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Paper presentation

Article

Neurotoxic Effects of Mixtures of Perfluoroalkyl Substances (PFAS) at Environmental and Human Blood Concentrations

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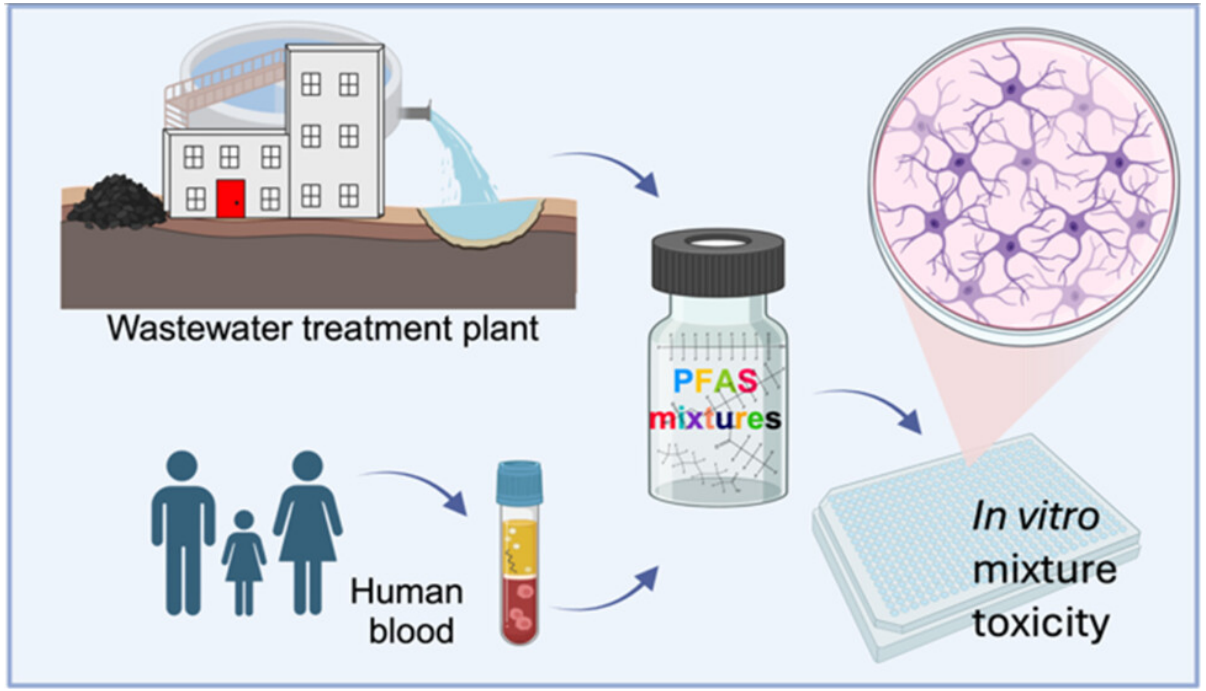

Atrayee Datta
16th November,2024

Terminologies

1. **PFAS** or Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are resistant to heat, water, oil, and grease, and hence have raised concerns regarding their impact on health and the environment.
2. **Cytotoxicity** is a measure of how a substance can cause damage to or kill cells in general.
3. **Neurotoxicity** specifically refers to the toxic effects of substances on nerve cells (neurons) and the nervous system. In this study neurotoxicity was assessed by:

    a. **Oxidative stress response** occurs when there is an imbalance between free radicals and antioxidants in the body, leading to cellular damage. It is a significant factor in various health issues.
    b. **Mitochondrial toxicity** refers to the harmful effects that substances can have on the mitochondria, leading to decreased energy production, impaired cellular metabolism, and increased production of reactive oxygen species (ROS).
    c. **Neurite outgrowth** refers to the process by which developing neurons extend their axons and dendrites, which are essential for forming connections with other neurons.

Background

ELSEVIER

Toxicology

Volume 457, 15 June 2021, 152789

Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure

The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences, 2024, **79**(3), 1–6
https://doi.org/10.1093/gerona/glad208
Advance access publication 31 August 2023
Research Report

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OXFORD

First Observations of a Potential Association Between Accumulation of Per- and Polyfluoroalkyl Substances in the Central Nervous System and Markers of Alzheimer's Disease

Nicolas Delcourt, PharmD, PhD,[1,2,*] Alix-Marie Pouget, PharmD,[1] Alicia Grivaud, PhD,[3] Leonor Nogueira, MD, PhD,[4] Frédéric Larvor, PhD,[3] Philippe Marchand, PhD,[3] Eric Schmidt, MD, PhD,[2] and Bruno Le Bizec, PhD[3]

ELSEVIER

Chemosphere

Volume 129, June 2015, Pages 239-245

Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers *in vitro*

Chemical Research in Toxicology

pubs.acs.org/crt Review

Neurotransmission Targets of Per- and Polyfluoroalkyl Substance Neurotoxicity: Mechanisms and Potential Implications for Adverse Neurological Outcomes

This manuscript is part of a special collection: Chemical Exposures and Impact on Human Health.

