

# Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

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United States  
Environmental Protection  
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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 (BMD)
- Summary
- SAB Charge
- Questions

# Background

- Universe of environmentally relevant PFAS is greater than 9,000 compounds.
- 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: PFOA, PFOS, PFBS, GenX chemicals; ATSDR: PFHxS, PFNA
  - In process assessments – EPA: PFBA, PFHxA, 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 EPA guidance.
- Based on common 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** approach.

## Guidelines for the Health Risk Assessment of Chemical Mixtures

Published on September 24, 1986, Federal Register 51(185):34014-34025

EPA/630/R-00/002  
August 2000

## Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures

Risk Assessment Forum Technical Panel



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# Dose Addition: Prediction of Mixture Effects

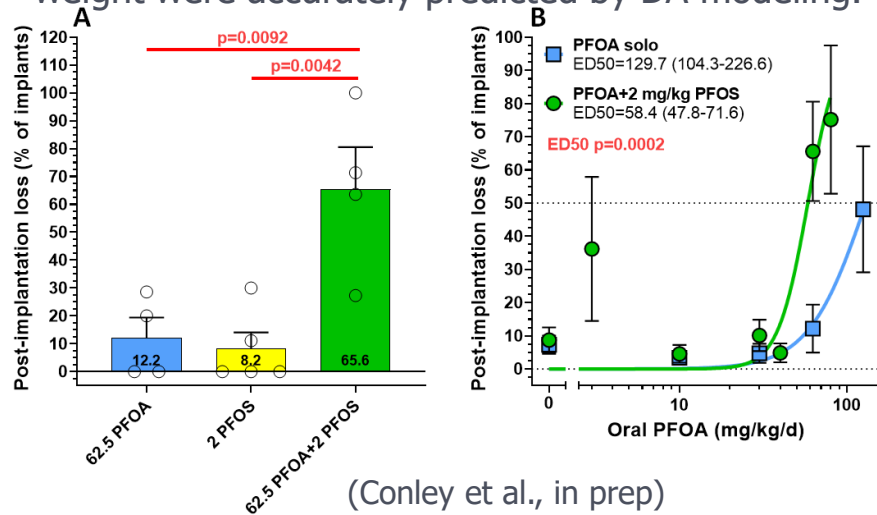
- The HI, RPF, and Mixture BMD approaches are all based on an assumption of dose additivity.
- 'Dose addition (DA) applies when mixture components act on similar biological systems and elicit a common response' (section 4.1.1, EPA, 2000).
- In contrast, 'response addition (RA) applies when mixture components 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 toxic mixture effects as compared to DA.
- Further, for combinations of individual chemical doses that do not produce a measurable response, RA models underpredict the cumulative action of mixture components.
- The National Research Council (2008) recommended that EPA focus cumulative assessment on the health outcomes, as opposed to the specific mechanism or mode of action, and supported DA as the most appropriate model for estimating cumulative 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 the same general population of cellular or nuclear receptors (e.g., PPARs, 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 thyroid hormone levels, lipid synthesis and metabolism, developmental toxicity, and immune and liver function.
- Limited studies of PFAS mixture effects supports the assumption of DA, for example:
  - An in vitro mixture study of PPAR $\alpha$  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 recent mammalian studies indicate that exposure to combined PFOA, PFOS, and PFHxS (Marques et al., 2021) and combined PFOA, PFOS, PFNA, PFHxS, and GenX chemicals (Roth et al., 2021) in mice produced numerous significant effects compared to control which were consistent with the spectrum of individual PFAS effects.

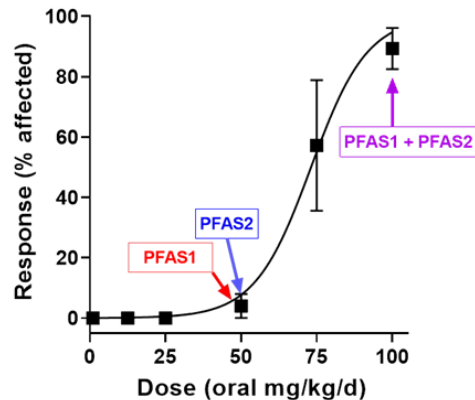
# Dose Additivity: PFAS Supporting Evidence

- In vivo mixture toxicity studies from EPA (Conley et al., in prep):
  - 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.



