HHS Public Access

Author manuscript

J Toxicol Environ Health A. Author manuscript; available in PMC 2022 May 05.

Published in final edited form as:

J Toxicol Environ Health A. 2021 July 03; 84(13): 553–567. doi:10.1080/15287394.2021.1901251.

Per- and polyfluoroalkyl mixtures toxicity assessment "Proof-of-Concept" illustration for the hazard index approach

M. M. Mumtaz, **M. C. Buser**, **H.R. Pohl**

Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States

Abstract

The 2018 ATSDR mixture framework recommends three approaches including the hazard index (HI) for environmental mixture toxicity assessment. Per- and polyfluoroalkyls (PFAS) are found in our environment and general populations. Recent experimental mixture toxicity studies of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and an assessment of 17 PFAS indicate the use of additivity for their joint toxicity assessment. The aim of this investigation was to detail the stepwise procedures and examine the extent and use of the HI approach for PFAS mixture assessment. Using estimated general public lifetime exposures (high, medium, and low), binary mixtures of PFOS and PFOA yielded, respectively, hazard indices (HIs) of 30.67, 8.33, and 3.63 for developmental toxicity; 10.67, 5.04, and 2.34 for immunological toxicity; 3.57, 1.68, and 0.78 for endocrine toxicity; 4.51, 1.73, and 0.79 for hepatic toxicity; and 15.08, 2.29, and 0.88 for reproductive toxicity. A heterogeneous mixture of PFOA, PFAS, dioxin (CDD), and polybrominated compounds (PBDE) for high exposure scenario yielded HIs of 30.99 for developmental, 10.77 for immunological, 3.64 for endocrine, 4.61 for hepatic, and 17.36 for reproductive effects. The HI values are used as a screening tool; the potential concern for exposures rises as HI values increase. For HI values >1, a follow-up including further analysis of specific exposures, use of internal dosimetry, and uncertainty factors is conducted before recommending appropriate actions. The HI approach appears suitable to address present-day PFAS public health concerns for initial assessment of multiple health effects, until further insights are gained into their mechanistic toxicology.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

Keywords

PFAS; mixtures; hazard index

CONTACT M. M. Mumtaz, mgm4@cdc.gov, Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States.

Introduction

Per- and polyfluoroalkyls (PFAS) are synthetic substances present in the environment. These compounds have existed in commerce for several decades, but more recently have become a focus of community concerns across the United States (ATSDR 2018a). These chemicals are a diverse group of approximately 5,000 chemicals with important structural variations that include perfluoroalkyl acids (PFAAs), perfluoroalkane Figure: sulfonic acids (PFSAs), perfluoroalkyl carboxylic acids (PFCAs), and some polyfluorinated substances that may degrade or metabolize to the widely reported important perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).

The major exposure pathways for PFOS for the general population in Europe and North America are food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets (Trudel et al. 2008). For PFOA, major exposure pathways are oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion (Trudel et al. 2008). Exposure pathways for other PFAS such as perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) are less extensively studied but expected to be similar to PFOA and PFOS.

The mechanisms underlying toxicity attributed to PFAS are not well understood. There is strong evidence that some effects observed in rodents, such as hepatotoxicity, immunotoxicity, and developmental toxicity, involve activation of peroxisome proliferator-activated receptor-α (PPARα); however, humans and nonhuman primates are less responsive to PPARα agonists than rodents (Corton et al. 2014). In addition, PPARα-independent mechanisms are also involved, and it is not known if species differences exist for these mechanisms (ATSDR 2018a).

The available epidemiology studies (Grandjean et al. 2012, 2017; Lenters, Portengen, and Rignell-Hydbom et al. 2016; Mi et al. 2020; NTP 2016; Olsen, Burris, and Burlew et al. 2000; Winquist and Steenland 2014) suggest the following associations between PFAS exposure and several adverse health effects:

- Increased cholesterol levels (PFOA, PFOS, PFNA);
- Changes in liver enzymes (PFOA, PFOS, PFHxS);
- Decreased vaccine response in children (PFOA, PFOS, PFHxS);
- Increased risk of high blood pressure or preeclampsia in pregnant women (PFOA, PFOS); and
- Decreases in infant birth weights (<20 g (0.7 ounces) fall in birth weight per 1 ng/ml elevation in PFOA or PFOS in blood).

The International Agency for Research on Cancer (IARC 2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B), and the United States Environmental Protection Agency (EPA (2016b); EPA 2016c) indicated that there was suggestive evidence of carcinogenic potential of PFOA and PFOS in humans. Increases in testicular and kidney

cancer were noted in highly exposed humans (Barry, Winquist, and Steenland 2013; Shearer et al. 2020; Vieira et al. 2013).

The actual composition of an exposure defines the focus and direction of a toxicity assessment. For meaningful real-life risk assessment, a thorough qualitative and quantitative analysis of environmental exposures is needed. Such exposures are often to chemical mixtures and not just limited to individual chemicals. Mixtures containing PFAS may be homogeneous, containing only PFAS, or heterogeneous, containing PFAS and other persistent organic pollutants (POPs).

Mixtures risk assessment approaches

The ATSDR (2018b) "Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors" recommends three broad data-driven approaches for toxicity assessment of environmental mixtures. The first two approaches are whole mixtures based and are used if the mixture or a similar mixture has actually been tested and data are available. The framework details a tiered workflow for the third and most-often used approach, the hazard index (HI) (Figure). This approach is based upon the toxicity of individual chemical components of the mixture and their respective exposure levels (Eq. 1). In the initial stage of the workflow, the problem formulation is undertaken. At this stage, all chemicals found at a waste site in various environmental media with completed exposure pathways (CEPs) are identified (ATSDR 2005). Completed exposure pathways represent unbroken chains linking source contaminant (s), fate and transport of contaminants through environmental media, a point of exposure, route of entry into the body (inhalation, ingestion, or dermal), and potentially exposed population. If the exposures are from multiple route mixture analyses are conducted for all the relevant routes. Having a CEP indicates that either a human population has been exposed in the past, is being exposed presently, or might be exposed in the future to these chemicals. Then, information is collected on potential adverse health effects associated with each chemical based upon toxicologic, epidemiologic, or health outcome data.

Tier 1 analysis: First the hazard quotient (HQ) – the ratio of estimated exposure dose of a chemical divided by its health guidance value (HGV) – is calculated for each single chemical. Health guidance values might be ATSDR's minimal risk level (MRL), EPA's reference dose (RfD), or any other appropriate values (e.g., state guidance values). EPA reported that most hazardous waste sites present elevated risks for fewer than 12 chemicals (EPA 2020). Chemicals are retained for further analysis if their HQ values approach or exceed 1. While chemicals with HQs <1 are expected to individually pose no health impacts, these substances may exert an impact when summing exposure occurs to multiple chemicals. Therefore, all agents with HQs ≥0.1 are retained for further analysis in tier 2.

Tier 2 analysis: HI is calculated for *preliminary* evaluation of the potential for noncancer toxic effects by combining the HQs of all individual chemicals retained in tier 1 (Eq. 1). The HI assumes dose addition, and a *preliminary* hazard index is a sum of HQs ≥0.1 of all known and measured chemicals for site-specific CEPs. This *preliminary* HI does not group chemicals based upon shared toxicity targets (i.e., common adverse effects) or modes of

action (MOAs). When a *preliminary* HI value exceeds 1, further evaluation is recommended in tier 3 analyses.

$$HI = \sum_{i=1}^{n} HQ_i = \frac{E_1}{HGV_1} + \frac{E_2}{HGV_2} + \ldots + \frac{E_n}{HGV_n} \quad \text{(Eq.1)}$$

Tier 3 analysis: Because the dose-additive HI approach does not account for possible interactions among mixture components, in this tier, an evaluation of interactions that might impact the joint toxicity of the mixture is conducted. A weight of evidence (WOE) methodology is used to assess the role of interactions in the overall expression of toxicity of the mixture. In a complex mixture, multiple components might exert common effects other than their individual critical effects (e.g., liver); thus, a secondary effect (e.g., reproductive or neurotoxicity) may become an adverse health effect of potential concern in a population. In such situations, target organ toxicity doses (TTDs) that are akin to effect-specific MRLs are developed. Subsequently, these are used to calculate effect-specific HQs and effect-specific HIs for a more complete characterization of health risk.