Josephine M. Brown-Leung and Jason R. Cannon*

The design of an environmentally relevant mixture of persistent organic pollutants for use in *in vivo* and *in vitro* studies

Hanne Friis Berntsen, Vidar Berg, Cathrine Thomsen, Erik Ropstad & Karin Elisabeth Zimmer

Pages 1002-1016 | Published online: 30 Aug 2017

Cite this article https://doi.org/10.1080/15287394.2017.1354439 Check for updates

Chemical Research in Toxicology

pubs.acs.org/crt Article

Evaluation of Per- and Polyfluoroalkyl Substances (PFAS) *In Vitro* Toxicity Testing for Developmental Neurotoxicity

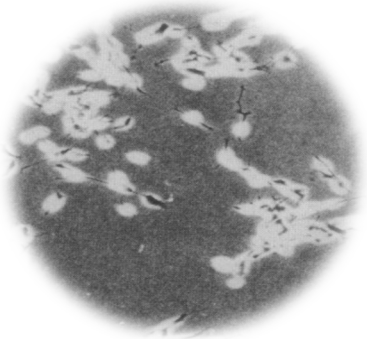
Kelly E. Carstens,* Theresa Freudenrich, Kathleen Wallace, Seline Choo, Amy Carpenter, Marci Smeltz, Matthew S. Clifton, W. Matthew Henderson, Ann M. Richard, Grace Patlewicz, Barbara A. Wetmore, Katie Paul Friedman, and Timothy Shafer

Aim of study

The present study, evaluated mixture toxicity of PFAS at concentration ratios relevant in the environment and in human blood, focusing on their impacts on two cell lines

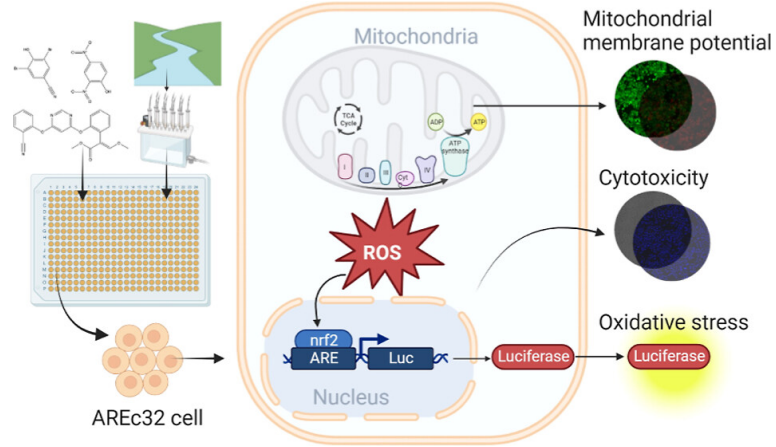
Neurotoxicity Assay

Human neuroblastoma (**SH-SY5Y**) cells differentiated into neuron cells were used as a screening tool to assess **cytotoxicity** and **neurite outgrowth**, serving as proxies for neurotoxicity.



MItoOxTox Assay

Oxidative stress response, mediated via the nuclear factor erythroid 2-related factor 2-Antioxidant Response Element (**Nrf2-ARE**) pathway, was quantified using the reporter protein **luciferase**, **while mitochondrial toxicity** was assessed using the **mitochondrial membrane potential** (MMP) indicator in the reporter gene cell line **AREc32**.



Lee, J.; König, M.; Braun, G.; Escher, B. I. Water Quality Monitoring with the Multiplexed Assay MitoOxTox for Mitochondrial Toxicity, Oxidative Stress Response, and Cytotoxicity in AREc32 Cells. *Environ. Sci. Technol.* **2024**, *58* (13), 5716–5726.

Study design

PFAS

Single PFAS

PFNA, PFDA, PFBA, PFOA, PFOS, HFPO-DA, PFHpA, PFHxA, PFHxS, PFBS, PFPeS, 6:2 FTS

Environmental Mixture

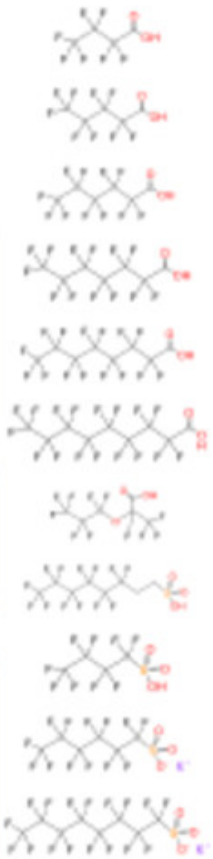
PFBA, PFHxA, PFOA, PFOS, PFHxS, PFBS, 6:2 FTS, HFPO-DA, PFPeA, PFNA, PFHpA

