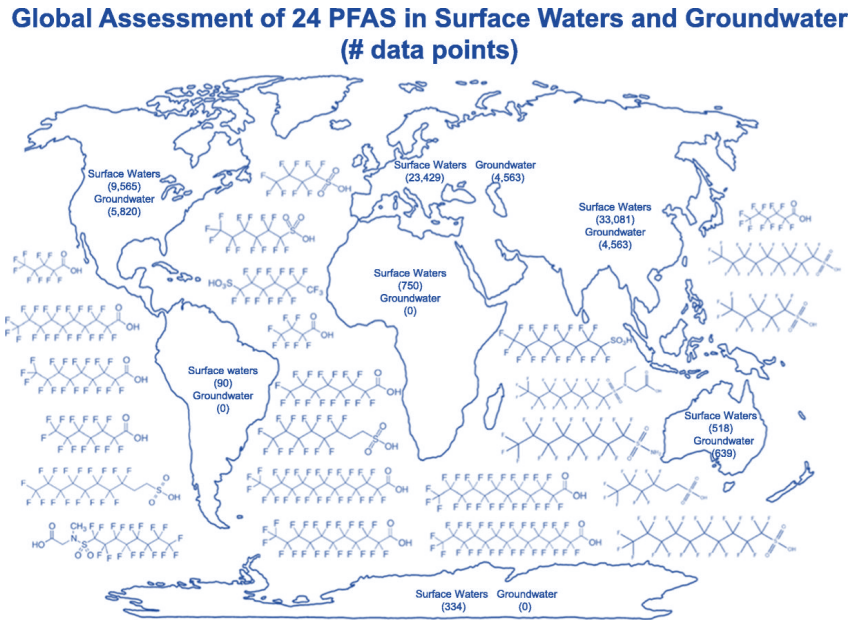
- No reported detections of FC10-diol, FC8-diol, or FC8-DOD in environment
- Literature pertains mostly to synthetic chemistry and film-forming properties
- Patents suggest potential use in medical and dental devices, photosensitive resins and films, conductive and electrode films, optical polymers, etc.
- Current information is too sparse to estimate environmental releases and loading

Assessment Relevance

- FC10-diol presently, the most estrogenic PFAS known.
  - Average *in vivo* BMC = 8.4 µg/L
  - Uncertainty factor ≈25 (only one vertebrate tested)
  - PNEC for estrogenic effects ≈ 336 ng/L

- Conservative assessment – assume all PFAS are as potent as FC10-diol
- Only considering estrogenic effects of PFAS

- PNEC based on short-term in vivo gene expression response is protective relative to effects on reproduction



Sims JL, et al. Global occurrence and probabilistic environmental health hazard assessment of per- and polyfluoroalkyl substances (PFASs) in groundwater and surface waters. Sci Total Environ. 2022. doi: 10.1016/j.scitotenv.2021.151535.

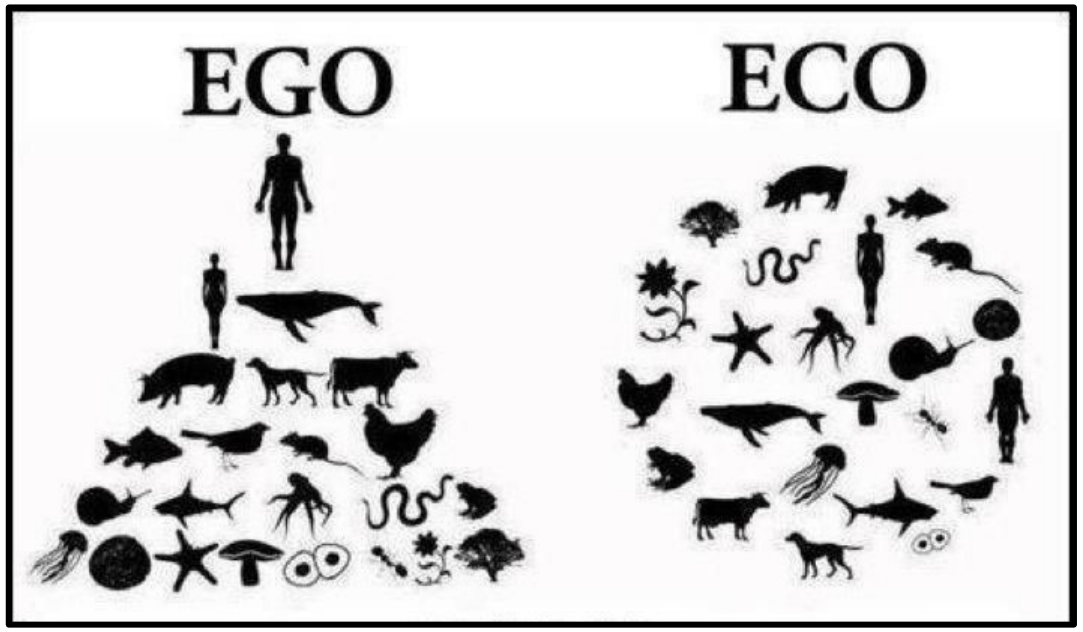
Only sites at or above the 99th percentile* were predicted to exceed the 336 ng/L PNEC, even assuming all PFAS are as estrogenic as FC10-diol

*Recognizing, that current probabilistic assessment is only based on 24 PFAS.

Villeneuve DL, et. al. *Verification of In Vivo Estrogenic Activity for Four Per- and Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists via New Approach Methodologies.* Environ Sci Technol. 2023 Mar 7;57(9):3794-3803. doi: 10.1021/acs.est.2c09315.

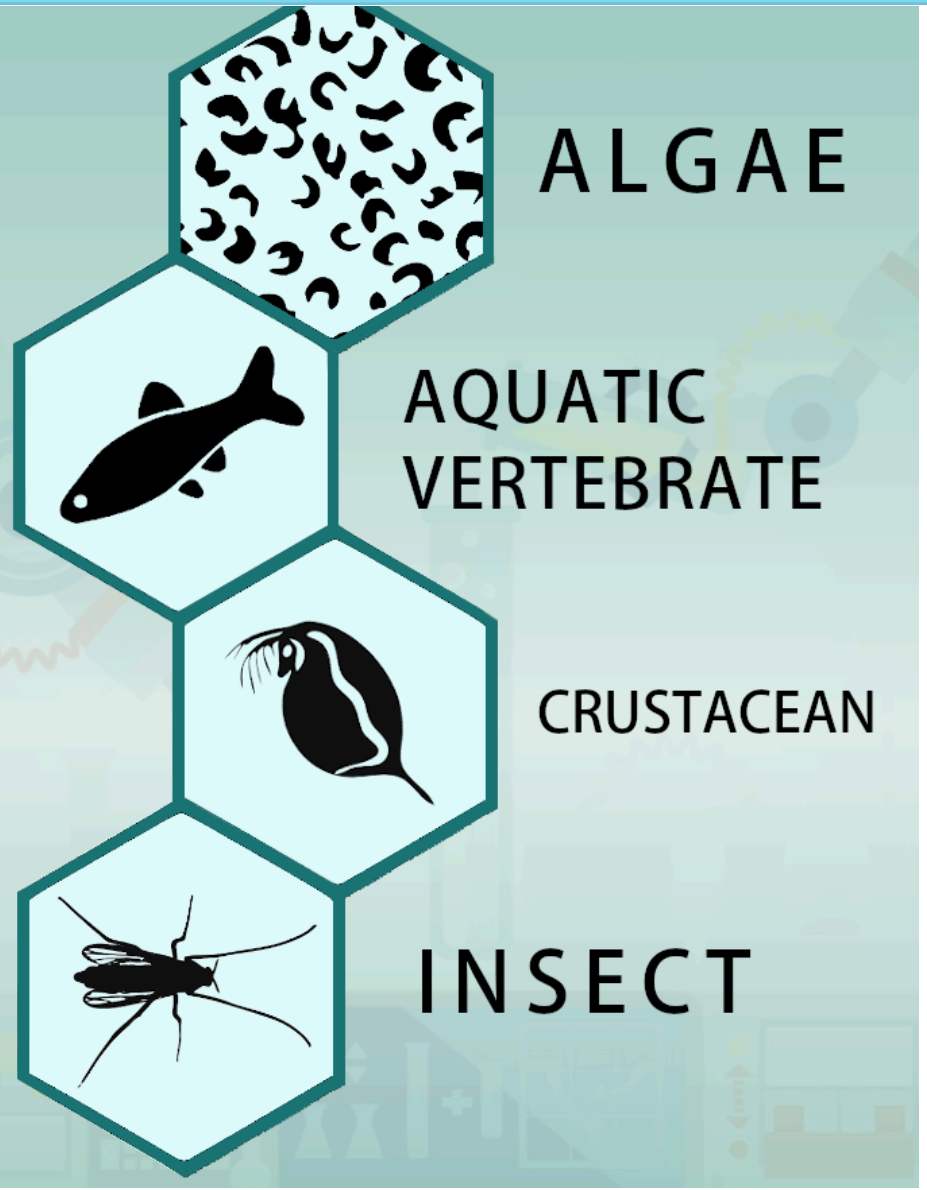
High Throughput Transcriptomics for Risk-Based Screening

