**Report on Comparison of Cell-based Bioactivity Concentrations and Human Population Blood Concentrations for Selected PFAS Compounds**

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Disclaimer

# Introduction

The US EPA Office of Pollution Prevention and Toxics (OPPT) has requested that the EPA Office of Research and Development (ORD) provide a summary of an ORD research effort comparing *in vitro* cell-based bioactivity concentrations with blood concentrations from human populations for a selected group of per- and polyfluoroalkyl substances (PFAS). The ratio of these two values, here called the Bioactive Concentration to Blood Concentration Ratio (BCBCR), is a similar concept to the Margin of Exposure (MoE) used in standard risk assessments. The *in vitro* data are taken from a battery of cell-based assays, mostly run in human cells. The key result from each assay is the concentration in cells that will cause a biological perturbation. In general, there is no direct link between the *in vitro* bioactivity detected by an assay and a specific apical *in vivo* toxicological effect. However, previous studies have demonstrated that *in vitro* bioactivity provides a conservative estimate of the dose causing toxicological responses in traditional animal-based studies (Paul-Friedman, Gagne et al. 2020). All of the *in vitro* bioactivity is publicly available through primary publications and the EPA CompTox Chemicals Dashboard (<https://comptox.epa.gov/dashboard>).

The BCBCR is the bioactivity concentration divided by the blood concentration, where both are expressed in units of ng/mL. A value less than 1.0 indicates that blood levels for an individual are higher than the concentration required to elicit some kind of biological perturbation in human cells. The BCBCR approach is treated as a screening-level assessment method that can be used to quickly assess multiple chemicals, and ones with the lowest BCBCR values can be prioritized for further assessment (US EPA 2012). The BCBCR approach described here is subject to a number of sources of uncertainty, some of which will result in an underestimation of risk. Therefore, it is appropriate to add a safety factor when using the BCBCR values, indicating that BCBCR values larger than 1.0 may still be of concern. For the illustration provided below, a safety margin of 100 is used, which is consistent with screening-level assessment practices under TSCA.

The complete version of this analysis is given in an ORD manuscript currently under internal EPA review, prior to submission to a peer-reviewed journal. In that manuscript, BCBCR values are analyzed for 31 PFAS. This is the set of chemicals for which published blood levels and *in vitro* bioactivity data are available. Hence, the data incorporated in this report have been extracted from the peer-reviewed literature and/or subject to EPA’s internal quality assurance and review, but the full analysis and procedures incorporated herein are still undergoing independent peer review. For this report, we focus on six PFAS out of the 31 that are of interest to OPPT. These are PFOA (Perfluorooctanoic acid), PFNA (Perfluorononanoic acid), PFDA (Perfluorodecanoic acid), PFUnDA (Perfluoroundecanoic acid), PFTriDA(Perfluorotridecanoic acid) and PFTeDA (Perfluorotetradecanoic acid).

# Methods

## Biomonitoring Data

Human biomonitoring data were collected from 247 published studies. For the chemicals described in this report, the biomonitoring data are provided in an accompanying spreadsheet with corresponding study references. Concentrations in several matrices were available in these studies (whole blood, serum, plasma from both adults and cord blood). All concentrations were converted to ng/mL. Additionally, all values were converted to plasma concentrations if they were originally derived from matrices other than plasma. Serum concentrations were assumed to be equivalent to plasma concentration, but whole blood values were divided by a chemical-specific blood-to-plasma partition coefficient taken from the *httk* R package (Pearce, Setzer et al. 2017). Each study reported one or more concentration metrics for the population tested (e.g., workers; situation-specific). The metrics are the 5th percentile, 10th percentile, 25th percentile, 50th percentile, 75th percentile, 90th percentile, 95th percentile, 98th percentile, 99th percentile, maximum, mean, median and minimum. For this report, we only include data for the metrics 95th percentile, 98th percentile, 99th percentile and maximum, to focus on the most highly exposed members of the populations. Data were manually extracted from source documents into a standard form. A selected set of records, including all with values >100 ng/mL, were checked for accuracy by a second reviewer. Each data set is characterized by the sampling location (country, state or region, city) and a brief statement about the cohort, especially whether they were suspected of being exposed to PFAS compounds (e.g., factory workers, individuals living in communities with PFAS-contaminated drinking water) or were a general population with no known source of PFAS exposure. All values are derived from the concentration distribution of a population and not for specific individuals. Each record in the accompanying spreadsheet indicates the chemical (name, Chemical Abstracts Registry Number or CASRN, the EPA DSSTox Substance ID or DTXSID, and a chemical abbreviation), the matrix (plasma, serum, whole blood, cord blood, cord plasma or cord serum), the population metric (95th, 98th, 99th, maximum), the concentration and units (always in ng/mL), the location of the population sample, whether the population was exposed or general, a source, name and brief description of the dataset, and a URL pointing to the data source, which could be an online report or a journal publication.

