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Hazard/Risk Assessment

Suggestions for Improving the Characterisation of Risk from Exposures to Per and Polyfluorinated Alkyl Substances (PFAS).

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Abstract: Many state and Federal environmental and health agencies have developed risk-based criteria for assessing the risk of adverse health effects of PFAS exposure to

humans and the environment. However, the criteria that have been developed vary; drinking water criteria developed for PFOA, for example, can vary by up to 750. This is due to differences and variability in the data and information used, study/endpoint selection, assumptions and magnitude of uncertainty factors used in the absence and extrapolation of critical effect data, differences in underlying approaches to addressing exposure within criteria development, and/or policy decisions on levels of acceptable risk. Here we have critically evaluated the methods used to develop these criteria while focussing on derivation and application of drinking water criteria and discuss a range of improvements to risk characterisation practice recently presented at a SETAC Focused Topic Meeting on PFAS conducted by the Society of Environmental Toxicology and Chemistry in Durham, North Carolina, USA 12-15 AUG 2019. Here we propose methods that consider maximizing the use of disparate data streams, seeking patterns, and proposing biological-based approaches to evidence integration towards informed criteria development.

Keywords: PFAS, risk characterisation, evidence integration, risk assessment, guideline values

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INTRODUCTION

It is within the responsibility of many government organizations to protect human health and the environment from the adverse effects from exposures to

chemicals. Legislation and regulations establish risk management frameworks traversing a broad range of potential public exposures, including consumer products, the environment and workplaces. The risk assessment process requires problem identification, hazard identification and assessment (deriving health-based guideline values; HBGVs), exposure assessment and risk characterisation. Risk characterisation methods can be used for both site-specific risk assessments as well as the development of site specific or population level risk-based criteria.

Per and polyfluorinated alkyl substances are an extremely variable in structure, category of compounds representing over 4,000 individual man-made molecules that have been used in a wide array of consumer and industrial products. Some of these compounds are resistant to environmental degradation, and have shown to accumulate in humans (Olsen et al. 2003; Vierke et al. 2012; Pérez et al. 2013). Human serum concentrations are ubiquitous but highly variable. This variability may be influenced by age and lifestyle but is most certainly due to environmental contamination in areas of manufacturing, use of fire-fighting foams, and in some agricultural use. However, even in areas with no manufacturing, elevated PFAS levels have been found suggesting diverse sources of exposure (Manzano-Salgado et al. 2016; Hu et al. 2018; Boronow et al. 2019). Recent testing of human sera show declining concentrations of key PFAS, such as PFOA and PFOS, in the general population (CDC 2016; Toms et al. 2019). Typical concentrations in the environment are also variable with most of the focus on drinking water concentrations in jurisdictions where groundwater and/or surface water used for drinking water supply is affected (Scher et al. 2018). In some jurisdictions, drinking water is less of an issue and soils, food, and biota with elevated PFAS concentrations are the primary driver for exposure (Vestergren and Cousins 2009; Thompson et al. 2011; Shan et al. 2016).

Many federal regulatory agencies have developed their own HBGVs which are then used to derive risk-based exposure criteria for environmental media such as drinking water, soil, and food that are intended to be protective of chronic exposure of the general population. However, there can be considerable variability in interpretation of toxicity data and how assumptions and measures to consider uncertainty are applied to develop these criteria with risk positions developed by different authorities varying by up to 750-fold for PFOA (Dourson et al. 2019). Similarly, these federal agencies have different ways to approach partitioning of background PFAS exposures in the development of these criteria, have differing background exposures, and finally have policy positions on acceptable risk levels that may differ. In addition to federal positions, numerous state or provincial authorities have added complexity and confusion to this array of risk positions, making international meetings, such as the one prompting this manuscript, important avenues for collaborative interactions and harmonization.

Here we have focussed on the risk characterisation process involved in the HBGV derivation and consider its application in drinking water criteria developed by various jurisdictions, identify differences and provide suggestions for improving these criteria. Many of the suggestions outlined here were based on discussions at a SETAC Focused Topic Meeting on PFAS conducted by the Society of Environmental Toxicology and Chemistry in Durham, North Carolina, USA 12-15 AUG 2019 and also from reviews of the development of criteria (e.g., (Cordner et al. 2019). It is important to note that the suggestions for improvement of the risk characterisation process are not based on consensus of the participants but are those of the authors and generally reflect some of the ideas and points made during the breakout session on risk characterization. Therefore, the following discussion focuses on the evidence

supporting the endpoints that have been used in HBGV derivations, role of weight of evidence approaches, contribution of exposure considerations, and suggestions for improvement.

DERIVATION OF HEALTH-BASED GUIDELINE VALUES (HBGVs)

In deriving HBGVs, the typical risk assessment approach begins with a hazard identification and characterization process which identifies the toxicological and epidemiological endpoints to be used in risk assessment following an assessment of strength of the respective studies. The purpose of the hazard identification step is to identify a point of departure (POD) which is the starting point used to estimate (usually by way of dosimetry) a safe or tolerable level of exposure to the chemical in question for chronic oral exposure to the general population to include sensitive subpopulations. These safe or tolerable level derivations are broadly referred to as HBGVs, however, depending on the organization and jurisdiction they may be referred to by various names including the Tolerable Daily (or Weekly) Intake (TDI or TWI), Reference Dose (RfD), Minimal Risk Level (MRL) and the Derived No-Effect Level (DNEL). In the derivation of HBGVs, the hazard identification work has been done and is documented by a number of jurisdictions. Further, a more detailed discussion on the toxicity of PFAS is presented in the summary paper (Roberts et al., in prep.).

Selection of the Critical Health Effect and POD

Since health-based guideline values are intended to be protective of chronic oral exposures to the general population (which includes sensitive subpopulations but not particularly hypersensitive individuals), an understanding of the critical health effects

and development of PODs are important. The critical effects are often the ones exhibiting a relevant adverse effect at the lowest exposure concentration for humans. Based on a rapidly growing dataset, PFOS and PFOA have potential to cause numerous adverse effects in humans and animals alike; however, the list of purported effects are long and difficult to interpret. This is because comparison of effects across species is problematic. The exposure-response relationships vary greatly across species which may be due to species differences in elimination kinetics, the mechanism of toxicity and how the exposure levels were measured between studies. In general, rodent studies have shown strong evidence of hepatotoxicity, immunotoxicity, and developmental toxicity, which may be linked to peroxisome proliferator-activated receptor-α (PPARα) dependent or PPARα independent toxicity mechanisms (Lau et al. 2010; DeWitt et al. 2016). Primates are thought to be less responsive to PPARα agonists which has led to the exclusion of some effect endpoints, observed in the rodents, in the derivation of health-based guideline values. These include endpoints like increased liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels which are thought to be peroxisome proliferation related in rodents (Hall et al. 2012; ATSDR 2018; EFSA 2020b). Although the number of studies and effect endpoints investigated for primates (non-human) is much lower, there is evidence of hepatotoxicity, endocrine- and reproductive toxicity (Griffith and Long 1980; Butenhoff 2002; Seacat 2002; Chang et al. 2017 Jan 23). Other evidence suggests functional similarities to fatty acids (some PFAS), for many of which functions are still under investigation (Fritsche 2006; Salama et al. 2015). Plots of the lowest- and no observed adverse effect levels (LOAEL; NOAEL) for intermediate or chronic PFOA (A) and PFOS (B) exposure durations, for a range of adverse effects (grouped by affected organ system or endpoint as per ATSDR 2018),

