Hazard Characterization and Grouping of PFAS using a Compendium of Human Cell Lines from Different Organs

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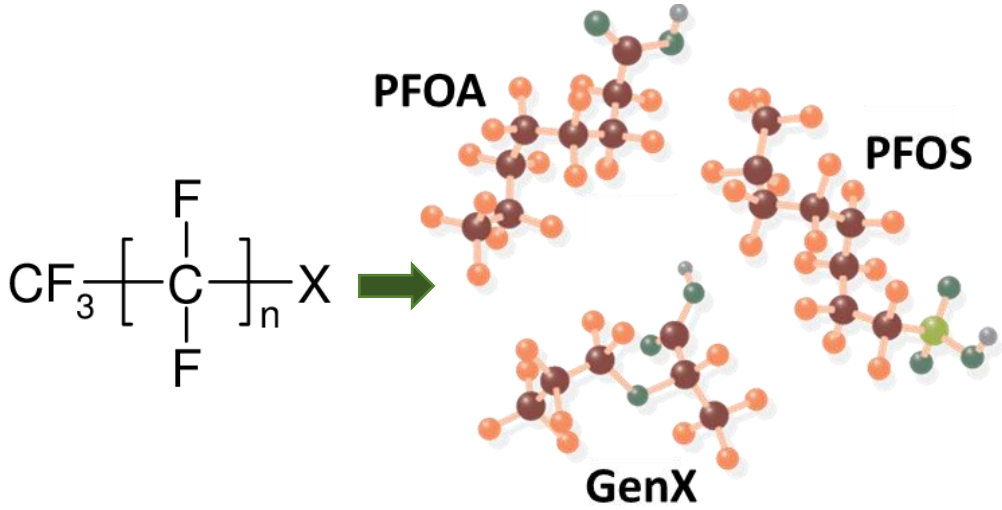
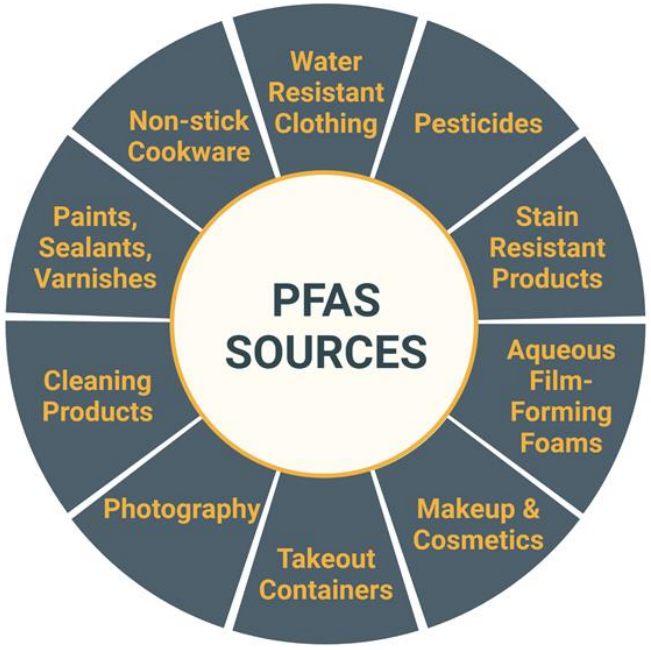
Structural Diversity



Use & Applications



Scope of PFAS

- Synthetic chemicals using since the 1940's in a variety of manufacturing facilities and consumer products
- **Defining PFAS:** any substance that contains at least one fully fluorinated methyl (CF3-) or methylene (-CF2-) carbon atom (without any H/Cl/Br/I attached to it)
- Currently more than **10,000 PFAS** in commerce


Portland Press Herald

A forever farm is no match for forever chemicals

A fourth-generation farmer running an organic dairy farm in Fairfield lost his livelihood when his milk and lands tested hot for PFAS contamination. 'It got us good.'

BY PENELOPE OVERTON STAFF WRITER

CONTAMINANTS

The Surprising Places PFAS Are Being Found

From the arctic to your blood, PFAS are ubiquitous on our planet

Ryan Moore

Dec. 7, 2021


Pain in the butt: Study finds forever chemicals present in toilet paper

CARLY WANNA
BLOOMBERG

So-called forever chemicals seem to be turning up everywhere. We wear them, clean our houses with them and, according to a new study, perhaps even wipe ourselves with them.

The report, published this week in Environmental Science & Technology Letters, has found evidence of per- or polyfluorinated chemicals - also known as PFAS - in toilet paper. An

more that it's this issue that is pretty ingrained into society, and we have to think how we can limit its uses across a wide range of products."

For the study, researchers analyzed samples of toilet paper from four regions - Africa, North America, South and Central America and Western Europe - between November 2021 and August 2022. They detected six types of PFAS in the toilet paper samples and said one chemical in particular, 6:2 di-PAP, was especially prevalent. No one

Rolls of toilet paper move alo
Shinei Co. factory in Fuji, Sh
Japan's gross domestic prod
three months to June, faster
revised report by the Cabinet
government spending, busin


The Washington Post
Democracy Dies in Darkness

EATING LAB

How can I avoid eating food with 'forever' chemicals?

It's hard to avoid PFAS, but experts say there are ways to mitigate your exposure to the man-made chemicals

By Teddy Amenabar

Updated June 9, 2023 at 1:51 p.m. EDT | Published June 6, 2023 at 2:49 p.m. EDT

PHYS.ORG

Topics

Week's top

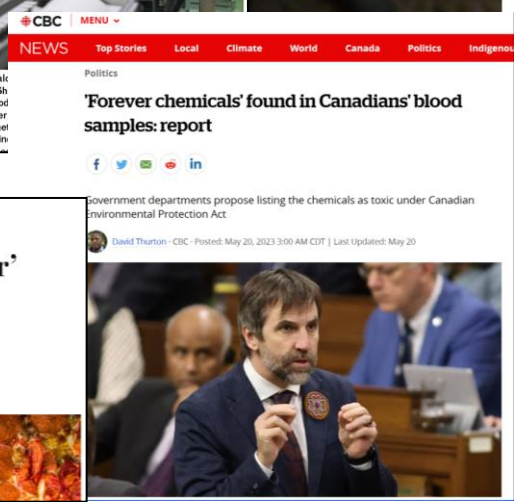
Nanotechnology | Physics | Earth | Astronomy & Space | Chemistry | Biology | Other Sciences

JANUARY 17, 2023

Editors' notes

Eating one wild fish same as month of drinking tainted water: study

by Daniel Lawler


CBC | MENU

NEWS | Top Stories | Local | Climate | World | Canada | Politics | Indigenous

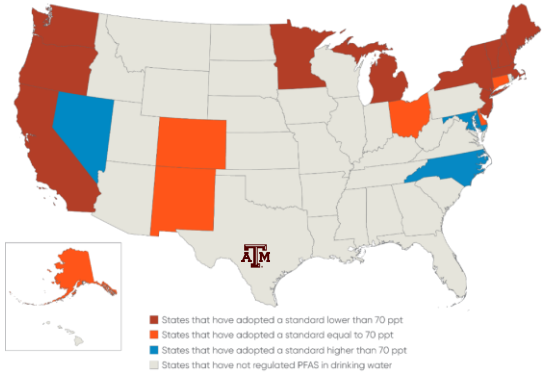
Politics

'Forever chemicals' found in Canadians' blood samples: report

overnment departments propose listing the chemicals as toxic under Canadian nvironmental Protection Act

David Thurton - CBC - Posted: May 20, 2023 3:00 AM CDT | Last Updated: May 20

Current Regulations & Restrictions



European Union

Pre- 2010

- PFOS have been included in the Stockholm Convention to eliminate their use
- PFOS restricted in the EU under the persistent organic pollutants (POPs) regulation

2010-2020

- Safety Threshold for PFAS in food
- PFOA banned under the POPs regulation
- Substances of Very High Concern under REACH
  - Based on persistence, mobility, and toxicity

2021-2024

- PFAS restriction proposal submitted to ECHA
  - Option A: Full Ban
  - Option B: Ban with use-specific derogations (proposed restriction)
- Restriction proposal for PFAS in firefighting foams
- PFHxS added to ban for POPs regulation
- Drinking Water Directive: limit of 0.5 ug/L for all PFAS

USA

- EPA published Significant New Use Rules impacting several hundred PFCAs and PFSAs
- EPA drinking water limit for PFOA and PFOS was set to 70 ppt
- Implemented 2010-2015 PFOA Stewardship Program
- 30/50 states have state-level restrictions with levels between the original proposed drinking water levels, and the newly proposed levels
  - California – 5.1 ppt for PFOA & Nevada – 667,000,000 ppt for PFBS
- EPA proposed National Primary Drinking Water Regulation
- EPA designates 2 PFAS: PFOA and PFOS as hazardous substances under CERCLA
- EPA updated drinking water advisories for PFOA and PFOS and a few alternatives
  - PFOS (0.02 ppt), PFOA (0.004 ppt), GenX (10 ppt), PFBS (2,000 ppt)
- EPA Unregulated Contaminant Monitoring Rule to establish nationwide monitoring for 29 PFAS

US Federal Government PFAS Programs

- **CDC/ATSDR** – PFAS assessments in communities near current or former military bases (both exposure and health surveys for 6 PFAS)
- **CDC/NHANES** – ongoing assessment of the U.S. population's exposure to environmental chemicals (biomonitoring for 17 PFAS in blood and urine)
- **White House Office of Science Technology Policy** – State of the Science Report to be used as a "PFAS roadmap" for Federal Agencies
- **U.S. EPA** – new data collection through ToxCast, ExpoCast, including IVIVE
- **NASEM** – examining health outcomes associated with PFAS on behalf of CDC

NATIONAL ACADEMIES Sciences Engineering Medicine

Centers for Disease Control and Prevention

ATSDR
AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

PFAS Data Gaps: How to Group PFAS?

National PFAS Testing Strategy: Identification of Candidate PFAS for Testing

and could further inform the Agency's future research, monitoring, and regulatory efforts. Given the large number of PFAS to which exposures may have occurred or that are currently ongoing, the Strategy is based on an approach that groups similar PFAS into categories. The categories serve as the basis for both identifying PFAS chemicals for testing as well as allowing EPA to establish toxicity levels for PFAS within the identified categories. Thus, rather than seeking data about each of the thousands of individual PFAS, which would require extensive resources in terms of time, costs, and animals, the Strategy aims to identify a representative substance(s) for each chemical category where categories have been constructed to span the landscape of PFAS of interest.

- Group similar PFAS into categories
- Substances that cover entire PFAS landscape
- Test representative substances for each category

Assessing Groups of PFAS

ECHA's database contains information of several thousand individual PFAS on the EU market. These belong to a variety of subgroups. Assessing and, where relevant, managing risks subgroup by subgroup would require a considerable amount of time. Therefore, ECHA acknowledges that a holistic group approach to regulatory assessment and risk management needs to be explored.

- Subgroup approaches are time intensive
- Holistic group approach to group substances

PFAS Report

Grouping by mechanism of action and/or structure will facilitate development of mechanistically and/or empirically based prediction models to estimate toxicity thresholds for additional PFAS.

- Grouping strategies based on structure and/or mechanism of action
- Subsequent predictive modeling for other PFAS

PFAS Data Gaps: Inter-Individual Variability

NATIONAL ACADEMIES Sciences Engineering Medicine Guidance of PFAS Exposure, Testing, and Clinical Follow-Up

The presence of PFAS in everyday consumer products may be an important source of exposure for the general population, but this likely varies greatly by individual (Rodgers et al., 2022). Consumer are not distributed uniformly across populations. Race, ethnicity, poverty, age, life stage, and other social factors can place people at disproportionately high risk for diseases with environmental causes as a result of hazardous exposures at increased levels compared to the general population (Gochfeld and Burger,

Note that interindividual variability in PFAS testing results may be a function of differences not only in exposure but also in pharmacokinetics with respect to excretory clearance. Such host factors as parity, breastfeeding status, menstrual status, age, genetic polymorphisms, concurrent acute or chronic disease, and medication use can affect pharmacokinetics.

- PFAS exposure varies by individual
- Race, ethnicity, life stage, other social factors can introduce additional risk for diseases as a result of hazardous exposures
- Inter-individual variability introduced by differences in exposure and pharmacokinetics

FY 2022 – 2026 EPA Strategic Plan

and evidence that inform local decisions. Finally, where feasible based on availability of data and methods, EPA will explicitly and consistently assess risks to childhood lifestages and other vulnerable populations as part of the Agency's approach for developing risk assessments and in its research agenda.