(Conley et al., in prep)

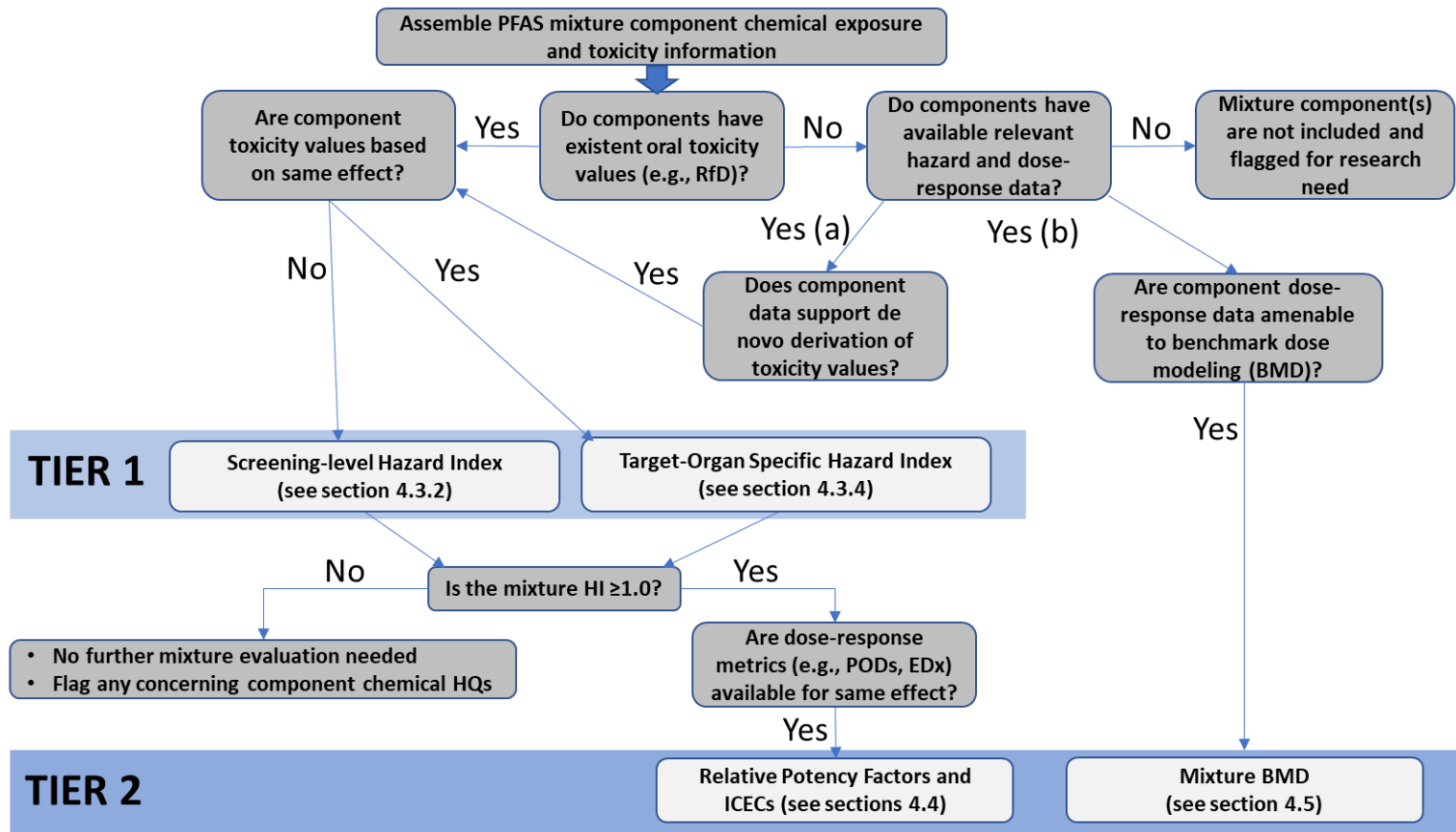
Example: PFAS1 and PFAS2 are equipotent  
At 50 mg/kg each causes a ~5% effect  
Response Addition prediction of mixture ~7%  
Dose Addition prediction of mixture ~95%