are depicted in Figure 1 for rodents and monkeys. It is noted this information has been collated from systematic reviews conducted by other organizations (EFSA 2018; ATSDR 2018) and is presented here merely as an overview of the range of effect data considered by agencies or authorities in the derivation of HBGVs. The dose response appears more variable for certain groupings (developmental-, hepatic- and immune effects), however it should be noted these groupings consist of observations from multiple study endpoints with varying sensitivity. Species differences in toxicodynamics may also exist, however, these cannot be determined without accounting for difference in kinetics. For this reason, the delivered external dose is likely a poor metric of comparison. Nonetheless the bulk of the toxicity data are available in this format.

Effects in humans are largely based on epidemiological studies which have suggested associations between exposure to PFOS and PFOA for a range of health outcomes including increased total and LDL cholesterol, increased ALT levels (indicator of adverse liver effects), reduced birth weight, and decreased vaccine response (lower antibody titres) (Gallo et al. 2012; Whitworth et al. 2012; Eriksen et al. 2013; Abraham et al. 2020 Mar 29). It is noted that some effects (e.g., alterations in serum lipids and immune effects) reported in epidemiological studies are associated with PFOS and or PFOA exposures lower than those reported to cause effects in other animals (non-human). Many of these study designs (cross-sectional studies) compare plasma levels of PFAS to current health conditions. There are varying views as to whether these associations are consistent or clinically significant (Chang et al. 2016; Convertino et al. 2018; ATSDR 2018). Recently, the EFSA (EFSA 2020b) published a scientific opinion in which they questioned the causality of the association between PFOS or PFOA exposure with increased cholesterol levels which is one of the most

commonly published associations and the basis of the 2018 TWIs for PFOS and PFOA. In 2020, the EFSA updated their opinion (draft and final) and derived a new TWI based on epidemiological evidence for reduced vaccine response (draft opinion based on reduced antibody titres against haemophilus influenzae type b and final based on antibody titres against diphtheria) to PFOS, PFOA and two other long chain PFAS (EFSA 2020a). The EFSA are not alone in concluding that immunosuppression is a critical health endpoint for guideline derivation (Gleason et al. 2018; DeWitt, Blossom, et al. 2019; DeWitt, Cox, et al. 2019; Minnesota DoH 2019). It is noted that opinions or views on this topic are likely to remain divided until more longitudinal studies are available (which can reduce the risk of bias and confounding).

Whether a POD is selected based on animal or human data, typically evidence from each (as well as mechanistic and *in vitro* data) is considered to increase confidence. When using animal data, evidence from human studies is sought to ensure the effect is biologically relevant, understand extent of species differences, and used to support the plausibility of the effect (in addressing confounders). Mechanistic and *in vitro* data are optimally used to bridge phylogenetic conserved pathways from controlled laboratory animal studies to human relevance.

The exposure-response metrics preferentially used for HBGV derivation are the no-observed-adverse-effect level (NOAEL) or the benchmark dose (e.g., BMDL10; (WHO and Food and Agriculture Organization of the United Nations 2009). The BMD is defined as the exposure level corresponding to a specific change in an adverse response (e.g., 5% or 10% increase in expected observation within a population (Davis et al. 2011)). While both metrics are suitable starting points for a POD, the BMD is less dependent on dose selection and uses all the data from a study

to plot the dose response curve and as such is the preferred metric for many regulatory agencies (including USEPA and the EFSA; Davis et al. 2011). In addition, the BMD method can account for variability in the dataset by calculating a confidence limit (BMDL; Davis et al. 2011). Although the BMD approach is often the preferred method for POD derivation, BMD modelling requires a robust dataset which may not be available for each effect endpoint (Haber et al. 2018). Optimally, BMD approaches that use expected toxicity concentration distribution profiles are highly recommended, such as Bayesian BMD models (Shao and Shapiro 2018). The selection of critical effects is shown in Table 1. This table is not intended to capture all available derivations but to provide a snapshot of the variety of values and data supporting decision points selected by regulators from around the world; more than one agency is shown from the European Union and United States as these regions have a high number of active health authorities/agencies.

Derivation of the Human Equivalent Dose or Concentration

Table 2 outlines the selection of parameters for deriving the human equivalent dose (HED). Depending on the POD selected, extrapolation from animal doses or serum concentrations to human equivalent data may be necessary. Most commonly this extrapolation is achieved using either a scaling method or pharmacokinetic (PK) modelling. The HBGV derivations reviewed as part of this study mainly relied on a combination of PK modelling and scaling equations. The scaling relationships are described by Equation 1 and Equation 2 for most agencies where CL is clearance (defined as the volume of serum in this case which is cleared of PFOS or PFOA per unit time), V_d is the volume of distribution (defined as the proportionality ratio of the

dose and serum concentration) and $t_{1/2}$ is the half-life (Toutain and Bousquet-Melou 2004; Bardal et al. 2011).

Equation 1. $POD_{HED} = POD \times CL$

Equation 2. $CL = V_d \times \left(\ln(2) / t_{\frac{1}{2}} \right)$

Another approach used by some agencies like the Canadian FPTC was to use the difference in clearance (CL_{animal}/CL_{human}) to calculate an uncertainty factor to reflect differences in interspecies toxicokinetics. An overview of the parameter values used in HED derivations are summarised in Table 2.

<Table 2.>

Overall, the differences in parameter selection are small, CL values varied by a factor of 1.4 for PFOA and by 1.8 for PFOS. Vd values varied only slightly and reflect that PFOS and PFOA are highly serum protein bound as reported previously (Jones et al. 2003; Beesoon and Martin 2015) (Table 2). Although there is relatively high variability in published cohort studies, there is relatively little variability in half-lives used for HED conversions (factor of approximately 1.6 for PFOS and 1.7 for PFOA). Published cohort studies can show a higher degree of variability which is thought to be due to differences in the study populations (like age of participants and level of exposure) and confounding from ongoing background exposures (Worley et al. 2017). Another source of variability in the HED derivations is the animal serum concentration used to represent the POD. Average serum concentrations were estimated using PK modelling which can vary depending on the model.