EPA will support the development of new science to address uncertainties related to the environmental health of children and vulnerable populations, including through intramural and extramural research. EPA

- Assess risks to childhood lifestages and vulnerable populations
- Support research that addresses uncertainties

ECHA EUROPEAN CHEMICALS AGENCY Annex XV Restriction Report

Goldenman et al. (2019) indicate that the contamination may be poorly reversible or even irreversible, and may reach levels that could render natural resources such as soil and water unusable far into the future, resulting in continuous exposure and unavoidable harmful health effects, particularly for vulnerable populations, such as children.

- Continuous PFAS exposure leading to unavoidable harmful health effects to the population including vulnerable individuals

PFAS Data Gaps: Cardiotoxicity

National PFAS Testing Strategy: Identification of Candidate PFAS for Testing

Testing for cardiac sensitization. Certain terminal categories consisting of short-chain volatile PFAS may be considered for testing for cardiac sensitization[28] because existing data for halogenated hydrocarbons indicate these compounds may lead to cardiac arrythmias and occasionally to sudden death resulting from sensitization of the heart muscle to endogenous compounds in the body (e.g., adrenaline).[29,30]

- Consider testing PFAS for cardiac sensitization
- Read-across indicates that exposure to PFAS may lead to cardiac arrhythmias

Guidance on PFAS Exposure, Testing and Clinical Follow-Up

The committee observed gaps in the evidence, rendering the evidence inadequate or insufficient, for many health effects including the following:

- immune effects other than reduced antibody response, and ulcerative colitis;
- cardiovascular outcomes other than dyslipidemia;

- Gaps in current data or inadequate/insufficient data for a lot of health effects
- Cardiovascular toxicity

Annex XV Restriction Report

Liver toxicity and metabolic disruption	Increased serum alanine transferase (ALT) which is a marker of liver toxicity and fatty liver diseases
	Increases total and LDL-cholesterol
	Increased risk of cardiovascular diseases

- Exposure to PFAS leading to increased risk of cardiovascular diseases

Where do we go from here?

- Test, test, test more!
- Test using *in vitro* battery (**not** *in vivo*)
  - Higher-throughput, time, cost, ethical reasoning
- Still, need to prioritize testing of representative PFAS based on hazard and/or exposure, reduce dimensionality
- Most available data cover a limited number of organs; need to address other “-icities” (including cardio)
- Can use *in vitro* models to address human variability

Solution: an optimized human cell-based battery of broad coverage assays

Rusyn Lab Case Studies: Using a Battery of hiPSC-Derived Cells for "Decision Making"

Grouping of UVCBs to waive (or minimize) animal testing requirements

Deriving "protective" points of departure and bioactivity-exposure ratios

Testing validity of dose reconstruction assumptions for chemical mixtures

Rapid hazard screening after disaster events that may involve re-distribution of chemicals

House et al 2022

House et al 2021

Tsai et al 2023

Research Article

Grouping of UVCB Substances with Dose-Response Transcriptomics Data from Human Cell-Based Assays

Research Article

Grouping of UVCB Substances with New Approach Methodologies (NAMs) Data

SOT | Society of Toxicology
academic.oup.com/toxsci

A tiered testing strategy based on *in vitro* phenotypic and transcriptomic data for selecting representative petroleum UVCBs for toxicity evaluation *in vivo*

Chen et al 2020

Ford et al 2022

Research Article

Rapid Hazard Characterization of Environmental Chemicals Using a Compendium of Human Cell Lines from Different Organs

toxics

Article

A Population-Based Human In Vitro Approach to Quantify Inter-Individual Variability in Responses to Chemical Mixtures

Hsieh et al 2021

Research

Risk Characterization and Probabilistic Concentration–Response Modeling of Complex Environmental Mixtures Using New Approach Methodologies (NAMs) Data from Organotypic *in Vitro* Human Stem Cell Assays

Jang et al 2022

toxics

Article

Cumulative Risk Meets Inter-Individual Variability: Probabilistic Concentration Addition of Complex Mixture Exposures in a Population-Based Human In Vitro Model

Chen et al 2021

SOT | Society of Toxicology
academic.oup.com/toxsci

Risk Characterization of Environmental Samples Using *In Vitro* Bioactivity and Polycyclic Aromatic Hydrocarbo

International Journal of Environmental Research and Public Health

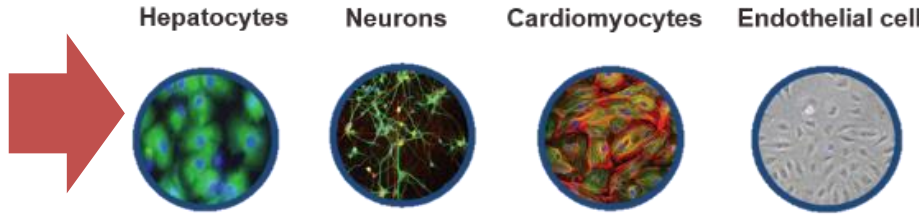
Chen et al 2021

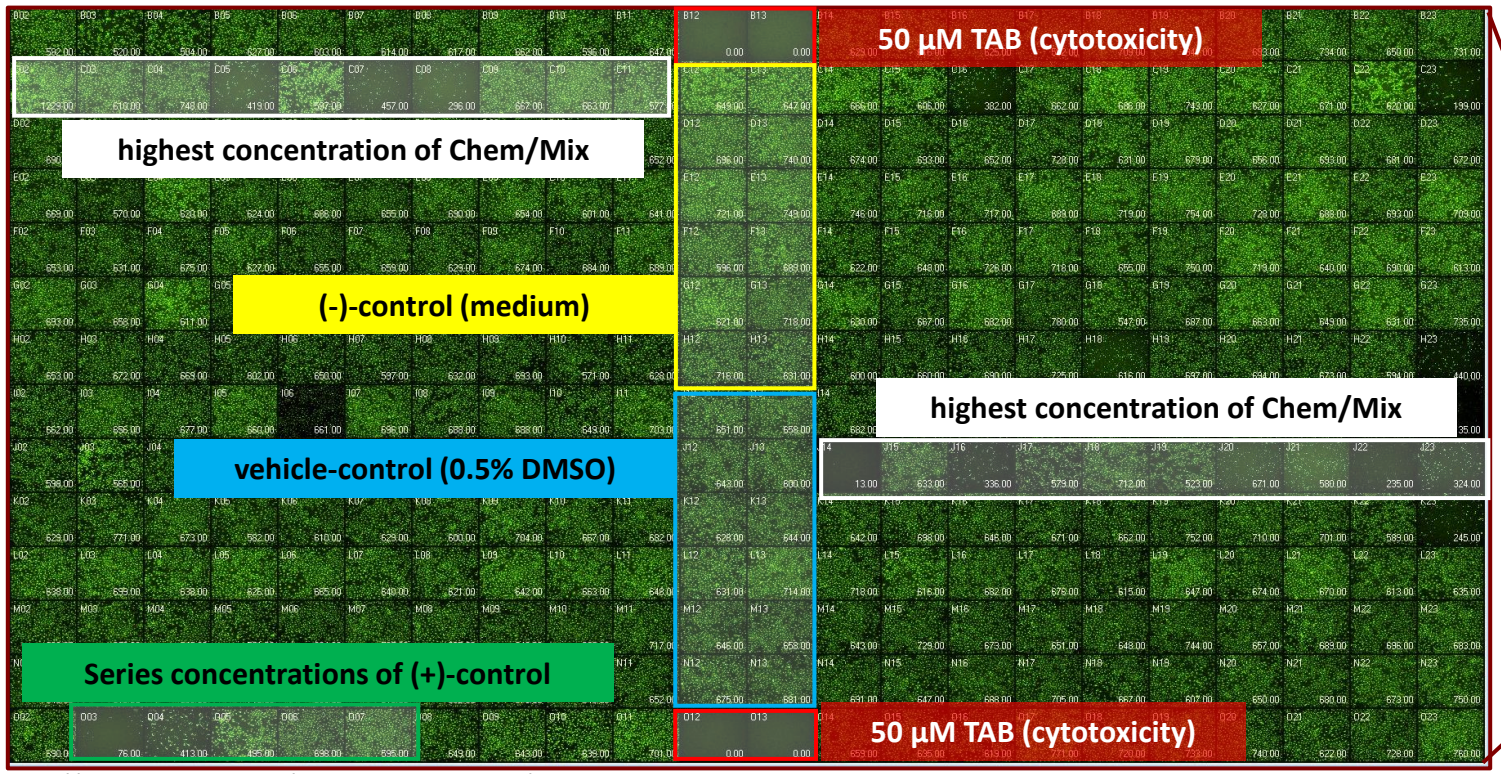
Article

Potential Human Health Hazard of Post-Hurricane Harvey Sediments in Galveston Bay and Houston Ship Channel: A Case Study of Using *In Vitro* Bioactivity Data to Inform Risk Management Decisions

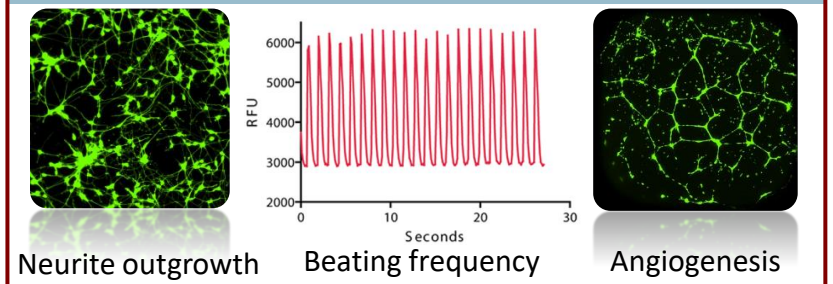
Rusyn Lab Case Studies: Using a Battery of hiPSC-Derived Cells for "Decision Making"

A compendium of Human iPSC-derived cells [96- or 384-well plates]





High content imaging-based phenotyping

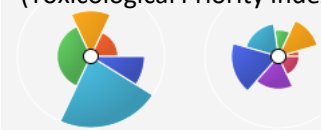


Neurite outgrowth | Beating frequency | Angiogenesis

Data Analysis

ToxPi GUI (v 2.0)

(Toxicological Priority Index)



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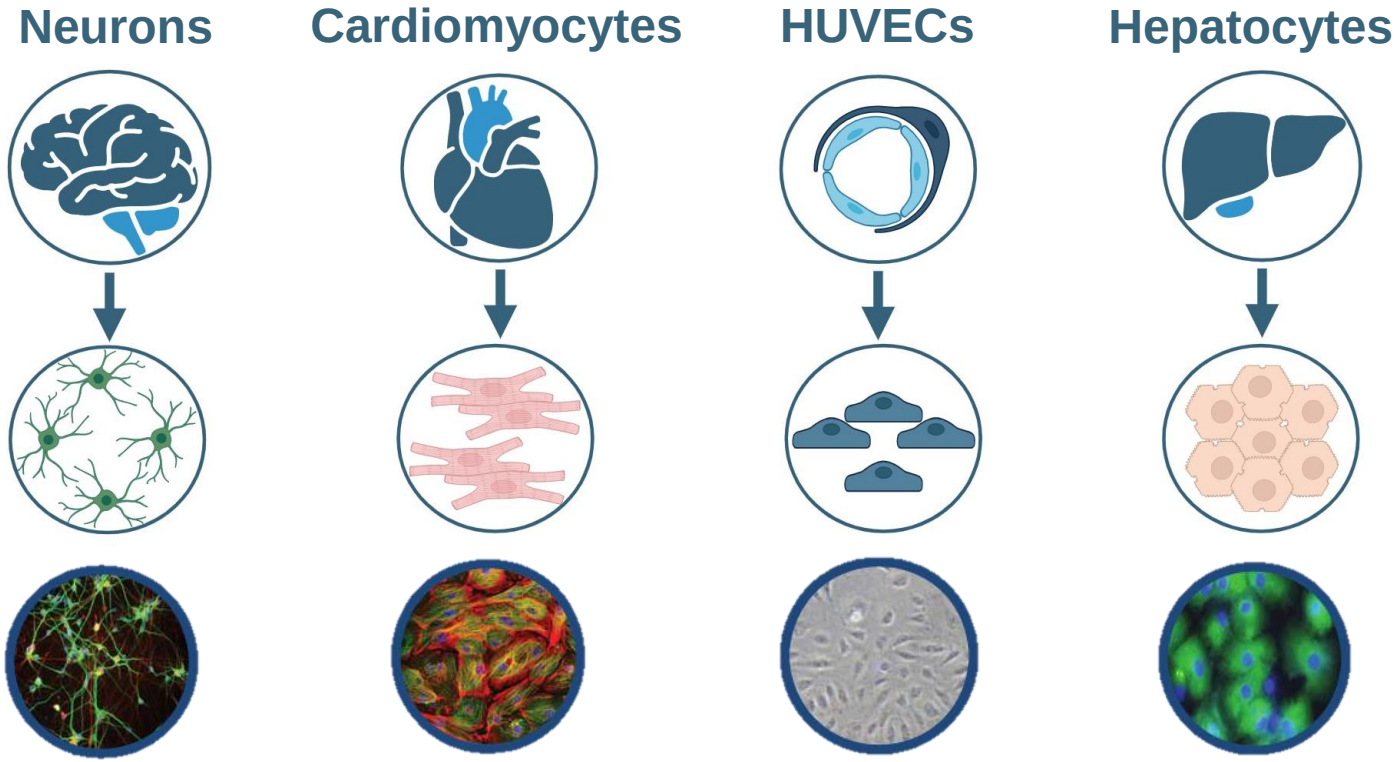
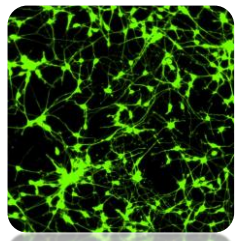
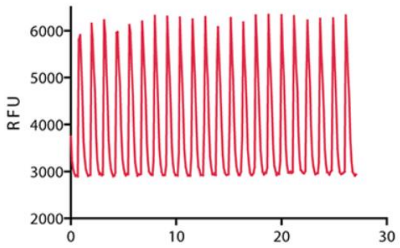
https://bioinformant.com/ipsc-disease-models/

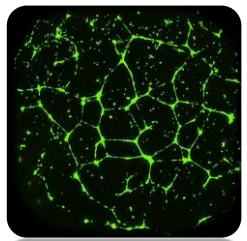
Using a Targeted Assay Battery of hiPSC-Derived Cells for "Decision Making"




Neurite outgrowth



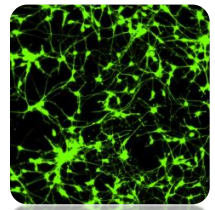
Beat frequency

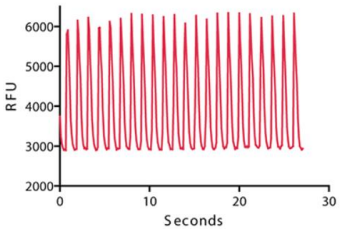

Angiogenesis

...

