OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Notification Level Recommendation

Perfluorohexane Sulfonic Acid in Drinking Water

March 2022

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

Notification Level (NL) Recommendation Documents provide information on health effects from contaminants in California drinking water. A recommended NL is a concentration of a contaminant in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis. The Office of Environmental Health Hazard Assessment (OEHHA) recommends these health-based advisory levels to the Division of Drinking Water of the State Water Resources Control Board (Water Board) for chemicals in drinking water that lack regulatory or maximum contaminant levels (MCLs). Based on these recommendations and other considerations, the Water Board establishes NLs and Response Levels. Health and Safety Code Section 116455 requires drinking water systems to notify their governing body, and recommends they notify consumers, when a detected chemical exceeds its NL. If a chemical is present in a drinking water source at the Response Level – a concentration considerably greater than the notification level – the Water Board recommends that the drinking water system take the source out of service.

When a risk assessment for a chemical of concern in drinking water is lacking, the Water Board may request that OEHHA develop an NL recommendation. NL recommendations are based on sensitive, well-conducted and scientifically valid studies. OEHHA considers the publicly available studies of health effects in humans and laboratory animals, as well as studies of toxicokinetics and mechanisms of toxicity.

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SUMMARY

This document presents a notification level (NL) recommendation by the Office of Environmental Health Hazard Assessment (OEHHA) to the State Water Resources Control Board (Water Board) for perfluorohexane sulfonic acid (PFHxS) in drinking water. OEHHA recommends that the Water Board establish a drinking water NL at 2 parts per trillion (ppt), equivalent to 2 nanograms per liter (ng/L), or at the lowest level at which the chemical can be reliably detected in drinking water using available and appropriate technologies. The NL recommendation is based on the noncancer effects of PFHxS, specifically, decreased thyroid hormone levels in male rats (NTP, 2019). There were insufficient data to evaluate the potential carcinogenicity of PFHxS.

INTRODUCTION

At the request of the Water Board, OEHHA has developed a recommendation for a drinking water NL for PFHxS. This document reflects OEHHA's focused review of the human and animal toxicity studies identified from the open literature.

PFHxS (CAS RN 355-46-4) and its potassium salt (potassium perfluorohexane sulfonate or K^+PFHxS; CAS RN 3871-99-6) are six-carbon fluorocarbons with a sulfonic acid functional group that act as anionic surfactants, and are used in textile products for their water-repellent properties and in fire-fighting foams (Boucher et al., 2018; Glüge et al., 2020). PFHxS and its potassium salt are members of a large class of chemicals known as per- and polyfluoroalkyl substances (PFAS). In the environment, the potassium salt is expected to rapidly ionize to PFHxS. Similar to other PFAS, PFHxS does not occur naturally, and its presence in the environment is due to anthropogenic activity. Due to its saturation with highly stable carbon-fluorine bonds, the PFHxS molecule is resistant to degradation. As a result, this compound persists in the environment and in biological organisms.

Environmental occurrence

PFHxS is ubiquitously present in the environment. It has been detected in air, soil, sediment, and precipitation, as well as in river, lake, sea and ground water (reviewed in (ECHA, 2019)). PFHxS has been detected in the low ppt (ng/L) range in drinking water from various European countries, China, Australia and Canada, with higher detections at contaminated sites, such as Ronneby, Sweden and Treviso, Italy (reviewed in (ECHA, 2019)). The highest detected level was 1,770 ppt, reported for the outgoing water from Brantafors waterworks in Ronneby, Sweden, in 2013 (Li et al., 2018). In the US, PFHxS was included in the Third Unregulated Contaminant Monitoring Rule (UCMR3) for public water systems (PWS) and was detected in 1.1% of the 4,864 PWS tested (Hu et al., 2016). The low detection frequency in this report was likely driven by the high minimum reporting level of 30 ppt for PFHxS.

In 2019-2020, the Water Board conducted a California state-wide PFAS monitoring program to sample PWS quarterly for four consecutive quarters at over 600 water system sites adjacent to nearly 250 airports with fire training areas and municipal solid waste landfills. The results of this monitoring program are available to the public.[1] With a detection limit of 1.4 ppt, PFHxS was detected in approximately 40% of samples. The average PFHxS levels in the sampled water (excluding non-detects) were 12.9-17.6 ppt, depending on the quarter.

PFHxS has been detected in various foods, with high concentrations found in fish, as recently reported by the European Food Safety Authority's (EFSA) Panel on Contaminants in the Food Chain (Schrenk et al., 2020). Higher human serum PFHxS levels appear to result from exposure to certain consumer products, such as carpeting and carpet applications (Beesoon et al., 2012; Zhu et al., 2021). Thus, humans are environmentally exposed to PFHxS from multiple sources.

Biomonitoring

PFHxS is commonly detected in human serum. Based on National Health and Nutrition Examination Survey (NHANES) data, PFHxS was detected in 96-99% of all serum samples in the general US population. Human serum PFHxS levels measured by NHANES remained at similar levels from 1999 through 2008, based on the geometric means (2.13 and 1.96 ng/ml for 1999-2000 and 2007-2008, respectively) and the upper 95th percentiles (8.70 and 9.80 ng/ml, respectively) reported (Kato et al., 2011). However, Jain (2018) reported a slight decrease in serum PFHxS levels in the NHANES-sampled general US population from 2003-2004 (geometric mean, 1.88 ng/ml) to 2013-2014 (geometric mean, 1.38 ng/ml), averaging to a 6.3% yearly decrease.

Yearly serum decreases over the same period were much higher for perfluorooctanoic acid (PFOA; 17%) and perfluorooctane sulfonic acid (PFOS; 33.5%), likely reflecting the industrial phase-out of these PFAS (Jain, 2018). A longer PFHxS half-life ($T_{1/2}$) in humans compared to that of PFOS (5.3 and 3.4 years, respectively (Li et al., 2018)) may also contribute to the lower yearly reductions of PFHxS in serum compared to PFOS in the US.

Results from the Childhood Autism Risk from Genetics and Environment (CHARGE) study indicated that in Northern Californian mothers with young children, serum PFHxS decreased from approximately 0.60 ng/ml in 2009 to approximately 0.35 ng/ml in 2016, with an average 8% yearly decrease rate (Kim et al., 2020). Information on serum PFHxS levels in California residents can also be found at the Biomonitoring California website.[2] Among the most recent studies (with sampling in 2016-2019), 99-100% of serum samples contained detectable PFHxS, with the geometric mean values ranging

[1] https://www.waterboards.ca.gov/pfas/drinking_water.html, accessed August 2021
[2] https://biomonitoring.ca.gov/results/chemical/2183, accessed August 2021.

0.613-1.29 ng/ml (90th percentiles:1.81-3.79 ng/ml). Further details about the studies can be found on the Biomonitoring California website.

Advisory levels and regulatory standards

Various agencies both within and outside the US have released reference levels for PFHxS, some specifically for human exposure in drinking water. The Minnesota Department of Health (MDH) developed a short-term, subchronic and chronic noncancer health-based value (nHBV) of 47 ppt for drinking water (MDH, 2020). This value was based on decreased free thyroxine (T4) in serum in a 28-day oral exposure study in rats (NTP, 2019). Co-critical effects were decreased free and total T4 and triiodothyronine (T3), changes in cholesterol levels, and increased hepatic local necrosis. To account for presumably increased PFHxS serum concentration in infants, this assessment applied a toxicokinetic (TK) model based on the PFOA model previously developed by MDH (Goeden et al., 2019).

The Michigan PFAS Action Response Team Science Advisory Workgroup (MPART SAW) adopted MDH's PFHxS assessment with slight variations in the PFHxS TK model parameters to develop the health-based value (HBV) of 51 ppt for drinking water.[3]

The New Hampshire Department of Environmental Services (NHDES) developed an MCL of 18 ppt for drinking water (NHDES, 2019) based on reduced litter size in a developmental study in mice (Chang et al., 2018) and the modified MDH PFHxS TK model to account for age-dependent changes in PFHxS serum levels with chronic exposure. Part of this risk assessment was published in a peer-reviewed journal (Ali et al., 2019).

Vermont, Connecticut, and Massachusetts developed regulatory values applicable to the sum of 4-6 PFAS, including PFHxS, and did not release PFHxS-specific values. These assessments were based on presumed similarities between the toxic effects of PFOA and PFOS to those of other PFAS in the group. EFSA also established a tolerable weekly intake (TWI) for the sum of four PFAS, including PFHxS, based on immune effects in a human study (EFSA, 2020).

As of January 2021, the United States Environmental Protection Agency (US EPA) has not set an MCL, Health Advisory (HA) level, or Regional Screening Level (RSL) for PFHxS. US EPA has not yet developed reference levels for PFHxS. However, in November 2019, the agency released the Systematic Review Protocol for assessing PFHxS under its Integrated Risk Information System (IRIS) program (US EPA, 2019). PFAS, including PFHxS, are not designated as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Effective January 1, 2020, PFHxS was added to US EPA's TRI (Toxics Release

[3] https://www.michigan.gov/documents/mdhhs/PFAS_-_Overview_of_Michigan_Values_FINAL_675761_7.pdf, accessed August 2021.

Inventory) list of chemicals subject to reporting.[4] PFHxS is among the more than 170 PFAS on this list.

The Agency for Toxic Substances and Disease Registry (ATSDR) released its *Toxicological Profile for Perfluoroalkyls* in May 2021. This document established the PFHxS minimal risk level (MRL) for oral exposures of intermediate duration at 2×10^{-5} mg/kg-day (ATSDR, 2021) based on the critical effect of thyroid follicular epithelial hypertrophy/hyperplasia reported in a subchronic rat study by (Butenhoff et al., 2009).

The Ohio Department of Health (ODH) set the action level for PFHxS at 140 ppt (ODH, 2019), based on the draft PFHxS ATSDR assessment (finalized in 2021 (ATSDR, 2021)) and on the EPA's Drinking Water Equivalent Level (DWEL) approach, which assumes that 100% of an individual's exposure to PFHxS comes from drinking water.

The European Chemicals Agency (ECHA) has not yet classified PFHxS or its salts as a hazard. However, as of March 2020, the proposal to restrict the manufacture, use, and placement on the market of PFHxS, its salts and related substances was being finalized (ECHA, 2020). PFHxS is listed as one of 20 PFAS in Annex III of the European Union (EU) Drinking Water Directive, and the sum of these 20 PFAS is not to exceed 0.1 µg/L (European Comission, 2020). While other EU countries variously group PFAS for regulatory purposes, France developed an individual indicative toxicity value (iTV) for PFHxS at 0.004 mg/kg-day (ANSES, 2017) based on increased absolute and relative liver weight in the Butenhoff et al. (2009) study in rats. The World Health Organization (WHO) does not currently list PFHxS or other PFAS in its Guidelines for Drinking Water Quality (WHO, 2017).

OEHHA's review of the PFHxS regulatory literature revealed that this contaminant of emerging concern is increasingly targeted by regulatory agencies in the US and around the world due to its persistence in the environment, bioaccumulation in humans and other organisms, and observed toxic effects in humans and animals.

SYSTEMATIC LITERATURE SEARCH

OEHHA performed a systematic literature search for epidemiologic studies on the human health effects of PFHxS. The following four sources were used: PubMed, Embase, review articles on PFAS, and the bibliographies of all relevant articles identified from the other three sources. The terms used in the PubMed and Embase searches are provided in Figure A1.1 in Appendix 1. The following inclusion and exclusion criteria were used to identify relevant studies:

- All human epidemiologic studies of PFHxS and an adverse human health effect were eligible for inclusion.

[4] https://www.epa.gov/toxics-release-inventory-tri-program/implementing-statutory-addition-certain-and-polyfluoroalkyl-0, accessed September 2021.

- All studies published before April 7, 2021, were included.
- Studies presenting results as mean differences, regression or correlation coefficients, relative risk estimates, or any other appropriate outcome metric were included.
- No restrictions were placed on the methods used to evaluate PFHxS exposure, although almost all studies used PFHxS concentrations in serum.
- Studies using cohort and case-control designs were included.
- Case-reports were excluded because of the lack of a comparison group. Ecologic and cross-sectional studies were considered, although the potential for ecologic fallacy or reverse causation was examined.
- Abstracts and studies without original data (e.g., editorials) were excluded.

Several studies reported results only for multiple PFAS combined. Although these studies were considered, they were not included in OEHHA's detailed evaluations since associations could not be specifically ascribed to PFHxS.

Studies identified from PubMed and Embase were first screened by titles and abstracts and then by full article review if needed. The literature search is summarized in Figure A1.2 in Appendix 1.

For animal toxicity studies, OEHHA conducted a systematic literature search in June 2021 of multiple open literature databases (PubMed, Embase, Scopus, and Toxnet) using a search string intended to identify all studies that mention PFHxS in the title or abstract. The search terms used for each database and the flowchart for selecting candidate critical studies are included in Appendix 1.

From the initial search of animal toxicity studies, OEHHA identified 2,105 individual studies. OEHHA uploaded the identified references into the DistillerSR systematic review software (Evidence Partners, Canada) and conducted inclusion/exclusion screening for relevant toxicological data against a PECO (populations, exposures, comparators, and outcomes) statement designed to capture oral animal toxicity studies (Appendix 1). Additional classification bins in the PECO statement included toxicokinetic evidence, in vitro and mechanistic studies, and human epidemiological studies.