Blood Mixture

PFOS, PFOA, PFHxS, PFNA

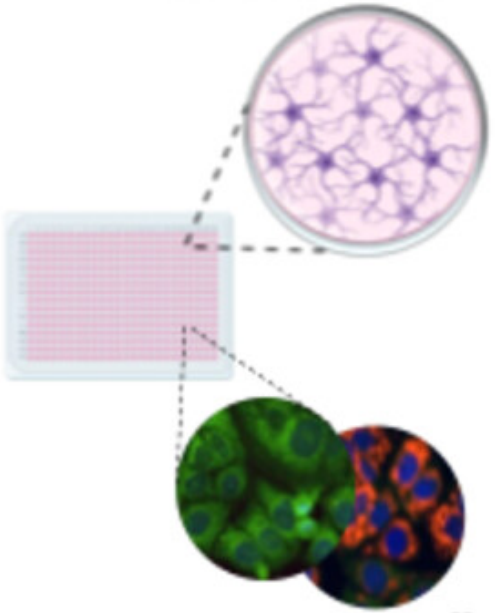
Biosolids & Mixtures

Primary sludge (PS) and waste activated sludge (WAS)
PFOS, PFOA, PFHxS



Exposure of 2 cell lines
Detection of cytotoxicity

Differentiated SH-SY5Y cells



AREc32 cells

Detection of specific effects

Neurite outgrowth

Oxidative stress response

Mitochondrial activity

Table 1. PFAS Included in This Study, Design of the Environmental Mixture (Envmix), the Blood Mixture (Bloodmix), the Mixtures of Wastewater Activated Sludge (WASmix) and Primary Solid (PSmix)

		environmental mixture (envmix)					
chemical name	abbreviation	concentration C_i (ng/L)	concentration C_i in molar units (pM)	molar fraction p_i in envmix	molar fraction p_i in bloodmix[a]	molar fraction p_i in WASmix[b]	molar fraction p_i in PSmix[c]
perfluorobutanoic acid	PFBA	8.1	38.1	0.139			
perfluoropentanoic acid	PFPeA	6.1	23.1	0.086			
perfluorohexanoic acid	PFHxA	5.6	18.3	0.066	0.127	0.207	
perfluoroheptanoic acid	PFHpA	7.4	20.3	0.075			
perfluorooctanoic acid	PFOA	11.0	26.6	0.098	0.289	0.181	0.249
perfluorononanoic acid	PFNA	8.0	17.2	0.064	0.107		
perfluorobutane sulfonic acid	PFBS	4.9	16.3	0.061			
perfluoropentane sulfonic acid	PFPeS	5.1	13.7	0.051			
perfluorohexane sulfonic acid	PFHxS	5.9	14.7	0.055			
perfluorooctanoic sulfonic acid	PFOS	20	42.3	0.150	0.477	0.612	0.751
6:2 fluorotelomer sulfonic acid	6:2 FTS	10	23.4	0.086			
2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid	HPFO-DA	5.8	17.6	0.065			

[a]Mean of detected concentrations in children's serum: 96 μg/L (4.7 pM) PFOA, 0.81 μg/L (1.8 pM) PFNA, 0.83 μg/L (2.1 pM) PFHxS and 3.90 μg/L (7.8 pM) PFOS. [b]Mean of detected concentrations in WAS: 4.2 ng/g_{solid} (10.1 pmol/g_{solid}) PFOA, and 15.3 ng/g_{solid} (30.6 pmol/g_{solid}) PFOS.[45] [c]Mean of detected concentrations in PS: 8.5 ng/g_{solid} (20.7 pmol/g_{solid}) PFHxA, 7.5 ng/g_{solid} (18.1 pmol/g_{solid}) PFOA, and 30.6 ng/g_{solid} (61.2 pmol/g_{solid}) PFOS.[45]

Methods

- Specificity ratio (SR)= $\frac{IC_{10}}{EC_{10}}$

IC_{10} is the inhibitory concentration for 10% cytotoxicity
EC_{10} is the effect concentration for 10% effect, i.e., for neurite outgrowth inhibition.

- Baseline toxicity ($IC_{10,\ baseline}$) is the minimum toxicity of any chemical, it can be predicted from its tendency to accumulate in biological membranes.

It is stimulated by the liposome-water distribution ratio. ($D_{lip/w}$)

- Toxicity Ratio (TR) is the measure of excess cytotoxicity.

$$TR = \frac{IC_{10,baseline}}{IC_{10}}$$

- The 10% inhibitory concentration for cytotoxicity of a mixture $IC_{10}(CA)$

$$IC_{10}(CA) = \frac{1}{\sum_{i=1}^{n} \frac{p_i}{IC_{10,i}}}$$

- Index of prediction quality (IPQ)
- Contribution of one component 'i' to the overall mixture effect.(Tox_i)
- The relative effect potency , relative to PFOA

$$REP_i = \frac{EC_{10,PFOA}}{EC_{10,i}}$$

- The concentration responsive curve (CRC) was calculated for any effect level below 10%

$$\text{effect } y(\text{mixture}) = \sum_{i=1}^{n} p_i \times \text{slope}_i \times C_{tot}$$

Results and discussion

Table 2. Liposome–Water Distribution Ratio of the Anionic PFAS Species, $D_{lip/w}$, and Cytotoxicity Inhibitory Concentrations IC_{10} for AREc32 and SH-SY5Y Cells and Effect Concentration EC_{10} for 10% Reduction of Neurite Length[a]