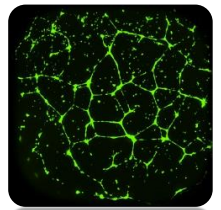
48 endpoints total across 6 cell types

High content imaging-based phenotyping


Neurite outgrowth



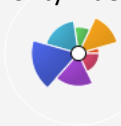
Beating frequency


Angiogenesis

Data Analysis

ToxPi GUI (v 2.0)

(Toxicological Priority Index)



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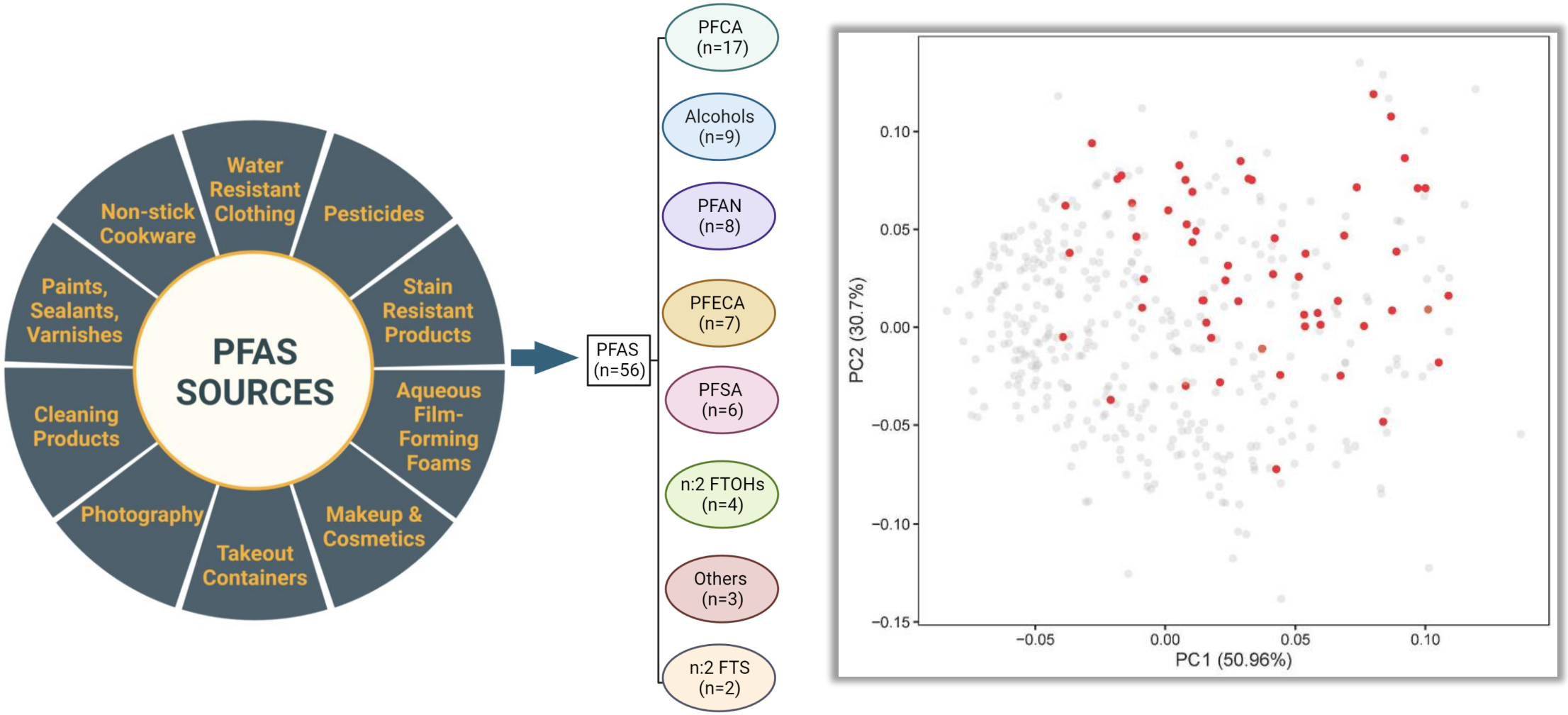
Addressing Regulatory Science Questions

1. Are PFAS subclasses the best way to group PFAS?
2. Can we rank PFAS to identify trends based on the overall bioactivity?
3. Does *in vitro* bioactivity data exceed PFAS exposure levels?
4. How do our PODs benchmark to those from the CompTox Dashboard?
5. Compared to other industrial chemicals, how bad are PFAS?
6. Are PFAS potentially hazardous to cardiomyocytes?
7. Are there particular subpopulations at risk for PFAS exposure?

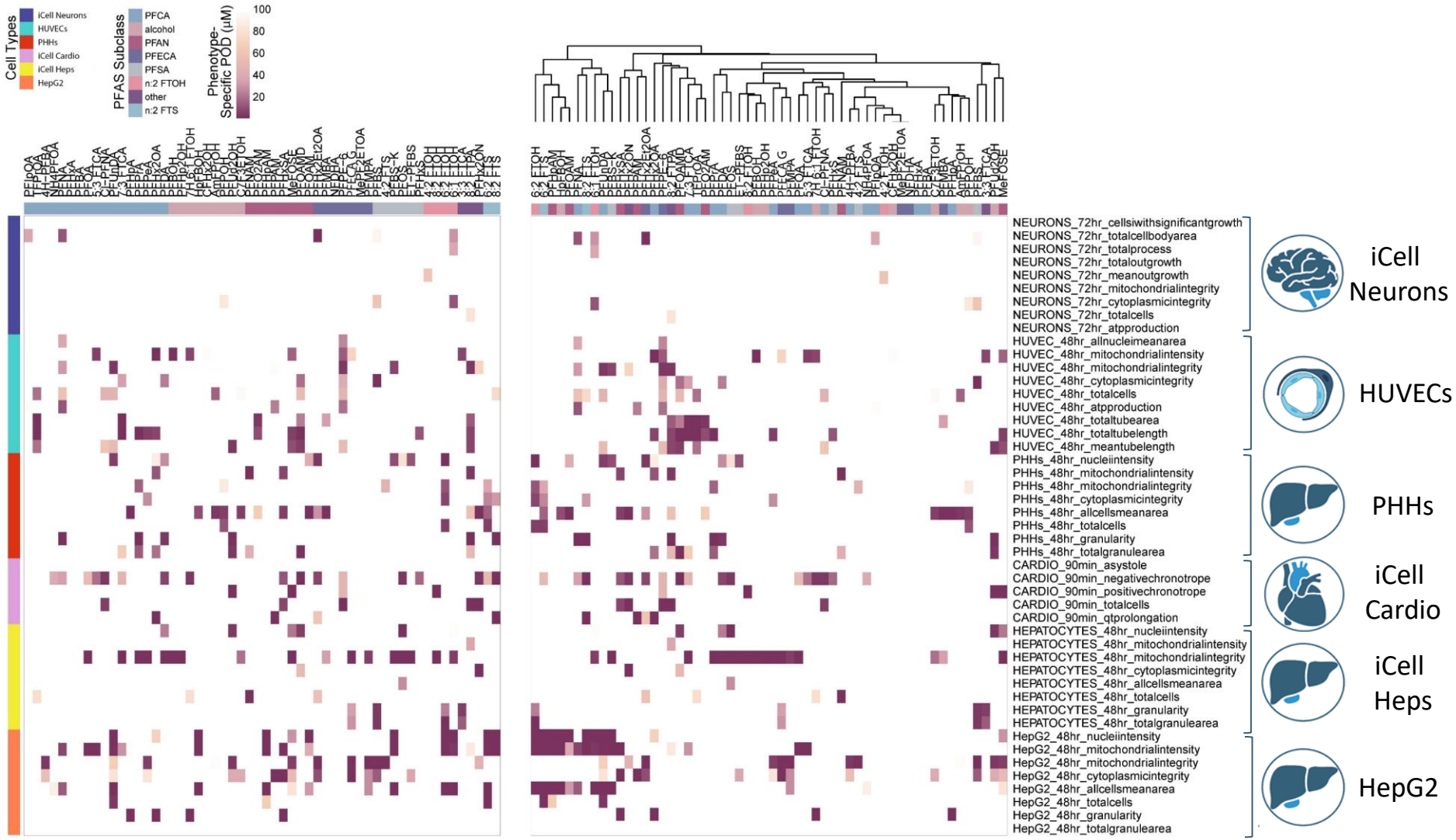
Conceptual Approach



Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

Are subclasses the best way to group PFAS?

Big reveal of the ending…



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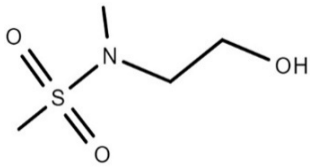
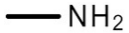
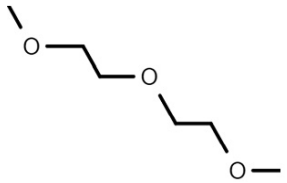
Are subclasses the best way to group PFAS?