The purpose of this study was to specifically show the application of the ATSDR (2018b) tiered "Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors" for PFAS mixture assessment. Based upon available data, case studies are presented to illustrate the stepwise procedures that might currently be employed. Thus, the HI approach is used to demonstrate the risk assessment of homogeneous and heterogenous binary and tertiary mixtures containing PFOS, PFOA, dioxins (CDDs), and polybrominated compounds (PBDEs) that have been found to co-occur (Lynch et al. 2019).

Methods

Exposure estimates

Daily oral exposures for PFOA and PFOS estimated by Trudel et al. (2008) were obtained from the perfluoroalkyls toxicological profile (ATSDR 2018a). For PFOS and PFOA, adult estimates for high-exposure scenarios were approximately 30 and 47 ng/kg/day, respectively. For medium exposure scenarios, these levels were 15 and 2.5 ng/kg/day and for low exposure scenarios 7 and 0.4 ng/kg/day, respectively (Trudel et al. 2008). These high-, medium-, and low-exposure scenarios are based upon the 95th percentiles of input parameters, medians, and 5th percentiles, respectively. Trudel et al. (2008). These three exposure scenarios were calculated to encompass a wide range of general population exposures. Further, these three scenarios as well as the contribution to variance in these estimates for each input parameter were calculated in order to reflect the variability and uncertainty of input parameters. Since PFOA and PFOS are no longer produced or used in the United States, current exposure levels may be lower than those predicted by Trudel et al. (2008). Exposure values for chlorinated dioxins (CDDs) and polybrominated compounds (PBDEs) that might potentially co-occur were obtained from their respective toxicological and interaction profile documents (ATSDR 2017, 1998). For CDDs, exposure was estimated at 1.7 pg/kg/day, and for PBDEs, exposure was estimated at 7.1 ng/kg/day. These exposure estimates may have changed since they were developed; however, this is a methods paper

meant to illustrate how the mixture risk assessment approach may be applied to PFAS using a hypothetical exposure scenario. Therefore, these exposure estimates are sufficient for the purposes of the present study.

Health Guidance Values (HGVs)

Minimal risk levels (MRLs) are ATSDR HGVs used to evaluate the toxicity and risk posed by priority environmental chemicals for acute (1–14 days), intermediate (15–364 days), and chronic (>365 days) exposure durations. These values are extensively peer reviewed by internal and external experts. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk for adverse non-cancer health effects over a specified duration of exposure. Thus, MRLs are based upon the concept that a threshold level of exposure exists, below which no non-cancer health effect is likely to occur. For the derivation of MRLs exhaustive literature searches are conducted to compile the database of the overall toxicity of a specific chemical. Identified studies that are of the highest quality are then categorized by each route and duration of exposure and organ system toxicity. Subsequently, the studies that present dose response data are closely reviewed to identify a critical study that includes data for the most sensitive effect at the lowest dose in humans or animals for the route and duration of exposure. Such an investigation provides the dose that is used as the point of departure (POD) to derive the MRL. All other appropriate studies identified are used as supporting evidence for MRL derivation. The POD is divided by uncertainty and modifying factors to calculate the MRL. Depending upon the available data, the POD might be a No Observed Adverse Effect Level (NOAEL), a Lowest Observed Adverse Effect Level (LOAEL), or a lower limit of the 95% confidence interval of the benchmark dose level (BMDL). Sometimes the POD might be determined based upon actual external exposure levels, default values, time weighted averages (TWA), through dosimetric adjustments, or even target organ or system-specific concentrations using physiologically based pharmacokinetic (PBPK) models.

Intermediate MRLs for PFOA, PFOS, CDDs, and PBDEs were extracted from their respective toxicological profiles to use in calculating HQs and HIs. The PODs for PFOA and PFOS are based upon developmental effects. Serum PFAS levels were utilized as PODs for derivation of MRLs because of large interspecies differences in the toxicokinetics of perfluoroalkyls for which mechanisms are not completely understood. The basic assumption in the use of serum PFAS levels as POD is that animals and humans may yield similar relative responses if serum levels are similar following exposure. Details of each individual MRL derivation are provided in the perfluoroalkyls toxicological profile (ATSDR 2018a).

Target Organ Toxicity Dose (TTD) for PFAS

During the mixture risk assessment process, on an as-needed basis, additional effect-specific values – the TTDs – are developed for potential secondary effects of a chemical. The TTD approach, which is a refinement of the HI approach, was devised in order to accommodate the assessment of mixtures whose components do not all exert the same critical effect (i.e., the most sensitive effect providing the basis of the public health guidance value), but may produce toxic effects in common target organs dependent upon exposure level (ATSDR 2018b; Mumtaz, Poirier, and Colman 1997). The TTD approach considers the

reality that most components of contaminated-site-related mixtures affect other target organs at doses higher than those that produce the critical effect of the guidance value. These other effects may vary from component to component and may be important in assessing the adverse health effects initiated by the mixture. Thus, multiple TTDs may be derived for each chemical for effects other than the critical effect for which MRL is derived. The TTDs are derived using the same steps as were employed for MRL derivation in order to ensure consistency across values. Similar to MRLs, TTD calculations use a conservative approach in order to be protective of human health. Therefore, exceeding one does not necessarily mean an effect will occur. The TTDs are utilized to calculate effect-specific HIs and HQs for evaluation of combined adverse health effects of mixtures. The TTDs do not undergo extensive rigorous review that MRLs do because they are developed on a case-by-case basis. The TTD values for secondary effects are typically higher than the MRL value of a chemical. If calculated TTDs are lower than MRLs, then ATSDR defaults to the MRL value when calculating HQs/HIs because there is more confidence in the MRL. The perfluoroalkyls toxicological profile served as the database to identify studies used for TTD derivation. The provisional, draft MRLs developed by ATSDR were intermediate-duration, oral values. In order for the derived TTD values to be comparable to the MRLs, intermediate-duration oral studies were selected that reported an adverse effect for the various endpoints used for TTD development. These studies demonstrated the lowest LOAEL values for various endpoints and were selected as the “critical studies” for the TTD derivations. Below is a brief description of how these values were derived; more detailed information may be found in Appendix A of the toxicological profile for perfluoroalkyls (ATSDR 2018a).

Calculation of internal dose metric

Time-weighted-average (TWA) serum PFOA and PFOS concentrations corresponding to external doses and exposure durations were predicted from a pharmacokinetic model (Wambaugh et al. 2013) using animal species-, strain-, and gender-specific parameters. When animal species, strain, or gender is unavailable in the Wambaugh et al. (2013) model, TWA serum concentrations are estimated from the measured serum concentrations from the principal study of the areas under the curve (AUC).

Human Equivalent Dose (HED)

HEDs were calculated based upon the assumption that humans might exhibit similar effects as the lab animal at a given serum concentration. HEDs were calculated that would result in steady-state serum concentrations (C_{ss}) of PFAS equal to serum concentration in animals that were selected as the POD. This was done using the first-order single-compartment model that is based upon serum elimination $t_{1/2}$ values, an assumed apparent volume of distribution (V_d), and gastrointestinal absorption fraction (AF) (Eq. 2):

$$HED = \frac{C_{SS} * k_e * V_d}{AF}; where k_e = \frac{\ln(2)}{t_{1/2}} \quad \text{(Eq.2)}$$

Model parameters used in these calculations are presented in Table 1.

Uncertainty and modifying factors

HED values are divided by relevant uncertainty factors (UFs) and modifying factors (MFs), when applicable, to arrive at the final TTD values. ATSDR uses UFs for extrapolation from animals to humans, use of a LOAEL, and human variability. Modifying factors are employed when necessary; this may be for database limitations or concerns for a more sensitive endpoint, among others.