Recent studies have shown that variability in half-lives for PFOA and PFOS may be related to population differences as well as the study design (follow up period) (Xu 2020). (Li et al. 2018a) reported mean PFOS and PFOA half-lives of 3.4- and 2.7 years respectively from a cohort of people exposed to PFAS in contaminated drinking water (106 people aged 4-84 in Sweden). Age and BMI were found (scientific meeting abstract) to contribute significantly to the retention as evaluated by plasma half-life, with faster elimination in younger participants and those with lower BMI, for the same Swedish cohort (Li et al. 2019). There is some evidence to suggest that elimination of PFOA may follow a non-linear trend, with faster elimination shortly after cessation of exposure (Xu 2020). Where elimination is non-linear, half-life estimates may vary with respect to the follow up period; Xu et al. (2020) reported a half-life of 1.77 years for PFOA based on a 5-month follow-up in workers exposed to PFOA in drinking water. In contrast, a patent application (Elcombe et al. 2013) has shown kinetic results in a phase 1 clinical trial of cancer patients that suggests a shorter half-life may be more appropriate for PFOA noting the unique nature of the cohort of this study. Nevertheless, this possibility might be worthy of further investigation.

Uncertainty in HED Determines HBGV

Uncertainty factors are used to address deficiencies in the database or extrapolations used to derive the HBGV (Dorne and Renwick 2005) (Table 3).

In the derivation of HBGVs, uncertainty factors have largely been applied based on default extrapolation factors (i.e., 10 for intraspecies variability and 2.5 or 3 for interspecies toxicodynamic variability) and in some instances additional uncertainty factors have been applied based on database limitations or exposure

extrapolations (Table 3). PK extrapolations were used to extrapolate human equivalent PoDs and as such no additional uncertainty factors were applied for interspecies toxicokinetics. A review of the reported LOAELs from animal studies largely agrees with intraspecies variability; however, interspecies variability may not be well represented with the default toxicodynamic factor of 3. Figure 2 provides a summary of dose-response (based on measured serum levels) data compiled from ATSDR (2018) and EFSA (2018, 2020) reviews for immune effects linked to PFOS exposure. At the species level (Panel A, Figure 2), data is lacking to comment on intraspecies variability however at the strain level (for mice; Panel B, Figure 2) B6C3F1 mice appear to show increased immune sensitivity to PFOS exposure. This was also noted in the recent EFSA (2020) review.

The intraspecies (or inter-individual) uncertainty factor is intended to adjust the point of departure to account for the difference between average- and sensitive subpopulations (Dankovic et al. 2015). Although animal data is of limited relevance to intraspecies (human-human) variability it may provide some insights on the magnitude of the toxic response particularly for endpoints where the mechanism of action is unknown. For interspecies variability, ideally, the uncertainty factor is based on comparison of animal and human studies, however human studies are rare, and as such comparisons between different animal species may serve as a surrogate to estimate interspecies variability (Bokkers and Slob 2007). The rationale for comparing dose-responses for different animals is that the magnitude of variability is likely similar to that observed between animals and humans (Martin et al. 2013). A metanalysis of relevant datasets may provide further insights into toxicodynamic variability which could be used to derive health endpoint specific uncertainty factors for PFAS.

Considering the differences in the starting points (POD), the derivation processes and how uncertainties have been addressed it is not surprising that HBGVs from around the world can vary by one to two orders of magnitude for the same compound. What is evident from these HBGV derivations is the uncertainty associated with each step:

- There is no consensus on a critical effect for either PFOA or PFOS however, there are two main target organs used to set HBGVs which are the liver and immune system. What is also confusing, is that some regulators inconsistently regard their relative toxicity (in terms of which compound is more toxic).
- The HED conversions differed primarily due to differences in half-lives and PK modelling parameters and attributes used to estimate animal serum concentrations at the POD. However, it is important to note that the importance of kinetics is relative to the window of effect at the tissue of interest. For example, developmental toxicity may be more related to the Cmax or average concentration during the appropriate window of concern (Dourson et al. 2019).
- The range in uncertainty factors applied for the same datasets also detracts from confidence in the overall derivations. This raises issues of public confidence and of risk communication.

INFLUENCE OF EXPOSURE SCENARIOS AND ASSUMPTIONS

The treatment of exposure within the derivation of drinking water criteria can have a significant influence on the final criteria derived, for example whilst a similar HBGV is adopted by US EPA (2016), Minnesota (2019 and Health Canada (2018) for PFOA, the drinking water criteria derived ranges from 35 ng/L to 200 ng/L. Table 4

provides an overview of considerations for quantifying exposures in the development of drinking water guidelines in the US, Australia and Canada to illustrate the impact of parameter choice in exposure quantification on the final criteria value.

Relative source contribution aims to consider what proportion of the health-based guideline value may be attributed to the specific environmental media such that it is protective for other background exposures (e.g., air, food, consumer products). Guidance on incorporating RSC varies with region. WHO (WHO 2017) guidelines note that where possible, RSC should be based on data from background exposures, and that in the absence of data, a default RSC of 20% can be used. In Australia, drinking water guideline development assumes 10% contribution from water consumption (for commercial chemicals), noting higher contributions may be relevant for some chemicals (NHMRC 2018). US EPA (US EPA 2000) advises RSC can be between 20% and 80% of the HBGV and includes a decision tree on how to identify an appropriate value, noting that the default value is 20%.

There is value in understanding the nature of exposure to understand if the RSC included in the derivation of a water criteria may be generally protective. Tap water exposures in the US have been estimated to contribute from 4.5% to 34% of total exposure for certain PFAS compounds in a nationwide study of a cohort of women aged 30 to 55 (Hu et al. 2019). PFOA was estimated to contribute approximately 12%, PFOS 4.5% to 5.7% and PFHxS 34% of the measured plasma concentrations (Hu et al. 2019). Outside of drinking water, diet has consistently shown to be a primary contributor of PFOS and PFOA to exposure for the general population, with estimates ranging from 66% up to 100% for PFOS exposure, though for other PFAS and in certain settings, indoor contributions such as dust play an

increased role (Lorber and Egeghy 2011; Gebbink et al. 2015; EFSA 2018; Sunderland et al. 2019). Most of the drinking water guideline derivations examined in Table 4 used the default RSC apart from the derivations by the NHMRC and Minnesota DOH which are discussed further here.

The NHMRC uses a more conservative default RSC, which assumes that 10% of the acceptable intake (HBGV) will arise from the consumption of drinking water for most chemicals including PFOS and PFOA (NHMRC 2018). This RSC assumption would imply that drinking water is a minor contributor to PFOS and PFOA exposure in Australia. This assumption can be tested using biomonitoring results, reverse dosimetry and water quality monitoring data. Thompson et al. (2010) used a pharmacokinetic modelling (simple one-compartment) approach to estimate intakes based on pooled serum samples collected from the general population (in south east Queensland, Australia). They estimated mean total daily intakes of 1.4 ng/kg bw/day for PFOS and 0.8 ng/kg bw/day for PFOA (for males and females of all ages) (Thompson et al. 2010a; Thompson et al. 2010b). In a separate study Thompson et al. (Thompson et al. 2011) collected and analysed drinking water samples from 34 locations across Australia and reported PFOS and PFOA to range from <0.66-16 ng/L and <0.5-9.7 ng/L respectively. Using the assumptions provided in Table 4, daily intakes attributable to drinking water were estimated to range from <0.004-0.45 ng/kg bw/day for PFOS and from <0.004-0.28 ng/kg bw/day for PFOA which make up from <1% to 35% of the mean total daily intakes estimated in Thompson et al. (2010) depending on location. It is noted out of the 34 locations all but two locations had RSCs <10% which would indicate that the assumption made by NHMRC is likely representative of general population exposure to PFOS and PFOA in Australia. Recently the Minnesota Department of Health (MDH) used biomonitoring data from

the general population (from national (NHANES) and local biomonitoring programs) to select appropriate and locally relevant RSCs for PFOS and PFOA. Using the USEPA Exposure Decision Tree, the MDH derived RSCs of 50% for infants and 20% for adults, for both PFOS and PFOA, based on a conservative estimate of background exposure (95^{th} percentile serum concentrations) and an RSC ceiling of 80% to ensure a margin of safety (Minnesota DoH 2018; Goeden et al. 2019; Minnesota DoH 2019).