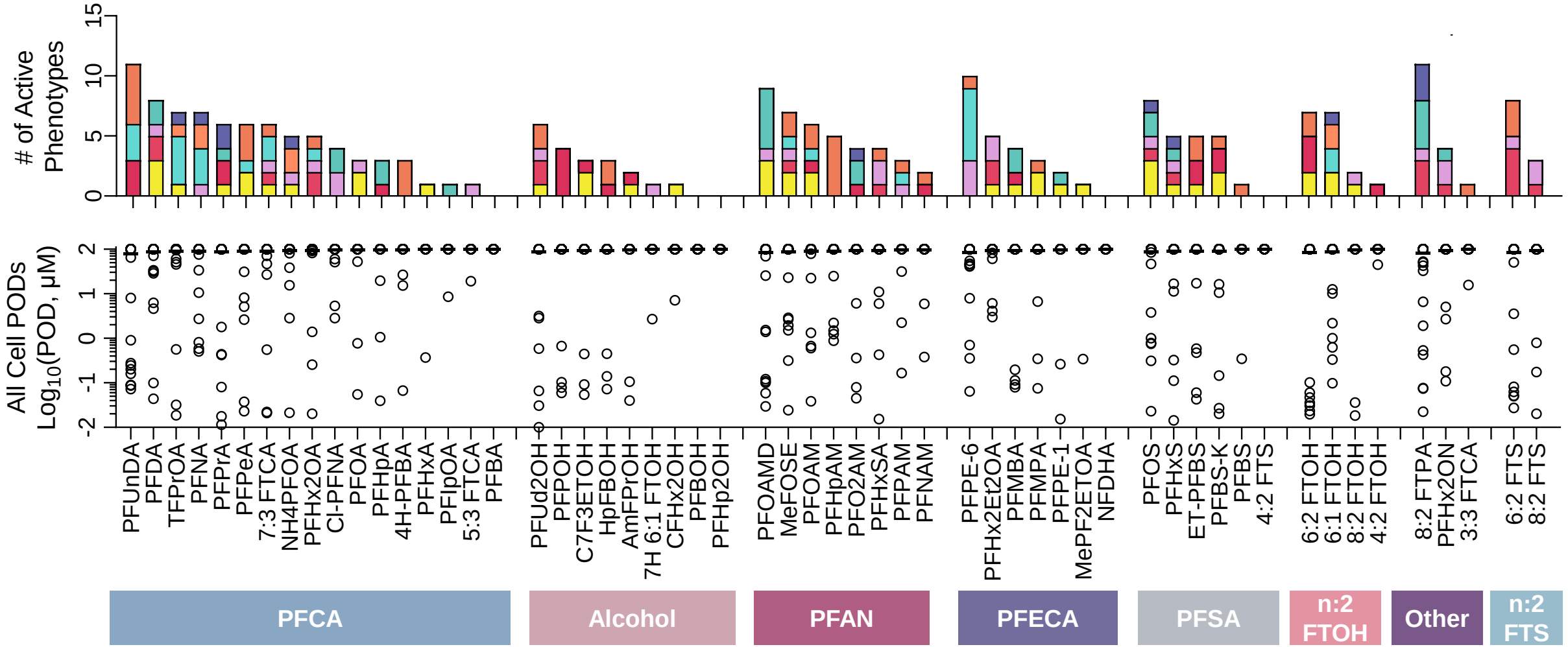
Big reveal of the ending…

Descriptor[a]	Structure[b]	Descriptor Meaning	ρ[c]	P_{adj}[d]	Chemicals with Descriptor	Cell Type (Phenotype)
SGR10703		N-attached double bonded heteroatoms				
SGR10587		Sulfonamide	-0.70	1.08E-05	MeFOSE PFHxSA	
SGR10099		Heteroatom-nitrogen bond				
SGR10668		Heteroatom-bonded methyl group	-0.70	1.08E-05	MeFOSE MePF2EtOA	PHHs (Mitochondria Intensity)
SGR10199		Two oxygens, 5 bonds apart	-0.57	3.11E-02	MeFOSE PFBOH MePF2EtOA	
SGR10032		Methyl group	-0.57	3.11E-02	MeFOSE, PFPE-1, PFMBA	
SGR10343		Any primary amine	-0.56	4.17E-02	AmFPrOH, PFHxSA, PFOAMD	iCell Heps (Cytoplasmic Integrity)
SGR10704		Polyethers	-0.69	2.84E-05	PFPE-6, C7F3ETOH	HUVECs (All Nuclei Mean Area)
SGR10013		Any carbon	-0.57	2.46E-02	All tested PFAS (C # varies)	
SGR10029		Any heteroatom	-0.57	2.79E-02	All tested PFAS (heteroatom # varies)	iCell Cardio (Min POD)
SGR10308		H-bond acceptors	-0.57	3.18E-02	All tested PFAS (# of H-bond acceptors varies)	

Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

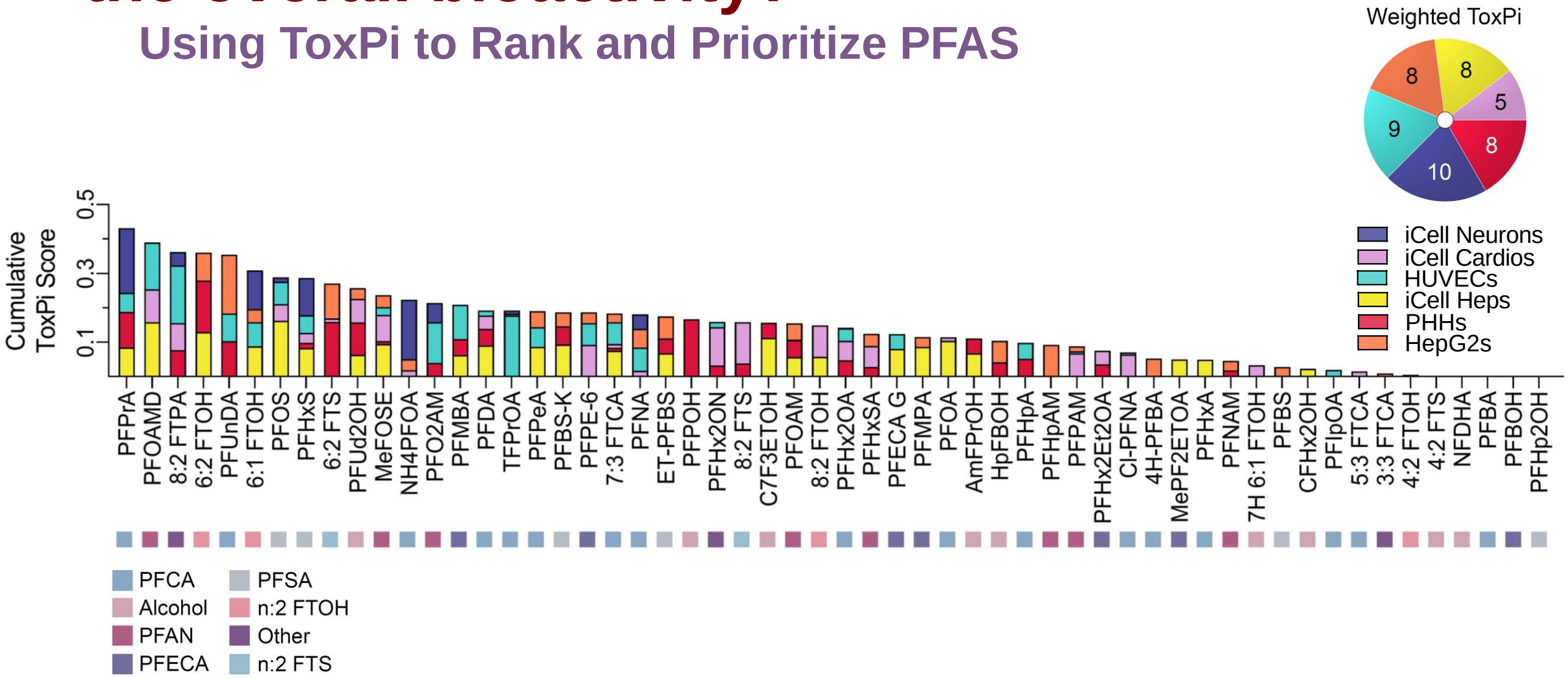
Can we rank PFAS to identify trends based on the overall bioactivity?

Good News! Little Bioactivity Observed across All Cell Types



Can we rank PFAS to identify trends based on the overall bioactivity?

Using ToxPi to Rank and Prioritize PFAS

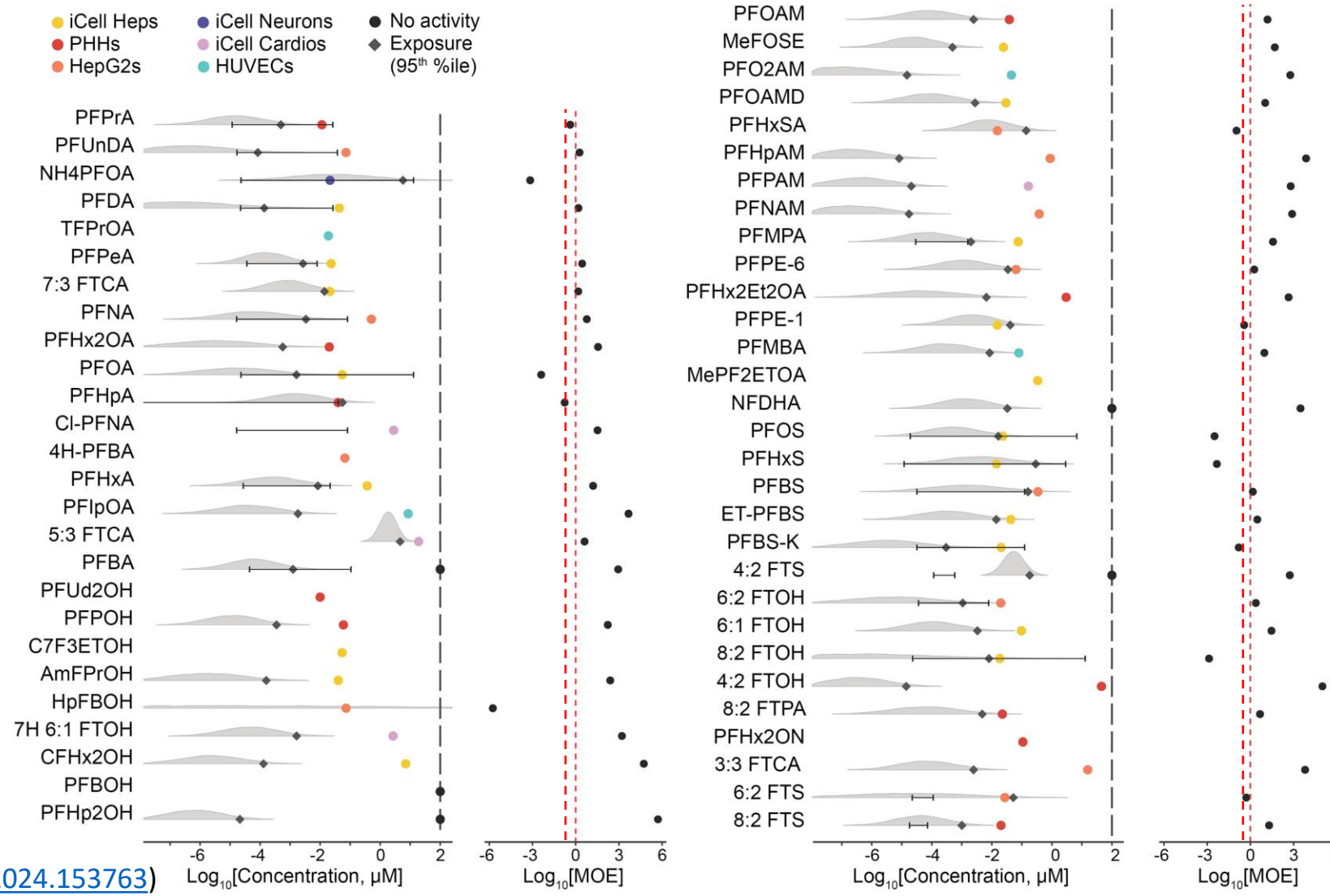


Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

Does *in vitro* bioactivity exceed PFAS exposure levels? *Using phenotypic PODs*

- Is there overlap between exposure and observed PODs?
- Human health risk assessment considers a margin ≥ 100 "protective"
- Compare exposure predicted data/blood level concentrations to the most sensitive *in vitro* POD

$$\text{Margin of Exposure (MOE)} \\ \text{Bioactivity Exposure Ratio (BER)} \\ \text{Margin of Safety (MOS)} = \frac{\text{POD or NOAEL}}{\text{Human Exposure}}$$

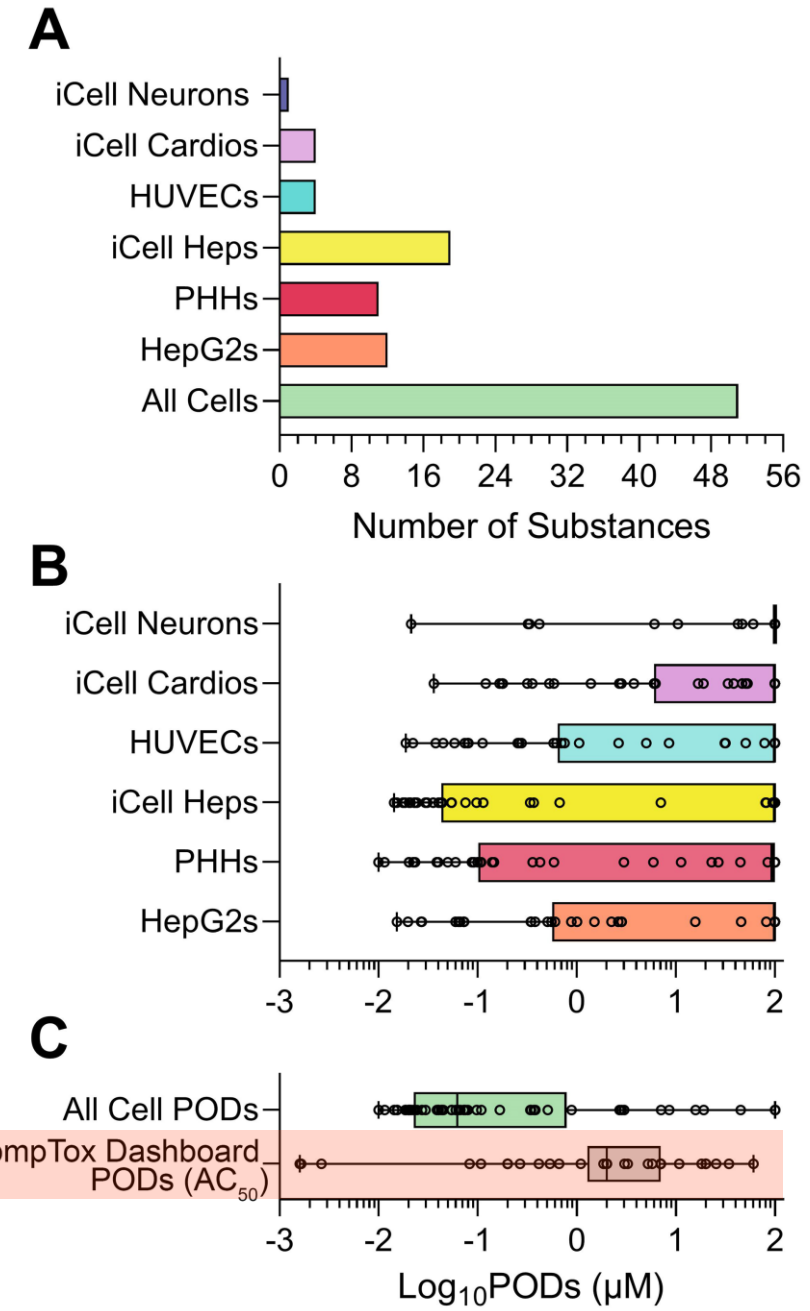


Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

How do our PODs benchmark to those from the CompTox Dashboard?