TTDs for CDDs and PBDEs

TTDs for CDDs and PBDEs were derived applying the relevant uncertainty factors (ATSDR 2017, 1998). In order for the derived TTD values to be comparable to MRLs and for these to be comparable to derived PFAS values, intermediate-duration oral studies were selected that reported adverse effect for the various endpoints used for TTD development.

Mixtures assessment

Five important health effects – developmental, reproductive, hepatic, immunological, and endocrine (i.e., thyroid effects) – were evaluated for illustrative mixtures containing PFAS. Illustrative mixtures are comprised of components that display a likelihood of co-exposure in the general population because they are omnipresent in the environment and often found in blood samples. The oral lifetime exposures obtained from the literature were used as the exposure levels and the individual chemical oral MRLs and TTDs were utilized as the guidance values for a binary mixture of PFOA and PFOS. The oral intermediate MRLs and TTDs were adopted for chronic HQ and HI calculations because of the long half-lives known for these chemicals. The elimination half-life in humans is estimated to be 3.5 years for PFOA and 4.8 years for PFOS (Olsen et al. 2007). An analysis was also conducted for a heterogenous mixture containing PFOA, PFOS, CDDs, and PBDEs because the likelihood of co-exposure exists for these chemical combinations. The 2,3,7,8-TCDD HGV was used to evaluate all CDDs. ATSDR has employed 2,3,7,8-TCDD as a representative for all CDDs in toxicity assessments. 2,3,7,8-TCDD is the most studied and one of the most toxic congeners and representative of CDDs as a group. This guidance is in accordance with the WHO (2005) Toxicity Equivalency Factors (TEFs) for dioxins. HGVs for lower brominated diphenyl ethers were chosen based upon the information in the Toxicological Profile for PBDEs (ATSDR 2017) indicating these compounds are more toxic than decaBDE. ATSDR derived separate MRLs for the two groups. ATSDR did not derive HGVs for individual lower BDE congeners. CDDs and PBDEs were selected to illustrate how ATSDR's mixture risk assessment process may be applied to a hypothetical heterogenous PFAS mixture. These substances exhibit similar characteristics as PFAS: (1) persistence in the environment, (2) tendency to bioaccumulate, (3) concern for potential harmful effects to humans, and (4) common toxicity endpoints.

Results

Details of MRLs extracted from the respective profiles are presented in Table 2. Briefly, an intermediate-duration oral MRL of 3×10^{-6} mg/kg/day was derived for PFOA based upon skeletal alterations at 13 and 17 months of age in the offspring of mice fed a diet containing PFOA on gestational day (GD) 1 through GD 21 (Koskela et al. 2016). This

MRL is based on a $LOAEL_{HED}$ of 0.000821 mg/kg/day and a total uncertainty factor (TUF) of 300 that included 10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability.

An intermediate-duration oral MRL of 2×10^{-6} mg/kg/day was derived for PFOS based upon delayed eye opening and transient decrease in F2 body weight during lactation in the offspring of rats administered PFOS via gavage in a 2-generation study (Luebker et al. 2005). This MRL is based upon a $NOAEL_{HED}$ of 0.000515 mg/kg/day and a TUF of 30 that included 3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability. A modifying factor of 10 was added for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

An intermediate-duration oral MRL of 2×10^{-8} mg/kg/day was derived for CDDs (specifically for 2,3,7,8-TCDD) based upon decreased thymus weight in guinea pigs (DeCaprio et al. 1986). The MRL is based upon a NOAEL of 7×10^{-7} mg/kg/ day and a TUF of 30, 3 for extrapolation from animals to humans with cross-species comparison and 10 for human variability.

An intermediate-duration oral MRL of 3×10^{-6} mg/kg/day was derived for PBDEs (specifically for lower-brominated diphenyl ethers) based upon decreased serum testosterone in male rats (Zhang et al. 2013). The MRL is based upon a LOAEL of 0.001 mg/kg/day and a TUF of 300, which included 3 for use in a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability (Table 2).

TTDs were derived for PFOA and PFOS for hepatic, immunological, reproductive, and endocrine endpoints in order to calculate HQs and HIs specific to those endpoints. As mentioned above, the investigations were selected similar to MRL selection (i.e., based upon the highest NOAEL or lowest LOAEL in the database for particular health endpoints). Details on the study, specific effects, POD, and UFs used in the derivation process, as well as the final TTD values are presented in Table 3. All calculated values are based upon intermediate-duration oral studies in animals. Briefly, an endocrine TTD for PFOA of 2×10^{-4} mg/kg/day was derived based upon the decreased serum TT4 and FT4 in monkeys (Butenhoff et al. 2002). The TTD was based upon a $NOAEL_{HED}$ of 0.0068 and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability). A hepatic TTD for PFOA of 4×10^{-5} mg/kg/day was derived based upon the increased severity of chronic inflammation in the liver in mice (Filgo et al. 2015). This TTD was based upon a $NOAEL_{HED}$ of 0.0012 and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability). For reproductive effects, a TTD for PFOA of 4×10^{-6} mg/kg/day was derived based upon delayed mammary gland development in dams (White et al. 2011). This TTD was based upon a $LOAEL_{HED}$ of 0.00033 mg/kg/day and a TUF of 90 (3 for animal to human extrapolation with dosimetric adjustment, 10 for human variability, and 3 for use of a minimal LOAEL). Finally, for immunological effects, a TTD for PFOA of 7×10^{-5} mg/kg/day was derived based upon the reduced antibody response in mice (DeWitt et al. 2016). This TTD was based upon a $NOAEL_{HED}$ of 0.0021 mg/kg/day and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability).

For PFOS, an endocrine TTD of 9×10^{-6} mg/kg/ day was derived based upon increased TSH and reduced total T3 in monkeys (Seacat et al. 2002). This TTD was based upon a $NOAEL_{HED}$ of 0.0026 mg/kg/day and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability) as well as a modifying factor of 10 for concerns that immunotoxicity may be a more sensitive endpoint. A hepatic TTD for PFOS of 9×10^{-6} mg/kg/day was derived based upon elevated liver weight, decreased serum cholesterol, hepatocellular hypertrophy, and lipid vacuolation in monkeys (Seacat et al. 2002). This TTD was based upon a $NOAEL_{HED}$ of 0.0026 mg/kg/day and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability) as well as a modifying factor of 10 for concerns that immunotoxicity may be a more sensitive endpoint. A reproductive TTD for PFOS of 9×10^{-6} mg/kg/ day was derived based upon a significant fall in serum estradiol in monkeys (Seacat et al. 2002). This TTD was based upon a $NOAEL_{HED}$ of 0.0026 mg/kg/day and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability) as well as a modifying factor of 10 to parallel the factor applied in MRL development for concern that immunotoxicity may be a more sensitive endpoint. An immunological TTD for PFOS of 3×10^{-6} mg/kg/day was derived based upon impaired response of RBC in mice (Dong et al. 2011). This TTD was based upon a $NOAEL_{HED}$ of 0.000083 mg/kg/day and a TUF of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability).