# Dose Additivity: A Reasonable Assumption for PFAS

- Precedents of prior research conducted on mixtures of various chemical classes with disparate molecular mechanisms but common key events or adverse outcomes support predictions of dose additive effects.
  - Experimental evidence from mixtures studies across several classes of chemicals (e.g., dioxins, pesticides, phthalates) that disrupt common pathways typically produce dose additive alterations.
- Thus, it is considered a reasonable health-protective assumption that PFAS which share common adverse outcomes will produce dose-additive effects from co-exposure.



# Tier 1: Hazard Index (HI)

- HI is a conservative, screening level 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

HQ<sub>i</sub> = Hazard Quotient for chemical i

E<sub>i</sub> = Exposure, i.e., dose (mg/kg/d) or occurrence concentration, such as in drinking water (mg/L), for chemical i

RfV<sub>i</sub> = 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

# Tier 1: 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 Screening-Level HI.



# Tier 1: Hazard Index (HI) Example [PFOA + PFOS]

## Relatively Lower Exposure

Chemical	Hypothetical Exposure Estimate (ng/L)	2016 EPA Health Advisory (ng/L)	Example HQ
PFOA	20	70	0.29
PFOS	20	70	0.29
SCREENING LEVEL HAZARD INDEX			<b>0.6</b>

## Relatively Higher Exposure

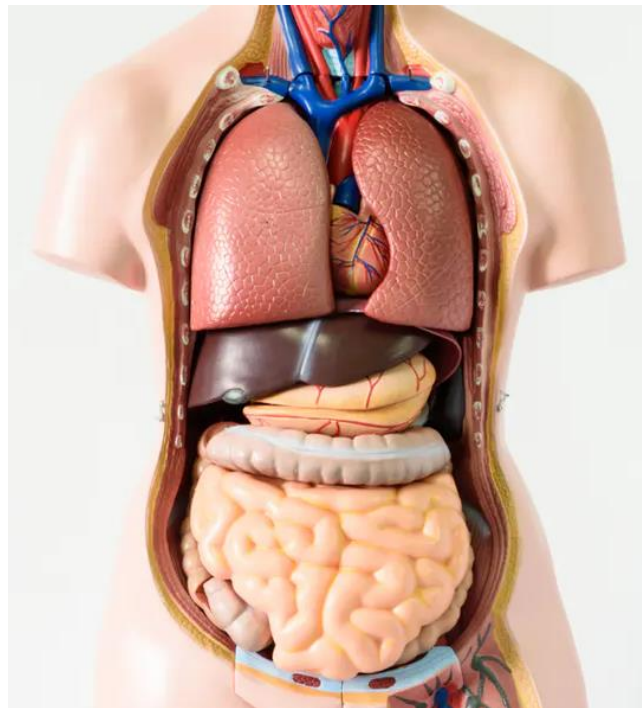
Chemical	Hypothetical Exposure Estimate (ng/L)	2016 EPA Health Advisory (ng/L)	Example HQ
PFOA	400	70	5.7
PFOS	400	70	5.7
SCREENING LEVEL HAZARD INDEX			<b>11</b>

(>1 indicates potential risk and need for further analysis)



# Tier 1: 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 calculated.
  - 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.



Komsan Loonprom/Shutterstock

# Tier 1: Screening-level HI and TOSHI



## Advantages

- Provides an 'indication' of human health risk associated with a PFAS mixture; easy to interpret and communicate results to stakeholders.
- Conservative 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 period 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).