In Vitro Assay Battery Sensitivity Comparison

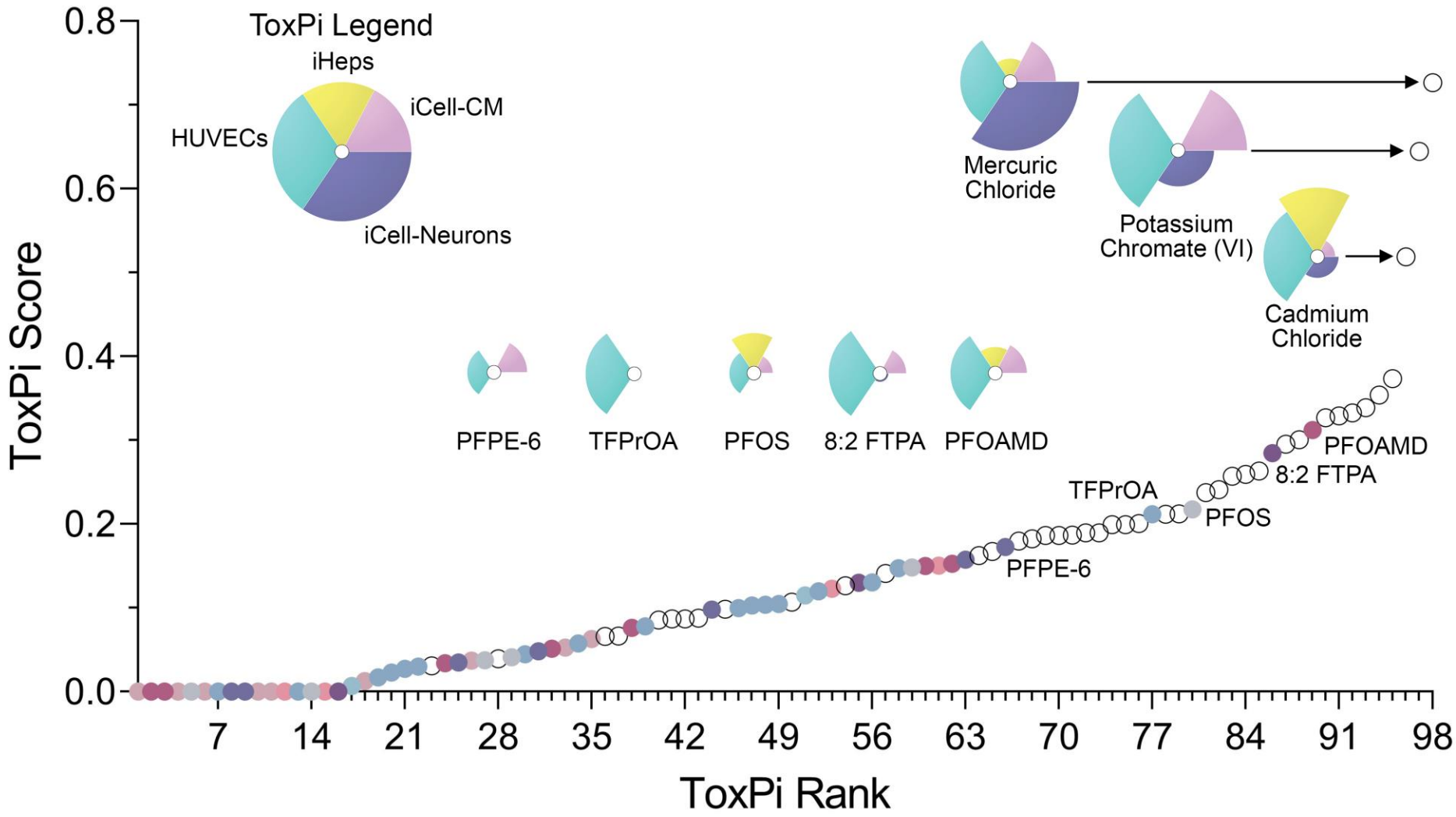
- Using the lowest AC_{50} value from the human cell-based assays in the EPA CompTox Dashboard
- EPA assays are more sensitive when comparing to individual cell types
- Comparing to all cell types, the 6 cell types are more sensitive than EPA AC_{50} values (n=20 *in vitro* models)



Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

Compared to other industrial chemicals: how “bad” are PFAS?

vs.



Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

Summary and Significance

- Cannot group by structure, other descriptors can be used for grouping
- Rank individual chemicals, but not subclasses
- Put *in vitro* data in the context of exposure, but still need more exposure data
- Optimized *in vitro* battery comparable sensitivity to ToxCast PODs
- PFAS not amongst the top ranked chemicals, in comparison to other industrial chemicals

Figures adapted from: Ford et al. 2024 (DOI: 10.1016/j.tox.2024.153763)

PFAS Data Gaps: Cardiotoxicity

National PFAS Testing Strategy: Identification of Candidate PFAS for Testing

Testing for cardiac sensitization. Certain terminal categories consisting of short-chain volatile PFAS may be considered for testing for cardiac sensitization[28] because existing data for halogenated hydrocarbons indicate these compounds may lead to cardiac arrythmias and occasionally to sudden death resulting from sensitization of the heart muscle to endogenous compounds in the body (e.g., adrenaline).[29,30]

- Consider testing PFAS for cardiac sensitization
- Read-across indicates that exposure to PFAS may lead to cardiac arrhythmias

Guidance on PFAS Exposure, Testing and Clinical Follow-Up

The committee observed gaps in the evidence, rendering the evidence inadequate or insufficient, for many health effects including the following:

- immune effects other than reduced antibody response, and ulcerative colitis;
- cardiovascular outcomes other than dyslipidemia;

- Gaps in current data or inadequate/insufficient data for a lot of health effects
- Cardiovascular toxicity

Annex XV Restriction Report

Liver toxicity and metabolic disruption	Increased serum alanine transferase (ALT) which is a marker of liver toxicity and fatty liver diseases
	Increases total and LDL-cholesterol
	Increased risk of cardiovascular diseases

- Exposure to PFAS leading to increased risk of cardiovascular diseases

PFAS Data Gaps: Inter-Individual Variability

NATIONAL ACADEMIES Sciences Engineering Medicine Guidance of PFAS Exposure, Testing, and Clinical Follow-Up

The presence of PFAS in everyday consumer products may be an important source of exposure for the general population, but this likely varies greatly by individual (Rodgers et al., 2022). Consumer are not distributed uniformly across populations. Race, ethnicity, poverty, age, life stage, and other social factors can place people at disproportionately high risk for diseases with environmental causes as a result of hazardous exposures at increased levels compared to the general population (Gochfeld and Burger,

Note that interindividual variability in PFAS testing results may be a function of differences not only in exposure but also in pharmacokinetics with respect to excretory clearance. Such host factors as parity, breastfeeding status, menstrual status, age, genetic polymorphisms, concurrent acute or chronic disease, and medication use can affect pharmacokinetics.

- PFAS exposure varies by individual
- Race, ethnicity, life stage, other social factors can introduce additional risk for diseases as a result of hazardous exposures
- Inter-individual variability introduced by differences in exposure and pharmacokinetics

FY 2022 – 2026 EPA Strategic Plan

and evidence that inform local decisions. Finally, where feasible based on availability of data and methods, EPA will explicitly and consistently assess risks to childhood lifestages and other vulnerable populations as part of the Agency's approach for developing risk assessments and in its research agenda.

EPA will support the development of new science to address uncertainties related to the environmental health of children and vulnerable populations, including through intramural and extramural research. EPA

- Assess risks to childhood lifestages and vulnerable populations
- Support research that addresses uncertainties

ECHA EUROPEAN CHEMICALS AGENCY Annex XV Restriction Report

Goldenman et al. (2019) indicate that the contamination may be poorly reversible or even irreversible, and may reach levels that could render natural resources such as soil and water unusable far into the future, resulting in continuous exposure and unavoidable harmful health effects, particularly for vulnerable populations, such as children.

- Continuous PFAS exposure leading to unavoidable harmful health effects to the population including vulnerable individuals

Why are we interested in population-based *in vitro* methods?

Traditional Toxicity Testing Methods: *In Vivo*

- Time and labor-intensive, expensive, and low throughput
- Challenges with extrapolation to humans
- Models often overlook inter-individual variability
- Ethical concerns

New Approach Methods for Toxicity Testing: *In Vitro*

- Faster, cheaper, and higher-throughput
- Ability to look at biologically-relevant phenotypes
- Can evaluate inter-individual and intra-species variability
- Reduces use of animal testing

Population-Based Human *In Vitro* Models

- Human lymphoblast cell lines (1,000+ donors)
- Human induced pluripotent stem cell-derived cardiomyocytes (~43 donors)
- Assess inter-individual and chemical-specific variability
- Translation to humans

Are PFAS potentially hazardous to cardiomyocytes?

Experimental Design and Quality Control Assessment

iPSC-derived cardiomyocytes from multiple donors (N=16)

⬇

Chemical Exposure to 56 Structurally-Diverse PFAS

⬇

Functional and Cytotoxicity Assays

⬇

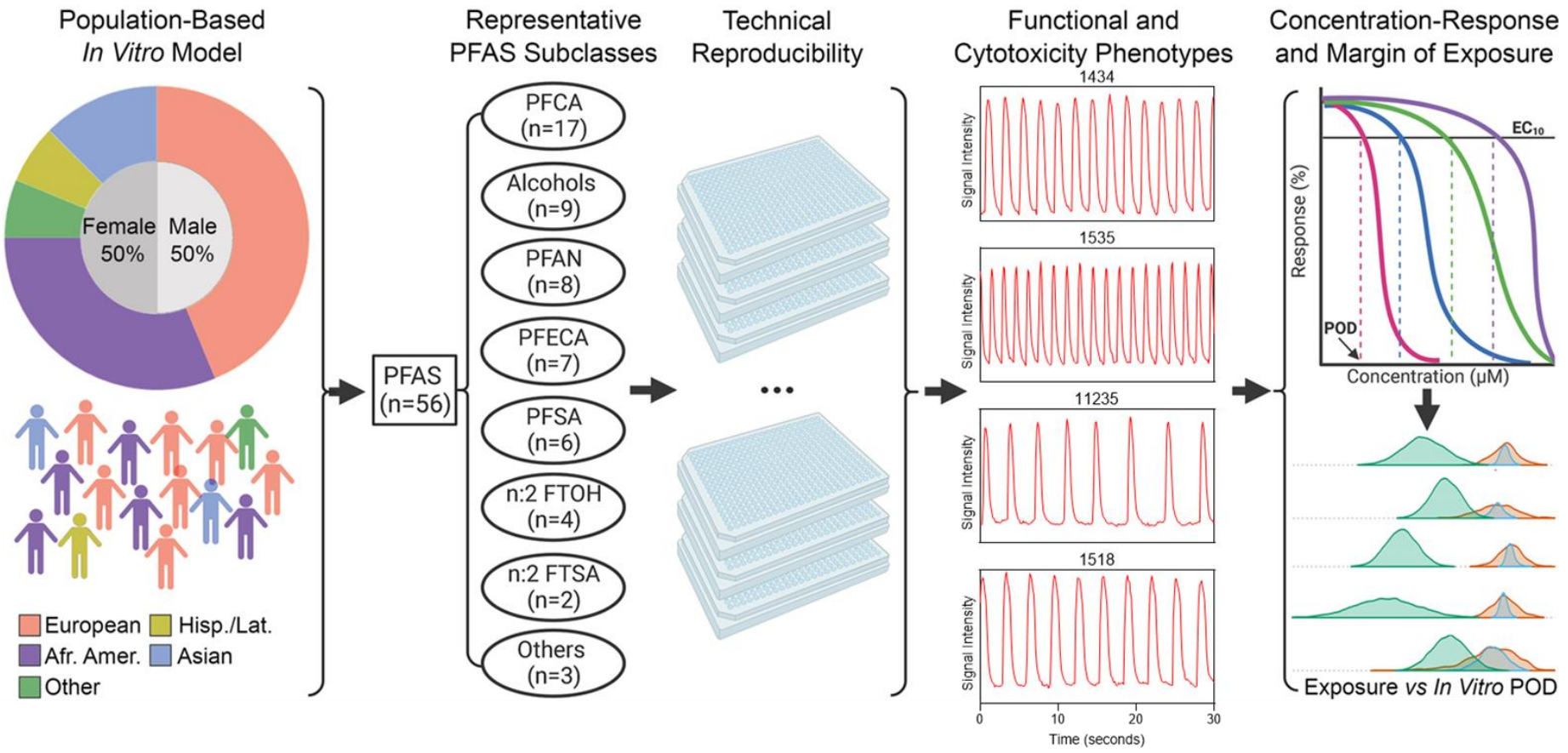
Cell-Line Specific Concentration Response Modeling

⬇

Cumulative Distribution of $TDVF_{05}$

⬇

Chemical-Specific MOEs



Are PFAS potentially hazardous to cardiomyocytes?