		AREc32 cytotoxicity			SH-SY5Y cytotoxicity			SH-SY5Y neurite outgrowth inhibition		
PFAS	log $D_{lip/w}$ [L_w/L_{lip}]	IC_{10}	SE IC_{10}	TR	IC_{10}	SE IC_{10}	TR	EC_{10}	SE EC_{10}	SR
PFBA	1.00[b]	3.92×10^{-3}	5.02×10^{-4}	2.06	1.95×10^{-3}	8.86×10^{-5}	3.97	2.13×10^{-3}	1.20×10^{-3}	0.92
PFPeA	1.75[d]	1.05×10^{-3}	8.06×10^{-5}	2.31	1.67×10^{-3}	1.02×10^{-4}	1.34	3.41×10^{-3}	1.51×10^{-3}	0.49
PFHxA	2.32[c]	2.82×10^{-4}	1.76×10^{-5}	4.02	1.23×10^{-3}	1.13×10^{-4}	0.82	1.23×10^{-3}	1.72×10^{-4}	0.99
PFHpA	2.91[c]	1.67×10^{-4}	9.32×10^{-6}	3.43	8.65×10^{-4}	7.85×10^{-5}	0.57	5.44×10^{-4}	9.83×10^{-5}	1.59
PFOA	3.52[c]	5.43×10^{-5}	3.03×10^{-6}	5.80	2.76×10^{-4}	2.66×10^{-5}	0.95	2.42×10^{-4}	1.70×10^{-5}	1.14
PFNA	4.25[c]	1.15×10^{-4}	1.20×10^{-5}	1.49	4.97×10^{-4}	5.62×10^{-5}	0.27	1.99×10^{-4}	2.49×10^{-5}	2.50
PFBS	3.51[c]	7.58×10^{-4}	5.25×10^{-5}	0.42	1.09×10^{-3}	6.00×10^{-5}	0.24	9.68×10^{-4}	3.77×10^{-5}	1.12
PFPeS	3.33[d]	2.82×10^{-4}	2.08×10^{-5}	1.33	4.92×10^{-4}	2.36×10^{-5}	0.64	5.72×10^{-4}	8.76×10^{-5}	0.86
PFHxS	4.13[c]	1.66×10^{-4}	1.27×10^{-5}	1.13	4.05×10^{-4}	3.85×10^{-5}	0.37	2.80×10^{-4}	4.60×10^{-5}	1.45
PFOS	4.89[c]	5.64×10^{-4}	5.82×10^{-5}	0.20	4.12×10^{-4}	3.85×10^{-5}	0.20	3.03×10^{-4}	5.12×10^{-5}	1.36
6:2 FTSA	3.87[d]	7.22×10^{-4}	4.66×10^{-5}	0.32	1.21×10^{-2}	2.30×10^{-3}	0.02	3.86×10^{-3}	8.05×10^{-4}	3.15
HFPO-DA	2.41[c]	4.22×10^{-4}	2.65×10^{-5}	2.40	1.18×10^{-3}	5.58×10^{-5}	0.76	2.80×10^{-3}	5.61×10^{-4}	0.42

[a]Full names of the abbreviated PFAS are given in Table 1. The toxic ratio TR is the ratio of the predicted IC_{10} of baseline toxicity and the measured IC_{10} (eq 5). The specificity ratio (SR) is the ratio of the predicted IC_{10} of baseline toxicity and the measured EC_{10} (eq 2). [b]Experimental log $D_{lip/w}$ from Droge.[52] [c]Experimental log $D_{lip/w}$ from Ebert et al.[53] [d]Predicted log $D_{lip/w}$ from Qin et al.[35]