Details of the study, specific effects, POD, and UFs used in the derivation process for CDDs and PDBEs (POPs) TTD values are presented in Table 4. All of the values derived are based upon intermediate-duration oral investigations in animals. For CDDs, TTD values for hepatic, reproductive, and developmental effects were all lower than the MRL of 2×10^{-8} mg/kg/day; therefore, per ATSDR guidance, there was a default to the MRL value when calculating HQs/HIs for these endpoints. For endocrine effects for CDDs, a TTD value of 3×10^{-8} mg/kg/day was derived based upon a 50% reduction in total serum T4 in male rats (Li and Rozman 1995). This TTD was based upon a NOAEL of 0.000003 mg/kg/day and a TUF of 100 (10 for animal to human extrapolation and 10 for human variability). For PBDEs, an endocrine TTD of 5×10^{-4} mg/kg/day was derived based upon cellular debris in the follicular lumen of thyroid and increased serum testosterone and E2 in mice (Maranghi et al. 2013). This TTD is based upon a LOAEL of 0.45 mg/kg/day and a TUF of 1,000 (10 for animal to human extrapolation, 10 for human variability, and 10 for the use of a LOAEL). A hepatic TTD for PBDEs of 5×10^{-4} mg/kg/day was derived based upon hepatocyte vacuolation, pyknotic nuclei in the hepatocytes, and periportal lymphocytic infiltration in mice (Maranghi et al. 2013). This TTD is based upon a LOAEL of 0.45 mg/kg/day and a TUF of 1,000 (10 for animal to human extrapolation, 10 for human variability, and 10 for the use of a LOAEL). An immunological TTD for PBDEs of 5×10^{-4} mg/kg/day was derived based upon follicular hyperplasia and lymphocytic infiltration in spleen, lymphocytic apoptosis, and Hassal's bodies in the thymus of mice (Maranghi et al. 2013). This TTD is based upon a LOAEL of 0.45 mg/kg/day and a TUF of 1,000 (10 for animal to human extrapolation, 10 for human variability, and 10 for the use of a LOAEL). Finally, a developmental TTD for PBDEs of 3×10^{-5} mg/kg/day was derived based upon impaired learning in mice offspring at post-natal week (PNW) 8 (Koenig et al. 2012). This

TTD is based on a LOAEL of 0.03 mg/kg/day and a TUF of 1,000 (10 for animal to human extrapolation, 10 for human variability, and 10 for the use of a LOAEL).

Using the exposure measurements extracted from ATSDR's toxicological profiles and the corresponding HGVs (either the MRLs or the calculated TTDs), HQs and HIs were calculated for a homogenous mixture of PFOA and PFOS for high, medium, and low exposure scenarios (Table 5). All HIs calculated for high and medium exposure scenarios were above 1, indicating potential concern. In addition, for developmental and immunological effects, the HIs for low exposure scenarios were also greater than 1; this indicates that further evaluation of the potential for developmental and immunological effects would be appropriate.

Table 6 presents the HQs and HIs for the heterogenous mixture of PFOA, PFOS, CDDs, and PBDEs. These values were based upon high exposure scenarios and the HGVs (either the MRLs or the calculated TTDs). Similar to the homogenous mixture, the HIs for all toxicity endpoints were greater than 1, indicating potential concern. The HI for developmental effects in the heterogenous mixture was the highest at 30.99. For comparison, the HI for high-exposure scenarios for developmental effects in the homogenous mixture was 30.67 (Table 5). This indicates that including CDDs and PBDEs in the mixture do not significantly contribute to the overall HI. This is also similar for the other endpoints.

Discussion

The overall goal of the mixture risk assessment at ATSDR is to evaluate the potential for harm from unintended exposure to environmental exposures and to inform the public of the consequence of such exposures. Environmental exposures are complex, consisting of chemicals (identified and unidentified), biological contaminants, and physical matter that might potentially produce adverse health effects. This project focused on the application of the ATSDR mixture guidance: specifically, the demonstration and use of the HI approach for risk assessment of some illustrative PFAS mixtures based upon hypothetical exposure scenarios.

It has become evident from biomonitoring studies that most humans are exposed to PFAS mixtures (CDC 2017). However, to date, few efforts have been undertaken to understand the mechanisms underlying the toxicity of the majority of these PFAS, particularly their mixtures (Kalloo et al. 2020). Epidemiological studies assessed and established associations between PFAS exposure and a wide range of potential adverse health effects (ATSDR 2018a; CDC 2017; ITRC (Interstate Technology & Regulatory Council) 2020; Preston et al. 2020). However, there are two major limitations to establishing the dose response relationships in these studies. First, the lack of adequate characterization of exposure to PFAS in the environment, and second, the possibility of exposures to mixtures including any other pollutants, such as POPs.

A plausible way to understand the dose–response relationships is through controlled experimental toxicology investigations that are mostly conducted in animals. Borg et al. (2013) performed a risk assessment for 17 PFAS for the general population and an

occupationally exposed group using the HI approach. Their assessment included broad assumptions, use of read-across and extrapolation of risk for some of the compounds. HIs were developed separately for “hepatotoxicity” and “reproductive toxicity” for the general population, and the results did not show cause for concern, except for a small sub-population of high fish consumers. However, Borg et al. (2013) indicated a possible concern for occupationally exposed groups.

Carr et al. (2013) studied *in vitro*, in a mouse cell line (COS-1), the relationship among mixtures of 4 PFAS: PFOA, PFNA, PFOS, and PFHxS. Data demonstrated that individual PFAS activate PPARα in the mouse cell line (COS-1). A non-linear logistic dose additivity model was employed to predict relative luciferase units, an indicator of PPARα activation. Less than dose additivity was reported for the mixture of 4 and also for binary combinations of the chemicals. Carr et al. (2013) concluded that dose additive models may be applied to assess the joint toxicity of mixtures containing these components with a caveat for potential antagonistic interaction. Wolf et al. (2014) continued *in vitro* testing with an aim to determine if binary combinations of PFOA and other PFAS initiate a dose additive effect on PPARα activation in the mouse one-hybrid *in vitro* model, COS-1 cells. The results supported dose additivity at the lower concentration ranges. Hu et al. (2014) examined the influence of individual and mixtures of PFAS in a human liver cell line (HL-7702) using the MTT colorimetric assay for assessing cell metabolic activity. Hu et al. (2014) concluded that three binary mixtures displayed synergistic effects; however, a mixture of 11 PFAS resulted in partial additivity.

PFAS has been associated with multiple toxicities that are induced by various mechanisms of actions, PPARα being one of them. PPARα mediates developmental and reproductive effects. PFAS induces the expression of PPARα and constitutive activated/androstane receptor (CAR)-related genes in the liver, indicating that hepatotoxic effects of PFAS may be initiated through multiple nuclear receptors (NTP 2019). Thus, even though these studies highlight the roles for PPARα and CAR, these do not capture the full suite of nuclear receptors that have been identified as potentially contributing to PFAS-induced toxicity. In addition to PPARα and CAR, *in vitro* reporter gene studies demonstrated that PFAS might bind to and activate the thyroid receptor. Therefore, it is unlikely that grouping strategies and mixture methods that focus on a specific nuclear receptor like PPARa might accurately predict human risk. Thus, PFAS risk assessments need to continue to include uncertainties until further insight is gained into the mechansims of PFAS-mediated toxicity (Goodrum et al. 2020).

The PFAS toxicological profile identified a need for studies evaluating potential interactions between perfluoroalkyl compounds and between perfluoroalkyl compounds and other chemicals (ATSDR 2018a). The Toxic Equivalency Factors (TEFs) approach is a potency-based scaling system, where potencies for individual components of a mixture are calculated with reference to an indicator chemical (Birnbaum and Staskal-Wikoff 2010). Attempts were made to derive TEFs for the PFAS, but several conditions required to justify this approach were not met, including knowledge of mechanistic differences in their toxicities (Peters and Gonzalez 2011). Hence, this approach has not been further developed or accepted for PFAS. A more generic method, the relative potency factors (RPFs) approach, might facilitate

mixture risk assessment (Bil et al. 2020). Several computational and statistical tools were also developed for advanced analysis to predict internal concentrations of PFAS (Braun et al. 2016; Worley and Fisher 2015; Worley et al. 2017a; Wambaugh et al. 2013; Lau et al. 2006; Luebker et al. 2005) to improve risk assessments.