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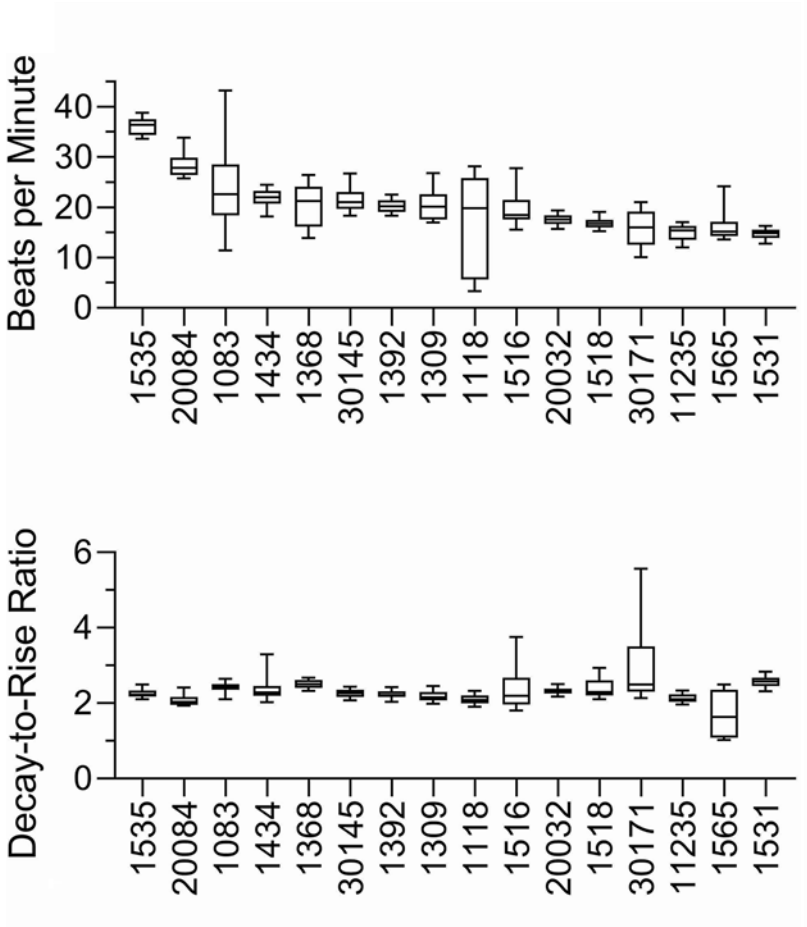
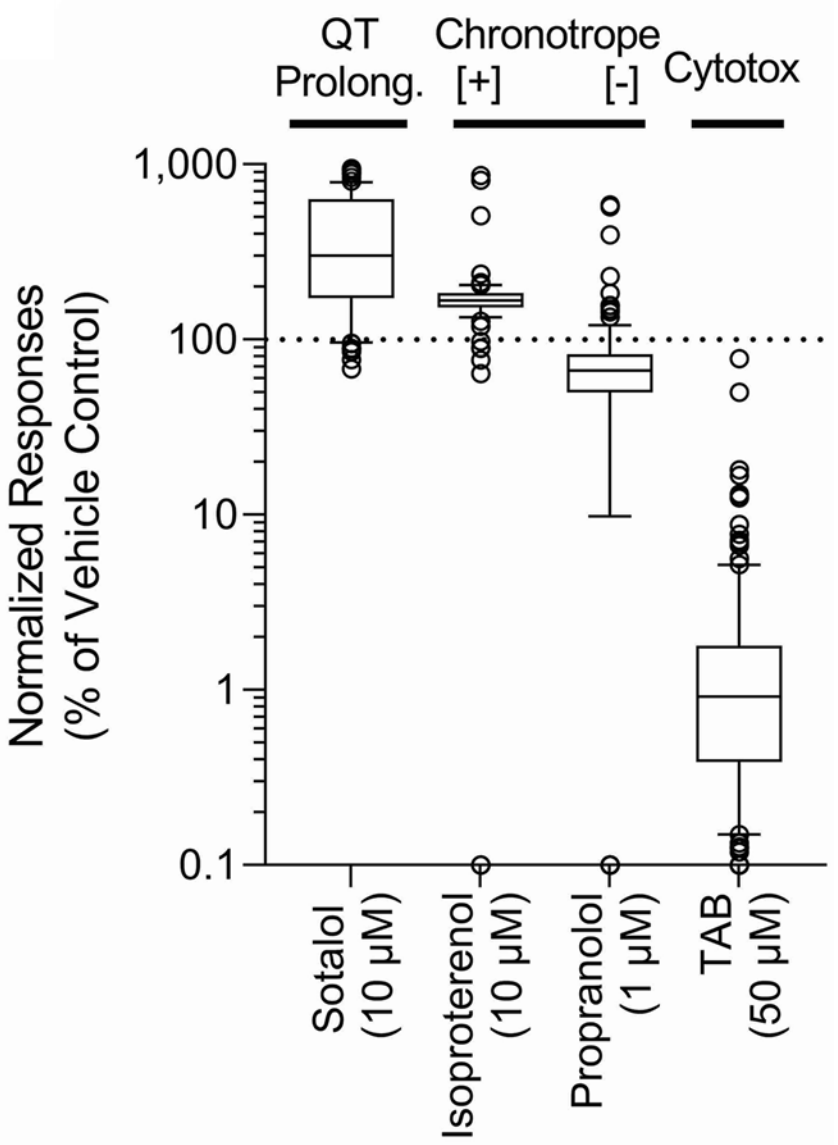
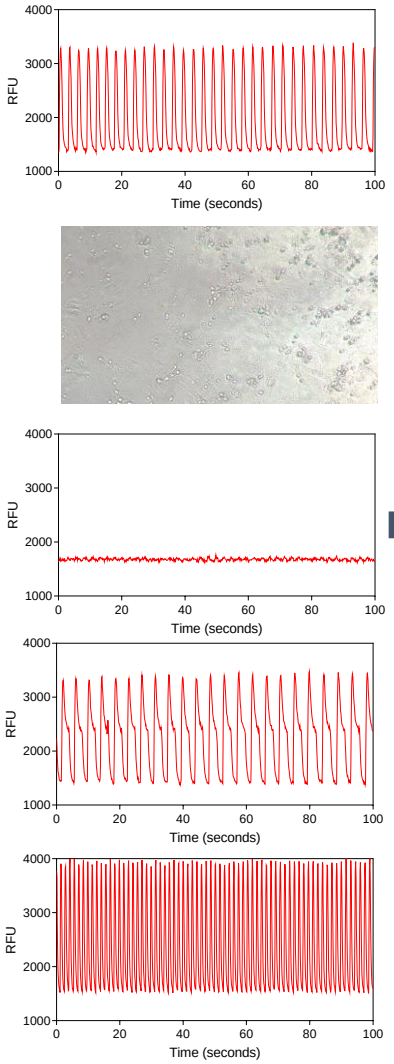
Chemical-Specific MOEs

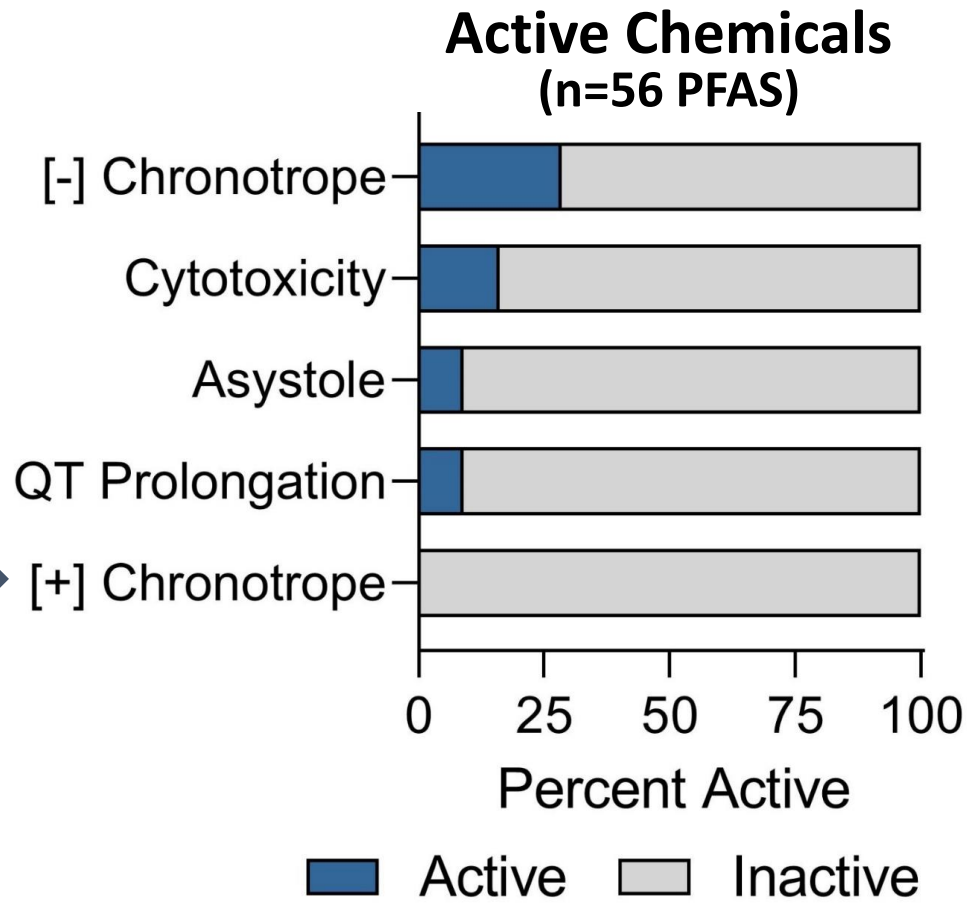




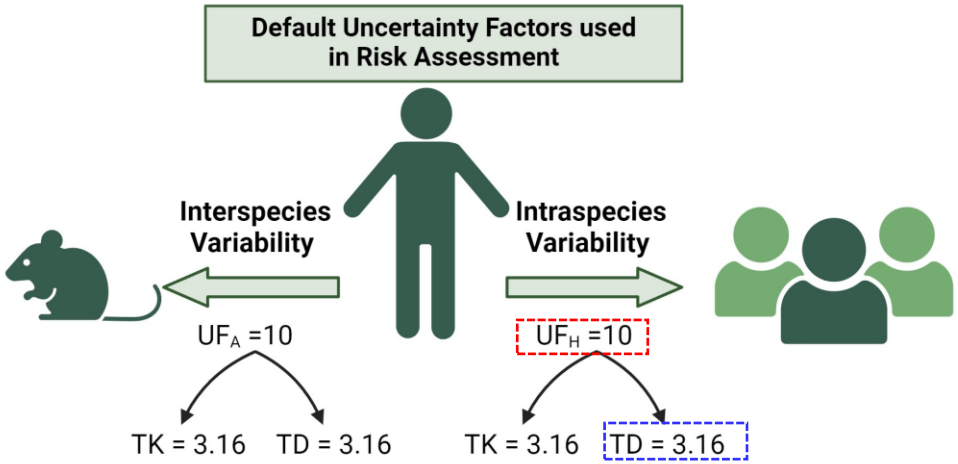
Are PFAS potentially hazardous to cardiomyocytes?