High throughput transcriptomics for risk-based screening



- Humans are just a tiny fraction of the biological diversity we are charged to protect.
- Many genes and pathways are conserved with humans/mammals, but…
- Unique physiology in other kingdoms, phyla, classes…
- How do we assure those pathways are covered?
- As we integrate NAMs into Next Generation risk assessment, want to make sure ECO is not an after thought.

Model Organisms



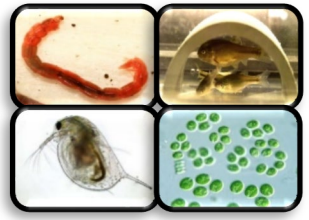
Genomically, physiologically, taxonomically and trophically diverse

- Primary producers (e.g., algae)
- Primary consumers (e.g., zooplankton, aquatic inverts)
- Secondary consumers (e.g., fish)

Commonly used for globally harmonized system for classification and labeling of chemicals for environmental hazard

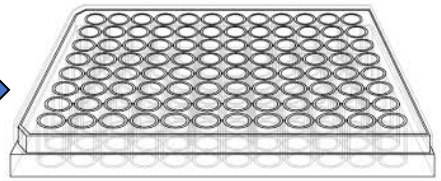
Aquatic organisms highly vulnerable to exposure

Toxicogenomic Approach



Load organisms

24 h exposure

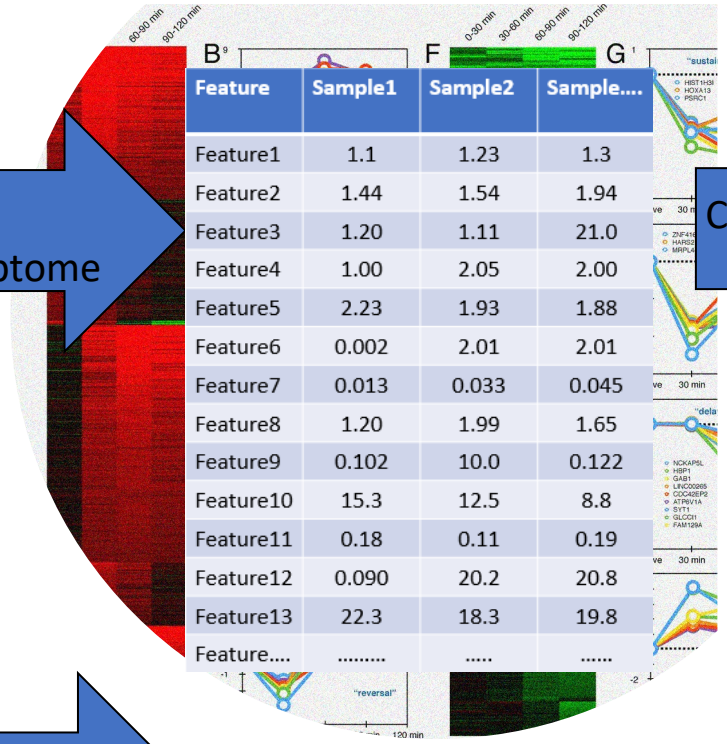


Phenotypic anchoring

Homogenate /extract

RNA

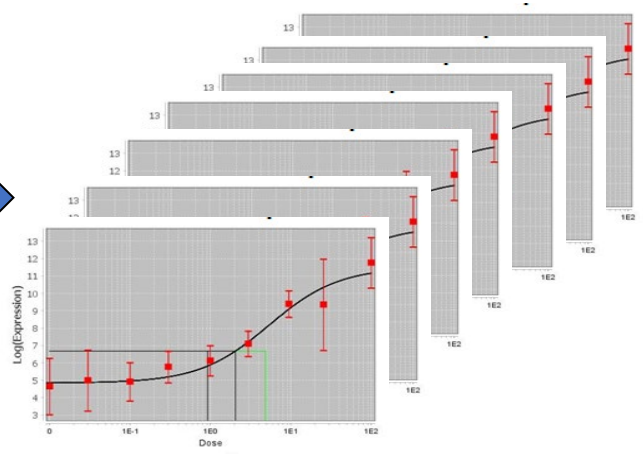
Whole transcriptome



Feature	Sample1	Sample2	Sample....
Feature1	1.1	1.23	1.3
Feature2	1.44	1.54	1.94
Feature3	1.20	1.11	21.0
Feature4	1.00	2.05	2.00
Feature5	2.23	1.93	1.88
Feature6	0.002	2.01	2.01
Feature7	0.013	0.033	0.045
Feature8	1.20	1.99	1.65
Feature9	0.102	10.0	0.122
Feature10	15.3	12.5	8.8
Feature11	0.18	0.11	0.19
Feature12	0.090	20.2	20.8
Feature13	22.3	18.3	19.8
Feature....	.....	.....	.....

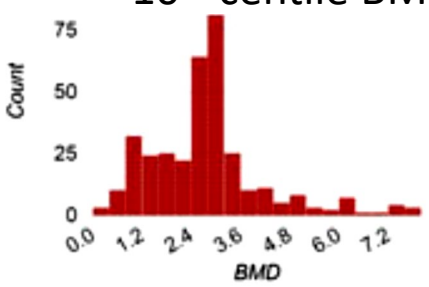
Curv

ve fitting



BMD Express

Distribution of BMDs



Point of departure

[tPOD]

Pathway BMDs

MoA inference

In principle

- Exposures that elicit concerted gene expression changes aren't necessarily adverse