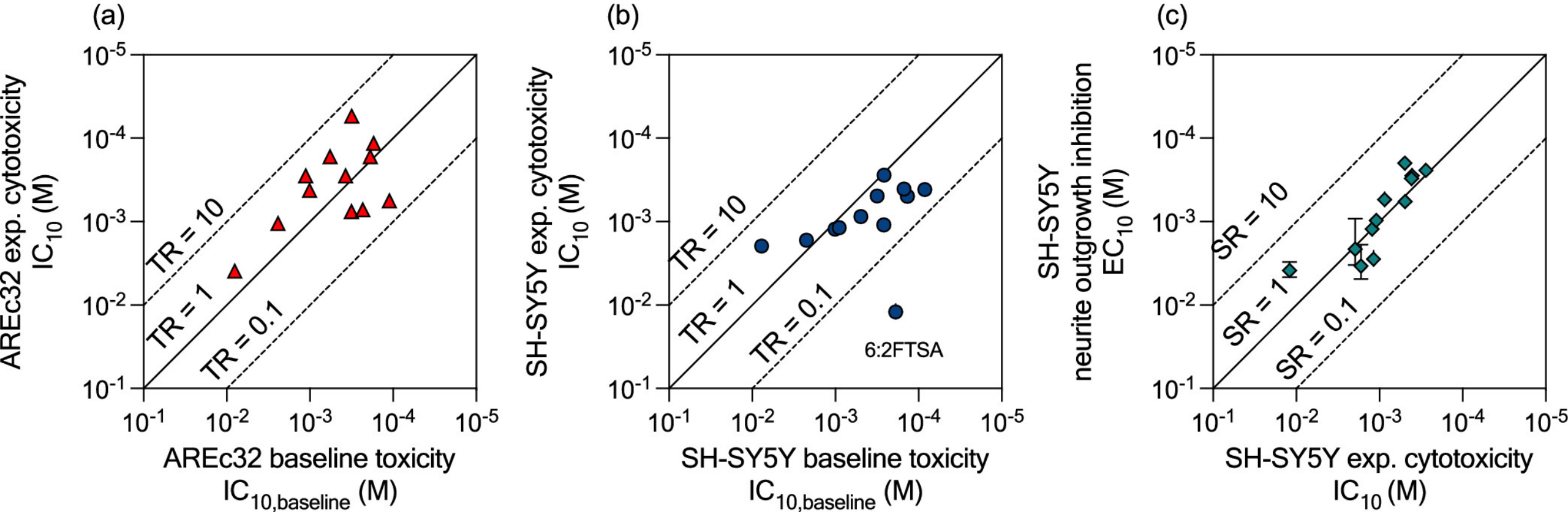
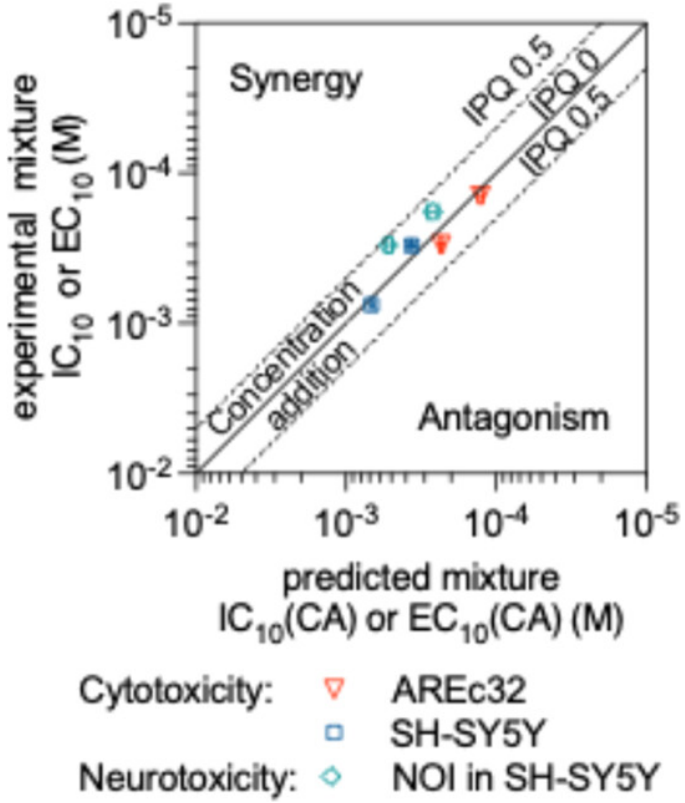

(a)
AREc32 exp. cytotoxicity
IC10 (M)
AREc32 baseline toxicity
IC10,baseline (M)
TR = 10
TR = 1
TR = 0.1
(b)
SH-SY5Y exp. cytotoxicity
IC10 (M)
SH-SY5Y baseline toxicity
IC10,baseline (M)
TR = 10
TR = 1
TR = 0.1
6:2FTSA
(c)
SH-SY5Y
neurite outgrowth inhibition
EC10 (M)
SH-SY5Y exp. cytotoxicity
IC10 (M)
SR = 10
SR = 1
SR = 0.1

Table 3. Cytotoxicity Inhibitory Concentrations IC_{10} for AREc32 and SH SY5Y Cells and Effect Concentration EC_{10} for 10% Reduction of Neurite Length (NOI) for the Two Designed Mixtures Envmix and Bloodmix (Table 1)[a]

		AREc32 cytotoxicity			SH-SY5Y cytotoxicity			SH-SY5Y neurite outgrowth inhibition		
	mixture	IC_{10}	SE IC_{10}	IPQ	IC_{10}	SE IC_{10}	IPQ	EC_{10}	SE EC_{10}	IPQ
envmix	CA prediction	2.30×10^{-4}	6.88×10^{-6}		6.77×10^{-4}	2.47×10^{-5}		5.24×10^{-4}	3.16×10^{-5}	
	experimental	2.98×10^{-4}	3.36×10^{-5}	0.28	7.52×10^{-4}	5.29×10^{-5}	0.11	3.01×10^{-4}	1.45×10^{-5}	0.42
bloodmix	prediction	1.27×10^{-4}	5.33×10^{-6}		3.66×10^{-4}	2.05×10^{-5}		2.66×10^{-4}	2.10×10^{-5}	
	experimental	1.41×10^{-4}	1.31×10^{-5}	0.11	3.03×10^{-4}	3.04×10^{-5}	0.17	1.80×10^{-4}	1.57×10^{-5}	0.32

[a]The mixture IC_{10} and EC_{10} were predicted with the mixture model of concentration addition (CA, eqs 6–9), and the index of prediction quality (IPQ) was calculated with eq 10.