Two independent reviewers conducted the Tier 1 (title/abstract) reference evaluation against the PECO statement. Tier 1 screening resulted in 20 unique references for PFHxS mammalian toxicity studies. Among these, there were four wildlife toxicological studies in mammals, four conference abstracts, one doctoral thesis, and one in vitro study. These entries were excluded from further review. The remaining eight studies in mice or rats were included in OEHHA's toxicological review. One additional study (Rosen et al., 2017) was further added to the list of animal toxicity studies when it was independently found to contain an in vivo toxicity component (changes in absolute and relative liver weights) that was not described in the abstract. Overall, OEHHA's search

strategy yielded nine animal toxicity studies that warranted further evaluation (see Figure A1.7 in Appendix 1).

TOXICOKINETICS

PFHxS demonstrates drastic interspecies differences in $T_{1/2}$, which are characteristic for longer chain PFAS, such as PFOA and PFOS (Pizzurro et al., 2019). Sex differences in PFHxS $T_{1/2}$ are also observed in rats and perhaps, monkeys and humans, but not in mice. In female and male rats, the oral PFHxS $T_{1/2}$ was 1.60-1.72 days and 15.9-34.1 days, respectively (Benskin et al., 2009; Kim et al., 2016; Kim et al., 2018). In female and male mice, the oral PFHxS $T_{1/2}$ was 24.9-26.9 days and 28.0-30.5 days, respectively (Sundström et al., 2012). With intravenous (i.v.) dosing, the PFHxS $T_{1/2}$ was 87 days in female monkeys and 141 days in male monkeys (Sundström et al., 2012). In humans, with an assumption of first-order kinetics, the PFHxS serum $T_{1/2}$ was 5.3-15.5 years (Li et al., 2018; Olsen et al., 2007; Worley et al., 2017). Li et al. (2018) reported a mean PFHxS serum $T_{1/2}$ of 4.7 years for women (95% CI, 3.9-5.9) and 7.4 years for men (95% confidence interval (CI), 6.0-9.7). This difference was statistically significant.

PFHxS transfer from mother to fetus is less efficient in humans compared to mice. The ratio of fetal-to-maternal serum PFHxS concentrations was 0.58 (range 0.35-1.28) in humans and 1.24 (no range provided) in mice (Pizzurro et al., 2019). However, likely due to efficient lactational transfer and slow elimination, PFHxS is increased in the serum of infants and young children relative to maternal levels. Studies indicate the PFHxS concentrations in children are up to 2-fold higher at 6 months (Fromme et al., 2010) and on average 1.9-fold higher (range 1.33-2.16) at 3 years of age (Kingsley et al., 2018; Mondal et al., 2014; Papadopoulou et al., 2016) relative to maternal levels.

There are several possible ways to adjust for interspecies TK differences in PFHxS risk assessments. The best-informed method is using a physiologically based pharmacokinetic (PBPK) model, when available. While Kim et al. (2018) developed a PFHxS PBPK model in the rat and then adjusted it for humans, this model was validated only with TK data from single-dose experiments. Lack of chronic exposure considerations in this model prevents OEHHA from incorporating it into this PFHxS risk assessment.

Using PFHxS serum concentrations in dose-response analysis would also account for interspecies TK differences. OEHHA chose this method as an appropriate TK adjustment since the candidate critical studies of PFHxS toxicity in animals all reported PFHxS serum concentrations (as described below). Given that this method yields a human reference (health-protective) concentration in units of serum concentration, further TK conversion to the applied human dose was needed.

Due to the long $T_{1/2}$ of PFHxS (5.3–15.5 years, according to three studies), its elimination in humans is readily approximated by a simple first-order elimination mechanism, corresponding to a one-compartment TK model. In such a model, PFHxS elimination can be described as $C_{serum} \times CL$, where C_{serum} is serum concentration, and

CL (clearance) is a TK parameter expressed in units of ml/kg-day. Assuming steady state, the input into the system, i.e., the applied dose, would equal the chemical elimination, and therefore: Dose = C_{serum} × CL.

There are two different ways to estimate human CL. In some cases, CLs for PFAS, such as PFOA and PFOS, were calculated using independently determined values for $T_{1/2}$ and volume of distribution (V_d), kinetic parameters that can be measured in human or animal studies (US EPA, 2016a, 2016b). However, this method may introduce high uncertainty, especially if the human V_d is unknown and an animal value is assumed in its place. Instead, human CL can be calculated directly, based on the estimated intakes and measured serum concentrations in certain situations of environmental contamination, where such estimates are possible.

For PFHxS, CL can be calculated based on epidemiological data from Ronneby, Sweden, where extremely high concentrations of PFHxS were present in drinking water due to contamination from a nearby airport from approximately 1985–2013. While the exact levels of exposure throughout this period are unknown, the PFHxS concentration was 1,700 ppt at the end of the exposure period (Li et al., 2018). Extensive biomonitoring started approximately 6 months after the last exposure, and between 2014-2016, 3,418 Ronneby residents participated.

Table 1 presents PFHxS serum levels in Ronneby residents stratified by estimated duration of exposure, which was determined as length of residence at an address serviced by the affected water system. Higher median serum levels were shown with longer presumed exposure. Exposed individuals are more likely to approach steady state serum levels when exposed for longer periods, in this case for ≥10 years. Thus, OEHHA used the ≥10-year exposure group as the basis for the PFHxS CL calculation. This group had many participants (N=1,176) and a lower percentage of individuals 66-94 years of age (24.3%) than the group exposed for at least 29 years (45.7%). Individuals in the 66- to 94-year-old age group tend to have increasing levels of PFHxS that cannot be described by the steady-state model. Such increases in this age group have been described in other population studies of PFAS and could be attributed to declining kidney function with age, and as a result, decreased PFAS elimination. Thus, selecting the group exposed for at least 10 years appeared to be a reasonable compromise by maximizing exposure without introducing excessive uncertainty due to a higher number of individuals for which a steady-state assumption cannot be made.

Table 1. Exposure[a] to PFHxS in residents of Ronneby, Sweden

Reference	N	Exposure characterization	Descriptive statistic	C_{serum} ng/ml
Li et al. (2018)	3,418	Original group: general population of Ronneby	Mean ± st.dev. Median	228 ± 232 152
Silva et al. (2020)	1,845	Exposed for at least one year ending in 2013, <2-year-olds excluded	Median[b]	245
	1,176	Exposed for at least 10 years ending in 2013	Median[b]	360
	506	Exposed for at least 29 years ending in 2013	Median[b]	481

Abbreviations: st.dev., standard deviation.
[a] Exposure is defined as residence at an address serviced by the affected water system.
[b] Separate values reported for men and women; averaged values presented here were calculated based on the reported percentages for each category.

The PFHxS drinking water concentration in Ronneby was 1,700 ppt in 2013 (Li et al., 2018). Assuming an average body weight (BW) of 70 kg, water consumption rate of 1.4 L/day and absorption efficiency (f_a) of 0.9:

$$\mathrm{CL} = \frac{\mathrm{C_w} \times 1.4\ \mathrm{L/d} \times \mathrm{f_a}}{\mathrm{C_{serum}} \times \mathrm{BW}} = \frac{1{,}700 \times 1.4 \times 0.9}{360 \times 70} = 0.085\ \text{ml/kg-day} = 8.5 \times 10^{-5}\ \text{L/kg-day}.$$

This CL value is similar to the 9.0×10^{-5} L/kg-day CL estimate derived by Minnesota and New Hampshire that was based on independent measurements of $T_{1/2}$ and V_d (MDH, 2020; NHDES, 2019). Thus, the 8.5×10^{-5} L/kg-day CL value is used for conversion to an applied dose in OEHHA's derivation of a health-protective concentration for PFHxS.

TOXICOLOGICAL EFFECTS IN HUMANS

A total of 3,432 publications were identified in OEHHA's PubMed and Embase searches. Of these, 219 publications reported outcomes other than developmental and reproductive toxicity (DART) and met the inclusion criteria described in Appendix 1. An additional three publications were identified from the bibliography and review article searches. These 222 publications reported on 208 different (non-DART) health outcomes. The most common outcomes were serum lipid and thyroid hormone levels. These were also the outcomes for which associations were most frequently reported. Other outcomes for which associations were frequently identified included vaccine responses and serum liver enzyme levels. These are discussed in further detail below.

On initial review, OEHHA found that the database of epidemiologic studies involving DART outcomes was very large and complex, including hundreds of individual studies, with many reporting findings for multiple different outcomes, in several different subgroups and strata. Because of this, findings involving DART outcomes were evaluated using a different process than that used for non-DART outcomes, as detailed in Appendix 1. Two hundred seventy-one DART publications were reviewed.

Lipids

Forty-five publications provided results on PFHxS and serum lipid levels. The primary lipids assessed were total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG). Six publications involved participants who were also included in another publication (Christensen et al., 2019; Fan et al., 2020; Huang et al., 2018; Jain and Ducatman, 2019; Nelson et al., 2010; Zare Jeddi et al., 2021). Clear or consistent associations were not seen in children, neonates, or pregnant women for any lipid. In studies of TC in adults, 11 found some evidence of an association with *increased* TC, one found evidence of an association with *decreased* TC, and 12 found no associations. Major differences in results were not seen by sex or study design, and study quality, based on the factors listed in Appendix 1, was similar across these studies regardless of whether an association was reported or the direction of the association. In most of the analyses finding an association for PFHxS, PFHxS was moderately correlated with other PFAS and effect sizes were markedly greater for another PFAS compared to PFHxS. This made it difficult to separate out the individual effect of PFHxS. Other common weaknesses in these studies included inadequate statistical power, limited evaluation of confounding, or lack of dose-response data. The results for LDL, HDL, and TG were similar to those for TC, with analyses involving mostly the same weaknesses.

Thyroid hormones

Thirty-nine publications provided information on PFHxS and thyroid hormones. The hormones assessed included thyroxine (T4), free thyroxine (fT4), triiodothyronine (T3), free triiodothyronine (fT3), and thyroid stimulating hormone (TSH). Overall, the vast majority of studies found no association between PFHxS and thyroid hormone levels. In almost all studies, thyroid hormone levels were measured at a single time point, and true associations may have been missed due to non-differential outcome misclassification (i.e., misclassification of the outcome that does not markedly vary by exposure). Two studies in pregnant women found statistically significant increases in TSH with increasing serum levels of PFHxS (Reardon et al., 2019; Wang et al., 2014). However, nine studies of TSH in pregnant women reported finding no association (Berg et al., 2015; Berg et al., 2017; Inoue et al., 2019; Itoh et al., 2019; Lebeaux et al., 2020; Preston et al., 2018; Wang et al., 2013; Webster et al., 2014; Xiao et al., 2020) or an association with *decreased* TSH (Aimuzi et al., 2020). Study quality did not vary markedly between the studies with and without reported associations. For other thyroid hormones and other population groups, consistent evidence of associations was not seen. Clear or consistent associations were also not seen for thyroid diseases like hypo- or hyperthyroidism.

Vaccine response

Ten publications provided information on PFHxS and vaccine response. Four of these involved cross-sectional or prospective data from two birth cohorts from the Faroe Islands (Grandjean et al., 2012; Grandjean et al., 2017a; Grandjean et al., 2017b;

Mogensen et al., 2015). Statistically significant decreases in diphtheria or tetanus vaccination responses were reported in some analyses, depending on the age at which the PFHxS exposure or vaccine response was assessed. However, in each of these instances, markedly greater effects were seen for other PFAS. Given the correlations seen between PFHxS and these other PFAS (correlation coefficients of about 0.5 (Grandjean et al., 2012), the associations reported in these publications could not be specifically attributed to PFHxS. Most of the studies from areas other than the Faroe Islands did not report clear or consistent associations between vaccine response and PFHxS.

Liver enzymes

Ten publications reported results for PFHxS and serum levels of liver enzymes or a related biomarker, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), or total bilirubin (TB). Consistent associations were not seen in neonates or children, and few studies evaluated associations separately in males and females. Although several studies identified associations between PFHxS and increases in ALT, AST, ALP, or TB in adults, these findings were not consistent across all studies. In most of the studies reporting associations, similar or greater effect sizes were seen for other PFAS, and detailed analyses aimed at separating out the effects of PFHxS were not reported. Few studies provided information on PFHxS and overt liver diseases, and these studies either involved small sample sizes, questionable comparison groups, and/or did not find clear associations.

Other outcomes

Several studies reported associations between PFHxS and glomerular filtration rate (GFR), body mass index (BMI), or related outcomes. However, concerns exist that these findings could be due to reverse causation, i.e., the possibility of the outcome affecting PFAS exposure (Dhingra et al., 2017; Inoue et al., 2020). Few studies investigated cancer outcomes, and clear or consistent associations were not seen. For all other non-reproductive and non-developmental outcomes, one of two situations occurred. Either an association was reported in only one study, and the result was not investigated or replicated in a second population (i.e., a lack of external consistency), or a small number of studies (generally two or fewer) found an association but many more studies of similar or greater quality found no associations.

DART

OEHHA identified 271 DART studies from the >3,000 studies screened. From these, OEHHA identified a number of studies reporting associations between PFHxS and developmental outcomes, including lower birth weight, childhood infections, pubertal development, body mass index, gestational age, postnatal growth, and others. However, several of these studies involved the same potential weaknesses described above, including lack of data on the effect of correlated exposures (i.e., to other PFAS),

small sample sizes and limited statistical power, the potential for reverse causation, or lack of clear dose-response data. Importantly, these weaknesses were not identified in all studies, and true causal associations cannot be ruled out for several DART outcomes at this time.