## *In Vitro* Bioactivity Data

The *in* *vitro* bioactivity data are derived from a set of ~150 PFAS compounds that were processed through eight sets of assays. All of the PFAS reported here passed analytical QC, which indicates that the samples tested had the intended chemical identity. The *in vitro* assays are described briefly here, and references provide more detail. The assays are grouped into “assay sets” where a set contains all assays from a single vendor or source, with distinct assay technology and/or bioactivity type and cell type. For each chemical there is an *in vitro* point of departure (POD) for each assay set. The set-level POD is the lower 5th percentile of the distribution of all PODs for that chemical and assay set for active assays. The minimum POD for the chemical is the minimum of the set-level PODs. All *in vitro* data is available through the CompTox Chemicals dashboard.

ACEA: (ACEA Biosciences, San Diego, CA (Rotroff, Dix et al. 2013, Houck, Patlewicz et al. 2021, Houck, Friedman et al. 2023)). This assay is a functional screen for estrogen receptor agonists. Chemicals active in this assay have potential endocrine activity.

ATG: (Attagene, Morrisville, NC (Romanov, Medvedev et al. 2008, Houck, Friedman et al. 2023). This platform measures a large number of ligand-activated nuclear receptor and other transcription factor activities representing diverse physiological processes, including metabolism and fatty acid regulation, endocrine activity, oxidative stress, and lipid peroxidation.

BSK: (BioSeek, now BioMAP, Diversity Plus Panel (Berg, Kunkel et al. 2005, Kleinstreuer, Yang et al. 2014, Berg 2017, Houck, Friedman et al. 2023)). This assay set consists of 12 human primary cell systems that model potential tissue and disease responses, including vascular, immune, skin, lung and general tissue responses, via stimulation of the mono- or co-culture systems to pathophysiologically-relevant states.

DNT: (Developmental neurotoxicity (Carstens, Freudenrich et al. 2023)). This assay battery was designed to detect chemicals with potential for developmental neurotoxicity.

HTPP: (High-throughput phenotypic profiling with the cell painting assay (Bray, Singh et al. 2016, Nyffeler, Willis et al. 2020, Nyffeler, Haggard et al. 2021). This high content imaging assay measures phenotypic changes in cell morphology in cells labeled with fluorescent markers for a variety of organelles (nucleus, nucleoli, endoplasmic reticulum, Golgi complex, plasma membrane, cytoskeleton, and mitochondria).

HTTr: (High-throughput transcriptomics with the TempO-Seq human whole transcriptome assay (Yeakley, Shepard et al. 2017, Harrill, Everett et al. 2021)). This assay measures gene expression changes using whole transcriptome targeted RNA-Seq in HepaRG (liver) and U-2 OS (bone osteosarcoma) cell lines.

Thyroid: This suite of assays covers critical pathways within the thyroid axis including deiodinase enzymes (Human Deiodinase 1,2 and 3 [DIO], Human Iodotyrosine deiodinase [IYD] (Olker, Korte et al. 2019, Olker, Korte et al. 2021); human thyroid peroxidase [TPO](Paul Friedman, Watt et al. 2016); and thyroid hormone plasma binding proteins transthyretin [TTR], and thyroxine binding globulin [TBG](Montano, Cocco et al. 2012). [Degitz et al. submitted].