Environmental mixture

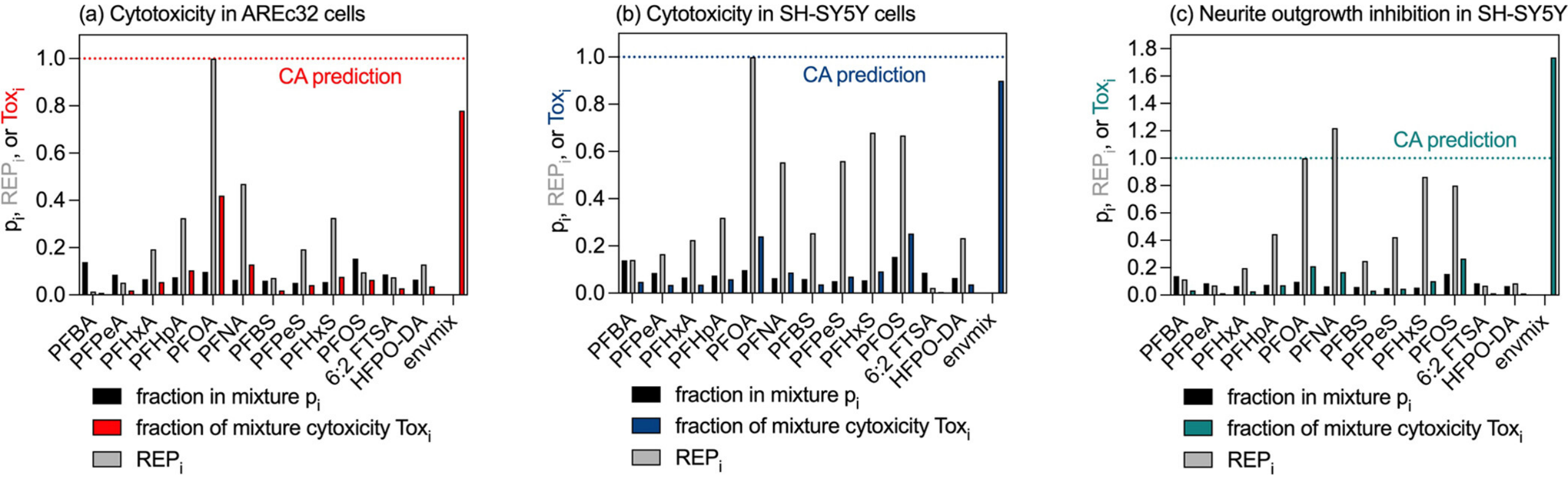


Figure: Comparison of the contribution of individual PFAS ‘i’ to the fraction in the mixture