In Vivo Phenotype	*In Vitro* Phenotype
Negative [-] Chronotrope	5% decrease in peak frequency
Cytotoxicity	10% decrease in total cells
Asystole	95% decrease in peak frequency
QT Prolongation	5% increase in decay/rise ratio
Positive [+] Chronotrope	5% increase in peak frequency



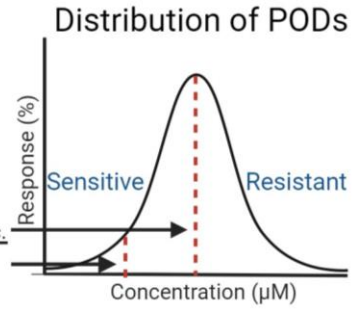


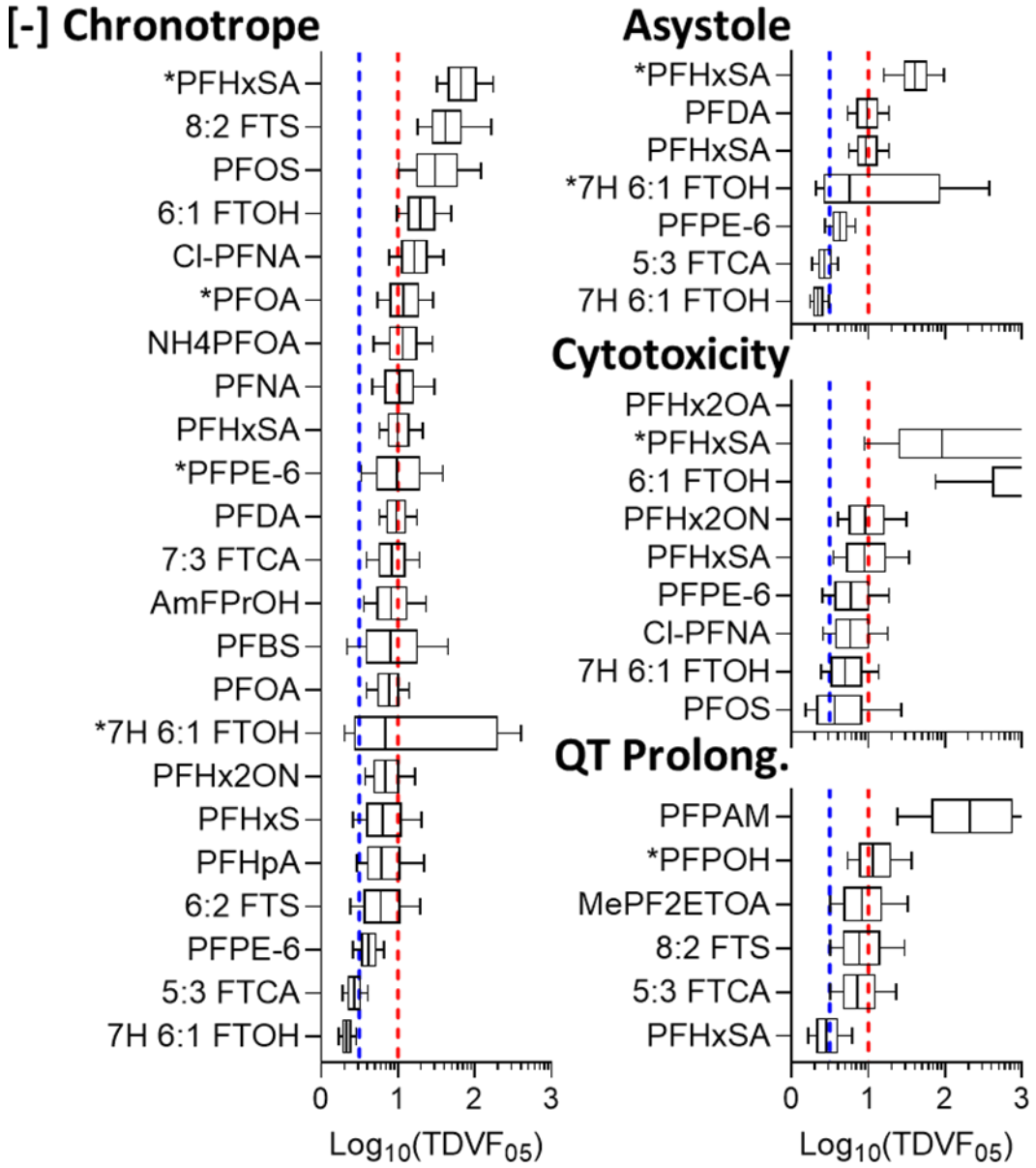
Are there particular subpopulations at risk for PFAS exposure?



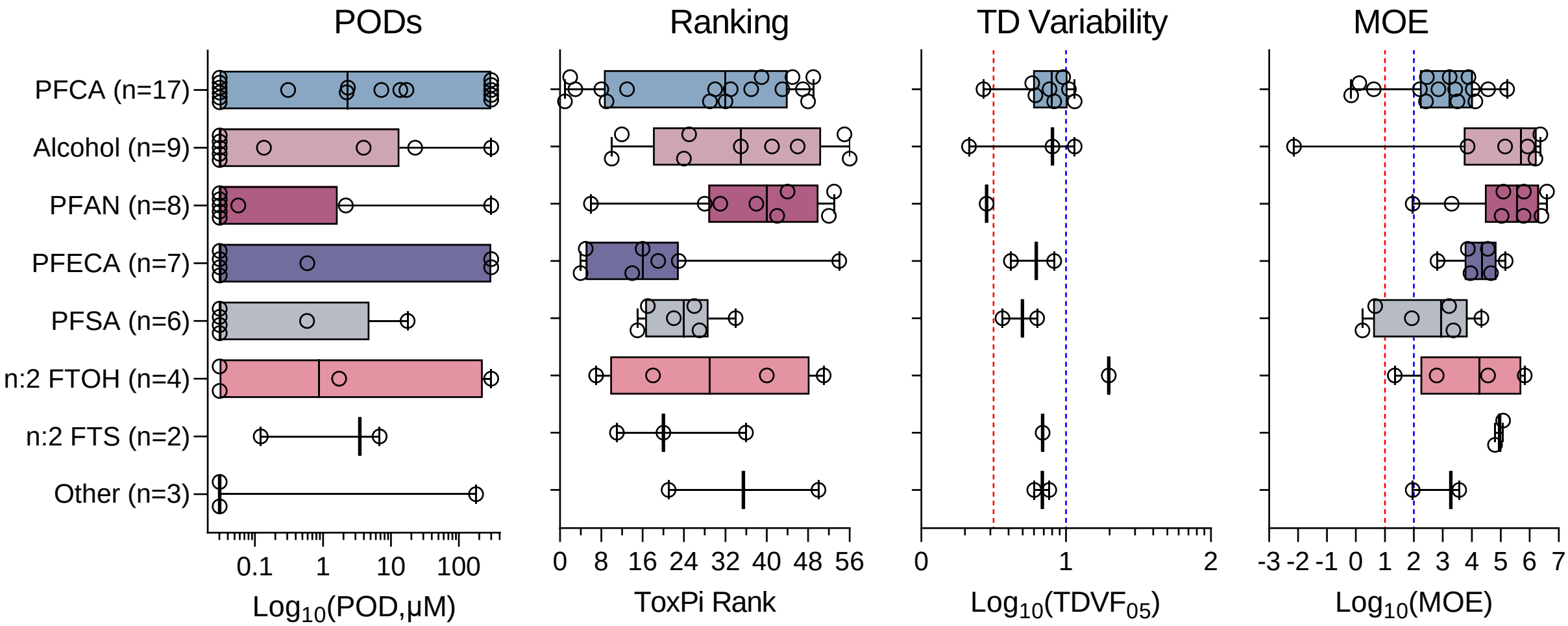
$$TDVF = \frac{POD_{med}}{POD_{sens.}}$$

$$TDVF_{05} = \frac{POD_{50th\ perc.}}{POD_{5th\ perc.}}$$





Are subclasses the best way to group PFAS?



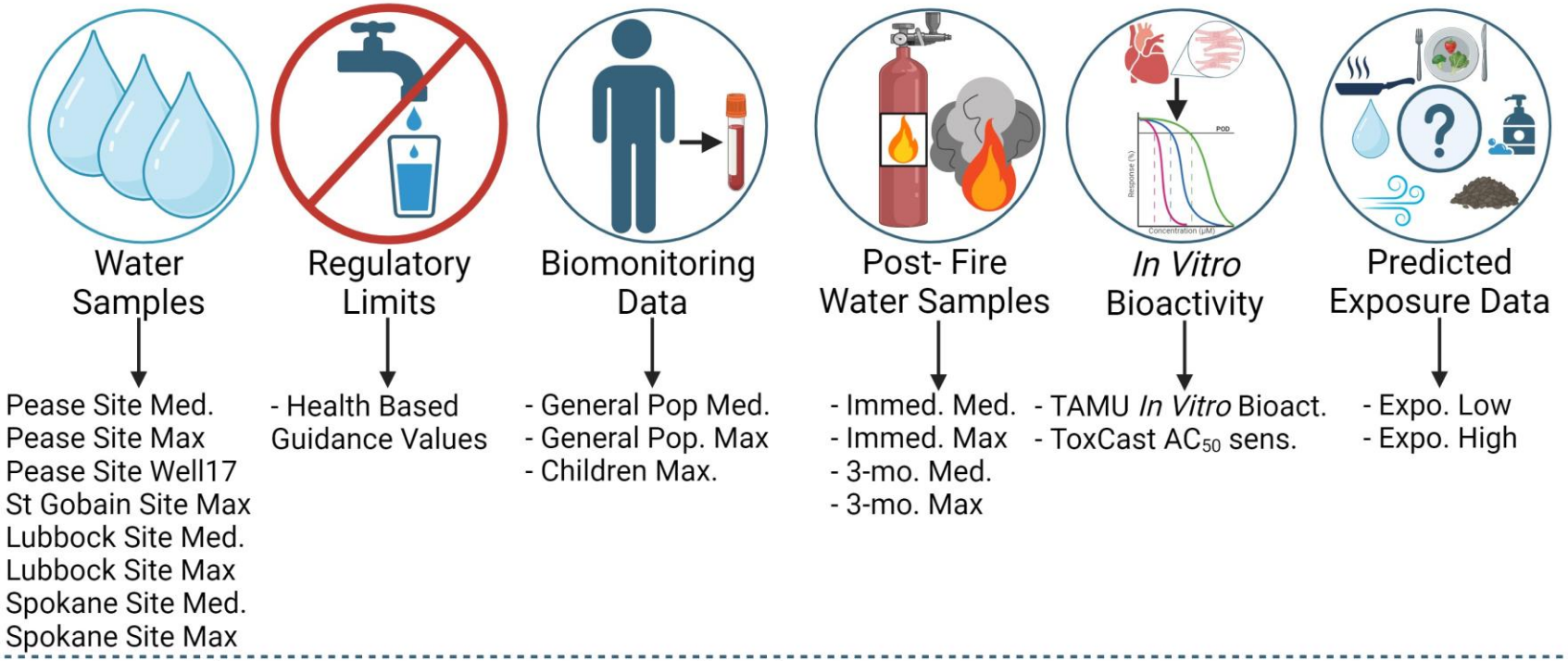
Summary and Significance

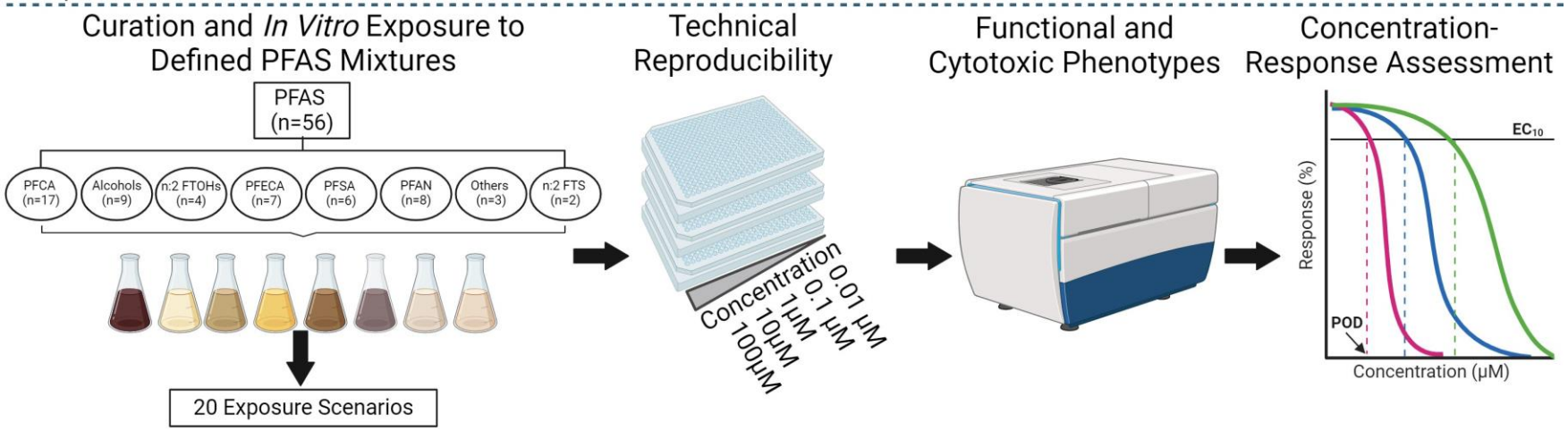
- Upon testing across various populations, no particular subpopulation was more/less sensitive
- Chemicals-specific TDVFs were typically HIGHER than the default uncertainty factor of $10^{1/2}$
- Cannot group by structure, but can prioritize chemicals
- We have hazard data, but need more measured exposure data to replace the predicted values

Summary & Takeaways

Regulatory Science Questions	Conclusions
1. Are PFAS subclasses the best way to group PFAS?	Cannot group by structure (shown by phenotypic and transcriptomic data)
2. Can we rank PFAS to identify trends based on the overall bioactivity?	Can rank individual chemicals, but not subclasses
3. Does *in vitro* bioactivity data exceed PFAS exposure levels?	We have hazard, but more exposure data is needed (not predicted)
4. How do our PODs benchmark to those from ToxCast?	Optimized *in vitro* battery comparable to ToxCast PODs
5. Compared to other industrial chemicals, how bad are PFAS?	PFAS not amongst top ranked chemicals, in comparison to other industrial chemicals
6. Are there particular subpopulations at risk for PFAS exposure?	Inter-individual variability was observed and can be quantified using *in vitro* methods

Where do we go from here?... Mixtures





How are mixtures currently assessed?