There are other new alternative methods (NAMs) such as read-across, structure–activity relationship (SAR), and quantitative structure–activity relationship (QSAR) that are being used for toxicity assessment of other environmental pollutants; however, minimum amount of data needed to use such techniques is not available for PFAS. Advanced complex methods continue to be developed for risk assessment of chemical mixtures that take into consideration multiple mechanisms and pathways that involve crosstalk of receptors (Sharma, Schumacher, and Kumar 2017). Theoretically, complex models and methods such as these may be conceptualized and developed; however, the general applicability of such models for effect predictions is limited because of lack of reliable exposure determinations. Currently available individual chemical toxicity data of the PFAS were used and applied the ATSDR framework to evaluate mixture toxicity. Illustrative binary mixtures of PFOA and PFOS and a quaternary mixture of PFOA, PFOS with CDD and PBDEs revealed the following:

The HIs for the binary mixture of PFOA and PFOS for five toxicity endpoints – developmental, reproductive, hepatic, immunological, and endocrine (i.e., thyroid) – were determined for high, medium and low lifetime exposures (Table 5). For developmental effects, HIs were 30.67, 8.33 and 3.63, respectively. If this illustrative example was a real-life exposure scenario, there would be a potential concern for developmental effects for individuals under the highest exposure scenario; additionally, there may be some concern for developmental effects following medium and low lifetime exposures that require further analysis. On the other hand, there would be less concern for reproductive and thyroid effects for similar lifetime exposure scenarios. The HIs for the heterogenous mixture of PFOA, PFOS, CDD and PBDEs were greater than 1 for all toxicity end points investigated (HI = 30.99 to 3.64) (Table 6). Public health assessors need to be cognizant of potential co-exposure of PFAS with other chemicals. However, some of these chemicals were phased out, and so their current exposure levels may be lower than those used in these examples (Lynch et al. 2019).

Environmental media found at National Priorities List (NPL) sites can contain mixtures of chemicals. Combined exposure to these chemicals may produce effects different from those expected following a single-chemical exposure. If the toxicity assessment is limited to the critical effects for which MRLs are available, there is a possibility risk characterization might not adequately account for complex environmental exposures. The TTD approach was proposed to evaluate other effects in addition to the critical effect (Mumtaz, Poirier, and Colman 1997). The HI approach integrates MRLs for critical effects and TTDs for secondary effects, thus enabling identification and characterization of multiple health effects as if the mixture has been experimentally tested to account for all pertinent biological effects. These types of analyses also provide health assessors and decision makers an awareness of the other potential health effects possible in a population. This information

may be utilized to calculate, relative contributions of each chemical component by route and identify initially if a particular route plays a major role.

The HI approach is intended to serve only as a screening tool to help decide if further evaluation of exposures is needed. Such analyses might include exposure-specific parameters, internal dosimetry, mechanism of action, dose response analysis, derivation of TTDs, and WOE analysis for chemical interactions (ATSDR 2018a). ATSDR does not use HI values for cleanup or action levels as some other organizations do. An HI of 30 is not quantitatively twice as hazardous as an HI value of 15, or a value of 15 is not 10-fold more hazardous than an HI of 1.5. It can be stated that a site with a hazard index of 30 might be relatively more hazardous than the one with a HI value of 15 when the mixtures consist of same chemical components. Exposures above the HI value of 1 do not infer that adverse health effects will occur.

There are different types of uncertainties inherent to the derivation of the MRLs and TTDs that get embedded in the HI values. Thus, not all MRLs, TTDs and HIs are similar in accuracy and precision and have varying degrees of associated uncertainties depending upon the method used to determine each POD. These methods vary from measurements of actual external exposures, use of default values, TWA, BMD analysis, dosimetric adjustments, and target organ or system-specific concentrations using physiologically based pharmacokinetic (PBPK) models. Thus, UFs might vary from 1 to 3,000. Most often, a total UF of <1,000 is used (https://www.atsdr.cdc.gov/mrls/mrllist.asp).

The HI is a practical field tool, hence, most often used in the health assessment of chemicals that utilize a larger database available on the toxicity of individual hazardous chemicals. However, the HI approach does not enable integration of nonadditive interactions that might alter the joint toxicity outcome. To address this issue, a binary WOE scheme for interactions is recommended in the tier 3 of the assessment process (ATSDR 2018b; Mumtaz and Durkin 1992). This scheme might be employed for PFAS, but currently there is a lack of interaction information to use this scheme. Finally, the HI approach is limited by exposure estimates and toxicological investigations for the chemicals of interest. For example, ATSDR derived MRLs for PFNA and PFHxS in addition to PFOA and PFOS, and there are toxicological studies that have investigated effects on various systems. However, there are no general population exposure estimates available for PFNA and PFHxS. This data gap precluded us from including them in this mixture analysis.

Conclusions

Major data gaps exist in our understanding of the mechanisms underlying PFAS-induced toxicity. However, CDC’s National Health and Nutrition Examination Survey (NHANES) reports the presence of some of these chemicals in the general U.S. population. There is a need to conduct toxicity assessments of these compounds to inform the public of the consequences of such exposures. Data gaps limit the assessment of toxicity resulting from environmental mixtures of PFAS and other pollutants. The HI approach is preferred when extensive toxicity information is not available on the whole mixture. The HI is the sum of the respective hazard quotients (HQs) of individual components of a mixture calculated

as the ratio between exposure (daily intake) and a limit value of a component. This is a practical approach that uses data from individual PFAS to assess their joint toxicity in mixtures.

This study provides a "proof-of-concept" illustration for the HI approach. Details of the stepwise procedure of the HI approach were provided using illustrative binary and quarternary PFAS mixtures. Further, it was also shown that this approach might be used to assess multiple toxicity endpoints of a given mixture. The HI values are not precise for quantitative assessments but might be employed for prioritization of exposures, particularly at waste sites. This approach may be further refined using data forthcoming from ongoing research efforts at several academic institutions and federal agencies such as the National Institute of Environmental Health Sciences (NIEHS) and EPA. As part of these efforts, differences in the pharmacokinetic and pharmacodynamic properties of PFAS, both *in vivo* and *in vitro*, are being actively investigated. The information generated might broaden our understanding and provide insight into the molecular, biological, metabolic, and physiological bases of observed toxicities. These factors are critical in quantifying joint toxicity and need to be incorporated into the health risk assessment process as more data become available. In summary, PFAS risk assessments continue to include uncertainties until further insights are gained into the mechansims underlying PFAS-mediated toxicity.

References

ATSDR. 1998. Toxicological profile for CDDS. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Agency for Toxic Substances Disease Registry https://www.atsdr.cdc.gov/toxprofiles/index.asp

ATSDR. 2005. Public health assessment guidance manual (update). U.S. Department of Health and Human Services, Agency for Toxic Substances Disease Registry. https://www.atsdr.cdc.gov/hac/phamanual/toc.html. October 2020.

ATSDR. 2017 . Toxicological profile for PBDEs. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Agency for Toxic Substances Disease Registry https://www.atsdr.cdc.gov/toxprofiles/index.asp

ATSDR 2018a. Toxicological profile for perfluoroalkyls, *draft for public comment*. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Agency for Toxic Substances Disease Registry https://www.atsdr.cdc.gov/toxprofiles/index.asp

ATSDR 2018b. Framework for assessing health impacts of multiple chemicals and other stressors. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Agency for Toxic Substances Disease Registry https://www.atsdr.cdc.gov/interactionprofiles/index.asp

Barry V, Winquist A, and Steenland K. 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ. Health Perspect. 121 (11–12):1313–18. doi:10.1289/ehp.1306615. [PubMed: 24007715]

Bil W, Zeilmaker M, Fragki S, Lijzen J, Verbruggen E, and Bokkers B. 2020. Risk assessment of per- and polyfluoroalkyl substance mixtures: A relative potency factor approach. Environ Toxicol Chem. 40 (3):859–870 *ePub before print*.