Blood mixture

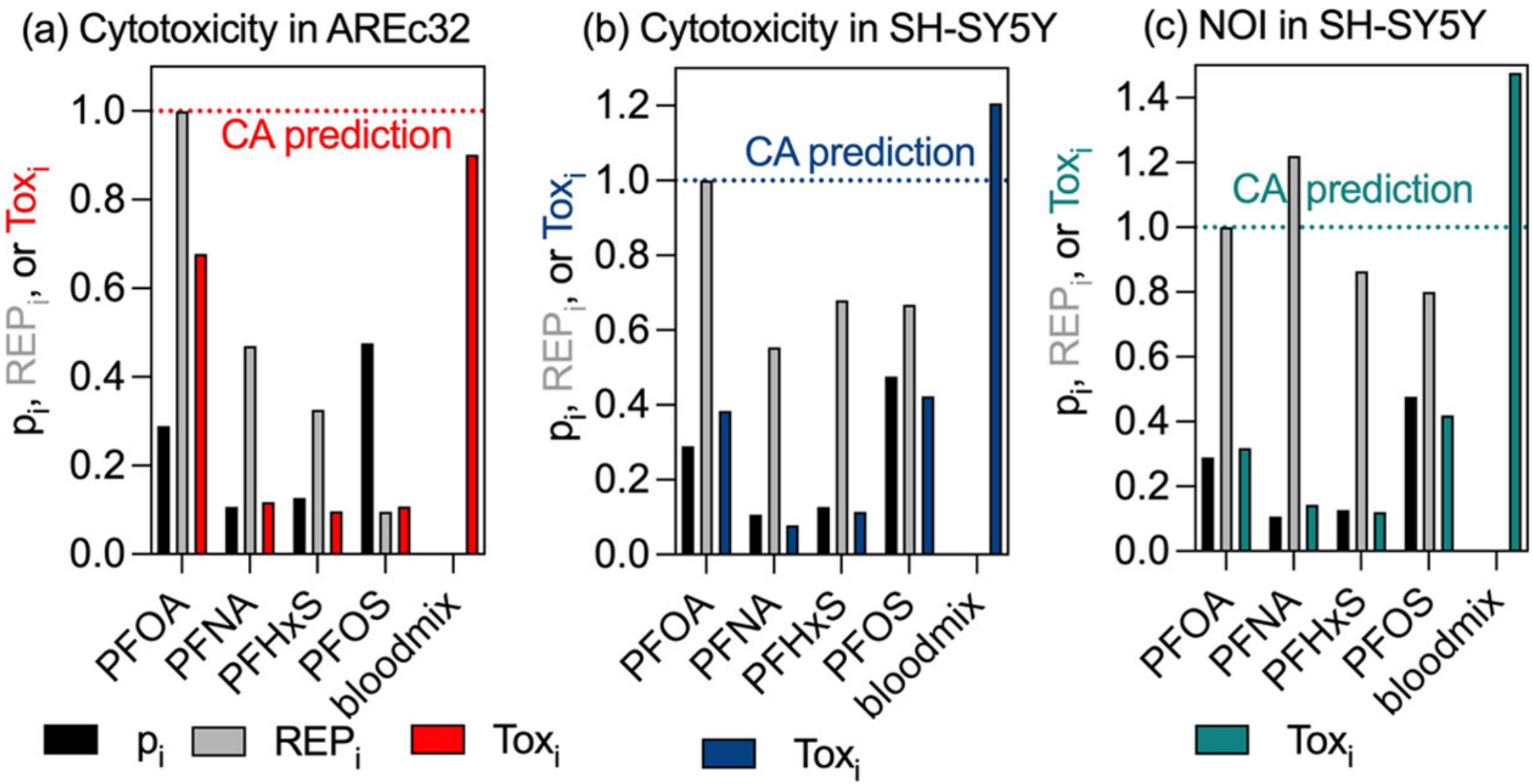
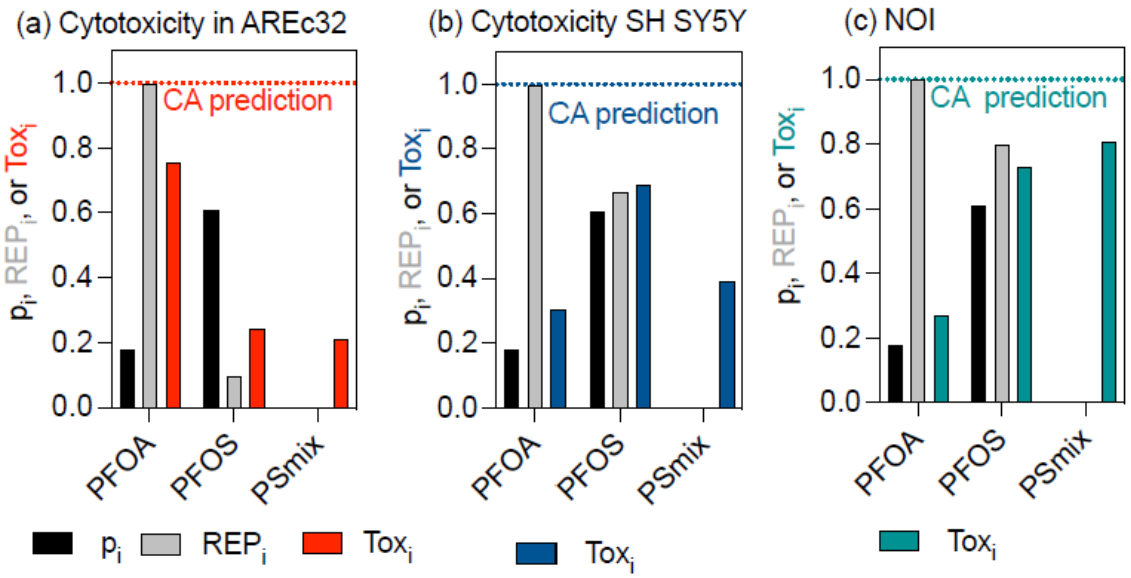
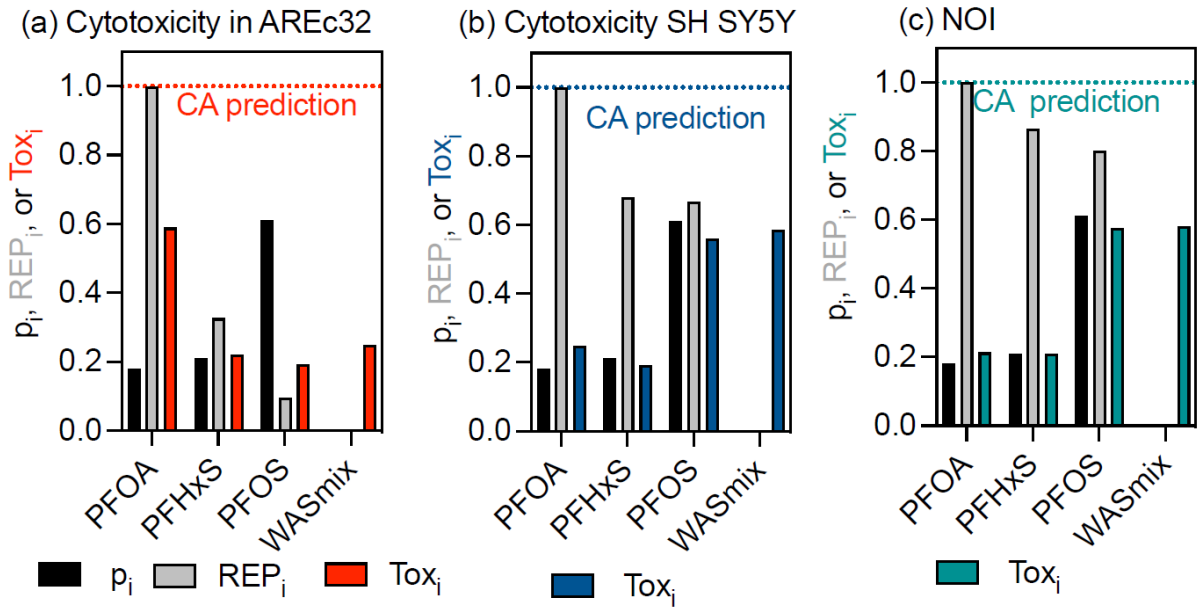