Estimates of exposure such as the MDH and Australian examples above are routinely undertaken using pharmacokinetic modelling approaches (Thompson et al. 2010a; US EPA 2016; Goeden et al. 2019; Sunderland et al. 2019) and may be useful for determination of locally relevant RSC. Other exposure parameters adopted in current drinking water criteria are usually based on the sensitivity of the receptor identified, corresponding to the relevant physiological age associated with the toxicological endpoint adopted within the health-based guideline value. The approach is commonly deterministic, incorporating default body weights relevant to a specific age range or point in time, for example 15 kg to represent a young child, 70 kg to represent an adult (NHMRC 2018). Minor variations are observed in water consumption rates which may represent regional differences at a high level, ranging from 0.6 to 0.78 L/day for young children consuming water to 1.5 to 2 L/day for adults. Criteria considering lactating mothers utilised the same ingestion rate of 0.054 L/kg-day as per combined direct and indirect community water ingestion at the 90th percentile for lactating women from NHANES. Whilst this approach may be generally appropriate to consider lifetime exposures, PFAS intake is likely to change considerably for infant exposures. Given that developmental toxicity is a sensitive endpoint, consideration of early life exposures is important in the development of HBGVs and risk-based exposure criteria (Post et al. 2017; Goeden et al. 2019). The

model adopted within the Minnesota drinking water guideline which captures the changing physiology of the breastfed child by considering incremental dose intake and subsequent serum levels from changing intake, bodyweight and volume of distribution over time, has also been adopted by several other states in the US (Goeden et al. 2019).

Whilst some assessments consider sensitivity analysis on water intake from national level exposure surveys, none appear to consider water intake in the context of required intake which can vary based on regional climate differences (Sawka et al. 2005).

SUGGESTIONS FOR REFINEMENT OF RISK CHARACTERISATION

There are 4 key areas where improvements can be made to current risk characterisation paradigms:

- the process by which key studies, outcomes, and points of departure are selected and integrated,
- determination of the appropriate toxicokinetics parameters for different critical effects,
- the application of uncertainty in HGBV derivation, and
- approaches to accounting for exposure in the context of guideline setting.

Selection of critical studies, toxic endpoint and point of departure - data integration

Systematic review processes are recommended to document the rigor of the literature search and to consider the quality, relevance, and biases in the reported data

(Rooney et al. 2014; Whaley et al. 2016). Quantitative methods are used often to score laboratory animal studies to determine their quality and relevance to criteria development (often termed weight-of-evidence (WoE) (Klimisch et al. 1997; Dekant and Bridges 2016). However, institutional biases often result in hesitation reporting negative (toxicity) data, and such data are often considered scientifically uninteresting (Fanelli 2012). However, the fact that both Type I and Type II (false positive and false negative, respectively) statistical error exist, quantitative weight of evidence combined with sound dose response relationships evaluation can serve to support studies most valuable and scientifically defensible from which to derive safe thresholds for exposure. Essentially, scores for controlled laboratory animal data that are highest would likely be considered more reliable as the basis from which to develop points of departure (PODs) and subsequently human equivalent doses or concentrations (HEDs) than those of lower scores. These PODs can be plotted on a scatter diagram to help assess the presence of patterns (i.e., at what oral dose thresholds occur). Coherence and corroboration is important and studies that may show PODs much lower in oral dose than others with lower WoE scores can be reliably discounted for each relevant toxic endpoint.

Selection of human-relevant toxic endpoints from controlled laboratory *in vivo* data is often not straightforward (as presented in earlier examples). What is considered "adverse" in humans may be different from that observed in rodents. Here, coherence of endpoint can be defended with *in vitro* and mechanistic information that support a shared biological pathway between species. Here also magnitude of response is important. The biological relevance of a statistical difference between treatments needs to be made otherwise the observation could be simply a response of uncertain biological significance. For example, if exposure results in a statistically

significant change in red blood cell count, it must also be shown that the decrease is below that which is within the natural variation of that species of that age and sex to be relevant.

Quantitative and qualitative methods also exist for evaluating epidemiological and other data streams (Hill 1965; Rooney et al. 2014; Fedak et al. 2015). Integrating these data (along with other corroborative information from in silico and read-across techniques) into those from controlled laboratory animal studies can be used together to best provide corroborative evidence for coherent criteria development. Optimally, HEDs developed from PBPK-adjusted controlled laboratory animal should be corroborated with human data and supported with mechanistic and read-across information (discussed further). Figure 3 outlines the process envisaged.

Toxicokinetics in derivation of HBGV

As mentioned previously, HED determination from animal studies tends to be deterministic. Effectively, the HED is an approximate interspecies dose conversion from a serum concentration which is linked to a critical effect or POD. The HED conversion (most commonly used) assumes steady state conditions exist and that clearance is linear. While at low doses elimination kinetics appear to be consistent with first order processes (with proportionate serum levels), those processes may not be consistent over time, life stage or gender (Roberts et al. 2016). For example, physiological changes may result in age specific parameters which influence clearance like the volume of distribution, glomerular filtration and excretion/reabsorption processes (Fernandez et al. 2011; Goeden et al. 2019). Gender specific differences in elimination kinetics are most apparent in rats (different half-lives for males and females) for some PFAS including PFOA and PFHxS (but not

PFOS). It remains unclear why, however it is postulated to be related to the differential expression of organic anion transporters responsible for renal reabsorption in the proximal tubules (Roberts et al. 2016). Gender specific differences in half-life are less apparent in mice, monkeys, and humans, however there is evidence from exposed populations that gender differences in elimination exist (Roberts et al. 2016; Li et al. 2018b).

While these differences in kinetics would be difficult to account for using the standard deterministic HED derivation approach they may be accounted for using a toxicokinetic or physiologically based pharmacokinetic (PBPK) modelling approach. Recently, an open-source PBPK model was published which used Markov chain Monte Carlo (MCMC) simulation to optimize model parameters and characterize uncertainty and the variability of parameters between species (Chou and Lin 2019). While this model accounts for interspecies differences, it acknowledges the need for further studies in order to consider different life stages and potential gender-related differences (Chou and Lin 2019). It is also important to keep in mind that PBPK extrapolations are from modelled estimates – they contain inherent assumptions and uncertainties as any extrapolation and should not be considered as fact.