Birnbaum LS, and Staskal-Wikoff DS. 2010. 5th international PCB workshop — Summary and implications. Environ Int 36 (8):814–18. doi:10.1016/j.envint.2010.06.011. [PubMed: 20728937]

Borg D, Lund B-O, Lindquist N-G, and Hakansson H. 2013. Cumulative health risk assessment of 17 perfluoroalkylated and polyfluorinated substances (PFASs) in the Swedish population. Environ. Int 59:112–23. doi:10.1016/j.envint.2013.05.009. [PubMed: 23792420]

Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, and Lamphear BP. 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age. The HOME study. Obesity 24:231–37. doi:10.1002/oby.21258. [PubMed: 26554535]

Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, Jung R, Jr KG, Lieder P, Olsen G, et al. 2002. Toxicity of ammonium perfluorooctanoate in male Cynomolgus monkeys after oral dosing for 6 months. Toxicol. Sci 69 (1):244–57. doi:10.1093/toxsci/69.1.244. [PubMed: 12215680]

Butenhoff JL, Kennedy GL, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, Gorman GS, Noker PE, and Thomford PJ. 2004. Pharmacokinetics of perfluorooctanoate in Cynomolgus monkeys. Toxicol. Sci 82(2):394–406. doi:10.1093/toxsci/kfh302. [PubMed: 15470233]

Carr CK, Watkins AM, Wolf CJ, Abbott BD, Lau C, and Gennings C. 2013. Testing for departures from additivity in mixtures of perfluoroalkyl acids (PFAAs). Toxicology 306:169–75. doi:10.1016/j.tox.2013.02.016. [PubMed: 23470359]

CDC. 2017. Fourth national report on human exposure to environmental chemicals, Updated Tables, Volume One, January.

Chang S, Noker PE, Gorman GS, Gibson SJ, Hart JA, Ehresman DJ, and Butenhoff JL. 2012. Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reprod. Toxicol 33 (4):428–40. doi:10.1016/j.reprotox.2011.07.002. [PubMed: 21889587]

Corton JC, Cunningham ML, Hummer BT, Lau C, Meek B, Peters JM, Popp JA, Rhomberg L, Seed J, and Klaunig JE. 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study. Crit. Rev. Toxicol 44:1–49.

DeCaprio AP, McMartin DM, O'Keefe PW, Rej R, Silkworth JB, and Kaminsky LS. 1986. Subchronic oral toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the guinea pig: Comparisons with a PCB-containing transformer fluid pyrolysate. Fundam Appl Toxicol 6 (3):454–63. doi:10.1016/0272-0590(86)90219-8. [PubMed: 3084328]

DeWitt JC, Williams WC, Nj C, and Rv L. 2016. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPAR α and T- and B-cell targeting. J Immunotoxicol 13 (1):38–45. doi:10.3109/1547691x.2014.996682. [PubMed: 25594567]

Dong GH, Liu MM, Wang D, Zheng L, Liang ZF, and Jin YH. 2011. Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. Arch. Toxicol 85 (10):1235–44. doi:10.1007/s00204-011-0661-x. [PubMed: 21327619]

EPA. 2016b. Drinking water health advisory for perfluorooctanoic acid (PFOA). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final-plain.pdf. March 3, 2017.

EPA. 2016c. Drinking water health advisory for perfluorooctane sulfonate (PFOS). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final-plain.pdf. March 3, 2017.

EPA. 2020. Regional Screening Levels (RSLs) - User's Guide. https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide (accessed September 10, 2020),

Filgo AJ, Quist EM, Hoenerhoff MJ, Brix AE, Kissling GE, and Fenton SE. 2015. Perfluorooctanoic Acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARalpha is not required. Toxicol Pathol 43 (4):558–68. doi:10.1177/0192623314558463. [PubMed: 25398757]

Goodrum PG, Anderson JK, Luz AL, and Ansell GK. 2020. Application of a framework for grouping and mixtures toxicity assessment of PFAS: A closer examination of dose-additivity approaches. Toxicol. Sci 179 (2):262–78. doi:10.1093/toxsci/kfaa123.

Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, and Budtz-Jørgensen E. 2017. Serum vaccine antibody concentrations in adolescents exposed to perfluorinated comopounds. Environ. Health Perspect 125 (1–7):077018. doi:10.1289/EHP275. [PubMed: 28749778]

Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, and Heilmann C. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. J. Am. Med. Assoc 307 (4):391–97. doi:10.1001/jama.2011.2034.

Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, and Koizumi A. 2005. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ. Res 99 (2):253–61. doi:10.1016/j.envres.2004.12.003. [PubMed: 16194675]

Hu J, Li J, Wang J, Zhang A, and Dai J. 2014. Synergistic effects of Perfluoroalkyl acids mixtures with J-shaped concentration-responses on viability of a human liver cell line. Chemosphere 96:81–88. doi:10.1016/j.chemosphere.2013.07.033. [PubMed: 23942018]

IARC. 2017. Agents classified by the IARC Monographs, Volumes 1–117. Lyon, France: International Agency for Research on Cancer.

ITRC (Interstate Technology & Regulatory Council). 2020. PFAS Technical and regulatory guidance document and Fact Sheets PFAS-1. Washington, D.C: Interstate Technology & Regulatory Council, PFAS Team. https://pfas-1.itrcweb.org/.

Kalloo G, Wellenius G, McCandless L, Calafat A, Sjodin A, Romano M, Karagas M, Chen A, Yolton K, Lanphear B, et al. 2020. Exposures to chemical mixtures during pregnancy and neonatal outcomes: The HOME study. Environ. Int 134:1–10. doi:10.1016/j.envint.2019.105219.

Koenig CM, Lango J, Pessah IN, and Berman RF. 2012. Maternal transfer of BDE-47 to offspring and neurobehavioral development in C57BL/6J mice. Neurotoxicol Teratol 34 (6):571–80. doi:10.1016/j.ntt.2012.09.005. [PubMed: 23022914]

Koskela A, Finnila MA, Korkalainen M, Spulber S, Koponen J, Håkansson H, Tuukkanen J, and Viluksela M. 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicol. Appl. Pharmacol 301:14–21. doi:10.1016/j.taap.2016.04.002. [PubMed: 27068293]

Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, and Stynar MJ. 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci 90 (2):510–18. doi:10.1093/toxsci/kfj105. [PubMed: 16415327]

Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BA, Lindh CH, Piersma AH, Toft G, Bonde JP, Heederik D, Rylander L, & Vermeulen R (2016). Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ. Health Perspect 124(3), 365–372. 10.1289/ehp.1408933 [PubMed: 26115335]

Li X, and Rozman KK. 1995. Subchronic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and their reversibility in male Sprague-Dawley rats. Toxicology 97 (1–3):133–40. doi:10.1016/0300-483X(94)02938-Q. [PubMed: 7716779]

Luebker DJ, Case MT, York RG, Moore JA, Hansen KJ, and Butenhoff JL. 2005. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 215 (1–2):126–48. doi:10.1016/j.tox.2005.07.018. [PubMed: 16146667]

Lynch KM, Fair PA, Houde M, Derek CM, Kannan K, Bossart GD, Bartell SM, and Gribble MO. 2019. Temporal trends in per- and polyfluoroalkyl substances in bottlenose dolphins (*Tursiops truncatus*) of Indian River Lagoon, Florida and Charleston, South Carolina. Environ. Sci. Technol 53 (24):14194–203. doi:10.1021/acs.est.9b04585. [PubMed: 31804805]

Maranghi F, Tassinari R, Moracci G, Altieri I, Rasinger JD, Carroll TS, Hogstrand C, Lundebye AK, and Mantovani A. 2013. Dietary exposure of juvenile female mice to polyhalogenated seafood contaminants (HBCD, BDE-47, PCB-153, TCDD): Comparative assessment of effects in potential target tissues. Food Chem. Toxicol 56:443–49. doi:10.1016/j.fct.2013.02.056. [PubMed: 23500779]

Mi X, Yang Y, Zeeshan M, Wang Z, Zeng X, Zhou Y, Yang A, Hu L, Yu H, Zeng X, et al. 2020. Serum levels of per- and polyfluoroalkyl substances alternatives and blood pressure by sex status: Isomers of C8 health project in China. Chemosphere 261:1–8. doi:10.1016/j.chemosphere.2020.127691.

Mumtaz MM, Poirier KA, and Colman JT. 1997. Risk assessment for chemical mixtures: Fine-tuning the hazard index approach. J Clean Technol Environ Toxicol Occup Med 6:189–204.