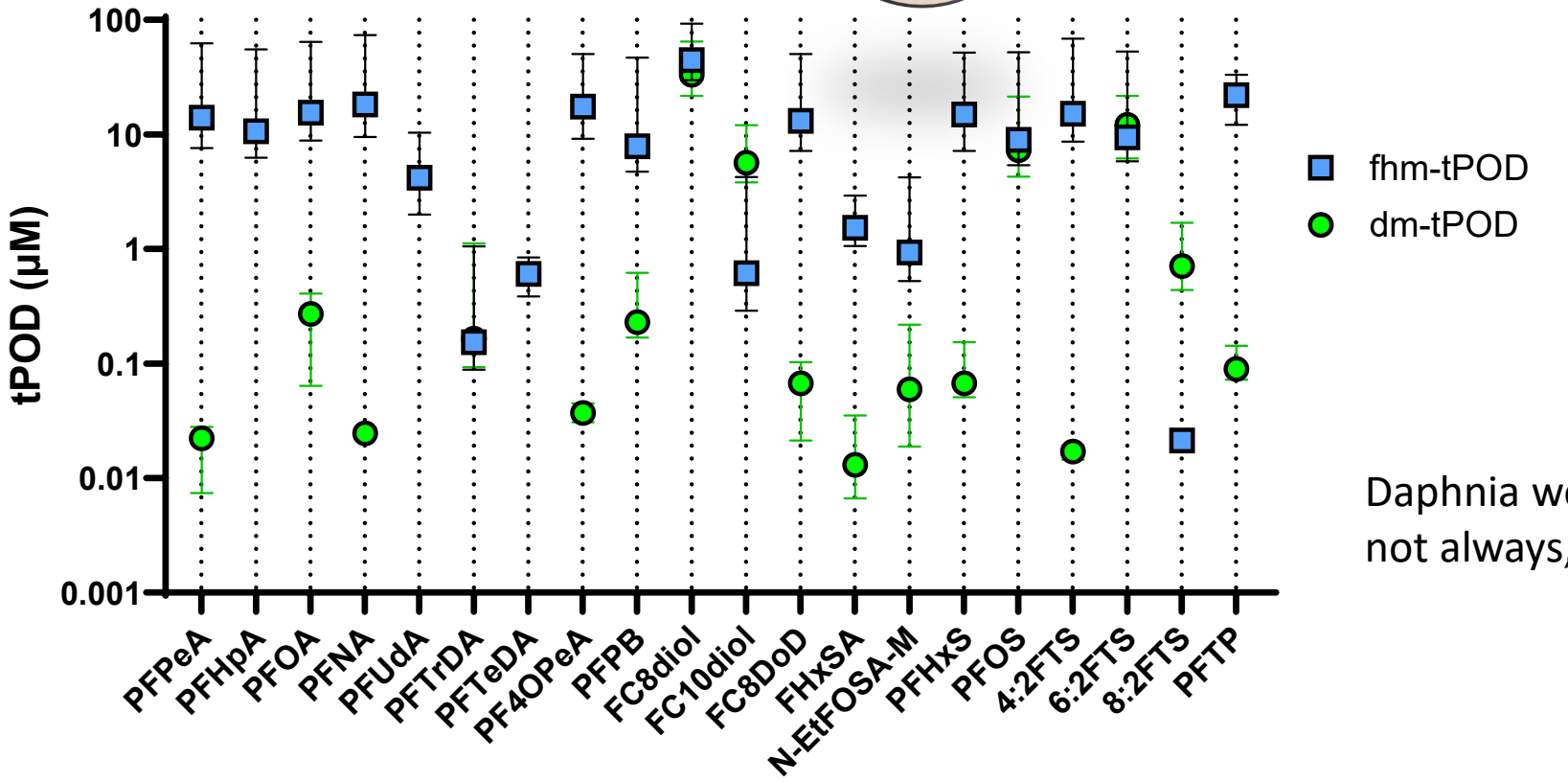
- Exposures that do not elicit concerted gene expression changes are unlikely to be hazardous, even over much longer exposure durations*

*Assuming potential bioaccumulation < margin of exposure

≈20 PFAS screened in HTTr assays with larval fathead minnows and *Daphnia magna*

tPOD IQR = 0.03-0.58 µM

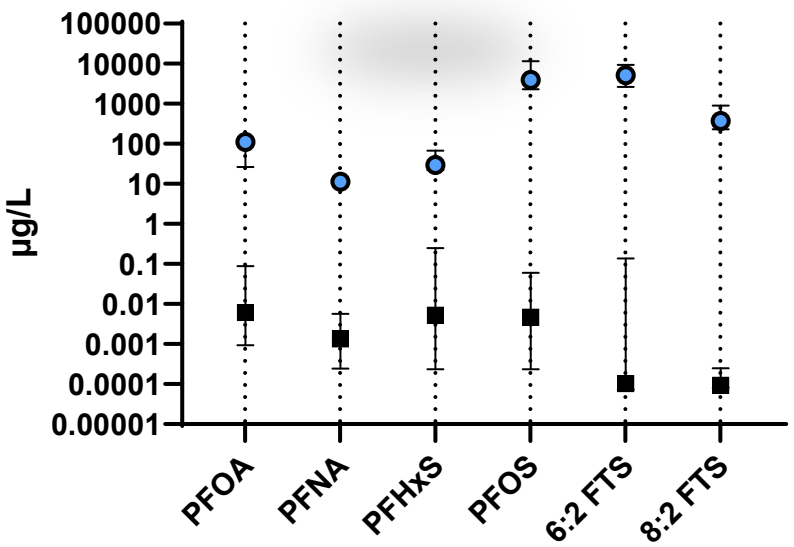
tPOD IQR = 1.4-15 µM

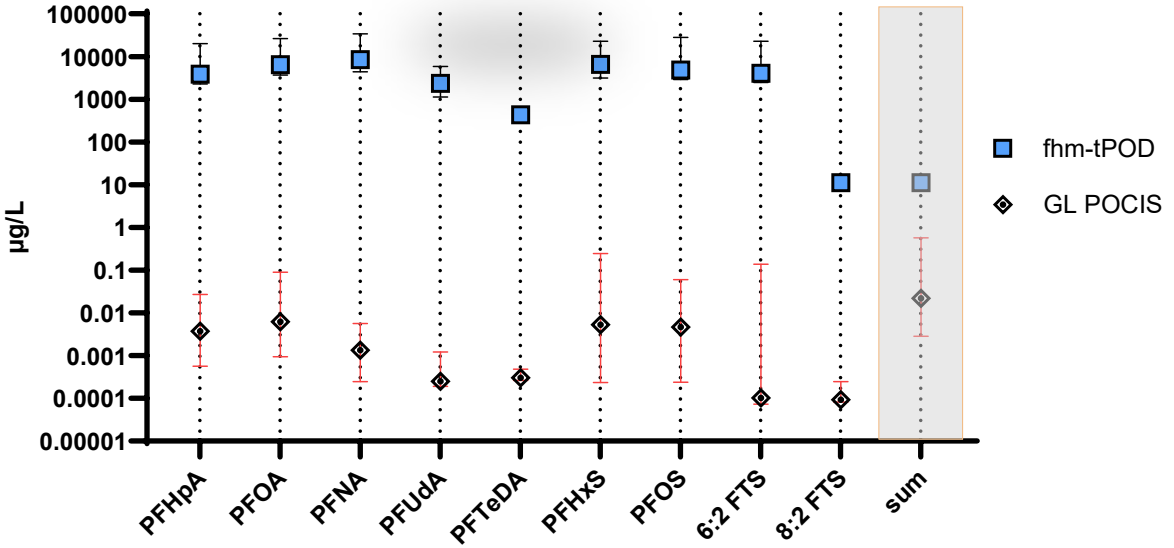


Daphnia were generally, but not always, more sensitive.

