Advancing the Science of PFAS Mixtures Assessment: Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

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Overview

- Background
- Purpose and general overview of the *Draft Framework*
- Dose additivity for PFAS
- Component-based approaches to assess mixtures:
  - Hazard Index (HI)
  - Relative Potency Factors (RPF)
  - Mixture Benchmark Dose (M-BMD)
- Summary
- Questions

Background

- Universe of environmentally relevant PFAS is greater than 12,000 structures
- PFAS have been found around the world in abiotic media, aquatic and terrestrial organisms, and humans
- Targeted and non-targeted analysis of environmental media, such as water, has revealed the co-occurrence of multiple PFAS
    - Third Unregulated Contaminant Monitoring Rule (UCMR 3): Two or more PFAS co-occurred in 48% of sampling events with PFAS detects; PFOA and PFOS co-occurred in 27% of sampling events
- Human biomonitoring data indicates multiple PFAS in blood (e.g., PFOA, PFOS, PFHxS, PFNA)

Background

- Human health risks associated with exposure to mixtures of PFAS has not been well characterized – few whole mixture studies; a formal PFAS mixtures assessment has not been conducted by federal government entities
- Toxicity information amenable to component-based mixtures assessment is available for several PFAS:
  - Final assessments – **EPA**: PFPrA, PFBA, PFBS, GenX chemicals, PFHxA; **ATSDR**: PFHxS, PFNA
  - In process assessments – **EPA**: PFOA, PFOS, PFHxS, PFNA, PFDA

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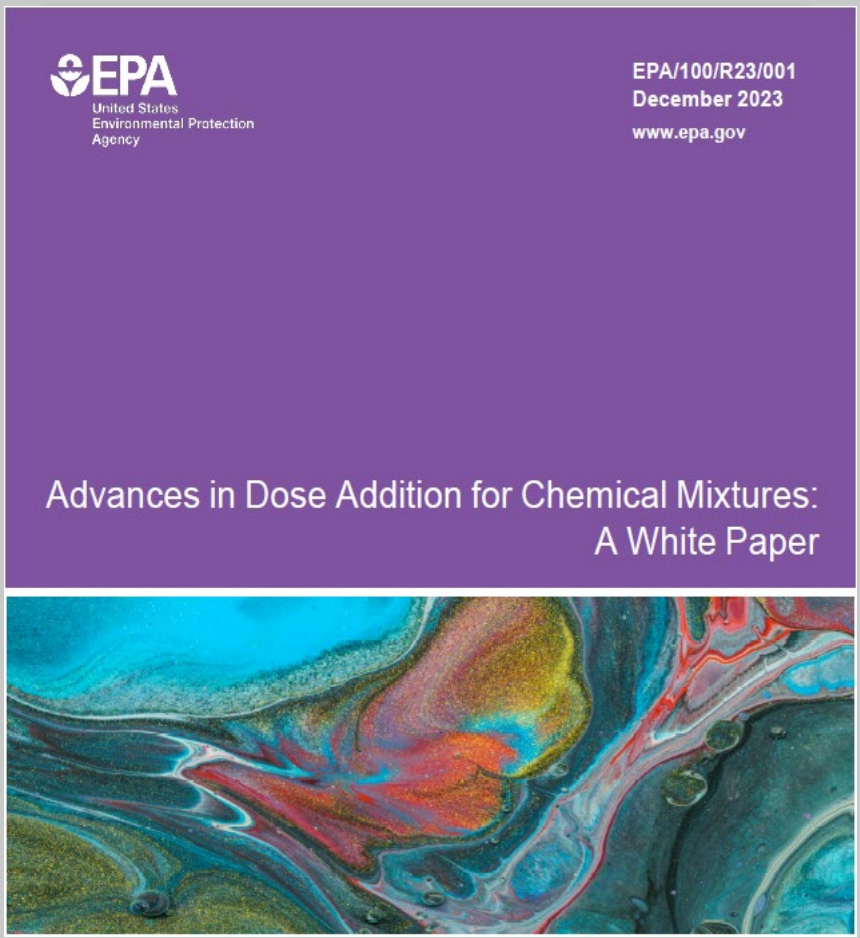
Key Aspects of the Framework