Zebrafish: This is a zebrafish embryotoxicity assay which measures lethality and malformations (hatching status, swim bladder inflation, edema, abnormal spinal or craniofacial structure, blood pooling, or changes in pigmentation), (Deal, Wambaugh et al. 2016, Poothong, Thomsen et al. 2017). [Britton et al. in preparation]

## BCBCR Calculation

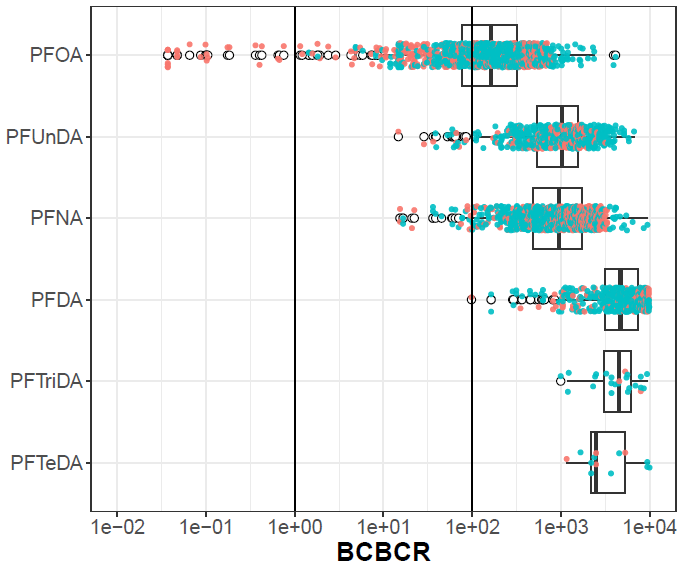
BCBCR values for each chemical and population were calculated as POD (*in vitro*)/plasma concentration. No attempt was made to extrapolate blood / plasma concentrations to concentrations in specific tissues.

# Results

All six PFAS chemicals have blood level data from at least one site in the US. The *in vitro* PODs are given in Table 1. In all but one case, the BioSeek assays gave the lowest POD. The BioSeek assays include human primary cell and co-culture models of autoimmune disease, chronic (vascular) inflammation, allergy, monocyte activation, lung inflammation and fibrosis, cardiovascular inflammation, and dermatitis, and wound healing (Houck, Friedman et al. 2023). The exception is PFDA where the most sensitive technology was the HTPP assay run in U-2 OS osteosarcoma cells. This assay measures changes in cell morphology under chemical exposure. The BCBCR values are illustrated in Figure 1. Vertical lines are drawn at BCBCR =1 and 100. Only PFOA crosses the BCBCR =1 line and only for exposed populations (orange points). PFOA, PFUnDA and PFDA show BCBCR values between 1 and 100 for both exposed and general populations. As mentioned above, a BCBCR of 100 is consistent with screening-level assessment practices under TSCA. A full discussion of the uncertainties in the BCBCR methods is included in the ORD manuscript currently under review.

**Table 1**: Chemical Level *in vitro* PODs in ng/mL.

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemical** | **POD (uM)** | **POD (ng/mL)** | **Most sensitive technology** |
| PFOA | 2.0 | 828.1 | BSK BT (Human CD19+B cells and PBMC/anti-IgM + TCR ligands) |
| PFNA | 2.0 | 928.2 | BSK KF3CT (Human keratinocytes and dermal fibroblasts/IL-1β, TNFα and IFNγ) |
| PFDA | 6.1 | 3140.8 | HTTP U2OS Osteosarcoma cells |
| PFUnDA | 0.6 | 310.3 | BSK LPS (HUVECs co-cultured with  monocytes and stimulated with bacterial endotoxin LPS) |
| PFTriDA | 2.4 | 1593.9 | BSK hDFCGF (Human dermal  fibroblasts stimulated with IL-1β + TNFα + IFNϒ and EGF + bFGF + PDGF-BB) |
| PFTeDA | 0.7 | 500.0 | BSK 4H (HUVECs stimulated with IL-4 + histamine) |



**Figure 1**: BCBCR values as a function of chemical and population. Each point is one population-metric value for one chemical. Points colored orange are from exposed populations and those colored blue are from general populations. The box and whiskers indicate the inner quartiles and 1.5 times the IQR, respectively. The open circles are points outside 1.5 times IQR.

# Summary

Here, we have briefly summarized a BCBCR analysis for a set of 5 PFAS of interest to OPPT. A key finding is that three of the six show BCBCR values <100, for both exposed and general populations. This indicates that, within the uncertainty of this approach, general populations have blood levels high enough to cause potential biological changes. Although it is not clear what the linkage is between the measured bioactivity and specific toxicological endpoints at the tissue and organ level, previous studies have demonstrated that *in vitro* bioactivity generally provides a conservative (i.e., lower) estimate of the dose that causes adverse effects in traditional animal-based toxicity studies (Paul-Friedman, Gagne et al. 2020).

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