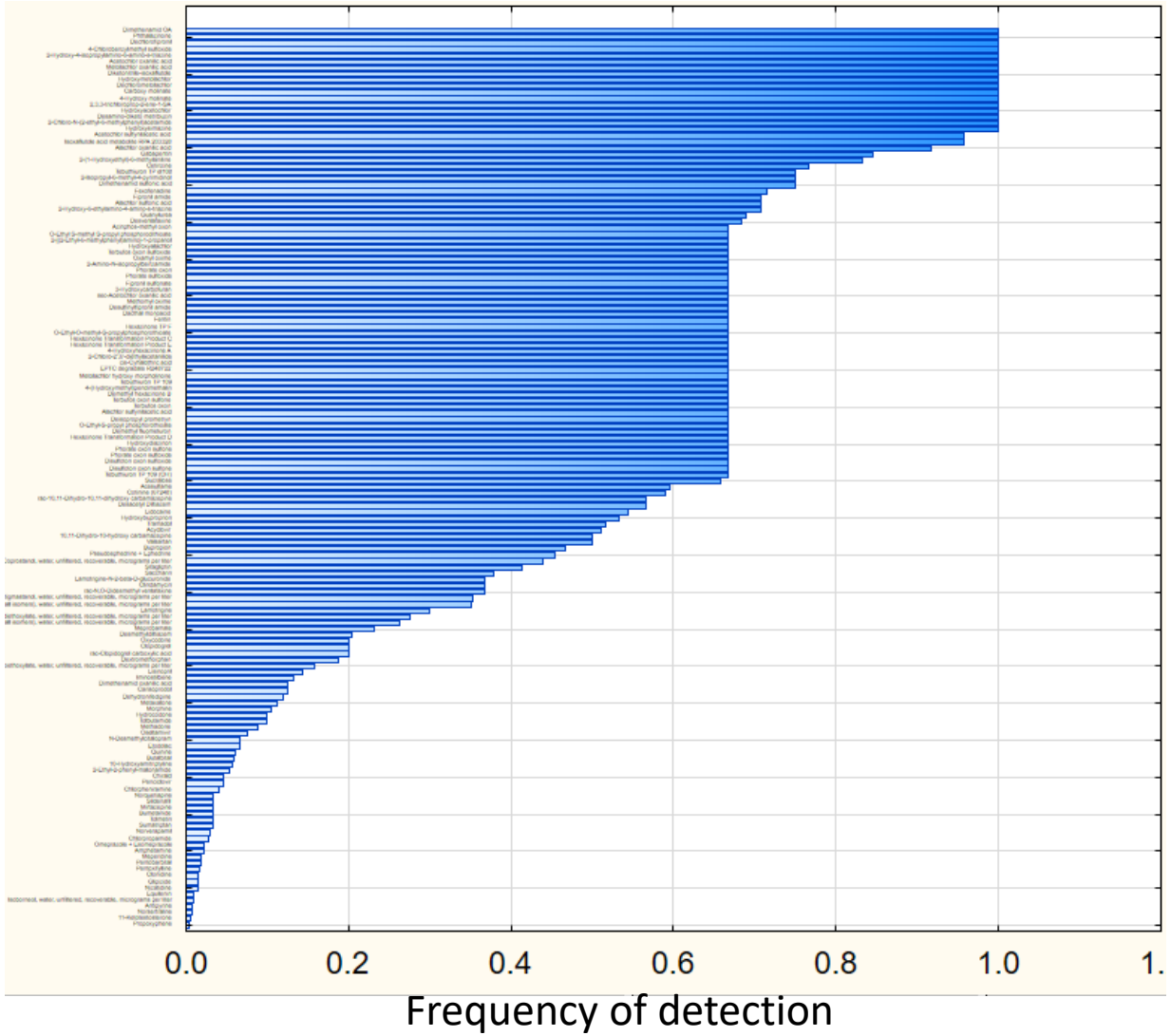
*tPODs are based on chemical concentrations in exposure water

PFAS concentrations in Great Lakes tributaries were << tPODs





Other Great Lakes CECs (monitored 2010-2018)

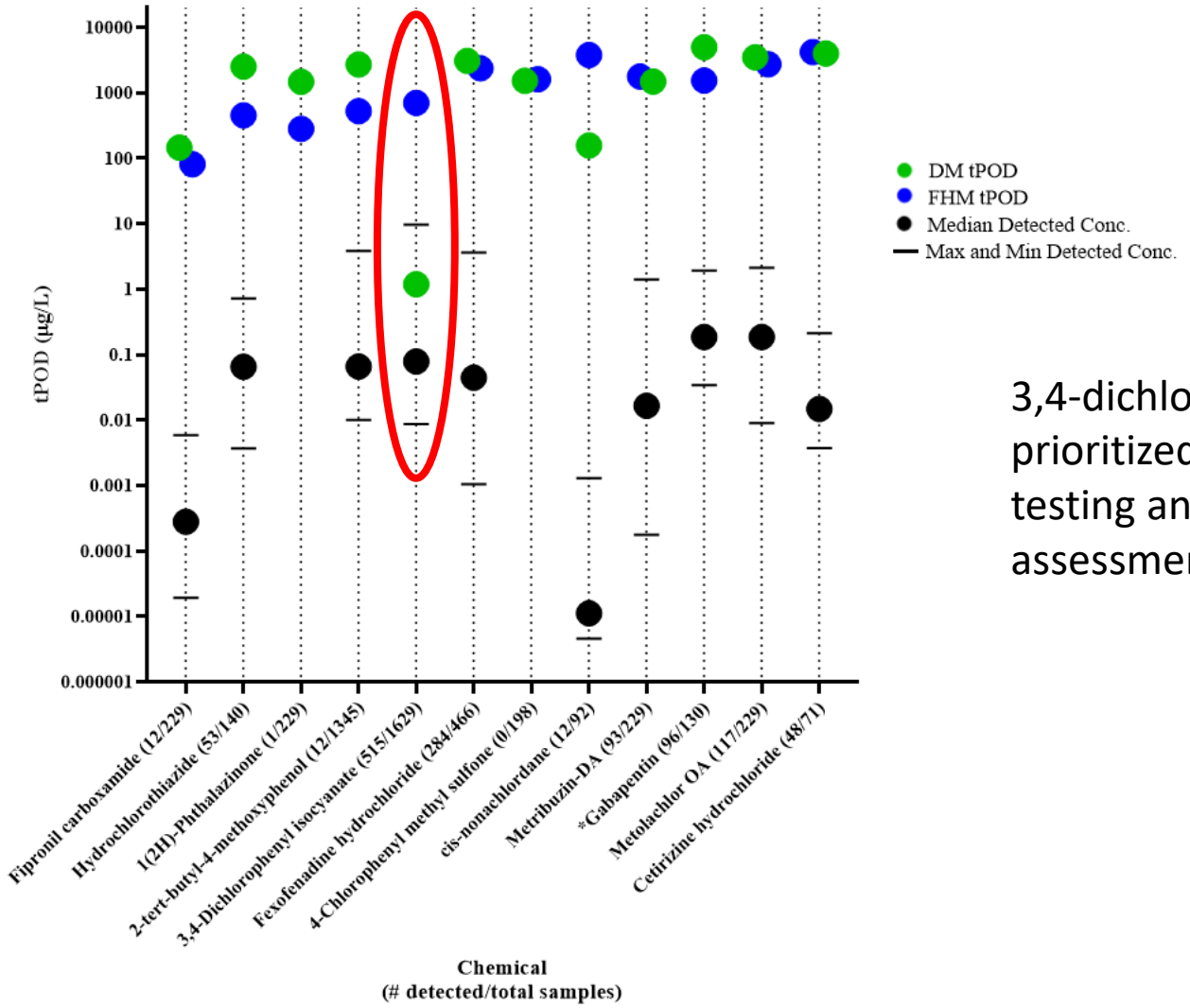


146 chemical for which no WQ benchmarks, ECOTOX, or ToxCast data were available

Pesticide degradates	69
PPCPs	66
Detergent metabolites	4
Flavors/fragrances	1
Hormones	2
Sterols	2
Other	2

Prioritized for hazard data collection based on detection frequency

Other Great Lakes CECs (monitored 2010-2018)



3,4-dichlorophenyl isocyanate prioritized for additional toxicity testing and site-specific effects assessment.

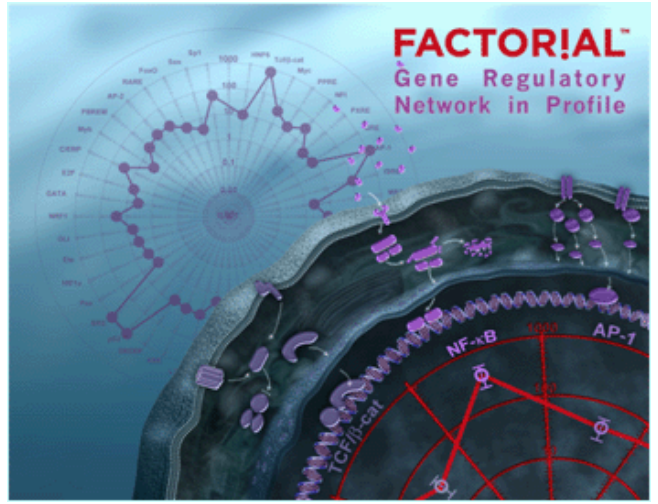
NAMs and AOPs for risk-based screening of complex mixtures

Case Study: South Platte River, CO

Jenna E. Cavallin, Jon Beihoffer, Brett R. Blackwell, Alexander R. Cole, Drew R. Ekman, Rachel Hofer, Aaron Jastrow, Julie Kinsey, Kristen Keteles, Erin M. Maloney, Jordan Parman, Dana L. Winkelman, Daniel L. Villeneuve