- Purpose: Provide a data-driven framework for estimating human health risks associated with oral exposures to mixtures of PFAS, consistent with existing U.S. EPA guidance
- Based on common profile of health outcomes/endpoints among PFAS
- Assumes dose additivity for chemicals with common health outcomes
- Relies on EPA component-based mixture assessment methods:
  - **Hazard Index,**
  - **Relative Potency Factors,** and
  - **Mixture Benchmark Dose**



Dose Addition: Prediction of Mixture Effects

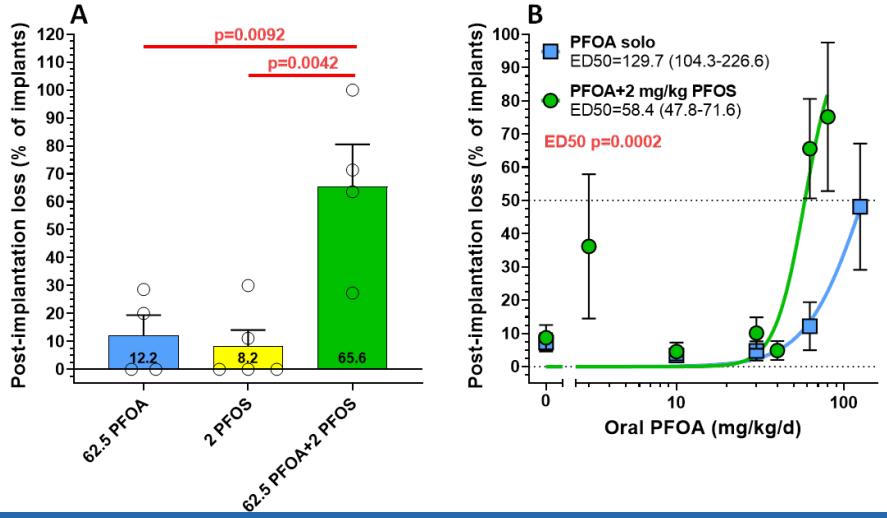
- The HI, RPF, and Mixture BMD approaches are all based on an assumption of dose additivity
- 'Dose addition (DA) applies when mixture components act on similar biological systems and elicit a common response' (section 4.1.1, EPA, 2000)
- In contrast, 'response addition (RA) applies when mixture components act on different systems or produce effects that do not influence each other' (section 4.1.1, EPA, 2000)
- In practice, for many toxicological effects DA and RA produce statistically indistinguishable predictions of mixture effects
- However, for some toxicologically-relevant endpoints which display steep dose-response curve slopes, RA models produce estimates of lower mixture potency and thus typically under-estimate joint toxicity of mixture components as compared to DA
- Further, for combinations of individual chemical doses that do not produce a measurable response, RA models underpredict the joint toxicity of mixture components
- The National Research Council (2008) recommended that EPA focus mixture assessment on commonality in health outcomes, as opposed to specific mechanism or mode of action, and supported DA as the most appropriate model for estimating mixture effects