Figure: Comparison of the contribution of individual PFAS ‘i’ to the fraction in the mixture

PSmix and WASmix





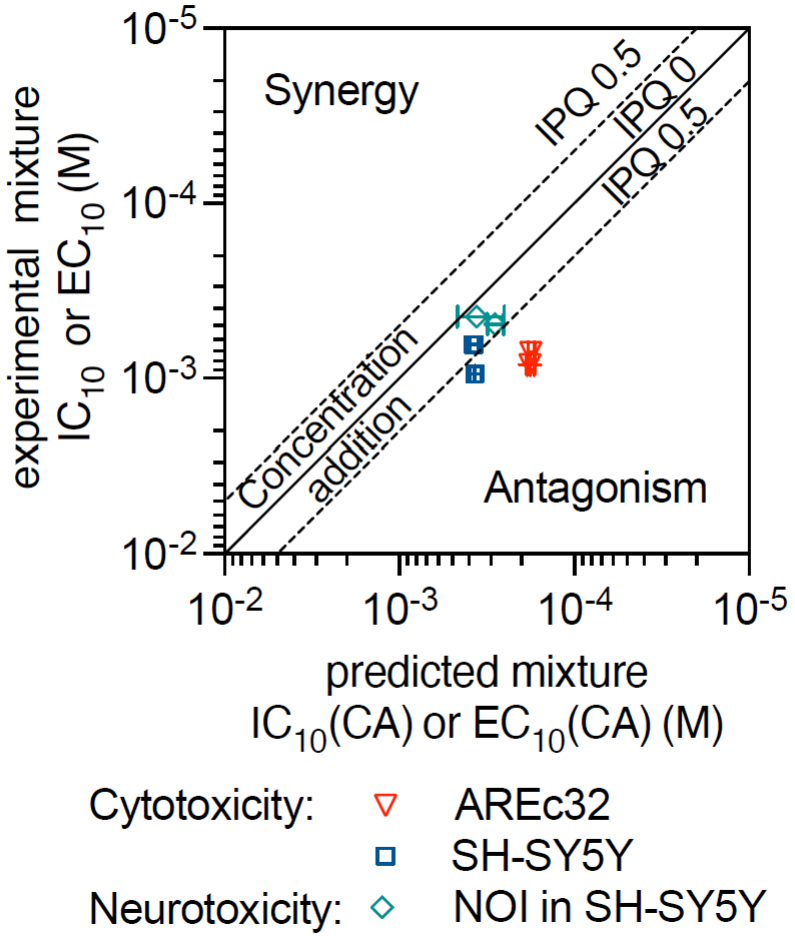


Figure: Comparison between the predicted and the baseline toxicity.
IPQ < 0.5 shows good prediction for NOI but cytotoxicity had a tendency towards antagonism

Findings

- The CRCs of the extracts of PS and WAS indicated activity in all end points of the MitoOXTox assay and the neurotoxicity assay. The extracts activated oxidative stress response and inhibited MMP, which are not activated by designed mixtures or individual PFAS.
- This may be due to many other biosolid extracts other than PFAS which can trigger such action.
- However, because of the high persistence of PFAS, it is likely that PFAS concentrations in environments where biosolids are applied are more important relative to the other biodegradable chemicals that also contribute to biosolids' toxicity.

Conclusion

- PFAS mixtures exhibit neurotoxic effects, particularly by inhibiting neurite outgrowth in neuron-like cells.
- Neurotoxicity is observed even at low, environmentally relevant concentrations.
- Mixture effects are additive, with toxicity levels aligning with the **Concentration Addition (CA)** model.
- Individual PFAS showed primarily cytotoxic effects but no significant oxidative stress or mitochondrial toxicity below cytotoxic levels.
- The study underscores the importance of assessing PFAS as mixtures rather than as isolated compounds for accurate risk evaluation.
- High-throughput screening (HTS) and new approach methodologies (NAMs) are validated as effective tools for evaluating mixture toxicity.

Thank you