While kinetics are essential for interspecies extrapolations, it is equally important to consider potential differences in the mechanism of toxicity which may also vary between species. Figure 1 demonstrates that some effects are relatively conserved across species (e.g., decreased body weight, developmental, immune, and hepatic effects) while some are not (renal or hematological). It is the opinion of the authors that extrapolation of effects across species would appear more reliable for effects that are not limited to observations to one species.

Assessment and Refinement of Uncertainty in the development of HGBV

Optimally, an HBGV is derived from the HED developed from *in vitro*, *in vivo*, and actual human data. If available, PBPK models can be used with *in vitro* or *in vivo* data and corroborated with human experience. Typically, HEDs also include application of UFs, if needed, to account for interspecies (UFA) and intraspecies (UFH) variation, subchronic to chronic exposure extrapolation (UFS), and LOAEL to NOAEL/BBMDL extrapolation (UFL). Traditional application of UFs relies on multiplicative compounding of individual UFs, which may result in an overly conservative composite UF as demonstrated by Swartout et al. (Swartout et al. 1998). An alternative approach, which was recommended by the National Academy of Sciences (NAS) (NRC, 2014) is to use Bayesian methods to apply UFs. Bayesian approaches incorporate an estimate of the appropriate adjustment based on prior knowledge as well as a level of uncertainty in that estimate, which are reflected as the log-normal distributions of the geometric mean (μ) and geometric standard deviation (σ) of the composite UF. Simon et al. (2016) provided a refinement of the method recommended by the NAS, which incorporates the μ and σ for each individual UF, rather than only considering these parameters for the overall composite UF. Our approach is adapted from the methods described by Simon et al., with the following formula for applying UFs to derive a candidate HBGV:

$$\ln(TRV) = \ln(HEC) - \sum \mu_{UF} - Z_a\sqrt{\sigma_{UFS}^2 + \sigma_{UFA}^2 + \sigma_{UFH}^2 + \sigma_{UFL}^2}$$

Where: Zα is the Z-score, which for the 95th percentile is 1.645.

The geometric means for all UFs except for UFL are assumed to equal 1 (μ = 0 for a log-normal distribution), indicating that these UFs address uncertainty only.

When $\mu = 0$, σ is calculated as the $\ln(UF)/Z\alpha$. Thus, at the 95% confidence level, a UF of 1 corresponds to $\sigma = 0$, a UF of 3 corresponds to $\sigma = 0.668$, and a UF of 10 corresponds to $\sigma = 1.4$. As described by Pieters et al. (1998), the geometric mean and standard deviation of the LOAEL/NOAEL ratio from 175 chronic studies are 4.5 and 1.7, respectively ($\mu = 1.504$ and $\sigma = 0.531$ on log-normal scale). Thus, these values are used for UFL instead of those adopted for the other UFs. As a result, the sum of μUF in this analysis is either 1.504 or 0, depending on whether or not the HED was derived from a LOAEL. This formula differs from that used by Simon et al. in two key ways:

1. When the HED was derived from a Bayesian Benchmark Dose (BBMD) analysis, Simon et al. (2016) used the BBMD, rather than the BBMDL, as the basis for the HED derivation and added a separate operator to account for the variance between the BBMD and BBMDL. Our current method uses the BBMDL as the basis for the HED derivation and thus does not incorporate this additional measure of variance. The use of the BBMDL as the basis for the HED will generally result in a slightly more conservative HBGV compared to the method employed by Simon et al., although the ratio of the BBMD/BBMDL can vary substantially based on a number of factors including the benchmark response level, the BBMD software model, the number of animals in each dose group, the variance of the dataset, and how close the benchmark response is to the actual data.

2. Simon et al. (2018) weighed the merits of applying the UFA either before or after incorporating PBPK modelling to derive the HED. Their analysis indicated that this decision had a modest impact on HBGV derivation,

and we could find no guidelines for best practice. Simon et al. applied the UFA prior to derivation of the HED, while our current approach applies all UFs after derivation of the HED. This approach is based on our preference to assess uncertainty in the HED value only after its derivation.

Although Pieters et al. (1998) also determined the ratio of subchronic/chronic NOAELs based on 149 studies (geometric mean and geometric standard deviation are 1.7 and 5.6, respectively), these values incorporate a relatively high geometric standard deviation, indicating that there is a great deal of uncertainty in this estimate, which may limit its utility. Similar estimations of data-derived ratios for subchronic-to-chronic and LOAEL-to-NOAEL extrapolations as those derived by Pieters et al. have also been determined by others (Hasegawa et al., 2010), and the use of either set of published values may be appropriate and should be evaluated before using. This evaluation could include an assessment of raw HEDs to human data to measure model fit for the following considerations:

a. Sometimes animal models are more sensitive than humans for biological reasons and may not require additional interspecies uncertainty factors.
b. HED could be further informed by *in vitro*/*in vivo* extrapolation and mechanistic data.

Some adverse effects may be realized by short-term repetitive exposures where lengthening exposure to chronic (>7 yrs) does not enhance probability for adverse effects at lower exposures, for example with developmental toxicity as demonstrated by Dourson et al. (2019). However, this may not be the case for the critical effects of chemicals with a long biological half-life. Several PFAS chemistries may fall into this category.

Considerations for accounting for exposure in deriving drinking water criteria

Whilst there are many uncertainties in establishing the HBGV, the approach to exposure can also drive considerable differences in the final environmental criteria applied. Adequately capturing exposure is a key component for risk characterisation within guideline development. Often the approach to exposure is based on existing default approaches but there is considerable variation to the level of sensitivity analysis included on exposure in the documentation supporting guideline derivation.

Relative source contribution often has the most significant impact for accounting for exposure. The RSC can include a percentage of total exposure assumed to come from exposure to drinking water at the criterion level or other levels depending on the situation. The RSC should consider the contribution of exposure from all sources including food and other non-drinking water sources so the overall exposure does not exceed relevant HBGVs. Often these sources are based on default values or estimated exposure concentrations. For example, if an exposure is considered low from food, an allowable drinking water criteria can be increased. The estimated consumption used in deriving drinking water criteria can again significantly alter the derived outcome.

The use of human serum data has been used to estimate existing contributions from the environment and better refine RSC but is reliant on the same pharmacokinetic parameters as those adopted in estimation of the HED. Targeting research to improve our understanding of pharmacokinetics and development of better models for PFAS will benefit our interpretation of background exposures.

Dose intake is a significant input determining whether concentrations reported in water are likely to present a risk to human health. The use of established default

parameters is usually determined from national exposure databases and is likely generally appropriate where we consider lifetime exposures. However, there are scenarios in which variable intake may pose a significant role, such as early life stages (Goeden et al. 2019), high physical demand or environmental scenarios which are known to require a higher or more varied water intake (Sawka et al. 2005). The relevancy of these fluctuations is not yet well established but may be important given the propensity for PFAS to bioaccumulate. Probabilistic methods to consider sensitivity of exposure parameter choice have been used in backward modelling scenarios (such as Hu et al 2018) however it less frequent to observe such detailed analysis in criteria development.