Dose Additivity: PFAS Supporting Evidence

- Mode of action (MOA) information for PFAS is complex and incompletely described
- PFAS tested to-date appear to interact with a diverse population of cellular or nuclear receptors (e.g., PPARs, ER, CAR, PXR, LXR, etc.), and receptor-independent binding partners/sites (e.g., thyroid hormone carrier proteins; organic anion transporters; etc.)
- PFOS, PFOA, and other PFAS disrupt signaling of multiple biological pathways resulting in common adverse effects on several biological systems including disruption of thyroid hormone economy, lipid synthesis and metabolism, developmental toxicity, and immune and liver toxicity
- Limited studies of PFAS mixture effects support the assumption of DA, for example:
    - An in vitro mixture study of PPARα activation demonstrated cumulative effects of combined exposure to binary combinations of PFOA and PFOS, PFNA, PFHxA, and PFHxS that conformed to DA models (Wolf et al., 2014)
    - Two more recent mammalian studies indicate that exposure to combinations of PFOA, PFOS, and PFHxS (Marques et al., 2021) and combined PFOA, PFOS, PFNA, PFHxS, and GenX chemicals (Roth et al., 2021) in mice produced hepatotoxicity and alterations in lipid homeostasis compared to controls which were consistent with the profile of individual PFAS effects (magnitude of effect was different)

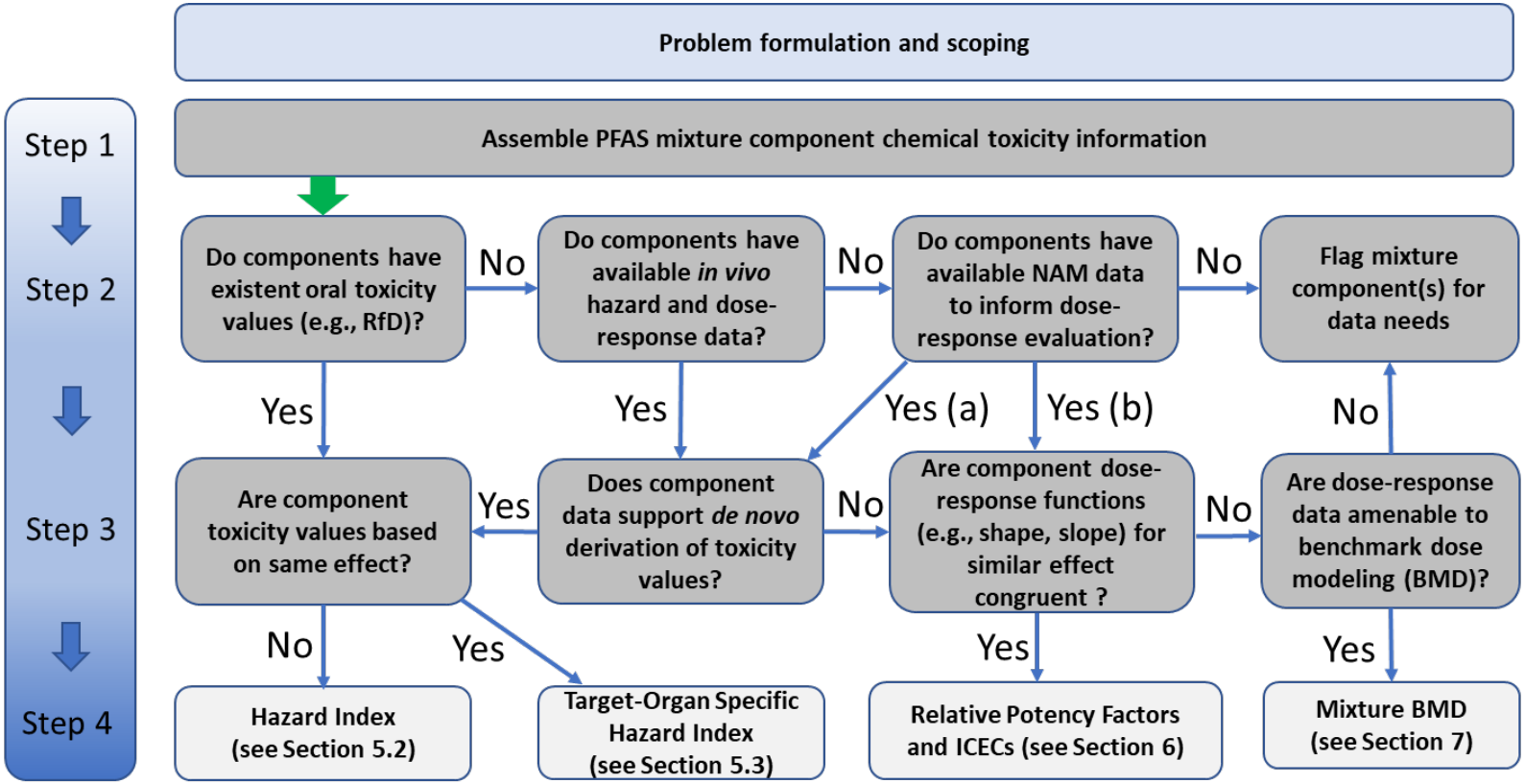
Dose Additivity: PFAS Supporting Evidence