Mumtaz MM, and Durkin P. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicology and Industrial Health 8 (6):377–406. doi:10.1177/074823379200800604. [PubMed: 7570620]

NTP. 2016. NTP monograph on immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). U.S. Department of Health and Human Services, National Institutes of Health, National Toxicology Program. https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf

NTP. 2019. NTP technical report on the toxicity studies of perfluoroalkyl carboxylates (Perfluorohexanoic Acid, Perfluorooctanoic Acid, Perfluorononanoic Acid, and Perfluorodecanoic Acid) administered by gavage to Sprague Dawley (Hsd: Sprague Dawley SD) Rats. National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, North Carolina.

Olsen GW, Burris JM, Ehresman DJ, Froelich JW, Seacat AM, Zobel LR, Butenhoff JL, Butenhoff JL, Froehlich JW, Seacat AM, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect 115 (9):1298–305. doi:10.1289/ehp.10009. [PubMed: 17805419]

Olsen GW, Burris JM, Burlew MM & Mandel JH 2000. Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drag Chem Toxicol. 23(4):603–20. doi:10.1081/DCT-100101973.

Peters JM, and Gonzalez FJ. 2011. Why toxic equivalency factors are not suitable for perfluoroalkyl chemicals. Chem. Res. Toxicol 24 (10):1601–09. doi:10.1021/tx200316x. [PubMed: 21913657]

Preston E, Webster T, Henn B, McClean M, Gennings C, Okend E, Rifas-Shimand S, Pearce E, Calafat A, Fleisch A, et al. 2020. Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: A mixtures approach. Environ. Int 139:1–11. doi:10.1016/j.envint.2020.105728.

Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, and Butenhoff JL. 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in Cynomolgus monkeys. Toxicol. Sci 68 (1):249–64.doi: 10.1093/toxsci/68.1.249. [PubMed: 12075127]

Sharma R, Schumacher M, and Kumar V. 2017. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. Environ. Int 99:1–14. doi:10.1016/j.envint.2016.09.016. [PubMed: 27697394]

Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, Freedman ND, Sampson JN, Silverman DT, Purdue MP, et al. 2020. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. J. Natl. Cancer Inst Epub ahead of print. doi:10.1093/jnci/djaa143.

Trudel D, Horowitz L, Wormuth M, Scheringer M, Cousins IT, and Hungerbühler K. 2008. Estimating consumer exposure to PFOS and PFOA. Risk Anal. 28 (2):251–69. doi:10.1111/j.1539-6924.2008.01017.x. [PubMed: 18419647]

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 2006 Oct;93(2):223–41. doi: 10.1093/toxsci/kfl055. Epub 2006 Jul 7. [PubMed: 16829543]

Vieira VM, Hoffman K, Shin HM, Weinberg JM, Webster TF, and Fletcher T. 2013. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: A geographic analysis. Environ. Health Perspect 121 (3):318–23. doi:10.1289/ehp.1205829. [PubMed: 23308854]

Wambaugh JF, Setzer RW, Pitruzzello AM, Liu J, Reif DM, Kleinstreuer NC, Ching N, Wang Y, Sipes N, Martin M, et al. 2013. Dosimetric anchoring of *in vivo* and *in vitro* studies for perfluorooctanoate and perfluorooctanesulfonate. Toxicol. Sci 136 (2):308–27. doi:10.1093/toxsci/kft204. [PubMed: 24046276]

White SS, Stanko JP, Kato K, Calafat AM, Hines EP, and Fenton SE. 2011. Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environ. Health Perspect 119 (8):1070–76. doi:10.1289/ehp.1002741. [PubMed: 21501981]

Winquist A, and Steenland K. 2014. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environ. Health Perspect 122 (12):1299–305. doi:10.1289/ehp.1307943. [PubMed: 25260175]

Wolf CJ, Rider CV, Lau C, and Abbott BD. 2014. Evaluating the additivity of Perfluoroalkyl acids in binary combinations on peroxisome proliferator-activated receptor-α activation. Toxicology 316:43–54. doi:10.1016/j.tox.2013.12.002. [PubMed: 24374136]

Worley RR, and Fisher J. 2015. Application of physiologically based pharmacokinetic modeling to explore the role of kidney transporters in renal reabsorption of perfluorooctanoic acid in the rat. Toxicol. Appl. Pharmacol 289(3):428–41. doi:10.1016/j.taap.2015.10.017. [PubMed: 26522833]

Worley RR, Moore SM, Tierney BC, Ye X, Calafat AM, Campbell S, Woudneh MB, and Fisher J. 2017. Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. Environ Int 106:135–43. doi:10.1016/j.envint.2017.06.007. [PubMed: 28645013]

Zhang Z, Zhang X, Sun Z, Dong H, Qiu L, Gu J, Zhou J, Wang X, and Wang SL. 2013. Cytochrome P450 3A1 mediates 2,2′,4,4′-tetrabromodiphenyl ether-induced reduction of spermatogenesis in adult rats. PLoS ONE 8 (6):e66301. doi:10.1371/journal.pone.0066301. [PubMed: 23762486]

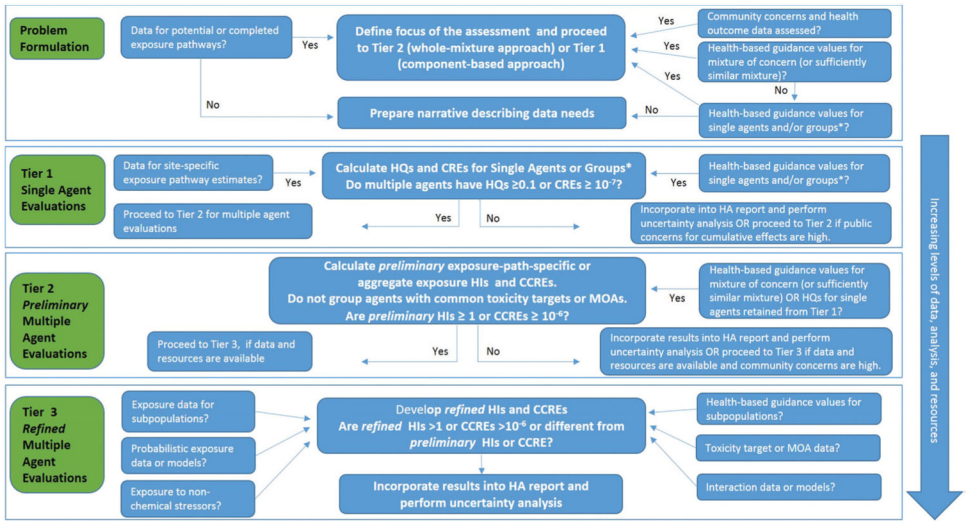

Figure:
The 2018 ATSDR mixture framework tiered approach (ATSDR 2018b).

Table 1.

First-order one-compartment model parameters used for PFOA and PFOS TTD calculations.

Parameter	PFOA	PFOS	Reference
Serum elimination half-life; $t_{1/2}$ (day)	1,400	2,000	Olsen et al. 2007
Serum elimination rate constant, k_e (day^{-1})	$4.95x10^{-4}$	$3.47x10^{-4}$	Calculated using Eq. 2
Gastrointestinal absorption fraction, AF	1	1	
Apparent volume of distribution, V_d (L/kg)	0.2	0.2	Butenhoff et al. 2004; Chang et al. 2012; Harada et al. 2005

Table 2.

ATSDR's intermediate-duration oral MRLs for PFAS and POPs.