In its most recent review, the European Food Safety Authority (EFSA, 2020) concluded that overall, there was not consistent or sufficient evidence of associations between PFHxS and DART outcomes, including birth weight, preterm birth, time to pregnancy, miscarriage, preeclampsia, neurobehavioral development, timing of puberty, male fertility, or childhood asthma and allergies. In its most recent review, ATSDR (2021) identified several epidemiologic studies reporting associations between PFHxS and outcomes such as reproductive hormone levels, certain sperm parameters, early menopause, infertility, longer time to pregnancy, birth weight, head circumference, and birth length. However, ATSDR also noted several potential weaknesses, including inconsistency of findings across different studies, associations reported in only a single study, questions regarding the adversity of some outcomes, limited evaluations of confounding, and reverse causation. Several recent review articles also described studies reporting associations between PFHxS and various DART outcomes (Ali et al., 2019; Chohan et al., 2020; Erinc et al., 2021; Fenton et al., 2021; Lee, 2018; Mokra, 2021; Sunderland et al., 2019). However, none of these reviews provided comprehensive evaluations of all studies or detailed analyses of causal inference or dose-response.

Summary

A large number of human epidemiologic studies have examined PFHxS and a variety of human health effects. For many outcomes, some high-quality studies reported associations, while others did not. For several outcomes, only a single study was identified, and the result has not yet been replicated or confirmed in a second study population. In some instances, when associations were reported for PFHxS, greater effect sizes were seen for other PFAS (usually PFOS or PFOA), and the individual effect of PFHxS could not be determined. Other limitations included inadequate statistical power, limited evaluation of confounding, or inappropriate comparison groups. In summary, an accurate assessment of the dose-response patterns of PFHxS in humans would be difficult to make based solely on the epidemiologic data available at this time.

TOXICOLOGICAL EFFECTS IN ANIMALS

OEHHA identified eleven published animal toxicity studies conducted with oral administration of PFHxS to mice or rats. These studies are summarized in chronological order in Table 2.

Table 2. Summary of PFHxS[a] studies and endpoints in animals

Sex/Species/ Reference	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/ LOAEL
Crl:CD(SD)IGS BR VAF/Plus Rat Male and female (15/sex/dose) Butenhoff et al. (2009)	0, 0.3, 1, 3, 10 mg/kg-day by gavage, from 14 days prior to cohabitation to SD44 (males); GD21, PND22 (females) or presumed GD25 (females, no litter); PND22 (F_1) F_1 were not dosed directly	Serum concentrations are reported for F_0 (m and f separately) and F_1 (pooled) for all doses at multiple time points	F_0 males: ↓ body weight gain; ↑ abs. and rel. liver weight; minimal to moderate hepatic hypertrophy with ↑ enlarged centrilobular hepatocytes; hematological changes; ↓ total cholesterol F_0 both sexes: minimal to moderate thyroid hypertrophy/ hyperplasia (follicular epithelium cells)[b]	NOAEL: 1 mg/kg-day for liver effects in males
E3L.CETP[c] C57/BL Mouse Male (4-8/dose) Bijland et al. (2011)	0, 6 mg/kg-day in the diet for 4–6 weeks	Experiment 1: 217.6 ± 13.3[d] (N=8, 6 weeks) Experiment 2: 197 ± 10.4[d] (N=6, 4 weeks) Experiment 3: 188.3 ± 31.5[d] (N=6, 4 weeks)	<u>Liver effects</u>: ↑ abs. liver weight; ↑ liver triglycerides; ↓ fecal bile acid secretion; ↓ hepatic VLDL production <u>Lipids and lipid-related:</u> ↓ plasma triglycerides; ↓ plasma cholesterol (HDL and non-HDL); ↓ plasma ApoAI; ↓ plasma free fatty acids; ↓ plasma glycerol; ↓ plasma VLDL clearance; ↓ plasma HDL-cholesterol clearance and catabolic rate	NA[e]
NMRI Mouse Male, female (4-6/dose/sex) Lee and Viberg (2013)	0, 6.1, 9.2 mg/kg by oral gavage (single exposure), sacrificed at 24 hours, 4 months	Not reported	Changes in specific protein levels in the hippocampus	NA[e]
NMRI Mouse Male, female (15/dose/sex) Viberg et al. (2013)	0, 0.61, 6.1, 9.2 mg/kg by oral gavage (single exposure) at PND10	Not reported	Changes in motor activity, spontaneous behavior and nicotine-induced behavior at 2–4 months after a single dose	NA[e]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/ LOAEL
SV129 Mouse (wt and PPARα-null) Male (4/dose) Das et al. (2017)	0, 10 mg/kg-day by oral gavage for 7 days	Not reported	↑ abs. and rel. liver weight; ↑ hepatocyte hypertrophy; ↑ liver steatosis; ↑ liver triglyceride levels; changes in gene expression	NA[e]
SV129S4 (PPARα-null) and SV129S1 (wt) Mouse Male (4/dose) Rosen et al. (2017)	0, 3, 10 mg/kg-day by oral gavage for 7 days	Not reported	↑ abs. and rel. liver weight; changes in gene expression	LOAEL: 3 mg/kg-day for rel. liver weight
Crl:CD1 (ICR) Mouse Male, female (10-30/sex/dose) Chang et al. (2018)	0, 0.3, 1, 3 mg/kg-day by gavage, from 14 days prior to cohabitation to SD42 (males); PND22 (females); PND36 (F_1) F_1 were dosed directly (PND22-PND36)	Serum concentrations are reported for F_0 (m and f separately) and F_1 (m and f separately, and pooled) for all doses at multiple time points	<u>Liver effects (F_0)</u>: ↑ abs. and rel. liver weight (m, f); cytoplasmic alternation, ground-glass (m, f); microvesicular fatty acid change (m); cytoplasmic vacuolation (f); hepatocellular centrilobular hypertrophy (m, f); single-cell necrosis (m) <u>Developmental effects</u>: ↓ number of pups/litter; ↓ live pups/litter; ↑ abs. and rel. liver weight in F_1; ↑ anogenital distance (at all doses, but no dose-response) <u>Serum (males)</u>: ↓ total cholesterol; ↓ bilirubin; ↑ alkaline phosphatase	NOAEL: 0.3 mg/kg-day for liver and developmental effects

Sex/Species/ Reference	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/ LOAEL
Wistar Rat Female (pregnant) (8–20/dose) Ramhøj et al. (2018); Ramhøj et al. (2020)[f]	Study 1: 0, 25, 45 mg/kg-day; Study 2: 0, 0.05, 5, 25 mg/kg-day; both studies by oral gavage for up to 35 days (GD7-PND22, dams; pups, not directly dosed, were sacrificed at PND17)	Not reported	↓ serum T4 (dams, F_1); ↓ serum T3 (dams, F_1); ↑ nipple retention (m F_1, in one of two studies) ↑ retroperitoneal fat pad and adrenals (m F_1, in one of two studies); ↑ liver weight (F_1); ↓ thyroid weight (f F_1); mild alterations in thyroid histology (m F_1); minor changes in F_1 motor activity and learning and memory in the radial arm maze	NOAEL: 0.05 mg/kg-day for decreased T4 (dams, F_1) and decreased rel. liver weight (F_1)
Sprague-Dawley Rat Male, female (10/sex/dose) NTP (2019)	0, 0.625, 1.25, 2.5, 5, 10 mg/kg-day (males); 0, 3.12, 6.25, 12.5, 25, 50 mg/kg-day (females) by oral gavage for 28 days	<u>Males</u>[g] 0.1022 ± 0.0144, 66.76 ± 3.5183, 92.08 ± 3.3479, 129 ± 5.5035, 161.7 ± 2.5124, 198.3 ± 4.9557 <u>Females</u>[g] 0.1744 ± 0.0223 37.03 ± 1.6509, 50.41 ± 1.5522, 63.820 ± 3.2015, 0.82 ± 3.7395, 95.51 ± 3.7455	<u>Liver effects:</u> ↑ abs. and rel. liver weight (m, f); hepatocellular hypertrophy (m); ↑ hepatic acyl-CoA oxidase activity (m) <u>Thyroid effects:</u> ↓ total T4 (m, f); ↓ free T4 (m, f); ↓ total T3 (m); <u>Hematology (males)</u>: ↓ reticulocyte count; ↓ plasma cholesterol; ↓ triglycerides; ↓ globulin; ↑ albumin/globulin ratio; <u>Nasal lesions (females)</u>: olfactory epithelium degeneration, hyperplasia, and suppurative inflammation <u>Other effects</u>: ↑ rel. kidney weight (m); ↓ abs. and rel. adrenal gland weight (m); ↑ rel. adrenal gland weight (f)	LOAEL: 0.625 mg/kg-day for decreased thyroid hormones in males

Sex/Species/ Reference	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/ LOAEL
C57BL/6J Mouse Male (6/dose) Pfohl et al. (2020)	0, 0.15 mg/kg-day in diet (high-fat (H) or low-fat (L)) for 29 weeks	L, serum: 62.0 ± 5.9[h] H, serum: 36.8 ± 4.3[h]	↑ abs. and rel. white adipose tissue weight (H); ↓ hepatic cholesterol (L); ↓ serum cholesterol (H); changes in blood lipids and phospholipids; changes in gene expression and protein expression	NA[e]

Abbreviations: abs., absolute; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ApoAI, apolipoprotein A-I; f, female; F_0, parental generation; F_1, first generation (pups); FOB, functional observational battery; GD, gestation day; HDL, high-density lipoprotein; LOAEL, lowest-observed-adverse-effect level; m, male; NA, not applicable; NOAEL, no-observed-adverse-effect level; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor α; SD, study day; rel., relative; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; VLDL, very low density lipoprotein; wt, wild type.

[a] Every study except Rosen et al. (2017) used the potassium salt of PFHxS (K^+PFHxS); Rosen et al. (2017) used the free acid of PFHxS.

[b] As determined by ATSDR (2021)

[c] E3L.CETP mice have attenuated clearance of apoB-containing lipoproteins and exhibit a human-like metabolism on a Western-type diet (Bijland et al., 2011).

[d] Average ± standard deviation, number of animals (N) is indicated

[e] LOAELs/NOAELs are not applicable (NA) for single-dose or single-exposure studies.

[f] Ramhoj et al. (2020) refers to Ramhoj et al. (2018) for experimental design and provides additional data for Study 2 originally reported in Ramhoj et al. (2018).

[g] Average ± standard error (N=10)

[h] Average ± standard error (N=3-6)

After reviewing the animal toxicity studies, OEHHA chose Butenhoff et al. (2009), Chang et al. (2018) and NTP (2019) as candidate critical studies. These studies employed multiple doses with higher numbers of animals per dose (10+), measured serum PFHxS concentrations, and reported multiple adverse effects at low doses, from which a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) could be identified. Although the Ramhoj et al. (2018, 2020) studies had the lowest NOAEL, the 100-fold difference between the two lowest doses does not provide clear information on where the effect might be in this wide range. Moreover, these studies did not report serum concentrations and were a compilation of separate experiments. The only available chronic PFHxS study (Pfohl et al., 2020) employed only a single dose group, focused on a limited number of endpoints (lipids) in mice, and did not report any adverse effects relevant for human health. Findings from this and the remaining animal studies were used as supporting evidence for critical effect determination. No animal cancer studies are available for PFHxS.

Butenhoff et al. (2009) is a DART study in rats. The most sensitive effects in this study, with a NOAEL of 1 mg/kg-day, appeared to be increased absolute and relative liver weights with minimal to moderate hepatic hypertrophy in males and thyroid hypertrophy/hyperplasia in both sexes (Table 2). While serum cholesterol was

decreased at all doses in males, with a LOAEL of 0.3 mg/kg-day, this was not considered as a candidate critical effect, as described in greater detail below. Hemoglobin was decreased at all but the lowest dose, but the maximum decrease was less than 7% compared to the control, and no clear dose-response was observed. There were no treatment-related changes in body weight, mating and fertility parameters, pregnancy status and outcomes, microscopic findings, organ weights (other than liver in males), histology (other than in liver), sperm parameters, primordial follicle counts, serum AST, serum ALT, F_1 liver weights, hematology parameters (females), or the functional observational panel (FOB). For its assessment, ATSDR (2021) selected thyroid hypertrophy and hyperplasia from Butenhoff et al. (2009) as the critical endpoint, with a NOAEL of 1 mg/kg-day. However, it is unclear how this NOAEL was determined.

Chang et al. (2018) is a DART study in mice. The most sensitive adverse effects in this study, with a NOAEL of 0.3 mg/kg-day, were increased absolute and relative liver weights, with corresponding hepatic hypertrophy, and developmental effects, such as decreased litter size, decreased number of live pups per litter and increased pup absolute and relative liver weights. There were no treatment-related effects on body weight, mating and fertility parameters, pregnancy outcomes (except litter size), microscopic findings, organ weights (except liver), histology (except liver), sperm parameters, serum AST, ALT, serum markers of kidney function, serum TSH, or the functional observation battery (FOB), motor activity, anogenital distance in female or all pups, areolate/nipple anlagen retention in male pups, pup survival, pup body weight, preputial separation (male F_1), vaginal opening (female F_1), thyroid weights (except a slight increase in female F_1, PND36), or thyroid histology.