- In vivo mixture toxicity studies from U.S. EPA (for example - Conley et al. 2023):
  - PFOS, HFPO dimer acid (also known as GenX chemicals), and Nafion byproduct 2 Mixture: Neonatal mortality, maternal gestational weight gain, pup body weight, and maternal thyroid hormone levels were accurately predicted by DA modeling.
  - PFOA and PFOS Mixture: Neonatal mortality, maternal gestational weight gain, maternal liver weight were accurately predicted by DA modeling.



Dose Additivity: A Reasonable Assumption for PFAS

- Prior research conducted on mixtures of chemicals across different classes with disparate molecular mechanisms or MOA/key events, but common adverse health outcomes, support dose additivity as predictive of mixture effect(s)
    - Previous mixtures studies across several classes of chemicals (e.g., dioxins, pesticides, phthalates) that disrupt common pathways typically produce dose additive alterations
- More recent PFAS mixture studies (e.g., Conley et al. 2023) indicate maternal and developmental tox associated with two or more components (with structural diversity) are consistent with dose additivity
- Thus, it is considered a reasonable health-protective assumption that PFAS which share common adverse outcomes will produce dose-additive effects from co-exposure
- EPA Science Advisory Board supported the assumption of dose additivity for PFAS mixtures in its 2021 review of the draft Framework


Problem formulation and scoping
Step 1
Assemble PFAS mixture component chemical toxicity information
Step 2
Do components have existent oral toxicity values (e.g., RfD)?
No
Do components have available *in vivo* hazard and dose-response data?
No
Do components have available NAM data to inform dose-response evaluation?
No
Flag mixture component(s) for data needs
Yes
Yes
Yes (a)
Yes (b)
No
Step 3
Are component toxicity values based on same effect?
Yes
Does component data support *de novo* derivation of toxicity values?
No
Are component dose-response functions (e.g., shape, slope) for similar effect congruent ?
No
Are dose-response data amenable to benchmark dose modeling (BMD)?
No
Yes
Yes
Yes
Step 4
Hazard Index (see Section 5.2)
Target-Organ Specific Hazard Index (see Section 5.3)
Relative Potency Factors and ICECs (see Section 6)
Mixture BMD (see Section 7)

Hazard Index (HI)

- The general HI is a health-protective approach that provides a risk "indicator" rather than a risk estimate for a mixture of component chemicals

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i}$$

- Where:

HI =	Hazard Index
HQi =	Hazard Quotient for chemical i
Ei =	Exposure, i.e., dose (mg/kg/d) or occurrence concentration, such as in drinking water (mg/L), for chemical i
RfVi =	Reference value (e.g., oral RfD or MRL [mg/kg/d]), or corresponding health-based, media-specific value (e.g., HBWC, such as a drinking water Health Advisory or MCLG in mg/L) for chemical i

Hazard Index (HI)

1. **Identify or Derive Chronic Oral RfDs of Mixture Components.**
    a) Federal human health assessment available;
    b) No federal human health assessment, but state or other assessment may be leveraged;
    c) No human health assessment available, but traditional hazard and dose-response (i.e., human epidemiological and/or experimental animal study) data are judged to support RfD derivation; or
    d) No assessment and no traditional hazard and dose-response data available; NAM data streams could be surveyed and leveraged for possible development of a NAM-based reference value
2. **Identify or Calculate HBWCs.**
3. **Select Exposure Estimates.**
4. **Calculate component chemical HQs and corresponding mixture HI.**