Current challenges in mixtures risk assessment:

1) Health effects
2) Unknown composition of the mixtures
3) Exposure assessment

- Existing risk assessment methods rely on data from individual chemicals
- No standardized approach to assess risk of mixtures

Two proposed methods:

1) Whole-mixture approach
2) Component-based approach

GUIDANCE

EFSA Journal

ADOPTED: 17 November 2021

doi: 10.2903/j.efsa.2021.7033

Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
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Abstract

This guidance document provides harmonised and flexible methodologies to apply scientific criteria and prioritisation methods for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. In the context of EFSA's risk assessments, the problem formulation step defines the chemicals to be assessed in the terms of reference usually through regulatory criteria often set by risk managers based on legislative requirements. Scientific criteria such as hazard-driven criteria can be used to group these chemicals into assessment groups. In this guidance document, a framework is proposed to apply hazard-driven criteria for grouping of chemicals into assessment groups using mechanistic information on toxicity as the gold standard where available (i.e. common mode of action or adverse outcome pathway) through a structured weight of evidence approach. However, when such mechanistic data are not available, grouping may be performed using a common adverse outcome. Toxicokinetic data can also be useful for grouping, particularly when metabolism information is available for a class of compounds and common toxicologically relevant metabolites are shared. In addition, prioritisation methods provide means to identify low-priority chemicals and reduce the number of chemicals in an assessment group. Prioritisation methods include combined risk-based approaches, risk-based approaches for single chemicals and exposure-driven approaches. Case studies have been provided to illustrate the practical application of hazard-driven criteria and the use of prioritisation methods for grouping of chemicals in assessment groups. Recommendations for future work are discussed.

Keywords: harmonised methodologies, human risk assessment, combined exposure to multiple chemicals, scientific criteria, grouping, assessment groups, dose addition

Requestor: EFSA

Question number: EFSA-Q-2019-00517

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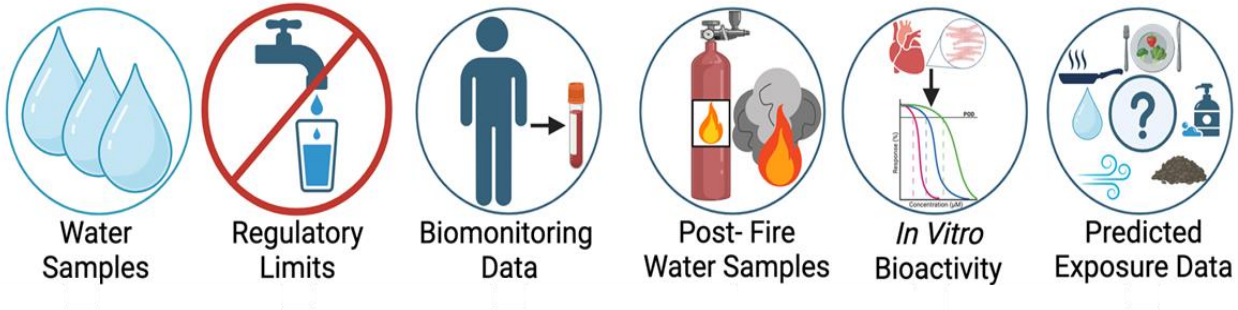
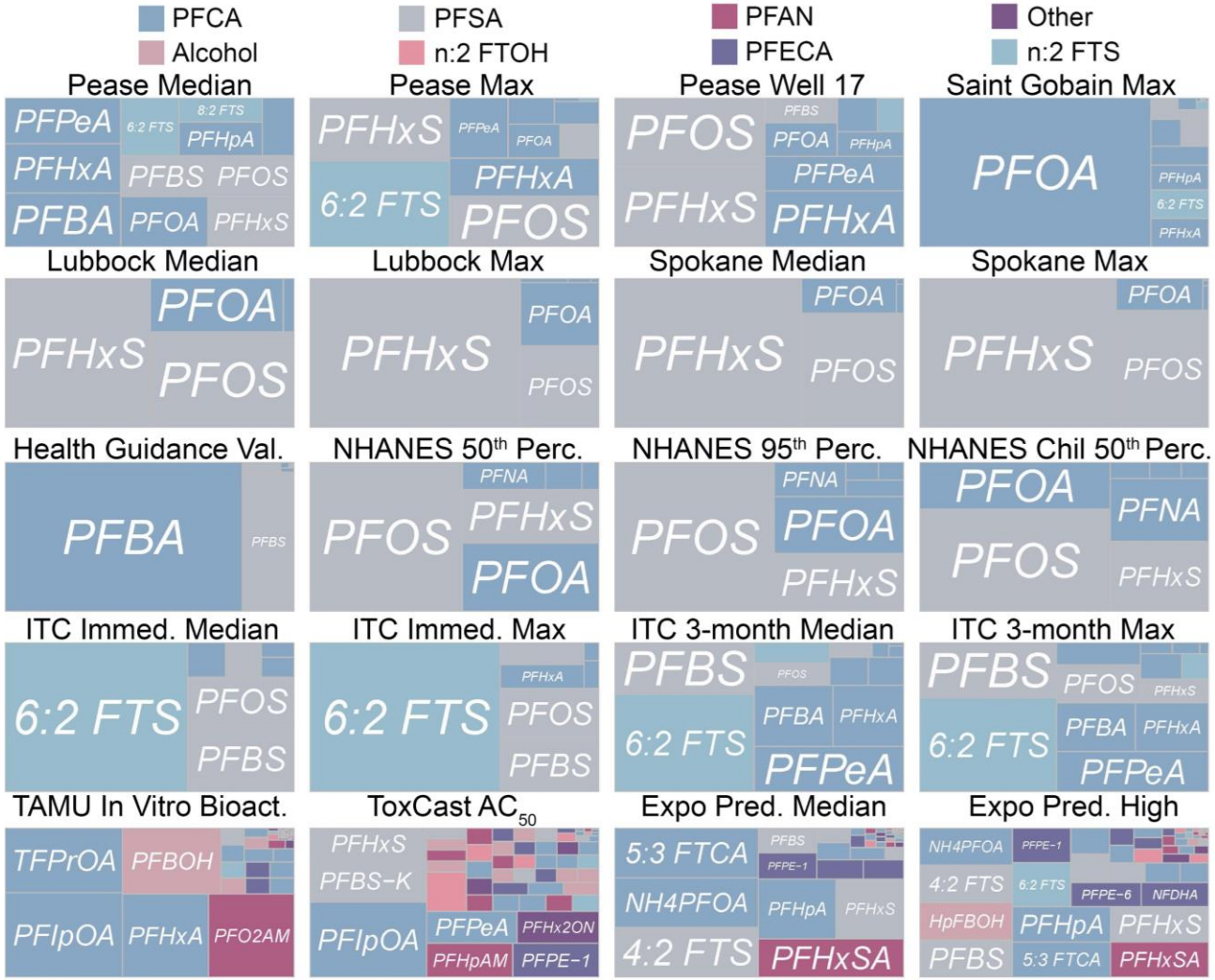
Where do we go from here?... Mixtures

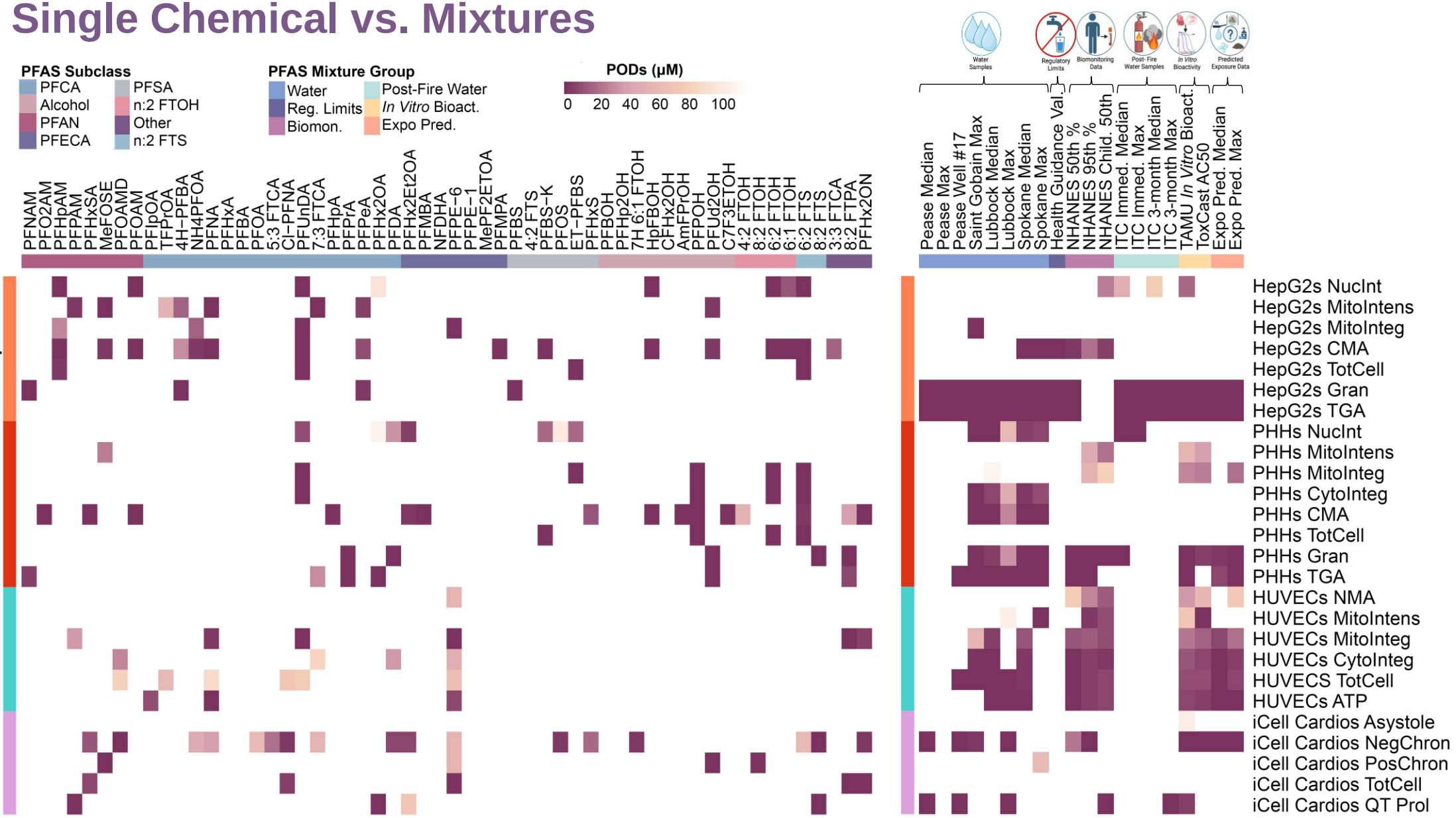


- Screening realistic exposure scenarios using available data
- Each box represents a mixture
- Colors = PFAS subclasses
- Sizes of boxes represent the portion of that chemical within the mixture



Bioactivity Comparison

Single Chemical vs. Mixtures



Thank you!

Questions?