The most sensitive adverse effects in a 28-day NTP (2019) study in rats were decreased levels in thyroid hormones (T4 and T3), with a LOAEL of 0.625 mg/kg-day, in male rats (Table 2). Total T4 was also decreased in female rats but at higher doses, with a NOAEL of 3.12 mg/kg-day. TSH levels were unchanged in both sexes. Consistent with other studies, absolute and relative liver weights were increased, with corresponding increases in liver pathology. There were no treatment-related changes in body weight. Decreased plasma cholesterol was observed at all but the lowest dose in males.

Overall, OEHHA identified four types of candidate critical effects: liver toxicity, thyroid toxicity, developmental toxicity, and perturbations of lipid homeostasis. These effects are discussed in more detail in the following sections and, when applicable, analyzed with benchmark dose modeling. It is OEHHA's policy to determine the point of departure (POD) from a toxicity study by fitting a dose-response model to the data using the US EPA Benchmark Dose Software[5] (BMDS version 2.7) when possible. The BMDS uses mathematical models to fit data and determines the dose (benchmark dose or BMD) corresponding to a pre-determined level of response (benchmark response or BMR). Typically, OEHHA uses a BMR of 5% above the background or the response of the

[5] Available at: https://www.epa.gov/bmds

control group for dichotomous data. For continuous data, a BMR of one standard deviation (SD) from the control mean is typically used when there are no data to indicate what level of response is biologically significant (OEHHA, 2008).

Liver toxicity

Increased absolute and relative liver weights were observed in each of the three candidate critical studies, as well as in additional mouse studies (Bijland et al., 2011; Das et al., 2017; Rosen et al., 2017) (Table 2). PFHxS serum concentrations were reported, and dose-response analysis was possible for both sexes in the NTP (2019) rat study and males in the Butenhoff et al. (2009) and Chang et al. (2018) studies. Female serum PFHxS concentrations in the latter two studies were also reported but demonstrated non-monotonous trends through pregnancy and lactation. Due to the lack of appropriate TK tools to estimate time averaged PFHxS concentration in the model of rat or mouse pregnancy, female liver weight datasets from Butenhoff et al. (2009) and Chang et al. (2018) were not included in the dose-response analysis.

The selected datasets for relative liver weight are plotted in Fig. 1A. An additional male mouse study (Pfohl et al., 2020), which is the only available chronic animal study for PFHxS, is included. In this single-dose study, there was no significant increase in relative liver weight compared to control, although the number of animals was low (N=6), and the variance was quite high. The top two curves in this graph (Fig. 1A) are from male mice, while the bottom three are from male and female rats. It appears that dose-response data for relative liver weights are consistent among species and the studies. Additional pathological findings were reported in the liver, including hypertrophy, histological changes, and single-cell necrosis (Table 2). These cumulative findings would constitute histological evidence of structural degenerative or necrotic changes, and therefore, the PFHxS-induced increase in relative liver weight is considered adverse (Hall et al., 2012).

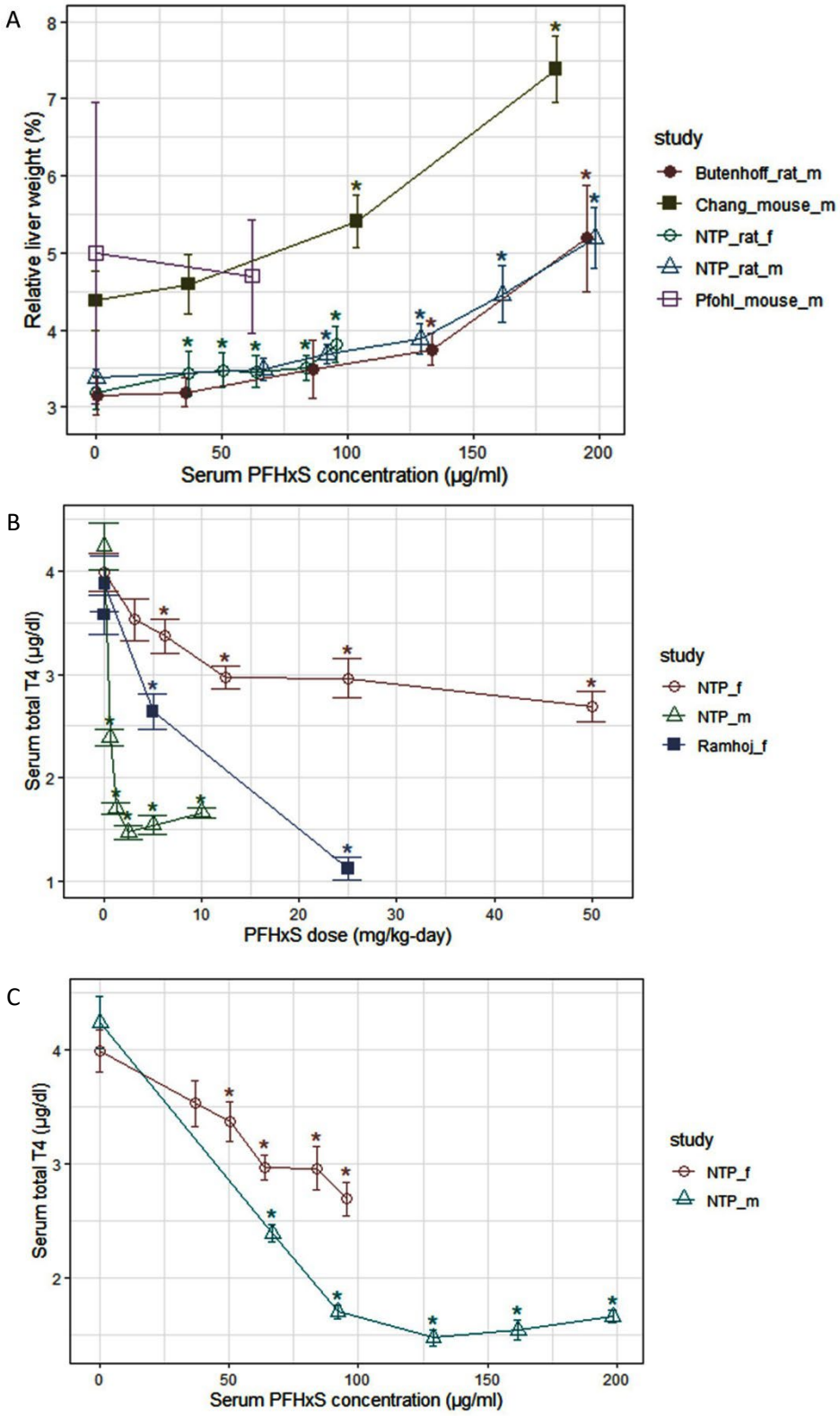

Figure 1. Combined dose-response for relative liver weight vs serum PFHxS concentration (A), and serum total thyroxine (T4) vs PFHxS dose (B) or serum concentration (C). Studies: Butenhoff et al. (2009); Chang et al. (2018); NTP (2019); Pfohl et al. (2020); Ramhøj et al. (2020). Abbreviations such as NTP_rat_f refer to the study, species, and sex (f, female; m, male). Values represent means ± standard deviations (A), or means ± standard errors of measurement (B, C) of N=10-30 (see Table 3 and references). Values that are significantly different from the corresponding control ($p<0.05$) are indicated with an asterisk.

The results for the dose-response analysis of increased relative liver weight datasets are presented in Table 3. Data were modeled over PFHxS serum concentrations, with a BMR of one standard deviation (1 SD). This default BMR was chosen because the threshold of concern for this endpoint is unknown. Among models with an acceptable fit ($p>0.05$), the model with the lowest Akaike information criterion (AIC) was chosen, and its BMD (benchmark dose) and BMDL (the lower limit of the one-sided 95% confidence interval on the BMD) were included in Table 3. Details of BMDS analyses are provided in Appendix 2. When BMDS modeling of a given dataset is possible, OEHHA uses the resulting BMDL as a POD for risk assessment. When no acceptable model is produced by BMDS, modifications to the analysis, such as dropping the high dose or using models with modeled variance, can be applied.

In the BMDS analysis of relative liver weight, the BMDLs ranged from 34.3–48.1 µg/ml (serum PFHxS concentration). One dataset did not produce a BMDL (poor model fit), so the corresponding NOAEL would be used as a POD.

Table 3. Dose-response modeling results for PFHxS candidate critical studies

Study Sex/Species (N) Duration	Serum Concentration (µg/ml)	Critical Effect	Critical Effect Value (% of control)[a]	NOAEL or LOAEL (serum conc., µg/mL; applied dose, mg/kg-day)	BMD/BMDL (µg/ml) p-value
Butenhoff et al. (2009) Male rats (15) 44 days	0.26[b] 35.54 86.4 133.5 195.2	↑ Rel. liver weight	100 ± 8 102 ± 6 111 ± 12 119 ± 7 165 ± 22	NOAEL: 81.0 µg/ml (1 mg/kg-day)	Poor model fit
Chang et al. (2018) Male mice (30) 42 days	0 36.9[b] 103.7 182.9	↑ Rel. liver weight	100 ± 9 105 ± 9 124 ± 8 168 ± 10	NOAEL: 36.9 µg/ml (0.3 mg/kg-day)	58.1/48.1 p = 0.7509
NTP (2019) Male rats (10) 28 days	0.1022 66.76 92.08 129 161.7 198.3	↑ Rel. liver weight	100 ± 3 103 ± 4 109 ± 4 114 ± 6 132 ± 11 154 ± 12	NOAEL: 66.8 µg/ml (0.625 mg/kg-day)	65.0/51.7 (modeled variance) p = 0.3283
NTP (2019) Female rats (10) 28 days	0.1744 37.03 50.41 63.82 83.82 95.51	↑ Rel. liver weight	100 ± 7 108 ± 9 109 ± 7 108 ± 7 110 ± 5 119 ± 7	NOAEL: 37.0 µg/ml (3.12 mg/kg-day)	44.7/34.3 p = 0.1131
NTP (2019) Male rats (10) 28 days	0.1022 66.76 92.08 129 161.7 198.3	↓ Total T4	100 ± 17 56 ± 6 40 ± 4 35 ± 5 36 ± 7 39 ± 4	LOAEL: 66.76 µg/ml (0.625 mg/kg-day)	39.67/28.63 (modeled variance) p = 0.1702

Study Sex/Species (N) Duration	Serum Concentration (µg/ml)	Critical Effect	Critical Effect Value (% of control)[a]	NOAEL or LOAEL (serum conc., µg/mL; applied dose, mg/kg-day)	BMD/BMDL (µg/ml) p-value
NTP (2019) Male rats (10) 28 days	0.1022 66.76 92.08 129 161.7 198.3	↓ Free T4	100 ± 18 47 ± 13 28 ± 5 21 ± 4 22 ± 5 22 ± 5	LOAEL: 66.76 µg/ml (0.625 mg/kg-day)	42.03/31.33 (modeled variance) p = 0.4261
NTP (2019) Male rats (10) 28 days	0.1022 66.76 92.08 129 161.7	↓ Total T3	100 ± 21 78 ± 16 69 ± 11 64 ± 9 62 ± 5	LOAEL: 66.76 µg/ml (0.625 mg/kg-day)	79.60/40.27 (high dose dropped, modeled variance) p = 0.6097
NTP (2019) Female rats (10) 28 days	0.1744 37.03 50.41 63.82 83.82 95.51	↓ Total T4	100 ± 15 88 ± 16 84 ± 13 74 ± 9 74 ± 15 67 ± 12	NOAEL: 37.03 µg/ml (3.12 mg/kg-day)	38.50/29.57 p = 0.823
NTP (2019) Female rats (10) 28 days	0.1744 37.03 50.41 63.82 83.82 95.51	↓ Free T4	100 ± 20.8 87 ± 21 84 ± 27 66 ± 10 70 ± 19 62 ± 17	NOAEL: 50.41 (6.25 mg/kg-day)	47.86/29.46 p = 0.4342

Abbreviations: LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; rel., relative; T3, triiodothyronine; T4, thyroxine.

[a] Values are mean ± standard deviation (N is study-specific).

[b] PFHxS serum concentrations were time-averaged based on reported serum levels.

Thyroid toxicity

Similar to findings with other PFAS, exposure to PFHxS resulted in decreased levels of thyroid hormones (T4, T3), while TSH levels were unchanged (NTP, 2019; Ramhøj et al., 2020). This endocrine disorder is termed hypothyroxinemia (i.e., 'low thyroxine' or T4). Maternal hypothyroxinemia can spontaneously occur in human pregnancy and has been linked to developmental and cognitive delays in offspring (Negro et al., 2011), making the in utero life stage particularly sensitive to additional disturbance. Importantly, decreased T4 levels in pregnancy were correlated with neurodevelopmental and cognitive deficits in children (Haddow et al., 1999). The underlying mechanisms of hypothyroxinemia appear distinct from perturbations of the HPT (hypothalamic-pituitary-thyroid) axis, where lower T3 and T4 levels would drive a compensatory increase in TSH.

While thyroid toxicity has been observed in rats exposed to PFHxS (NTP, 2019; Ramhøj et al., 2018; Ramhøj et al., 2020), one could argue that the shorter plasma half-life of T4 in rodents, due to the absence of the high affinity thyroxine-binding globulin (TBG) in the adult stage, predisposes the rodent thyroid to derangement by chemicals. However,

mice appear less susceptible than rats (Brändli-Baiocco et al., 2018). On the other hand, due to human and rodent physiological particularities, “the dynamic reserve capacity of T4 between humans and rodents near birth and in early postpartum might not be significantly different,” as US EPA (2021) concluded in the recent toxicity assessment for PFBS (perfluorobutane sulfonic acid). Overall, the endpoints for decreased T3 and T4 levels in rats (NTP, 2019) constitute adverse effects.