Hazard Index (HI) Example

Relatively Lower Exposure

Chemical	Hypothetical Exposure Estimate (ng/L)	Health Based Water Conc. (ng/L)	Example HQ
PFAS 1	20	50	0.4
PFAS 2	20	500	0.04
GENERAL HAZARD INDEX			**0.44**

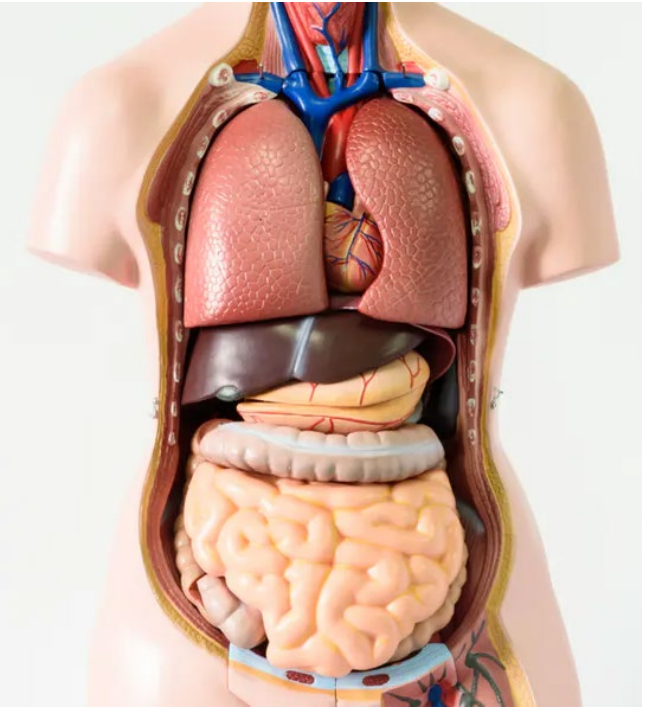
Relatively Higher Exposure

Chemical	Hypothetical Exposure Estimate (ng/L)	Health Based Water Conc. (ng/L)	Example HQ
PFAS 1	400	50	8
PFAS 2	400	500	0.8
GENERAL HAZARD INDEX			**8.8**

(HI >1 indicates potential health risk)

Target Organ Specific Hazard Index (TOSHI)

- Toxicity values (e.g., RfDs) are aggregated by the "same" target organ endpoint/effect, and HQ (and HI) values are developed for each effect domain independently (e.g., liver-specific HI, thyroid-specific HI)
- The disadvantage of a TOSHI is that it can only be performed for those PFAS for which a health effect specific RfD (e.g., target-organ toxicity dose or TTD) is available
    - For example, for some PFAS a given health effect might be poorly characterized or not studied at all, or, as a function of dose may be one of the less(er) potent effects in the profile of toxicity for that particular PFAS



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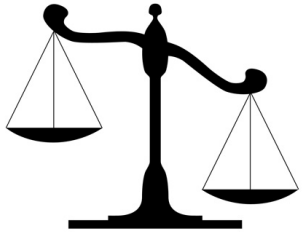
General HI and TOSHI



Advantages

- Provides an 'indication' of human health risk associated with a PFAS mixture; easy to interpret and communicate results to stakeholders
- General HI is health-protective indicator of mixture risk, as each component chemical HQ is based on its health-protective RfV

Challenges

- Risk 'indicator', not an estimate of the concentration of the mixture in water that may result in adverse health outcomes after a specific duration of exposure
- Requires derivation of a health-based, media-specific concentration like a drinking water Health Advisory or MCLG, in addition to the underlying oral RfV (e.g., RfD)