CONCLUDING REMARKS

There are a number of areas where the current approaches and methods to improve risk characterisation in the derivation of HGBV can be adopted. Such methods and approaches need to be documented to enable the range of stakeholders, but especially regulators, to be able to provide clear communication about risks and why HBGVs, and subsequent guidelines for PFAS, may differ from another jurisdiction. Transparency is part of the risk assessment and characterisation process however communication of the rationale for differences in criteria to end users requires attention as does the application of variable default assumptions and weight of evidence approaches which can have large differences on HBGVs.

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Figure:

Figure 1: Plots of NOAELs and LOAELs reported for mice (triangle markers), rats (square markers) and monkeys (circle markers) grouped by affected organ system or endpoint based on ATSDR 2018 for **A.)** PFOA; and **B.)** PFOS. The size of the marker

indicates the length of the study in days; included in these plots are studies of intermediate (15-364 days) and chronic (≥365 days) duration.

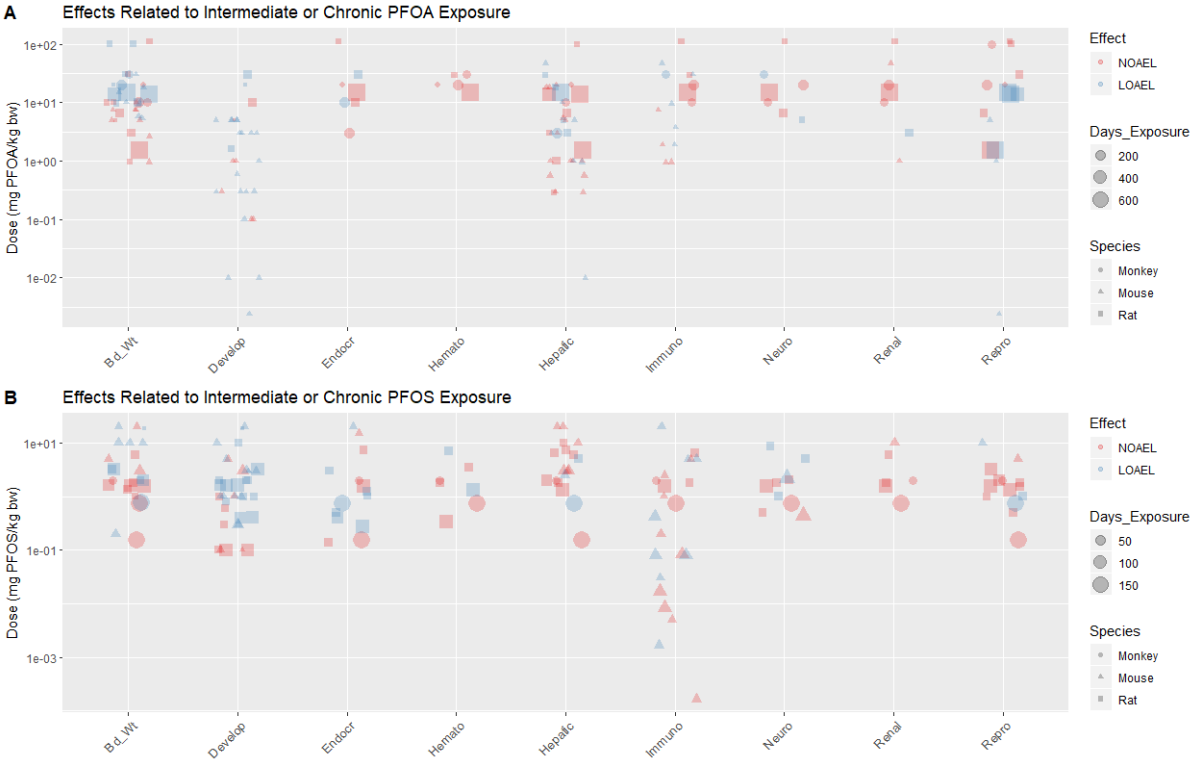

Figure 2: Plots of NOAELs and LOAELs reported for for "immune" effects based on ATSDR 2018 and EFSA 2020 for PFOS. The size of the marker indicates the length of the study in days; included in these plots are studies of acute (≤14 days), intermediate (15-364 days) and chronic (≥365 days) duration. **A.)** Provides an overview of reported NOAELS and LOAELs at the species level for mice (triangle markers), rats (square markers) and monkeys (circle markers). **B.)** Provides an

overview of reported NOAELS and LOAELs for different mouse strains including BALB/c (triangle markers), C57BL/6 (square markers) and B6C3F1 (circle markers).

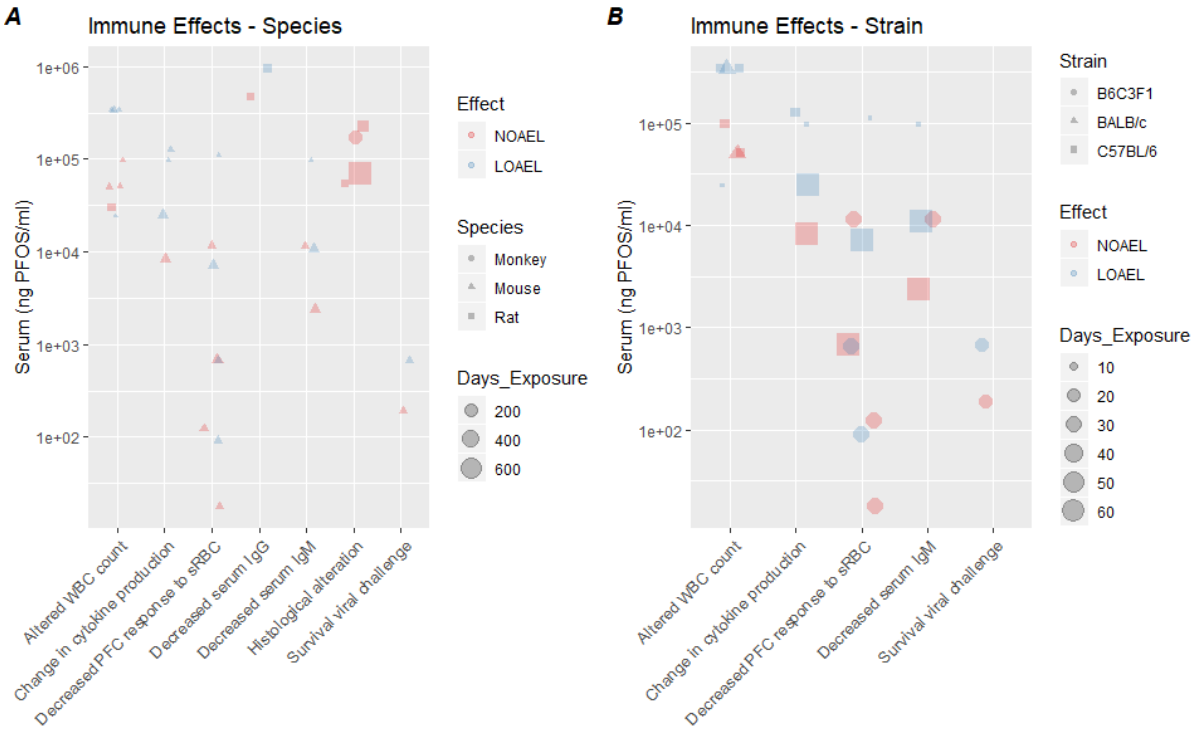

Figure 3: Data integration schematic for various lines of evidence towards health criteria development.