OEHHA compared the dose-response for total T4 based on applied doses (Fig. 1B) and serum concentrations (Fig. 1C) for the available studies (NTP, 2019; Ramhøj et al., 2020). Ramhøj et al. (2020) did not report serum concentrations, and only dose-based analysis was possible for this study. While the dose-response curves based on applied dose (two for female rats and one for male rats, Fig. 1B) diverged widely, the dose curves for the two available datasets (rats of either sex) based on serum concentrations were close to each other (Fig. 1C). Thus, dose-response based on serum concentrations appeared to smoothen the observed difference in dose-based curves and to account for sex-specific TK differences. This demonstrates the advantage of dose-response analysis based on serum concentrations. Alternatively, using applied doses would require TK adjustments and PBPK (physiologically based pharmacokinetic) models to account for dynamic changes in pregnancy. OEHHA concluded this approach would markedly increase uncertainty and did not include studies that did not report serum concentration, such as Ramhøj et al. (2020), among candidate critical studies.

The results of dose-response analyses for decreased T3 and T4 levels (NTP, 2019) are presented in Table 3. Data were modeled using PFHxS serum concentrations. The BMR was set at the default value of 1 SD because the level of maternal hypothyroxinemia resulting in neonate neurodevelopmental and cognitive damage is unknown. The resulting BMDLs for T4 values all grouped around 30 µg/ml, and the BMDL for total T3 in male rats was 40 µg/ml (Table 3).

In addition to decreased T3 and T4 levels in adult and neonate rats (NTP, 2019; Ramhøj et al., 2018; Ramhøj et al., 2020), thyroid effects of PFHxS exposure included thyroid hypertrophy and hyperplasia (Butenhoff et al., 2009), which was used as the basis for the ATSDR PFHxS assessment (ATSDR, 2021). This histopathological result is presented in Table 4 (reproduced from Table 4 in Butenhoff et al. (2009)).

Table 4. Thyroid hypertrophy/hyperplasia (follicular epithelium) of the F_0 generation male rats (Butenhoff et al., 2009)

	K^+PFHxS dose (mg/kg-day)				
	0	0.3	1.0	3.0	10.0
Number of rats evaluated	10	10	10	10	10
Minimal	0	1	1	2	0
Mild	2	2	1	2	3
Moderate	0	0	0	0	4
Total incidence	2	3	2	4	7

Regarding this dataset, ATSDR (2021) states, "In a developmental toxicity study, increased incidences of thyroid follicular cells hypertrophy, and hyperplasia were observed in F_0 male rats administered ≥3 mg/kg/day." However, datasets for other thyroid toxicity endpoints, such as decreases in thyroid hormones, appear to be more consistent and more sensitive than the thyroid hypertrophy/hyperplasia finding in Butenhoff et al. (2009).

Developmental toxicity

Two PFHxS studies found developmental effects, including decreased litter sizes, decreased live litters and increased absolute and relative liver weights in F_1 mice (Chang et al., 2018), and decreased thyroid hormones with changes in thyroid weight (females) or histology (males) in F_1 rats (Ramhøj et al., 2020). Decreased litter size and decreased live litters are severe adverse effects, and the corresponding data (Chang et al., 2018) are presented in Table 5. The statistical analysis is from the original report.

Table 5. Pregnancy outcomes in mice exposed to PFHxS (Chang et al., 2018)

Dose (mg/kg-day)	N	Number of implantation sites[a]	Number of pups born per litter[a]	Mean live litter size[a]
0	27	12.9 ± 1.65	12.3 ± 1.86	12.3 ± 1.86
0.3	29	12.4 ± 2.06	11.8 ± 2.28	11.8 ± 2.28
1	28	11.9 ± 1.99	10.8 ± 2.23*	10.6 ± 2.39*
3	25	11.7 ± 1.95	10.9 ± 1.73*	10.8 ± 1. 72*

[a] Data are presented as mean ± standard deviation.
*Statistically significantly different from control at p ≤0.05 using Dunnett's test.

The NOAEL for litter-related endpoints (based on reported statistical differences, Table 5) was 0.3 mg/kg-day, which corresponded to the dam serum concentration of 16.8 µg/ml on GD18 (gestation day 18). 'Number of pups born per litter' and 'mean live litter size' had similar NOAELs based on the reported statistical differences with control. These two effects are referred to as 'decreased litter size' for simplicity in the rest of this document.

Despite being reported as means and standard deviations, the underlying data for litter sizes are not normally distributed since they are necessarily bounded (litter size cannot exceed a certain value). Therefore, further statistical analysis requiring the assumption of normally distributed data (such as BMDS modeling) was not appropriate. Since individual litter data for this dataset were not available, alternative modeling approaches were not feasible. Therefore, OEHHA used the NOAEL of 16.8 µg/ml serum concentration for decreased litter size as a candidate critical POD for PFHxS.

The New Hampshire Department of Environmental Sciences chose decreased litter size in Chang et al. (2018) as a critical effect in its PFHxS assessment, but in contrast with OEHHA, they conducted the dose-response modeling analysis of this dataset with the BMDS continuous model suite (Ali et al., 2019; NHDES, 2019). The BMDL with the benchmark response set at a standard deviation of 0.5 was 13.9 µg/ml (Ali et al., 2019), close to the NOAEL chosen by OEHHA for this study (16.8 µg/ml).

The absolute and relative liver weights in the F_1 generation were also decreased, with a NOAEL of 1 mg/kg-day (Chang et al., 2018). These data could not be modeled due to the lack of individual animal data. Therefore, the sex-specific PODs for this health effect are mean PFHxS serum concentrations at the NOAEL, 52.0 and 64.6 µg/ml for female and male pups, respectively.

Absolute anogenital distance (AGD) and AGD adjusted to the cube root of body weight were significantly increased at all doses for male pups, while no changes were observed in females (Chang et al., 2018). This is an unusual finding. With regard to one-generation reproductive toxicity studies, the Organization for Economic Co-operation and Development (OECD) states, “A statistically significant change in AGD that cannot be explained by the size of the animal indicates an adverse effect of exposure and should be considered in setting the NOAEL” (OECD, 2013). In practice, such changes encompass decreased AGD in males and increased AGD in females, and the underlying endocrine mechanisms are well understood (Schwartz et al., 2019). Given the small extent of increased AGD (<5%) with the male F_1 pups, lack of dose-response, and lack of effect in females (Chang et al., 2018), OEHHA did not consider this endpoint for POD determination.

For the toxicological review of developmental effects, OEHHA also considered thyroid-related findings in F_1 rats reported by Ramhøj et al. (2020). In this study, T4 levels in pups closely mirrored those in the dams. Significant differences compared with controls were observed at the mid- and high-doses, although the extent of T4 decreases was greater in dams compared to pups. Due to the absence of reported serum concentrations for PFHxS, this study was not considered for a candidate POD. However, because thyroid hormone levels were affected by PFHxS in pups and dams at the same doses as those applied only to dams, it appears that pups are not more sensitive than dams for this endpoint.

Lipids

Following PFAS treatment, changes in lipid homeostasis are among the most sensitive biological effects in animals. Decreased cholesterol was the most sensitive effect of PFHxS in rats (Butenhoff et al., 2009; NTP, 2019). In animal models, PFHxS appeared to promote liver steatosis by increasing lipid accumulation and synthesis in the liver while interfering with lipid transport out of the liver (Bijland et al., 2011; Das et al., 2017; Pfohl et al., 2020). In agreement with this mechanism, serum triglycerides and cholesterol were decreased in animal studies (Bijland et al., 2011; Butenhoff et al., 2009; Das et al., 2017; NTP, 2019; Pfohl et al., 2020). In contrast, most human studies found increased or unchanged levels of cholesterol due to PFHxS exposure. PFHxS-dependent lipid effects were drastically attenuated in PPARα-null mice, indicating the central role of this receptor in underlying mechanisms (Das et al., 2017). Differences between human and rodent PPARα are thought to contribute to large differences in lipid homeostasis between human and animal models of PFOA and PFOS (Fragki et al., 2021). While less information is available for PFHxS, it is likely similar to PFOA and PFOS in adverse effects on lipid homeostasis, such as changes in cholesterol levels. Therefore, these endpoints in animals may not quantitatively predict human toxicity and health-protective concentrations.

CRITICAL EFFECT DETERMINATION AND HEALTH-PROTECTIVE CONCENTRATION CALCULATIONS

OEHHA develops health-protective concentrations (HPCs) that are expected to result in no adverse effects from daily exposure over a lifetime. For noncancer effects, HPC derivation starts with the PODs derived from the most sensitive animal or human studies, i.e., those studies that observe health-adverse effects at the lowest doses. This dose is converted to an acceptable daily dose (ADD), which is then back-calculated to the HPC in tap water. Because there were no studies of the carcinogenicity of PFHxS, only a noncancer HPC was derived.

OEHHA evaluated the health outcomes of the most sensitive animal toxicity studies available in the literature for HPC derivation. In the three selected candidate critical studies, the most sensitive health outcomes included effects on the liver, thyroid, and developing offspring following oral exposure to PFHxS. The ranked list of candidate PODs from these studies is presented in Table 6. No endpoints were included from Butenhoff et al. (2009) since the most sensitive POD in this study was 81 µg/ml (NOAEL for increased relative liver weight in male F_0 rats), exceeding other candidate PODs.

OEHHA considered other animal studies and health outcomes (e.g., lipids, thyroid hypertrophy/hyperplasia). However, those endpoints were not as sensitive as those listed in Table 6 and an HPC based on those effects would not adequately protect against these more sensitive effects. For the studies/endpoints where OEHHA could not develop BMDLs, NOAEL values were used as PODs.

Table 6. Candidate critical PODs for PFHxS animal studies (serum concentrations)

Study	Sex/Species	Endpoint	POD (µg/ml)	POD type
Chang et al. (2018)	Female mouse	↓ Litter size	16.8	NOAEL
NTP (2019)	Male rat	↓ Total T4	28.6	$BMDL_{1SD}$
NTP (2019)	Female rat	↓ Free T4	29.5	$BMDL_{1SD}$
NTP (2019)	Female rat	↓ Total T4	29.6	$BMDL_{1SD}$
NTP (2019)	Male rat	↓ Free T4	31.3	$BMDL_{1SD}$
NTP (2019)	Female rat	↑ Rel. liver weight	34.3	$BMDL_{1SD}$
NTP (2019)	Male rat	↓ Total T3	40.3	$BMDL_{1SD}$
Chang et al. (2018)	Male mouse	↑ Rel. liver weight	48.1	$BMDL_{1SD}$
NTP (2019)	Male rat	↑ Rel. liver weight	51.7	$BMDL_{1SD}$
Chang et al. (2018)	F_1 female mouse	↑ F_1 rel. liver weight	52.0	NOAEL

Abbreviations: 1SD, one standard deviation; F_1, first generation (pups); rel., relative; T3, triiodothyronine; T4, thyroxine.

Critical Effect Determination

Table 6 lists the PODs (either as NOAELs or BMDLs) derived from the studies OEHHA identified as suitable for quantitative dose-response analysis and HPC derivation. The results from animal toxicity studies of PFHxS in mice and rats indicate adverse pregnancy outcomes (litter size), changes in thyroid hormone levels, and liver toxicity as the most sensitive endpoints. The most sensitive PODs for the three types of endpoints range from 16.8–34.3 and differ only about 2-fold among each other.

Adverse liver effects, exemplified by increased relative liver weight, are among the most common and consistent findings reported in PFHxS animal toxicity studies (Fig. 1A). Each of the three candidate critical studies (Butenhoff et al., 2009; Chang et al., 2018; NTP, 2019) reported increased relative liver weight in both sexes of either mice or rats (Table 2). As described in the *Liver Toxicity* section, multiple adverse effects in the liver were reported, supporting the classification of the observed relative weight increase as adverse. Thus, PODs for increased relative liver weight were suitable candidates for PFHxS HPC derivation.

Decreased number of live pups per litter (or litter size, for short) is a severe adverse health effect. While OEHHA's analysis of human DART studies did not reveal obvious trends for adverse pregnancy outcomes due to PFHxS exposure, adverse effects of PFHxS in human reproductive and developmental health cannot be excluded. Epidemiological analysis of pregnancy is particularly challenging because pregnancy changes the elimination rate of PFHxS, making estimates of PFHxS exposure prior to and throughout pregnancy difficult. Nonetheless, the POD for decreased litter size is a suitable candidate for PFHxS HPC derivation.

The third type of candidate critical endpoint for POD derivation is decreased thyroid hormone levels. Thyroid hormones are critically important for human health. Hypothyroidism (deficiency of thyroid hormones) results in severe adverse effects, including growth retardation, neurological abnormalities and sometimes impaired hearing in infants and children, impaired cardiovascular function (bradycardia, increased peripheral resistance, diminished cardiac output), impaired pulmonary function, impaired peristalsis, impaired renal function and anemia in adults (Gardner and Shoback, 2017). PFHxS-driven decreases in thyroid hormones appear to be less severe health effects compared to classical hypothyroidism in that they do not increase the levels of thyroid-stimulating hormone (TSH). Still, decreased T4, even without the compensatory increase in TSH (hypothyroxinemia), was correlated with neurodevelopmental and cognitive deficits in children (Negro et al., 2011; Haddow et al., 1999). For PFHxS, there are no developmental studies of thyroid hormone levels in animals, and no mouse studies reporting T4 or T3 levels. Despite this uncertainty, the POD for decreased T4 in male rats is a suitable candidate for PFHxS HPC derivation, due to severity of possible developmental consequences of decreased T4 in humans.