Relative Potency Factors (RPF)

- For PFAS shown to induce the same health effect, a RPF represents the relative difference in potency between a mixture **index chemical** (IC) and other members of the mixture
    - The IC is the most well characterized toxicologically; may not necessarily be the most toxic member of a mixture
- The assumption under dose additivity is that the toxicity of each mixture component induces health effects via a common pathway/MOA and can operationally be considered a fixed concentration or dilution of the IC (EPA, 2000)
    - EPA, 2000 states: "The common mode-of-action assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)."
    - *This suggests that although the common MOA assumption for application of RPFs is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components*

Relative Potency Factors (RPF)

- RPFs are calculated using a common dose-response metric (e.g., human equivalent NOAEL, BMD_X, ED_X) such as points-of-departure (POD) for the IC and all other members (*i*) of the mixture:

$$RPF = \frac{POD_{IC}}{POD_i}$$

- IC equivalent concentrations (ICEC) are then calculated by multiplying each respective RPF_j by the corresponding mixture component chemical's concentration (d_j). The total mixture ICEC ($ICEC_{MIX}$) is then obtained by taking the sum of the component chemical ICECs (including that of the IC):

$$ICEC_{MIX} = \sum_{j=1}^{n} d_j * RPF_j$$

- Historically, a numerical estimate of risk for non-cancer health effects associated with exposure to the mixture of concern is then obtained by mapping the $ICEC_{MIX}$ onto the dose-response function for the IC [i.e., $y_{MIX} = f(ICEC_{MIX})$]
- In the context of water-specific application(s), the $ICEC_{MIX}$ could be compared directly to the HBWC for the IC to determine potential concern for the mixture

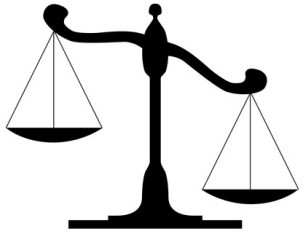
Relative Potency Factors (RPF)

Example: **Developmental** Effect RPFs and ICECs for PFAS Mixtures (Lower/Higher Exposures)

Mixture Component	POD_{HED} (mg/kg-day); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)	PFAS 2 ICEC (ng/L)
PFAS 1	0.001 ($NOAEL_{HED}$)	0.5	20	10
PFAS 2 (IC)	0.00051 ($NOAEL_{HED}$)	1	20	20
PFAS 3	0.21 ($NOAEL_{HED}$)	0.002	15	0.04
PFAS 4	0.07 ($NOAEL_{HED}$)	0.007	25	0.2
Mixture Total PFAS 2 ICEC (ppt)				**30**

Mixture Component	POD_{HED} (mg/kg-day); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)	PFAS 2 ICEC (ng/L)
PFAS 1	0.001 ($NOAEL_{HED}$)	0.5	400	200
PFAS 2 (IC)	0.00051 ($NOAEL_{HED}$)	1	400	400
PFAS 3	0.21 ($NOAEL_{HED}$)	0.002	300	0.7
PFAS 4	0.07 ($NOAEL_{HED}$)	0.007	500	2.1
Mixture Total PFAS 2 ICEC (ppt)				**603**

Relative Potency Factors (RPF)



Advantages

- No RfD needed, only effects/endpoints and associated dose-response metrics (e.g., NOAEL, BMD_X, ED_X)
- RPF method facilitates calculation of an actual mixture toxicity dose or concentration estimate

Challenges

- "Apples to apples" comparison (e.g., study design/duration, test species, effect, etc.) is optimal, but not always possible
- RPFs were generally intended for use when mixture components are demonstrated to have similar/same MOA; this information is generally unavailable for PFAS