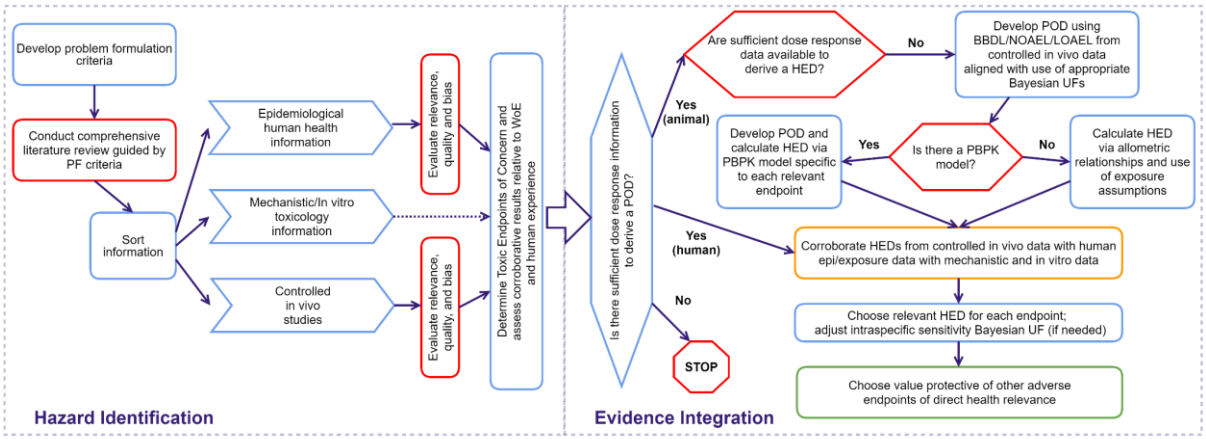

Table 1. Overview of jurisdictional selection of critical effects and points of departure for HBGV

	Agency	Comp.	Exposure period	Toxicological endpoint	Point of Departure	Critical effect study
POD based on Human studies	**EFSA (Final)** (EFSA 2020)	Sum of PFOA, PFOS, PFNA, PFHxS	Chronic	BMDL10 for inverse association between serum levels for sum PFASs and antibody titres against diphtheria	17.5 ng/mL	(Abraham et al. 2020 Mar 29)
	EFSA (draft) (EFSA 2020)	Sum of PFOA, PFOS, PFNA, PFHxS	Chronic	NOAEC for inverse association between serum levels for sum of PFASs and antibody titres against haemophilus influenzae type b	31.9 ng/mL	(Abraham et al. 2020 Mar 29)
	EFSA 2018 (EFSA 2018)	PFOA	Chronic	BMDL5 for disease risk factors related to ↑ serum cholesterol in humans	Values expressed as plasma concentrations: - PFOA conc. (9.2–9.4 ng/mL) - PFOS conc. (21–25 ng/mL) Corresponding to median daily intakes (calculated with a PBPK-model for humans): - PFOA 0.8 ng/kg bw-day - PFOS 1.8 ng/kg bw-day	(Steenland et al. 2009; Nelson et al. 2010; Eriksen et al. 2013)
		PFOS				
POD based on Animal studies	**Swedish EPA 2012** (Borg et al. 2012)	PFOA	Intermediate (gestation)	LOAEL for delayed mammary gland development and growth in mice	10000 ng/kg bw/day (150 ng/ml serum)	(Macon et al. 2011)
		PFOS	Intermediate	NOAEL for immunomodulation in mice (↓ sheep red blood cell (SRBC) specific IgM levels)	166 ng/kg bw-day (17.8 ng/g serum)	(Peden-Adams et al. 2008)
	Danish EPA 2015 (Larsen and Giovalle 2015)	PFOA	Acute to Intermediate	BMDL10 for liver effects	456000 ng/kg bw-day based on 13-week diet study	(EFSA 2008; US EPA 2014)
		PFOS	Chronic	BMD10 for liver effects in rats	33000 ng/kg bw-day	(Thomford 2001)
	New Jersey DEP 2017 and 2018 (Gleason et al. 2017; Gleason et al. 2018)	PFOA	Acute	BMDL10 for increased relative liver weight	4351 ng/ml serum concentration resulting in BMDL 10% decrease	(Loveless et al. 2006)
		PFOS	Intermediate	NOAEL for immunomodulation (↓SRBC specific IgM levels in mice)	674 ng/ml serum level	(Dong et al. 2009)

Minnesota DoH 2018 and 2019 (Minnesota DoH 2018; Minnesota DoH 2019)	PFOA	Intermediate (gestation)	LOAEL for ↓ ossification, accelerated PPS in male offspring, trend for ↓ pup body weight, and ↑ maternal liver weight in mice	38000 ng/ml serum concentration (average serum concentration for maternal animals estimated by US EPA for exposure at 1000000 ng/kg bw-day)	(Lau et al. 2006)
	PFOS	Intermediate	NOAEL for immunomodulation (↑ IL-4 and ↓ SRBC specific IgM levels) in mice	2360 ng/ml serum conc.	(Dong et al. 2011)
ATSDR 2018 (ATSDR 2018)	PFOA	Intermediate (gestation)	LOAEL for neurodevelopmental and skeletal effects in mice	300000 ng/kg -day (predicted serum concentration 8290 ng/ml)	(Onishchenko et al. 2011; Koskela et al. 2016)
	PFOS	Intermediate	NOAEL for ↓ pup body weight in rats	100000 ng/kg bw-day (predicted serum concentration 7430 ng/ml)	(Luebker et al. 2005)
US EPA 2016 (US EPA 2016a; US EPA 2016b)	PFOA	Intermediate (gestation)	LOAEL for ↓ pup ossification and accelerated puberty in male mice	1000000 ng/kg -day (predicted serum concentration 38000 ng/ml)	(Lau et al. 2006)
	PFOS	Intermediate	NOAEL for ↓ pup body weight in rats	100000 ng/kg bw-day	(Luebker et al. 2005)
Canada 2016 (Health Canada 2018a; Health Canada 2018b)	PFOA	Intermediate	BMDL10 for liver effects (hepatocellular hypertrophy)	50000 ng/kg bw-day	(Perkins et al. 2004)
	PFOS	Chronic	NOAEL for hepatocellular hypertrophy in rats (accounting for impurity of PFOS)	21000 ng/kg bw-day	(Butenhoff et al. 2012)
FSANZ 2016 (Roberts et al. 2016)	PFOA	Intermediate (gestation)	NOAEL for fetal toxicity in mice (↓ ossification and accelerated puberty in male mice)	1000000 ng/kg -day (predicted serum concentration 35100 ng/ml)	(Lau et al. 2006)
	Sum of PFOS, PFHxS[1]	Intermediate	NOAEL for ↓ parental and offspring body weight gains in a multigeneration reproductive toxicity study in rats	100000 ng/kg bw-day	(Luebker et al. 2005)
RIVM, 2018 (Zeilmaker et al. 2018)	PFBS, PFHxS, PFHpS, PFOS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA	multiple	BMD analysis for common phenomenological effect of liver toxicity, as revealed by liver hypertrophy (hepatocellular, centrilobular) and accompanying liver enlargement, i.e. absolute and relative liver weight.	relative potency factors for 12 PFAS (equivalency to PFOA)	(Seacat et al. 2003; Perkins et al. 2004; Butenhoff et al. 2009; Haas MC 2009; Lieder et al. 2009; Loveless et al. 2009; Mertens et al. 2010; Butenhoff et al. 2012; Hirata-Koizumi et al. 2012; Takahashi et al. 2014; Hirata-Koizumi et al. 2015; Kato et al. 2015)