NAMs-based Bioactivity Screening Attagene trans-Factorial™ Assay

- HepG2 cell-based assay; mRNA reporter assay
- Provides an assessment of multiple gene regulatory pathways in live cells
- Endpoints cover a range of biological processes
  - Xenobiotic metabolism (AhR, PXR, PPAR, FXR, LXR)
  - Endocrine activity (ER, AR, GR, TR)
  - Variety of Transcription Factors (NRF2,MRE, HSF1, TP53)

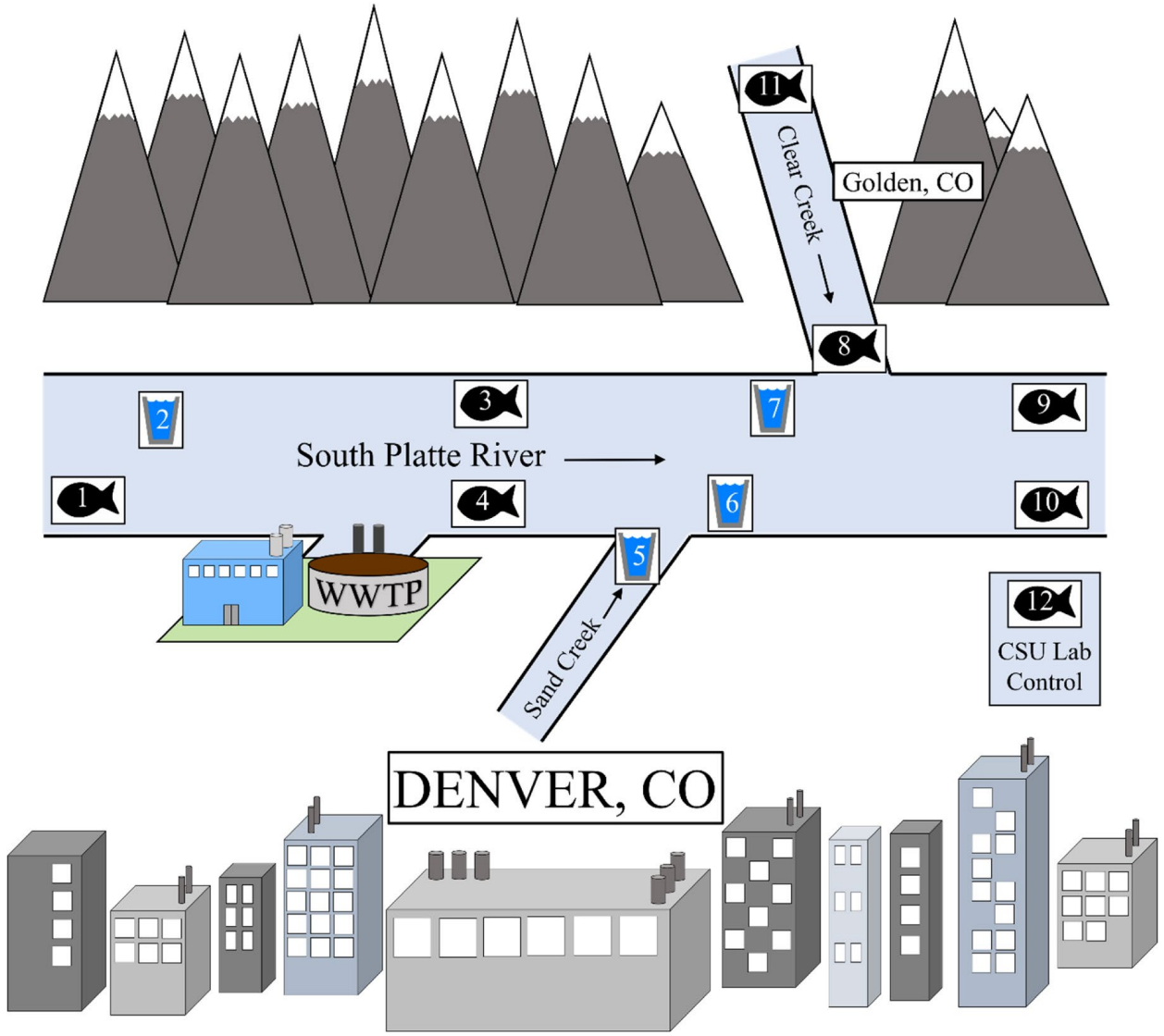


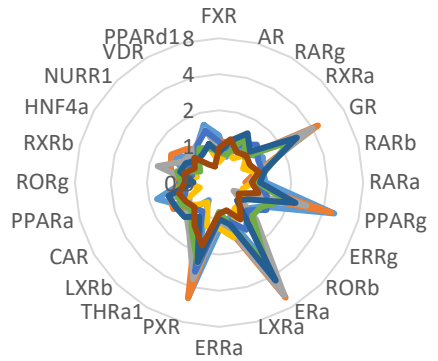
Romanov et al., 2008, Nat. Methods; 5(3):253-60
http://www.attagene.com/technology.php