Thus, OEHHA identified decreased litter size in mice (Chang et al., 2018), increased relative liver weight in female rats (NTP, 2019) and decreased total T4 in male rats (NTP, 2019) as suitable candidates for the PFHxS HPC derivation. These endpoints target different life-stages and would have different underlying mechanisms of toxicity. The corresponding POD serum concentrations (C_{serum}) are 16.8 mg/L, 34.3 and 28.6 mg/L, respectively.

Health-Protective Concentration Calculation

Human Point of Departure (POD)

To derive a human POD from the animal POD, OEHHA derived a human clearance (CL) value of 8.5×10^{-5} L/kg-day as described in the *Toxicokinetics* section.

For decreased litter size in mice (Chang et al., 2018):

$$POD_{human} = C_{serum} \times CL = 16.8 \frac{mg}{L} \times 8.5 \times \frac{10^{-5} L}{kg - day} = 0.00143 \frac{mg}{kg - day}$$

For increased relative liver weight in female rats (NTP, 2019):

$$POD_{human} = C_{serum} \times CL = 34.3 \frac{mg}{L} \times 8.5 \times \frac{10^{-5} L}{kg - day} = 0.00292 \frac{mg}{kg - day}$$

For decreased total T4 in male rats (NTP, 2019):

$$POD_{human} = C_{serum} \times CL = 28.6 \frac{mg}{L} \times 8.5 \times \frac{10^{-5} L}{kg - day} = 0.00243 \frac{mg}{kg - day}$$

Acceptable Daily Dose (ADD)

An ADD is an estimated maximum daily dose of a chemical (in mg/kg-day) that can be consumed for an entire lifetime without adverse effects. To determine the ADD, the POD is adjusted by factors to account for uncertainties in the risk assessment, such as differences between animals and humans (interspecies extrapolation), and differences among humans (intraspecies variation, including sensitive subgroups) in response to a chemical exposure. Additionally, factors may be applied to extrapolate from subchronic to chronic exposure duration, from LOAEL to NOAEL when a NOAEL or BMDL is not available, and also when the toxicity database is incomplete. These factors combined are referred to as the composite uncertainty factor (UF).

When developing health-protective concentrations for noncancer effects based on animal toxicity studies, OEHHA generally applies a composite UF of 300, consisting of 10 for interspecies extrapolation (√10 for toxicokinetics and √10 for toxicodynamics) and 30 for intraspecies variability (10 for toxicokinetics and √10 for toxicodynamics) (OEHHA, 2008). A detailed description of these factors is presented in Appendix 3.

When calculating the ADD for PFHxS, OEHHA applied an interspecies UF of √10 to account for potential differences in toxicodynamics when extrapolating data from animal studies to humans. Because PFHxS is not known to be metabolized in animals or humans, and because a toxicokinetic adjustment was applied to the animal POD to derive a human equivalent dose, the toxicokinetic components of the interspecies and intraspecies UFs were reduced by √10 each. Therefore, the intraspecies UF_H was reduced from OEHHA's default of 30 to 10 to account for human variability.

A subchronic UF of 10 is typically applied when the study duration is <8% of the animal's lifetime to account for the potential exacerbation of toxicity following chronic exposure (OEHHA, 2008). The critical study for increased relative liver weight in female rats and decreased total T4 in male rats (NTP, 2019) had a duration of <8% of the animal's lifetime, thus requiring a UF of 10. However, this factor was not applied with the POD for reduced litter size because the developmental effect occurred during a critical window of susceptibility during gestation.

The available animal toxicity studies for PFHxS are limited. There are no studies of potential immunotoxicity or carcinogenicity. The lack of such studies is a concern because immunotoxicity and positive results in cancer bioassays have been observed for other PFAS such as PFOS and PFOA. Thus, OEHHA is applying a database deficiency UF of √10 to account for these uncertainties.

The composite UFs for the PODs based on decreased litter size in mice, increased relative liver weight in female rats and decreased total T4 in male rats are summarized in Table 7.

Table 7. Uncertainty factors for candidate critical endpoints

Uncertainty factors	Candidate critical endpoint		
	↓ Litter size female mouse Chang et al. (2018)	↑ Relative liver weight female rat NTP (2019)	↓ Total T4 male rat NTP (2019)
Intraspecies UF_H	10	10	10
Interspecies UF_A	√10	√10	√10
Subchronic UF_S	1	10	10
Database deficiency UF_D	√10	√10	√10
Composite UF	100	1,000	1,000

OEHHA's practice is to choose a candidate critical study with lower overall uncertainty if the candidate studies are of comparable experimental design and quality. However, the candidate critical PODs for PFHxS are from animal studies of distinct design that target different life stages. Were OEHHA to choose the POD for decreased litter size solely based on the lower total UF, the resulting HPC may not be sufficiently health-protective for the endpoints of increased relative liver weight or decreased T4. Thus, neither of the candidate critical PODs can be chosen based solely on comparing the composite UFs, and all PODs are applied for HPC calculation for comparison.

To calculate the ADD, divide the POD_{human} by the composite UF.

For decreased litter size:

$$ADD = {0.00143 \frac{mg}{kg-day}}\Big/{100} = 14.3 \text{ ng/kg-day}$$

For increased relative liver weight:

$$ADD = {0.00292 \frac{mg}{kg-day}}\Big/{1{,}000} = 2.9 \text{ ng/kg-day}$$

For decreased total T4:

$$ADD = {0.00243 \frac{mg}{kg-day}}\Big/{1{,}000} = 2.4 \text{ ng/kg-day}$$

Relative Source Contribution (RSC)

In estimating health-protective concentrations of chemicals in drinking water for noncancer endpoints, OEHHA considers the relative source contribution (RSC), which is the proportion of the ADD that comes from tap water as part of total exposure from all

sources, including food and ambient air. When developing an appropriate RSC value for a chemical, OEHHA follows the US EPA Exposure Decision Tree Approach (US EPA, 2000). While there are limited data quantifying the levels of PFHxS in some exposure sources, including diet and indoor dust (EFSA, 2012; Poothong et al., 2020), PFHxS is a ubiquitous environmental contaminant with multiple potential exposure sources. Due to insufficient human data to assess the PFHxS exposure in California from all sources other than tap water, a default RSC of 20% was selected, consistent with the US EPA (2000) guidance.

Drinking Water Intake (DWI)

To calculate a drinking water HPC, the ADD is converted to a concentration in tap water that accounts for the total exposure to the chemical, including intake from ingestion, inhalation, and dermal contact with contaminants in tap water. Inhalation exposure can take place when a chemical volatilizes out of the water during cooking or showering. Dermal absorption of the chemical can occur during bathing and other household uses of tap water.

The HPC calculation requires the drinking water intake equivalent (DWI), which is expressed in the units of liters or liter equivalents per kilogram of body weight per day (L/kg-day or L_{eq}/kg-day, respectively). Liter equivalents represent the equivalent amount of tap water one would have to drink to account for the exposure to a chemical in tap water through oral, inhalation, and dermal routes. For oral intake rates, OEHHA uses age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994–1996, 1998 dataset). These age-specific oral intake rates are normalized to body weight and expressed as L/kg-day. For developmental effects, the oral DWI is that of the pregnant woman, 0.047 L/kg-day. For general toxicity endpoints reflecting lifetime exposure, the oral DWI is weight-averaged over life stages and equals 0.053 L/kg-day. Because infants have been identified as a sensitive group for the effects of decreased total T4, OEHHA is applying the 0- to 6-month infant DWI of 0.237 L/kg-day to derive the HPC based on this endpoint.

PFHxS exposure from tap water is expected to be predominantly from oral exposure. The Henry's Law solubility constant (H^{cp}) for PFHxS was estimated to be 5.1×10^{-1} mol/m^3-Pa (Sander, 2015). In the CalTOX 4.0[6] multimedia total exposure model developed for the California Department of Toxic Substances Control by the Lawrence Berkeley National Laboratory, inhalation exposure to contaminants is not likely to be significant relative to ingestion exposure when K/RT is less than 0.1. In this equation, $K=1/H^{cp}$ is the Henry's Law volatility constant, R = 8.314 m^3-Pa/K-mol is the universal gas constant, and T is the temperature (CalEPA, 1993). At room temperature, K/RT for

[6] Available at: https://dtsc.ca.gov/caltox-download-instructions/

PFHxS is 8×10^{-4}, much lower than 0.1. Thus, inhalation exposure to PFHxS from tap water during household uses is negligible.

There are no in vivo or in vitro studies of dermal absorption of PFHxS and most other PFAS. It was demonstrated in vitro that application of PFOA in aqueous solution over 2 days transferred negligible amounts through human skin (Fasano et al., 2005). Application of PFOA dissolved in acetone resulted in approximately 25% absorption of the applied dose at 25 hours, but practically no absorption occurred in the first 5 hours of application (Franko et al., 2012). Based on the similar physicochemical properties of PFHxS and PFOA, and with the lack of any PFHxS-specific data, OEHHA concludes that dermal absorption of PFHxS from tap water under conditions of household use is unlikely. Thus, inhalation and dermal exposures to PFHxS due to tap water use are expected to be negligible. Accordingly, the DWI values for the PFHxS PODs are set to the oral drinking water intakes.

Health-Protective Concentration (HPC)

HPC = ADD × RSC ÷ DWI, where:

ADD = acceptable daily dose,

RSC = relative source contribution of 0.2, and

DWI = daily water intake rate of 0.047 L/kg-day (decreased litter size), 0.053 L/kg-day (increased relative liver weight), or 0.237 L/kg-day (decreased total T4)

For decreased litter size:

HPC = 14.3 ng/kg-day × 0.2 ÷ 0.047 L/kg-day = 60 ng/L or 60 ppt (rounded)

For increased relative liver weight:

HPC = 2.9 ng/kg-day × 0.2 ÷ 0.053 L/kg-day = 11 ng/L or 11 ppt

For decreased total T4:

HPC = 2.4 ng/kg-day × 0.2 ÷ 0.237 L/kg-day = 2 ng/L or 2 ppt

The lowest HPC of **2 ppt**, based on decreased total T4 from the NTP (2019) study in male rats, would be health-protective for other adverse effects, including increased liver weight in rats and decreased litter size in mice. Adjusting for the difference in molecular weight between PFHxS and K^+PFHxS does not change the HPC. This value is lower than the regulatory standards and advisory levels developed by other states due to differences in critical endpoints and studies used, and/or differences in risk assessment methods, such as specific TK adjustments, exposure scenarios for the sensitive life stage, specific RSC values and water intake values.

OEHHA recommends that the Water Board establish the NL for PFHxS in drinking water at the HPC of 2 ppt, or at the lowest level at which PFHxS can be reliably detected in drinking water using available and appropriate technologies.

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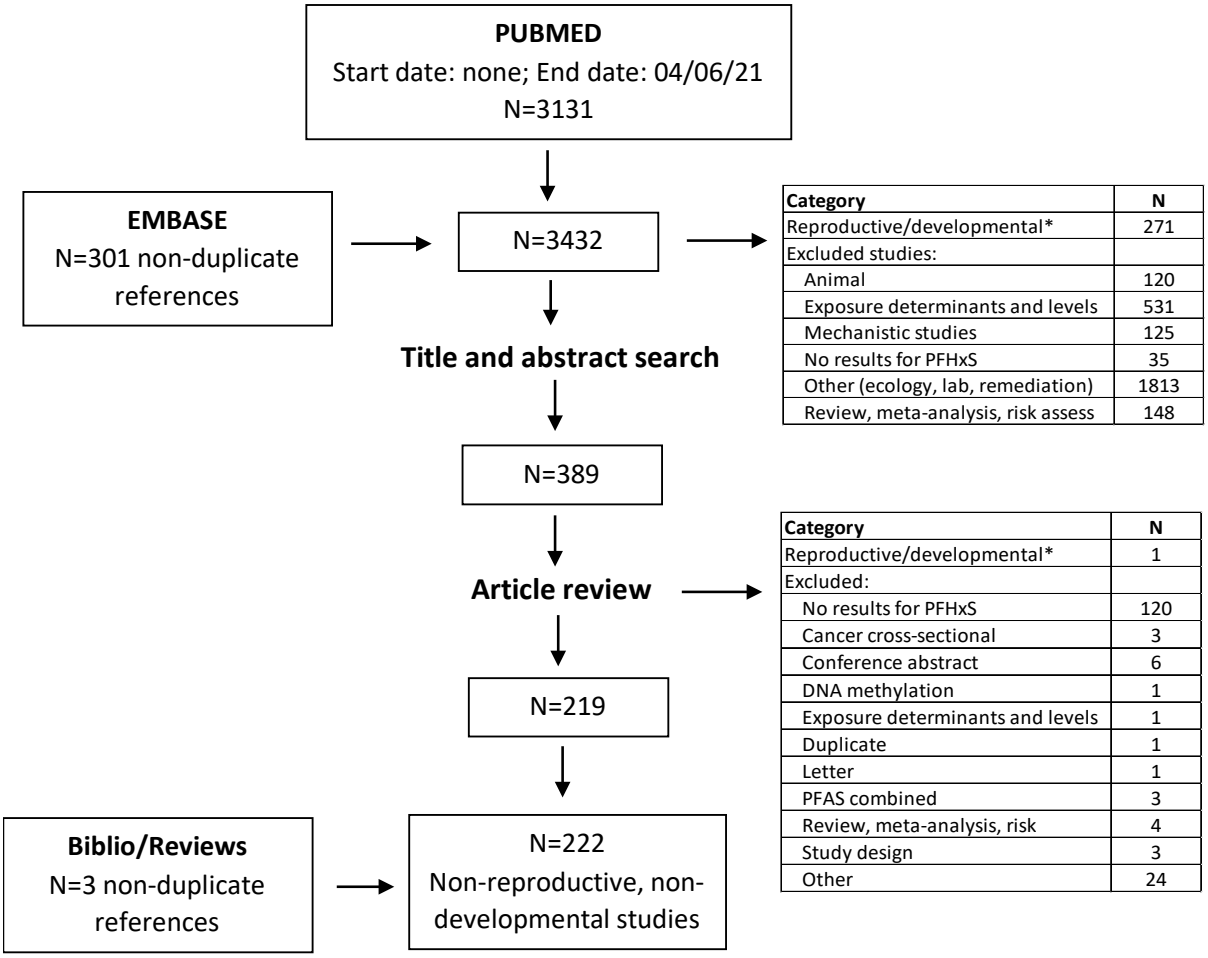
APPENDIX 1. LITERATURE SEARCH STRATEGY