Dose Addition Mixture BMD Approach

- Employs a dose additive model-based calculation of a mixture BMD based on a defined benchmark response (e.g., BMR_{10}) for a PFAS mixture with a specific mixing-ratio of component chemicals (described in EPA 2000 and NAS 2008)
- Based on BMDs for each of the PFAS in the mixture for the common health endpoint(s) being modeled (e.g., $BMDL_{10}$ for liver necrosis across all components
- End result is a mixture POD that is specific to the assortment and ratios of component PFAS in a specific mixture

$$t_{add} = \left(\sum_{i=1}^{n} \frac{a_i}{BMD_i}\right)^{-1}$$

where t_{add} is the total mixture dose in mg/kg/d, a_i represents the fixed proportions of the component PFAS in the mixture, and BMD_i is the i^{th} chemical BMD (e.g., $BMDL_{10}$ modeled at a BMR_{10}).

Dose Addition Mixture BMD Approach

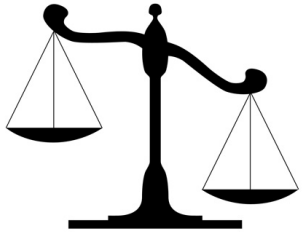
Mixture BMD Approach: Hypothetical Water Sample

	Measured Water Concentration (ng/L)	Mixing Ratio (Proportion)	Thyroid BMD (mg/kg/d)	Liver BMD (mg/kg/d)	Developmental BMD (mg/kg/d)
PFAS 1	10	0.02	0.24	0.044	0.01
PFAS 2	10	0.02	0.24	0.013	0.0051
PFAS 3	50	0.11	2.1	720	2.1
PFAS 4	400	0.85	70	0.1	0.7
Mixture Total	**470**	1.0			
Dose Addition Mixture BMD			4.16	**0.094***	0.132

$$t_{add} = \left(\sum_{i=1}^{4} \frac{a_i}{BMD_i}\right)^{-1} = \left(\frac{0.02}{0.044} + \frac{0.02}{0.013} + \frac{0.11}{720} + \frac{0.85}{0.1}\right)^{-1} = 0.094 \text{ mg/kg/d}$$

*The lowest mixture BMD is converted to a mixture RfD and corresponding HBWC if dealing specifically with water, for comparison to the measured concentration (i.e., 470 ng/L).

Dose Addition Mixture BMD Approach



Advantages

- No *a priori* requirement for having formal human health assessment values, such as oral RfDs or chemical-specific HBWCs, for any of the individual PFAS in the mixture
- Avoids any potential confusion that could arise from putting the mixture POD in the units of a single chemical (i.e., the IC from the RPF approach)

Challenges

- Need effect data for at least one common endpoint from the profile of PFAS effects for all components of the mixture (similar to RPF)
- Mixture BMD and subsequent mixture-HBWC is unique for each specific mixture based on PFAS assortment and ratios; PFAS mixtures may change over time in environmental media

Opportunities for NAMs in PFAS Mixture Assessment

- Several related research efforts are in-progress in the U.S. EPA to inform a large(r) hazard and dose-response landscape for PFAS; such data may be critical to informing data-poor mixture PFAS
- The expressed objective is to identify dose-response metrics that could be used in one or more of the component-based mixture assessment approaches
    - Molecular/cellular POD(s) (e.g., transcriptomic pathway-based; in vitro cell bioactivity)
    - Read-across/surrogate chemical POD(s)
- Considering data-poorness of the PFAS universe, integration of NAMs could be critical
- Requires expertise in the interpretation of NAM platform/data
- As with traditional human health assessment data, variabilities and uncertainties in NAM data would need to be transparently communicated in a corresponding mixture risk assessment

Summary

- The *Draft Framework* presents a data-driven, practical approach to using component-based mixtures evaluation of two or more PFAS, under an assumption of dose additivity
- Provides rationale and analyses demonstrating why dose additivity is a reasonable assumption for PFAS
- Designed to accommodate component PFAS with varying levels of toxicity information
- Includes descriptions and illustrative examples using the Hazard Index (HI), Relative Potency Factor (RPF) and Mixture Benchmark Dose (M-BMD) approaches

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Questions?

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