Fraction of Chemicals Measured

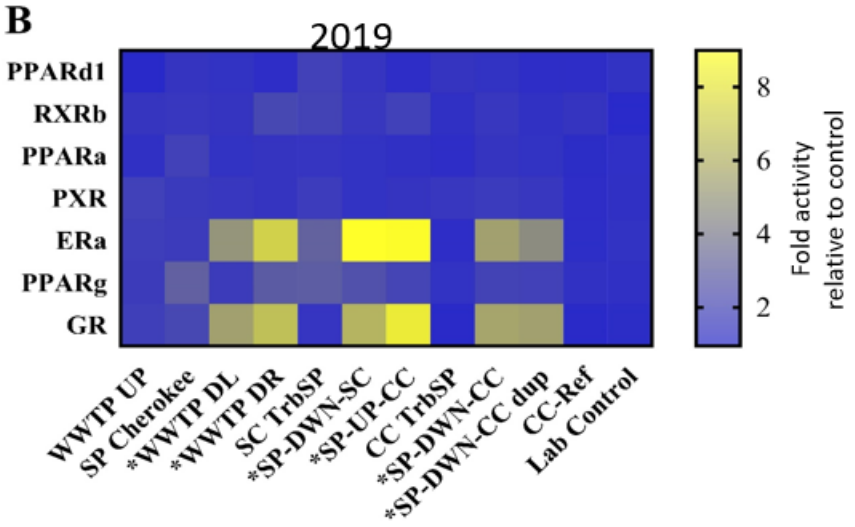
Unmeasured Fraction of Chemicals

Detected Activities





PXR, ERa, PPARg, GR

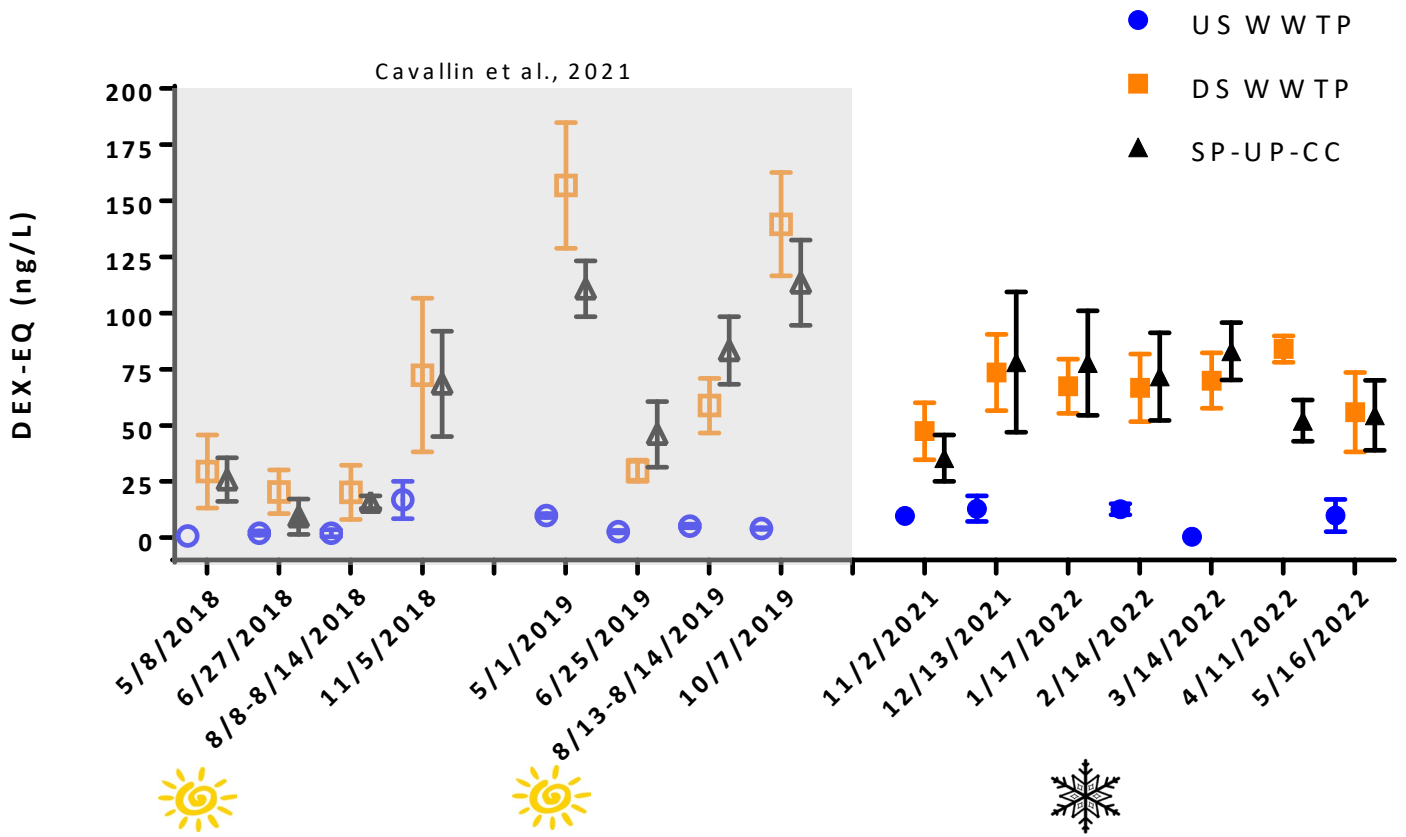


Targeted follow-up monitoring

GR activity

- The GR activity below the WWTP generally remained stable throughout the winter months (Dec.-March) with a mean (±SD) of 69±3.1 ng DEX-EQ/L.

- On average, total DEX-EQ throughout the fall/winter months was greater than those measured during the summer (June and August samples) in 2018 and 2019.



Hazards and Risk

Hazard to fish survival

https://aopwiki.org/aops/334

GR Activation* → Increase, *cripto-1 Expression* → Inhibition, Activin Signaling → Inhibition, fin regeneration → Reduced swim performance → Reduced survival → Population trajectory, decreased

Still orders of magnitude below concentrations that caused adverse effect in laboratory studies

Mean 69.3 ng Dex-EQ/L

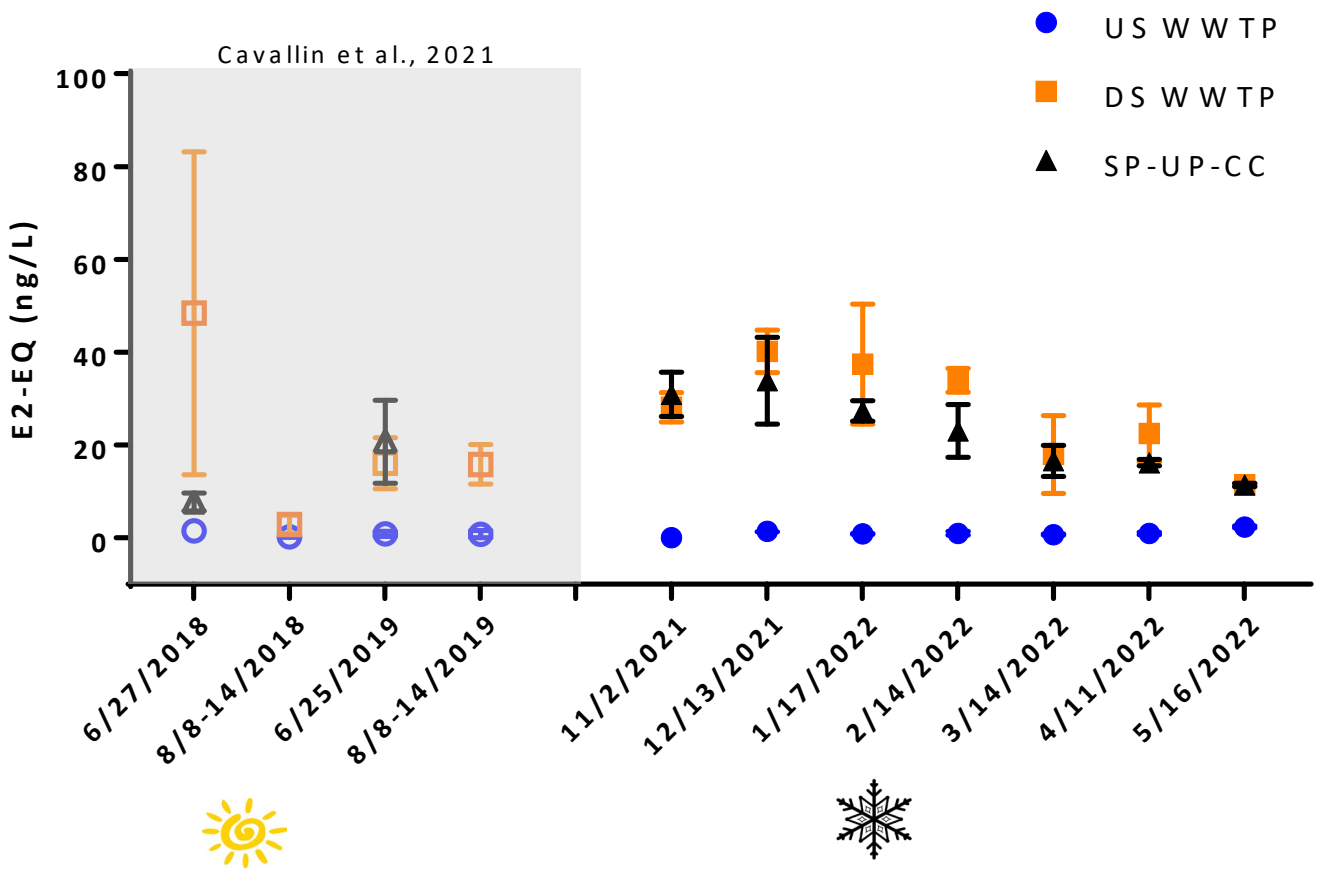
Minimal risk to fish in situ*

* With uncertainties

Targeted follow-up monitoring

ER activity

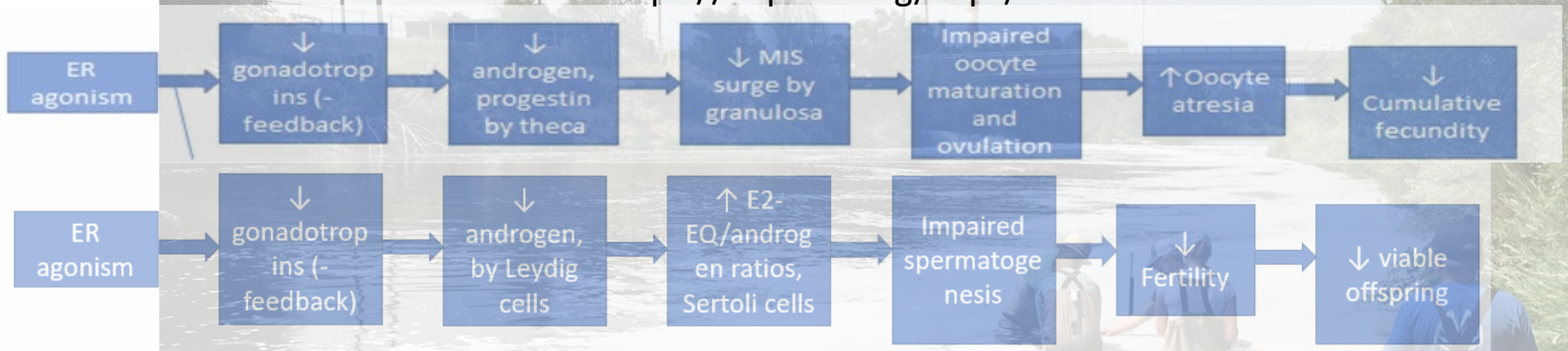
- The highest ER activity downstream of the WWTP was detected in December and steadily declined throughout the winter months.
- There was no ER activity above detection limits upstream of the WWTP.