Human Evidence

Figure A1.1. Literature search terms used to identify studies of PFHxS and human health effects

("perfluorohexane sulfonate"[tiab] OR PFHxS OR "perfluorohexanesulfonic acid"[tiab] OR "perfluorohexanesulfonate"[tiab] OR "PFHS cpd"[tiab] OR "perfluorohexane sulfonic acid"[tiab] OR "perfluorohexane-1-sulphonic acid"[tiab] OR "perfluorohexanesulphonic acid"[tiab] OR "perfluorohexane-1-sulfonic acid"[tiab] OR "1 Perfluorohexanesulfonic acid"[tiab] OR "Perfluoro-1-hexanesulfonate"[tiab] OR "Perfluorohexanesulfonic acid"[tiab] OR "Perfluorohexylsulfonate"[tiab] OR "tridecafluoro-1-Hexanesulfonic acid"[tiab] OR PFAS*[tiab] OR perfluoroalkyl*[tiab] OR perfluorocaprylic[tiab] OR perfluorocarbon*[tiab] OR perfluorocarboxyl*[tiab] OR perfluorochemical*[tiab] OR (perfluorinated[tiab] AND (C8[tiab] OR carboxylic[tiab] OR chemical*[tiab] OR compound*[tiab] OR octanoic[tiab])) OR PFAA*[tiab] OR "fluorinated polymer"[tiab] OR "fluorinated polymers"[tiab] OR (fluorinated[tiab] AND (polymer[tiab] OR polymers[tiab])) OR (fluorocarbon[tiab] AND (polymer[tiab] OR polymers[tiab])) OR Fluoropolymer*[tiab] OR (fluorinated[tiab] AND telomer*[tiab]) OR fluorotelomer*[tiab] OR fluoro-telomer*[tiab] OR fluorosurfactant*[tiab] OR "FC 143"[tiab] OR FC143[tiab] OR 335-67-1 [rn] OR Pentadecafluoroctanoate*[tiab] OR Pentadecafluorooctanoate*[tiab] OR pentadecafluoroctanoic[tiab] OR pentadecafluorooctanoic[tiab] OR "pentadecafluoro-1-octanoic"[tiab] OR "pentadecafluoro-n-octanoic"[tiab] OR "perfluoro-1-heptanecarboxylic"[tiab] OR perfluorocaprylic[tiab] OR perfluoroheptanecarboxylic[tiab] OR perfluoroctanoate[tiab] OR perfluorooctanoate[tiab] OR "perfluoro octanoate"[tiab] OR "perfluorooctanoic acid"[nm] OR perfluoroctanoic[tiab] OR perfluorooctanoic[tiab] OR "perfluoro octanoic"[tiab] OR "perfluoro-n-octanoic"[tiab] OR "perfluorooctanoyl chloride"[tiab] OR PFOA[tiab] OR APFO[tiab] OR 1763-23-1[rn] OR 307-35-7[rn] OR "1-octanesulfonic acid"[tiab] OR "1-perfluorooctanesulfonic"[tiab] OR "1-perfluoroctanesulfonic"[tiab] OR "heptadecafluoro-1-octanesulfonic"[tiab] OR "heptadecafluoro-1-octane sulfonic"[tiab] OR "heptadecafluorooctanesulfonic"[tiab] OR "heptadecafluorooctane sulfonic"[tiab] OR "heptadecafluoroctane sulfonic"[tiab] OR "perfluoroalkyl sulphonate"[tiab] OR perfluoroctanesulfonate[tiab] OR perfluorooctanesulfonate[tiab] OR "perfluoroctane sulfonate"[tiab] OR "perfluorooctane sulfonate"[tiab] OR "perfluoro-n-octanesulfonic"[tiab] OR perfluoroctanesulfonic[tiab] OR perfluorooctanesulfonic[tiab] OR "perfluorooctane sulfonic acid"[nm] OR "perfluoroctane sulfonic"[tiab] OR "perfluorooctane sulfonic"[tiab] OR perfluoroctanesulphonic[tiab] OR perfluorooctanesulphonic[tiab] OR "perfluoroctane sulphonic"[tiab] OR "perfluorooctane sulphonic"[tiab] OR perfluoroctylsulfonic[tiab] OR PFOS [tiab]) AND (("Epidemiologic Studies"[mh] OR "epidemiology"[sh] OR "Meta-Analysis"[pt] OR "Case Reports"[pt] OR Seroepidemiologic-Stud*[tiab] OR retrospective-stud*[tiab] OR prospective-stud*[tiab] OR Mortality[tiab] OR longitudinal-stud*[tiab] OR follow-up stud*[tiab] OR ecological-study[tiab] OR ecological-studies[tiab] OR Cross-Sectional Stud*[tiab] OR Correlation-stud*[tiab] OR cohort*[tiab] OR case-control*[tiab] OR cancer-registr*[tiab] OR case-series[tiab] OR case-referent[tiab] OR record-link*[tiab] OR workmen*[tiab] OR Worker*[tiab] OR persons[mh] OR age groups[mh]) OR ((metaanalysis[tiab] OR case-report[tiab] OR metaanalyses[tiab] OR meta-analysis[tiab] OR child*[tiab] OR elderly[tiab] OR aged[tiab] OR pediatric[tiab] OR paediatric[tiab] OR infant*[tiab] OR neonat*[tiab] OR preschool[tiab] OR teenage*[tiab] OR adolescen*[tiab] OR boy[tiab] OR boys[tiab] OR girl[tiab] OR girls[tiab] OR youth[tiab] OR student*[tiab] OR juvenile[tiab] OR persons[tiab] OR community[tiab] OR population[tiab] OR patients[tiab]) NOT medline[sb]))

Figure A1.2. Literature search for epidemiologic studies of PFHxS and human health effects*



Category	N
Reproductive/developmental*	271
Excluded studies:	
Animal	120
Exposure determinants and levels	531
Mechanistic studies	125
No results for PFHxS	35
Other (ecology, lab, remediation)	1813
Review, meta-analysis, risk assess	148

Category	N
Reproductive/developmental*	1
Excluded:	
No results for PFHxS	120
Cancer cross-sectional	3
Conference abstract	6
DNA methylation	1
Exposure determinants and levels	1
Duplicate	1
Letter	1
PFAS combined	3
Review, meta-analysis, risk	4
Study design	3
Other	24

*Human reproductive and developmental studies were reviewed using a different process than human non-reproductive and non-developmental studies.

Evaluations of study results, study quality, and causal inference

For outcomes other than developmental and reproductive toxicity (DART), OEHHA evaluated each study result, the quality of each study overall, and the major aspects of causal inference using an updated version of the Hill criteria (Hill, 1965). These criteria are listed below. When available, results by age group (neonates, children, and adults), sex, and in pregnant women were evaluated separately. Because the latency of PFHxS is unknown, cross-sectional and prospective analyses were also evaluated separately. In several instances, two or more publications involving the same endpoints included the same participants. To prevent "double counting," the results from a single publication were selected based on the following criteria. First, publications presenting separate results for males and females were selected over those that did not. Second, if none of the overlapping publications presented sex-specific results, the publication with

the larger sample size was selected. Selection based on the quality of the exposure or outcome assessment, publication date, or the methods used to control for potential confounding was also considered, but these factors generally did not vary across overlapping publications.

• Study design • Temporality • Chance • Effect size • Dose-response	• Blinding • Detection levels • Range of exposure • Exposure assessment • Selection bias	• Outcome assessment • Confounding • Subgroups • Overlap • Consistency	• Clear results • Fasting • Outliers

Evaluations of DART outcomes involved a narrower, more focused process than that described above. Each study was reviewed briefly to determine whether the study likely reported evidence of an association. When two or more studies reported likely associations for a specific outcome, those studies were selected for a more detailed review. This selection was based on study size (e.g., >200 participants), design (e.g., prospective studies were generally selected over cross-sectional studies), and the potential for accurate dose-response information. Analyses and conclusions regarding developmental outcomes from authoritative bodies (EFSA, 2020; ATSDR, 2021) and relevant review articles were also considered. Epidemiologic studies involving reproductive outcomes were not reviewed in detail by OEHHA. Rather, conclusions for these outcomes were based on those from other authoritative bodies (EFSA, 2020; ATSDR, 2021), general trends seen in OEHHA's review of other outcomes (e.g., common biases and errors), and information from relevant review articles in peer-reviewed journals.

Criteria used to evaluate the results, study quality, and causal inference of the epidemiologic studies of PFHxS and non-DART outcomes

Study design: Study designs included retrospective and prospective cohort studies, case-control studies, ecologic studies, and cross-sectional studies. Each study was evaluated with regards to the weaknesses that may occur with its design, including reverse causation and ecologic fallacy.

Temporality: Studies were evaluated as to the likelihood the exposure occurred before the outcome.

Chance: Each study result was evaluated for whether it was statistically significant. Statistical significance was defined as a p-value <0.05, or 95% confidence intervals (CI) that excluded 1.0 for relative risk estimates or 0 for mean differences or correlation or regression coefficients. OEHHA acknowledges that these definitions are somewhat arbitrary, that some results representing true effects may not meet these definitions, and that some results meeting these definitions may not represent true effects. As such, no

conclusions were based solely on statistical significance. The sample size was also considered under this criterion.

Magnitude of the association or effect size: Results that were not statistically significant but with large effect sizes (e.g., relative risk estimates >1.2, mean differences >5%, correlation coefficients >0.20, or regression coefficients indicating an effect size >5% between high and low exposure groups) were noted. Identifying large effect sizes in this manner is similar to the “Large magnitude” criterion used by the National Toxicology Program (NTP, 2019) and the “Strength of the association” criterion used by Hill (1965). OEHHA acknowledges that the specific criteria used to define a large effect size can be somewhat arbitrary and can sometimes be difficult to quantify. Importantly though, these criteria were not used as the sole determinants of causality.

Dose-response: If an association was identified, the shape of the dose-response curve was evaluated. When patterns were not monotonic or were not consistent from one study to the next, OEHHA explored whether there might be a potential reason for this.

Selection bias: Most studies involved convenience sampling. Each study was evaluated based on the likelihood that a biased selection process (e.g., selection based on both exposure and outcome in a biased manner) may have occurred.

Blinding: Studies were evaluated for whether the researchers measuring the exposure were blinded to the outcome status of the participants, and whether the researchers measuring the outcome were blinded to the exposure status of the participants.

Detection levels: Information was collected on the percentage of participants who had detectable levels of PFHxS. A low percentage could limit the precision of study findings.

Range of exposure: When possible, data on the distribution of PFHxS levels among the study participants were examined. In general, true effects are easier to identify when the contrast in exposure within the study population is large.

Exposure and outcome methods: The methods used to assess the PFHxS exposure levels and the outcomes of interest were evaluated. In general, OEHHA evaluated whether these were validated, generally accepted, or otherwise reasonable methods for assessing exposure and outcome.

Confounding: Each study was evaluated for whether it controlled or otherwise accounted for (e.g., through restriction, matching, or stratification) the factors most likely to cause confounding. This includes potential confounding by other PFAS. When provided, adjusted and unadjusted study results were also noted and compared.

Subgroups: Results stratified by sex and age (neonates, children and adolescents, and adults), and results in pregnant women and other subgroups (e.g., obese participants) were examined when provided.

Overlap: Several publications provided results for the same outcome in the same study population. To prevent "double counting," a single study was selected based on the following priorities. First, publications presenting results stratified by sex were chosen over those that did not. Second, publications with larger sample sizes were selected over those with smaller sample sizes. Selection based on the quality of the exposure assessment, the quality of the outcome assessment, or the adequacy of control for potential confounders was also considered, but these factors were not found to vary substantially across overlapping publications.

Consistency: Both internal (within a study) and external (between studies) consistency were evaluated. An example of internal consistency is when analyses using continuous variables lead to similar results as analyses using categorical variables. An example of external consistency is when two studies in separate populations report similar findings.