## Tier 2: Relative Potency Factors (RPF)

- For PFAS shown to induce the same/similar 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, ideally with health effects assessment; 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 effects via a similar pathway of biological perturbation 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 metric for application of RPFs is optimal, there is flexibility in the level of biological organization at which “similarity” can be determined among mixture components.
- Illustrative examples in the *Draft Framework* are based on shared, common apical effects to calculate RPFs across three effect domains: developmental, liver, and thyroid.



## Tier 2: 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 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):

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

- 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. For example, if the IC's dose response model is denoted  $f(ICEC_{MIX})$ , then the RPF based response to the mixture is estimated as:

$$y_{MIX} = f(ICEC_{MIX})$$

## Tier 2: Relative Potency Factors (RPF)

- In the context of the *Draft Framework*, there are important modifications or adaptations of this approach to note that include:
  - (1) Use of ICECs, which are water-specific correlates to index chemical equivalent doses (ICEDs) (EPA, 2000), and
  - (2) Using effect-specific HBWCs for the IC (e.g., 70 ppt for PFOS-induced developmental effects [decreased body weight in offspring]) as a benchmark point to compare a mixture ICEC to rather than directly mapping the mixture ICEC onto the IC dose-response.

# Tier 2: Relative Potency Factors (RPF)

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

Mixture Component	POD <sub>HED</sub> (mg/kg-day); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)	PFOA ICEC (ng/L)
PFOA	0.001 (NOAEL <sub>HED</sub> ) <sup>b</sup> (EPA, 2016)	0.5	20	10
PFOS (IC)	0.00051 (NOAEL <sub>HED</sub> ) (EPA, 2016)	1	20	20
PFBS	0.21 (NOAEL <sub>HED</sub> ) (EPA, 2021)	0.002	15	0.04
GenX chemicals	0.07 (NOAEL <sub>HED</sub> ) (EPA, 2021)	0.007	25	0.2
<b>Mixture Total PFOA ICEC (ppt)</b>				<b>30</b>

Mixture Component	POD <sub>HED</sub> (mg/kg-day); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)	PFOA ICEC (ng/L)
PFOA	0.001 (NOAEL <sub>HED</sub> ) (EPA, 2016)	0.5	400	200
PFOS (IC)	0.00051 (NOAEL <sub>HED</sub> ) (EPA, 2016)	1	400	400
PFBS	0.21 (NOAEL <sub>HED</sub> ) (EPA, 2021)	0.002	300	0.7
GenX chemicals	0.07 (NOAEL <sub>HED</sub> ) (EPA, 2021)	0.007	500	2.1
<b>Mixture Total PFOA ICEC (ppt)</b>				<b>603</b>



## Tier 2: 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, as opposed to the HI which is considered an indicator of potential hazard/toxicity.

### 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.

## Tier 2: 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 endpoint(s) being modeled.
- End result is a mixture POD that is specific to the assortment and ratios of 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,  $a_i$  are the fixed proportions of the component PFAS in the mixture, and  $BMD_i$  is  $i^{\text{th}}$  chemical BMD (e.g.,  $BMDL_{10}$  modeled at a  $BMR_{10}$ ).

# Tier 2: 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	<b>470</b>	1.0			
Dose Addition Mixture BMD			4.16	<b>0.094*</b>	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-HBWC for comparison to the measured concentration (i.e., 470 ng/L).

## Tier 2: 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 constellation 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.

# 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.
- Designed to accommodate component PFAS with varying levels of toxicity information.
- Provides rationale and analyses demonstrating why dose additivity is a reasonable assumption for PFAS.
- Includes descriptions and illustrative examples using the Hazard Index (HI) approach (Tier 1) and Relative Potency Factor (RPF) and Mixture Benchmark Dose (BMD) (Tier 2) approaches.



# SAB Charge

1. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, **an assumption of dose addition** for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.
  - A. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.
  - B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

# SAB Charge

2. Section 4.3 (**Hazard Index; HI**) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).
  - A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.
  - B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

# SAB Charge

3. Section 4.4 (**Relative Potency Factor; RPF**) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and GenX chemicals.
  - A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
  - B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

# SAB Charge

4. Section 4.5 (**Mixture BMD**) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.
  - A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.
  - B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

# Questions?

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