Hazards and Risk

Hazard to fish reproduction

https://aopwiki.org/aops/445



WWTP DL: median 26 ; max 50 ng E2-EQ/L

Risk to fish in situ*

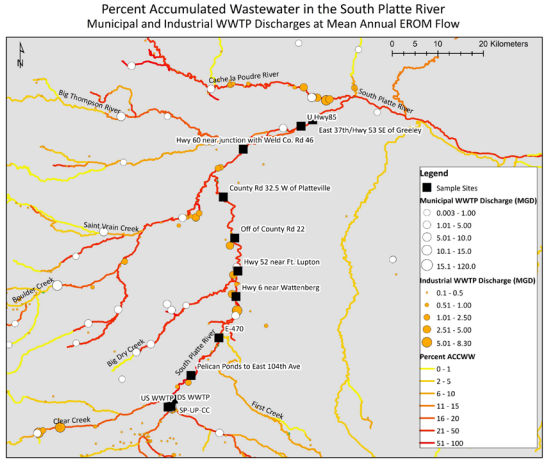
Exceeds concentrations of prototypical stressor that caused adverse effects in laboratory studies

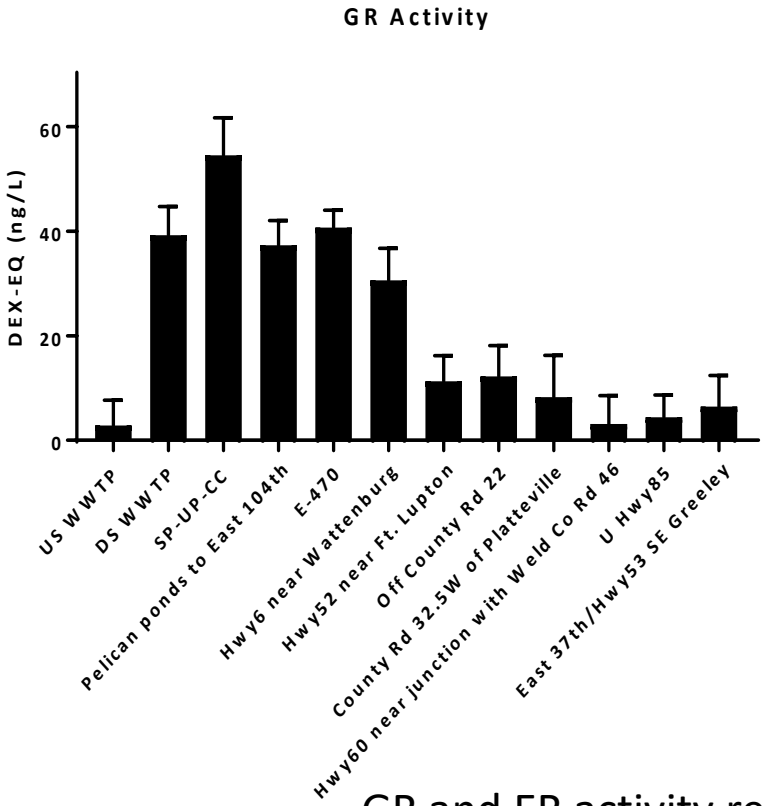
Exceeds effects-based trigger values[#] for estrogenic compounds

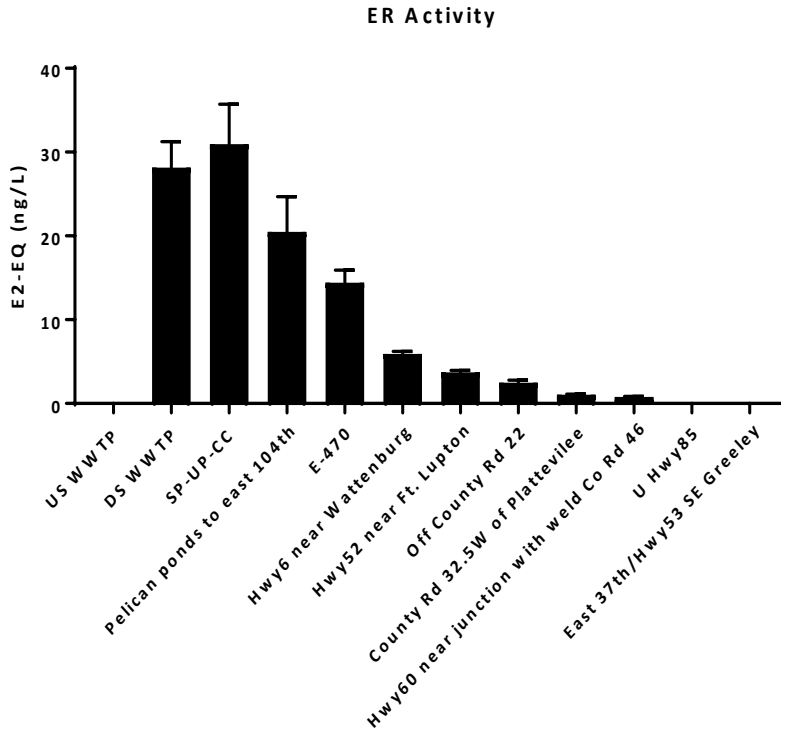
* With uncertainties

[#]Escher BI, et al. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. Sci Total Environ. 2018 Jul 1;628-629:748-765. doi: 10.1016/j.scitotenv.2018.01.340.

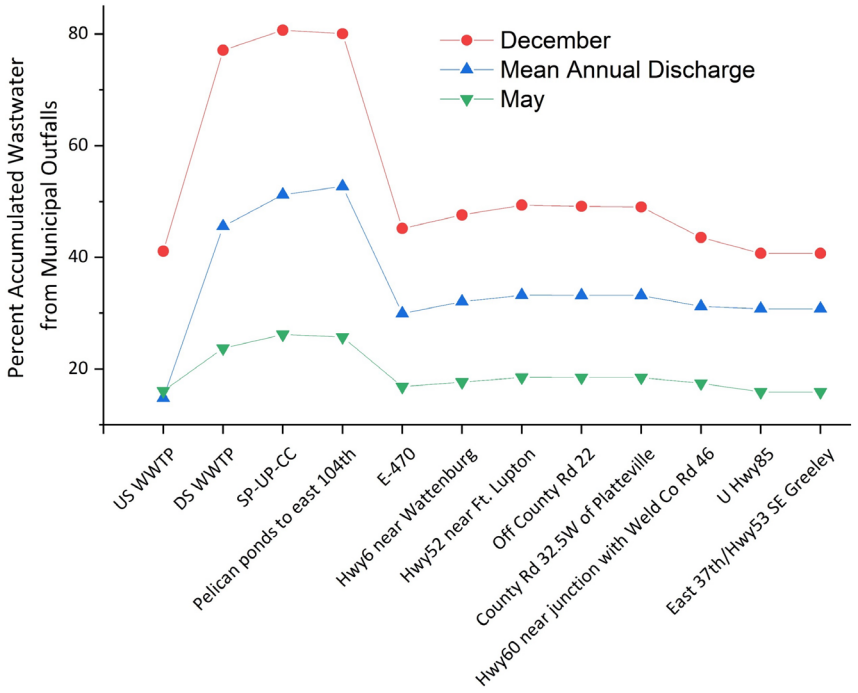
Hydrologic/Wastewater Modeling







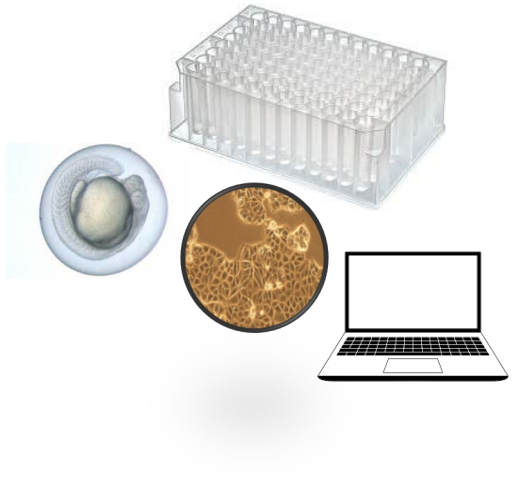
GR and ER activity remained elevated until ~30 km downstream of the WWTP