POD based on Animal studies

[1]FSANZ determined that there is not an adequate database to establish a HBGV for PFHxS however due to similarities in pharmacokinetic parameters the approach adopted in Australia is to apply the PFOS HBGV to PFHxS exposures as well. In practice this means PFOS and PFHxS exposures are summed and the total compared with the HBGV for PFOS.

Table 2. Jurisdiction selection of clearance volume and point of departure (POD) for HED Derivation.

Agency	Comp.	CL (L/kg bw-day)	Vd (L/kg)	t1/2 (days)	POD_{SC} (µg/ml)[7]	POD_{HED} (ng/kg bw-day)[8]
New Jersey DEP 2017, 2018	PFOA	0.00014	0.17[2]	839.5 (mean adults)[3]	4.35	609
	PFOS	0.000081	0.23[2]	1971 (mean adults)[6]	0.67	54.6
Minnesota DoH 2018, 2019	PFOA	0.00014[1]	0.17[2]	840 (mean adults)[3]	38	5300
	PFOS	0.00013[1]	0.23[2]	1241 (mean all ages)[4]	2.36	307
ATSDR 2018	PFOA	0.000099[1]	0.2[5]	1400 (mean adults)[6]	8.29	821
	PFOS	0.000069[1]	0.2[5]	2000 (mean adults)[6]	7.43	510
US EPA 2016	PFOA	0.00014[1]	0.17[2]	839.5 (mean adults)[3]	38	5300
	PFOS	0.000081[1]	0.23[2]	1971 (mean adults)[6]	6.26	510
FSANZ 2016	PFOA	0.00014[1]	0.17[2]	839.5 (mean adults)[3]	35.1	4900
	PFOS	0.000081[1]	0.23[2]	1971 (mean adults)[6]	7.14	600

[1]Where CL was not provided it was estimated using Equation 2 for comparison; [2] Thompson et al. 2010; [3] Bartell et al. 2010; [4] Li et al. 2018; [5] Based on review of multiple studies; [6] Olsen et al. 2007; [7] Animal serum concentration at the POD (measured or modelled); [8] Human equivalent POD

Table 3. A summary of uncertainty factors applied to derive HBGV by each regulatory agency

Agency	HBG Type	Comp.	HBGV (ng/kg bw-day)	POD_{HED} (ng/kg bw-day)	Uncertainty Factors				
					Total	Intraspecies	Interspecies	LOAEL to NOAEL	Other
EFSA	T	Sum	0.63[2]	NA	None applied, because the BMDL10 is based on infants which are				

2020 (Final)	WI /7	PFAS[1]			expected to be a sensitive population group.				
EFSA 2020 (draft)	T WI /7	Sum PFAS[1]	1.16[2]	NA	None applied NOAEC is based on infants which are expected to be a sensitive population group.				
EFSA 2018	T WI /7	PFOA	0.8	NA	None applied BMD modelling based on large epidemiological studies from the general population (including potentially sensitive subgroups) and risk factors for disease rather than disease outcomes.				
		PFOS	1.8	NA					
Swedish EPA 2012	D NE L	PFOA	2.0 ng/ml serum	NA	75	10	2.5	3	
		PFOS	0.12 ng/ml serum	NA	150	10	2.5		6 (subchronic to chronic)
Danish EPA 2015	TD I	PFOA	100	3000	30	10	3		
		PFOS	30	NA	1,230	10	41 for PK and 3 for PD		
New Jersey DEP 2017, 2018	Rf D	PFOA	2	609	300	10	3		10 (database uncertainty)
		PFOS	1.8	54.6	30	10	3		
Minnesota DoH 2018, 2019	Rf D	PFOA	17.6	5300	300	10	3		10 (database uncertainty)
		PFOS	3.1	307	100	10	3		3 (database uncertainty)
ATSDR 2018	M RL	PFOA	3	821	300	10	3	10	
		PFOS	2	510	300	10	3		10 (for immunotoxicity)
US EPA 2016		PFOA	20	5300	300	10	3	10	
	Rf D	PFOS	20	510	30	10	3		
Canada FPTC 2016, 2018	TD I	PFOA	21	521	25	10	2.5		
		PFOS	60	1500	25	10	2.5		
FSANZ 2016		PFOA	160	4900	30	10	3		
	TD I	Sum PFOS, PFHxS	20	600	30	10	3		

[1]Sum of PFOA, PFNA, PFHxS and PFOS
[2]Modelled using physiologically based pharmacokinetic model

Table 4. Example of exposure parameters applied in deriving drinking water criteria

Agency	**Comp.**	**EPA 2016**	**Minnesota DoH 2019**	**New Jersey DEP**	**Health Canada**	**Australia NHMRC 2018**

				2017	2018	
HBGV (ng/kg bw-day)	PFOA	20	18	2	21	160
	PFOS	20	3.1	1.8	60	20
Target Pop.	PFOA	Lactating women	Infant	Adult	Adult	Adult
	PFOS					
Ingestion Rate	PFOA	0.054 (L/kg bw-day)	Modelled[1]	2 (L/day)	1.5 (L/day)	2 (L/day)
	PFOS					
BW	PFOA	NA	Modelled[1]	70 kg	70 kg	70 kg
	PFOS					
Relative Source Contribution (RSC)	PFOA	20%	50% (20%)[2]	20%	20%	10%
	PFOS					
Derivation Method	PFOA	Standard[3]	TK Model[4]	Standard	Standard	Standard
	PFOS					
Drinking water criteria (ng/L)	PFOA	70	35	14	200	560
	Sum of PFOS and PFHxS	70	0.015	13	600	70

[1]Kinetic parameters based on age

[2]50% RSC for infants and 20% RSC for steady state

[3]The standard drinking water advisory approach: drinking water criteria = (HBGV x BW x RSC)/Ingestion Rate

[4]The Minnesota DoH used a toxicokinetic model to simulate serum PFOS and PFOA concentrations resulting from exposure to drinking water and milk for infants. The advisory level is intended to maintain serum concentrations at or below an RSC of 50% for breast-fed infants.

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