	Endpoint	Effect	POD (mg/kg/day)	UF/ MF	MRL (mg/kg/ day)	References
PFOA	Developmental	Skeletal effects in mice	0.000821 ($LOAEL_{HED}$)	300[a]	$3x10^{-6}$	Koskela et al. 2016; ATSDR 2018a
PFOS	Developmental	Delayed eye opening and decreased pup weight in rats	0.000515 ($NOAEL_{HED}$)	30/10[b]	$2x10^{-6}$	Luebker et al. 2005; ATSDR 2018a
CDDs[c]	Immune	Decreased thymus weight in guinea pigs	0.0000007 (NOAEL)	30[d]	$2x10^{-8}$	DeCaprio et al. 1986; ATSDR 1998
PBDEs[e]	Reproductive	Decreased serum testosterone in male rats	0.001 (LOAEL)	300[f]	$3x10^{-6}$	Zhang et al. 2013; ATSDR 2017

[a] 10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability

[b] 3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability; 10 MF for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

[c] Based on 2,3,7,8-TCDD

[d] 3 for extrapolation from animals to humans with cross-species comparison; 10 for human variability

[e] Lower brominated diphenyl ethers

[f] 3 for use of a minimal LOAEL; 10 for extrapolation from animals to humans; 10 for human variability

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; MF = modifying factor; MRL = minimal risk level; NOAEL = no-observed-adverse-effect level; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; UF = uncertainty factor;

Table 3.

Calculated TTDs for PFAS.

Endpoint	Effect	POD (mg/kg/day)	UF/MF	TTD (mg/kg/day)	Reference
PFOA					
Endocrine	Decrease in serum TT4 (27–35%) and FT4 (30–38%)	0.006783 ($NOAEL_{HED}$)	30[a]	$2x10^{-4}$	Butenhoff et al. 2002
Hepatic	Increased severity of chronic inflammation in the liver	0.00124 ($NOAEL_{HED}$)	30[a]	$4x10^{-5}$	Filgo et al. 2015
Repro	Delayed mammary gland in dams	0.00033 ($LOAEL_{HED}$)	90[b]	$4x10^{-6}$	White et al. 2011
Immune	Reduced antibody response	0.00211 ($NOAEL_{HED}$)	30[a]	$7x10^{-5}$	DeWitt et al. 2016
PFOS					
Endocrine	Increased TSH and decreased total T3	0.00262 ($NOAEL_{HED}$)	300[c]	$9x10^{-6}$	Seacat et al. 2002
Hepatic	Increased liver weight, decreased serum cholesterol, hepatocellular hypertrophy, lipid vacuolation	0.00262 ($NOAEL_{HED}$)	300[c]	$9x10^{-6}$	Seacat et al. 2002
Repro	Significant decrease in serum estradiol on days 62 (48%), 91 (42%), and 182 (96%); no histological alterations	0.00262 ($NOAEL_{HED}$)	300[c]	$9x10^{-6}$	Seacat et al. 2002
Immune	Impaired response to sRBC in mice exposed for 60 days	0.000083 ($NOAEL_{HED}$)	30[a]	$3x10^{-6}$	Dong et al. 2011

[a] 3 for animal to human extrapolation with dosimetric adjustment; 10 for human variability

[b] 3 for animal to human extrapolation with dosimetric adjustment; 10 for human variability; 3 for use of a minimal LOAEL

[c] 3 for animal to human extrapolation with dosimetric adjustment; 10 for human variability; 10 MF for concern that immunotoxicity may be a more sensitive endpoint

J Toxicol Environ Health A. Author manuscript; available in PMC 2022 May 05.

Table 4.

Calculated TTDs for POPs.

Endpoint	Effect	POD (mg/kg/day)	UF/MF	TTD (mg/kg/day)	Reference
CDDs					
Endocrine	50% reduction in total serum T4	0.000003 (NOAEL)	100[a]	$3x10^{-8}$	Li and Rozman 1995
Hepatic	Default to MRL value of 2×10^{-8} mg/kg/day				
Repro	Default to MRL value of 2×10^{-8} mg/kg/day				
Developmental	Default to MRL value of 2×10^{-8} mg/kg/day				
PBDEs					
Endocrine	Cellular debris in the follicular lumen of thyroid; increased serum testosterone and E2	0.45 (LOAEL)	1000[b]	$5x10^{-4}$	Maranghi et al. 2013
Hepatic	Hepatocyte vacuolation, pyknotic nuclei in the hepatocytes, periportal lymphocytic infiltration	0.45 (LOAEL)	1000[b]	$5x10^{-4}$	Maranghi et al. 2013
Immune	Follicular hyperplasia and lymphocytic infiltration in spleen; lymphocytic apoptosis and Hassal's bodies in thymus	0.45 (LOAEL)	1000[b]	$5x10^{-4}$	Maranghi et al. 2013
Developmental	Impaired learning in offspring at PNW 8	0.03 (LOAEL)	1000[b]	$3x10^{-5}$	Koenig et al. 2012

[a] 10 for animal to human extrapolation; 10 for human variability

[b] 10 for animal to human extrapolation with dosimetric adjustment; 10 for human variability; 10 for use of a LOAEL

Table 5.

HQs and HIs for a homogenous mixture of PFOA and PFOS for three levels of exposure scenarios.

		Developmental		Immunological		Endocrine		Hepatic		Reproductive	
	Exposure Estimate	**HGV**	**HQ**	**HGV**	**HQ**	**HGV**	**HQ**	**HGV**	**HQ**	**HGV**	**HQ**
High											
PFOA	$4.7x10^{-5}$	$3x10^{-6}$	15.67	$7x10^{-5}$	0.67	$2x10^{-4}$	0.235	$4x10^{-5}$	1.175	$4x10^{-6}$	11.75
PFOS	$3x10^{-5}$	$2x10^{-6}$	15	$3x10^{-6}$	10	$9x10^{-6}$	3.33	$9x10^{-6}$	3.33	$9x10^{-6}$	3.33
HIs			**30.67**		**10.67**		**3.57**		**4.51**		**15.08**
Medium											
PFOA	$2.5x10^{-6}$	$3x10^{-6}$	0.833	$7x10^{-5}$	0.036	$2x10^{-4}$	0.0125	$4x10^{-5}$	0.0625	$4x10^{-6}$	0.625
PFOS	$1.5x10^{-5}$	$2x10^{-6}$	7.5	$3x10^{-6}$	5	$9x10^{-6}$	1.67	$9x10^{-6}$	1.67	$9x10^{-6}$	1.67
HIs			**8.33**		**5.036**		**1.68**		**1.73**		**2.29**
Low											
PFOA	$4x10^{-7}$	$3x10^{-6}$	0.133	$7x10^{-5}$	0.0057	$2x10^{-4}$	0.002	$4x10^{-5}$	0.01	$4x10^{-6}$	0.1
PFOS	$7x10^{-6}$	$2x10^{-6}$	3.5	$3x10^{-6}$	2.33	$9x10^{-6}$	0.778	$9x10^{-6}$	0.78	$9x10^{-6}$	0.78
HIs			**3.63**		**2.34**		**0.78**		**0.79**		**0.88**

J Toxicol Environ Health A. Author manuscript; available in PMC 2022 May 05.

Table 6.

HQs and HIs for heterogeneous mixture of PFOA, PFOS, CDDs, and PBDE for high-exposure scenarios.

			Developmental		Immunological		Endocrine		Hepatic		Reproductive	
		Exposure Estimate	HGV	HQ	HGV	HQ	HGV	HQ	HGV	HQ	HGV	HQ
High												
	PFOA	$4.7x10^{-5}$	$3x10^{-6}$	15.67	$7x10^{-5}$	0.67	$2x10^{-4}$	0.235	$4x10^{-5}$	1.175	$4x10^{-6}$	11.75
	PFOS	$3x10^{-5}$	$2x10^{-6}$	15	$3x10^{-6}$	10	$9x10^{-6}$	3.33	$9x10^{-6}$	3.33	$9x10^{-6}$	3.33
	CDDs	$1.7x10^{-9}$	$2x10^{-8}$	0.085	$2x10^{-8}$	0.085	$3x10^{-8}$	0.057	$2x10^{-8}$	0.085	$2x10^{-8}$	0.085
	PBDEs	$7.1x10^{-6}$	$3x10^{-5}$	0.24	$5x10^{-4}$	0.014	$5x10^{-4}$	0.014	$5x10^{-4}$	0.014	$3x10^{-6}$	2.37
	HIs			**30.99**		**10.77**		**3.64**		**4.61**		**17.36**