GR and ER activity profiles generally parallel the % ACCWW

NAMs and AOPs can facilitate more efficient ecological risk assessment

NAMs and AOPs

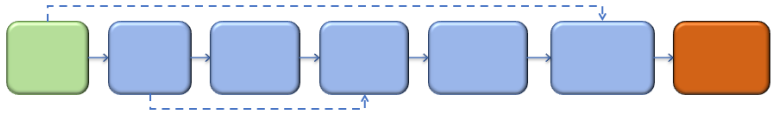


NAMs →

Which chemicals should be tested *[in vivo]*?
And of these, which should be tested first?

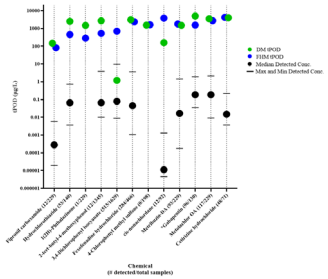
- Chemical-specific bioactivity (observations)
- Ranking potency

AOPs →



For what endpoints [*in vivo*]?
Based on what rationale?

- Anticipated hazards based on existing knowledge
- Not chemical-specific (search by bioactivity / effect)
- Guide the next tier(s) of testing



Exposure →

Contextualize with respect to risk

Conclusions

- We have been actively applying NAMs and AOPs
    - Pathway-based bioactivities
    - Transcriptomics

- Current role is to prioritize chemicals, sites, and/or endpoints for subsequent testing

- Building confidence in the methods and models that may eventually facilitate replacement with predictive approaches

- Need to continue conducting applied case studies to define the strengths and limitations of NAMs and AOPs

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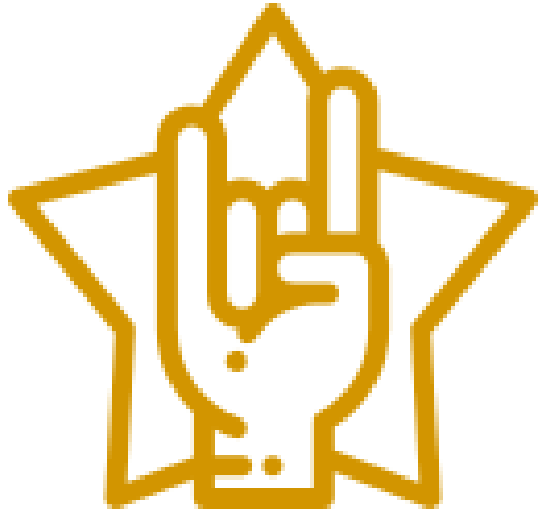

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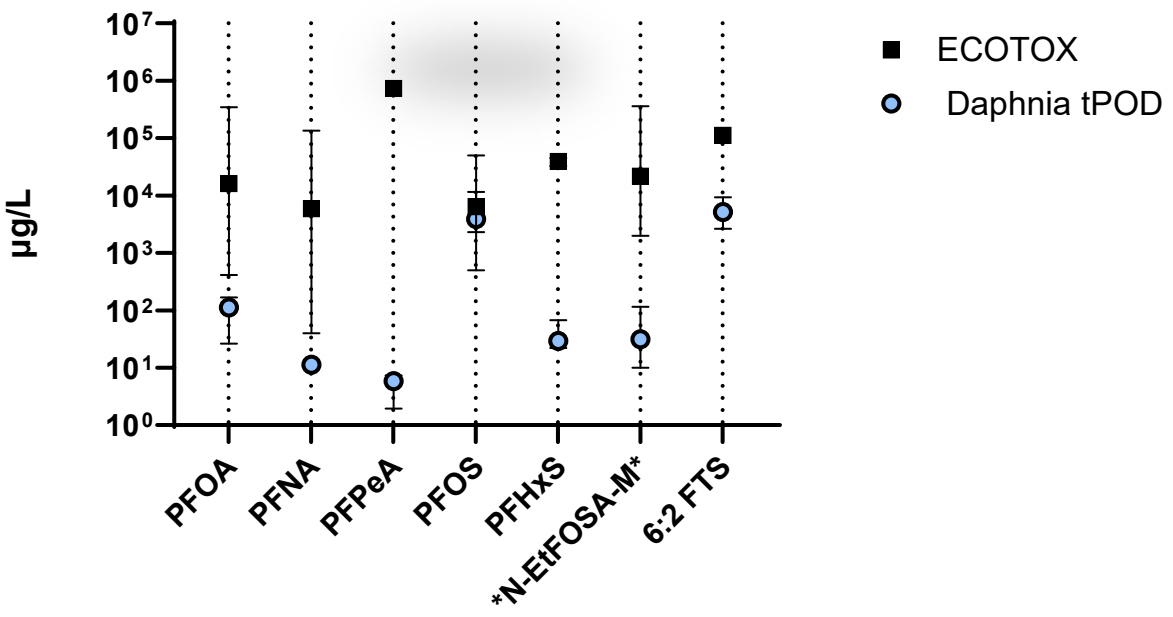
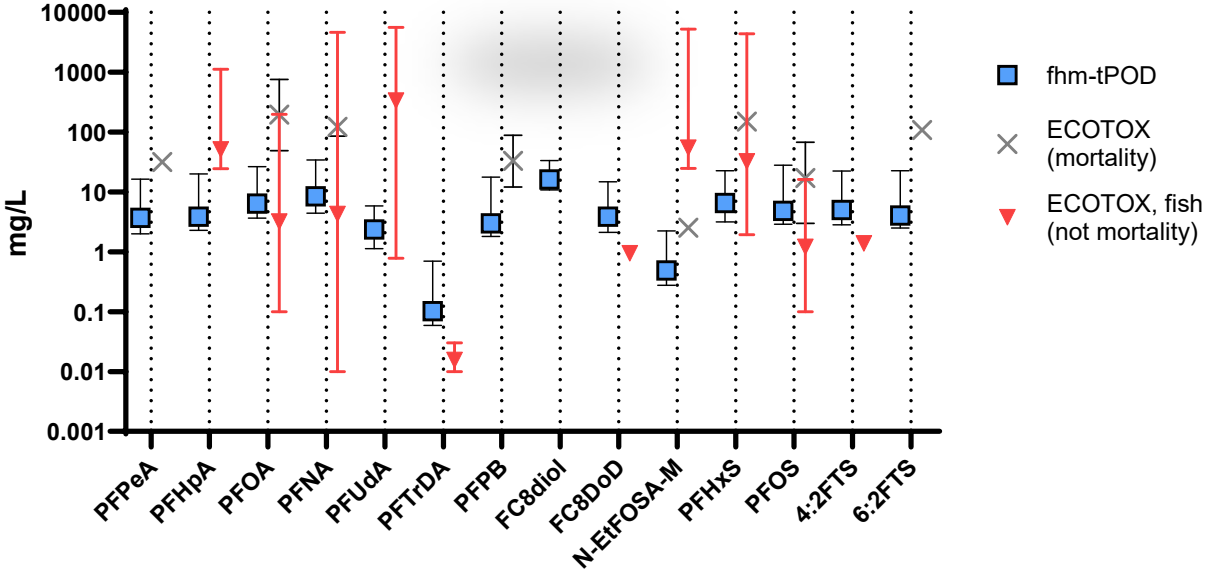
tPODs for data-rich PFAS in range similar to or less than sub-lethal effect concentrations in ECOTOX knowledgebase





Human cell-based tPODs were reasonably protective for fish, with a few exceptions, but not for *Daphnia magna*