Clear results: OEHHA evaluated whether the authors presented results in a manner that was readily understandable or otherwise clear and thorough.

Fasting: OEHHA evaluated whether or not participants were fasting prior to outcome assessment. This criterion was considered for outcomes such as serum lipid levels, which can be heavily influenced by recent meals.

Outliers: Several studies evaluated whether a small number of participants with outlying values may have exerted a major effect on study results. These data were considered in OEHHA's evaluations when provided.

Animal Evidence

Figure A1.3. PubMed – Search executed 10.23.20, 2.26.21 and 6.11.21

Search Terms	Results
355-46-6[rn] OR perflexane[nm] OR 355-46-4[tiab] OR PFHS[tiab] OR PFHxS[tiab] OR Perfluorohexane[tiab] OR perfluorohexanesulphonate[tiab] OR perfluorohexanesulfonate[tiab] OR Perfluorohexanesulfonic[tiab] OR perfluorohexanesulphonic[tiab] OR T-7485[tiab] OR T7485[tiab] OR T-7706[tiab] OR T7706[tiab] OR TCR-83[tiab] OR TCR83[tiab] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tiab] OR "1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro"[tiab] OR "tridecafluorohexane-1-sulfonic"[tiab] OR "perfluoro-n-hexane"[tiab]	1064 (updates: 59, 41)

Figure A1.4. EMBASE – Search executed 10.23.20, 2.26.21 and 6.11.21

Search Terms	Results
'perfluorohexanesulfonic acid'/de OR '355-46-4':ti,ab OR 'PFHS':ti,ab OR 'PFHxS':ti,ab OR 'Perfluorohexane':ti,ab OR 'perfluorohexanesulfonate':ti,ab OR 'perfluorohexanesulphonate':ti,ab OR 'Perfluorohexanesulfonic':ti,ab OR 'perfluorohexanesulphonic':ti,ab OR 'T-7485':ti,ab OR 'T7485':ti,ab OR 'T-7706':ti,ab OR 'T7706':ti,ab OR 'TCR-83':ti,ab OR 'TCR83':ti,ab OR '1 1 2 2 3 3 4 4 5 5 6 6 6-Tridecafluorohexane-1-sulfonic acid':ti,ab OR '1-Hexanesulfonic acid 1 1 2 2 3 3 4 4 5 5 6 6 6-tridecafluoro':ti,ab OR 'tridecafluorohexane-1-sulfonic':ti,ab OR 'perfluoro-n-hexane':ti,ab	336 (updates: 24, 20)

Figure A1.5. SCOPUS – Search executed 10.23.20, 2.26.21 and 6.11.21

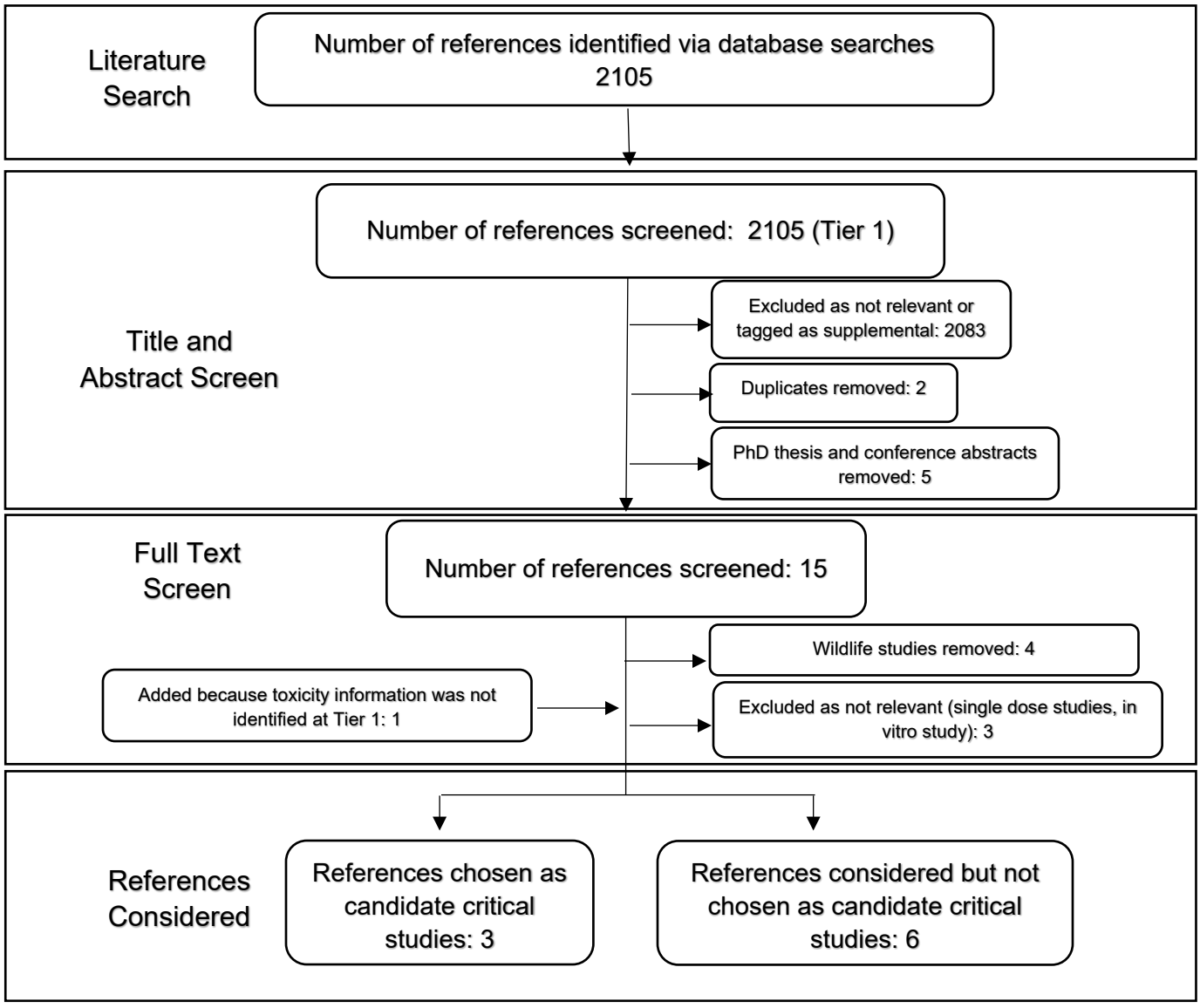
Search Terms	Results
CASREGNUMBER ("355-46-4") OR TITLE-ABS ("355-46-4" OR "PFHS" OR "PFHxS" OR "Perfluorohexane" OR "perfluorohexanesulfonate" OR "perfluorohexanesulphonate" OR "Perfluorohexanesulfonic" OR "perfluorohexanesulphonic" OR "T-7485" OR "T7485" OR "T-7706" OR "T7706" OR "TCR-83" OR "TCR83" OR "1 1 2 2 3 3 4 4 5 5 6 6 6-Tridecafluorohexane-1-sulfonic acid" OR "1-Hexanesulfonic acid 1 1 2 2 3 3 4 4 5 5 6 6 6-tridecafluoro" OR "tridecafluorohexane-1-sulfonic" OR "perfluoro-n-hexane")	508 (updates: 13, 14)

Figure A1.6. PECO statement used for Tier 1 literature screening

PECO element	Evidence
Populations	**Human:** Studies of any population and lifestage (occupational or general population, including children and other sensitive populations) will be tagged as "potentially relevant supplemental information – human studies." **Exclude**: biomonitoring studies and exposure studies (unless specifically relevant to California). **Animal:** Non-human mammalian animal species of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Zebrafish studies will be tagged as "potentially relevant supplemental information." **Mechanistic:** Studies of any human or animal (mammalian and non-mammalian) cell type, and mechanistic/genomic/in silico data with any biological significance will be tagged as "potentially relevant supplemental information."
Exposures	**Relevant forms:** Perfluorohexanesulfonic acid (CAS 355-46-4) in free form or any salt, and any synonyms. **Human:** Any exposure to PFHxS via any route. **Animal:** Any exposure to PFHxS via the oral route. Studies involving intraperitoneal or dermal exposures, or exposure to mixtures will be tagged as "potentially relevant supplemental information." **Mechanistic:** Any cell type exposed to PFHxS alone. Studies involving exposures to mixtures will be tagged as "potentially relevant supplemental information."
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) or PFHxS, or exposure to PFHxS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control. **Mechanistic:** A concurrent control group of cells exposed to vehicle-only treatment or untreated control.

Outcomes	All health outcomes (both cancer and noncancer) and toxicokinetics. **Exclude:** ecological studies, animal biomonitoring studies, and reviews.
PBPK models	Studies describing PBPK models for PFBS will be included. Studies describing toxicokinetic data and ADME will also be included.

Figure A1.7. Flowchart of literature screen (animal toxicity studies)

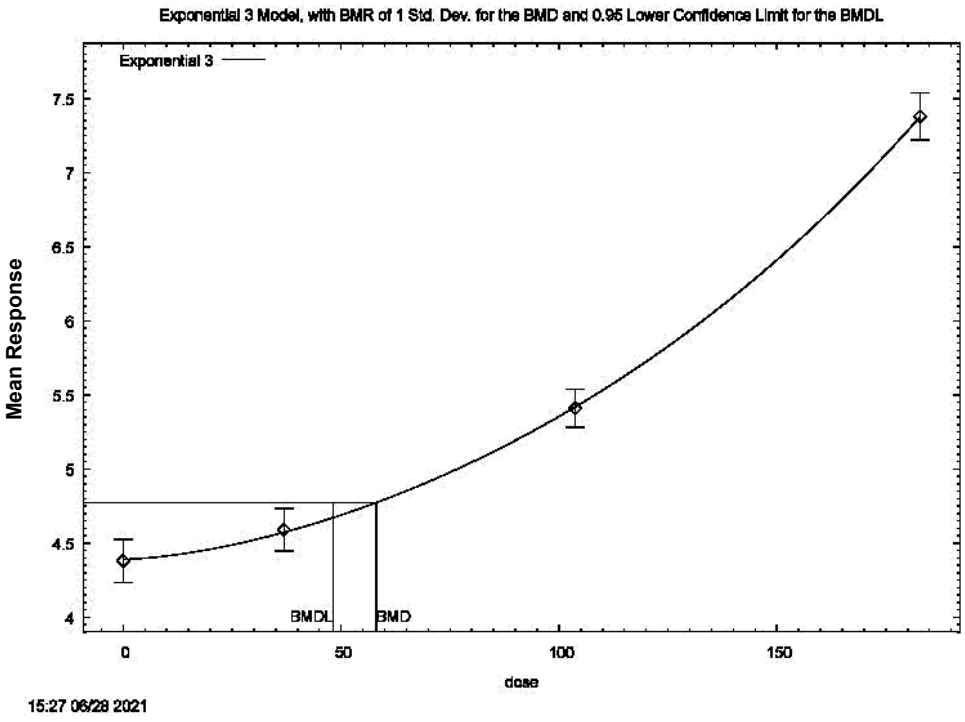


APPENDIX 2. BENCHMARK DOSE MODELING

This appendix provides the BMD modeling outputs for PFHxS toxicity data that were amenable to dose-response modeling. All models were run with default parameters and a benchmark response of 5% for dichotomous data or one standard deviation from the control mean for continuous data unless otherwise noted.

The model for decreased total T3 in male rats (Figure A2.6) was run with modeled variance (instead of the default constant variance) and without the high dose. When comparing outputs of different models for the same endpoint/dataset, model selection criteria are as follows: scaled residual ≤ the absolute value of two, goodness of fit p-value ≥0.05,[7] the Akaike's information criterion (AIC), and visual inspection of the dose-response curve. The lower limit of the 95% confidence interval of the BMD resulting in the benchmark response, the BMDL, was selected as the POD. The model selected for each study to derive candidate PODs is presented below.

Figure A2.1. Exponential 3 model output for increased relative liver weight in male mice (Chang et al., 2018)



[7] US EPA's Benchmark Dose Technical Guidance (2012) suggests using a goodness of fit p-value ≥ 0.1; however, models with less adequate fit (goodness of fit p-value ≥ 0.05) may be used when other criteria are taken into account, such as variability in the endpoint and visual fit.

Model Run Output for Figure A2.1: Exponential 3 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:

Model 3: Y[dose] = a * exp[sign * (b * dose)^d]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 58.0926
BMDL at the 95% confidence level = **48.1368**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-1.92238	-1.92322
rho	0*	0*
a	4.39089	4.21492
b	0.00362241	0.0028854
d	1.59297	1

* Indicates that this parameter has been specified

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
3	0	4.391	0.3824	-0.156
	36.9	4.573	0.3824	0.2504
	103.7	5.418	0.3824	-0.115
	182.9	7.378	0.3824	0.02207

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
3	55.34272	4	-102.6854

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)
Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)
Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)
Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	284.9	6	< 0.0001
Test 2	1.642	3	0.65
Test 3	1.642	3	0.65
Test 4	27.95	2	< 0.0001
Test 5a	0.1008	1	0.7509
Test 5b	27.85	1	< 0.0001
Test 6a	52.65	1	< 0.0001
Test 6b	-24.7	1	N/A
Test 7a	0.495	0	N/A
Test 7b	-0.3942	1	N/A
Test 7c	52.16	1	< 0.0001

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is less than .1. Model 4 may not adequately describe the data; you may want to consider another model.

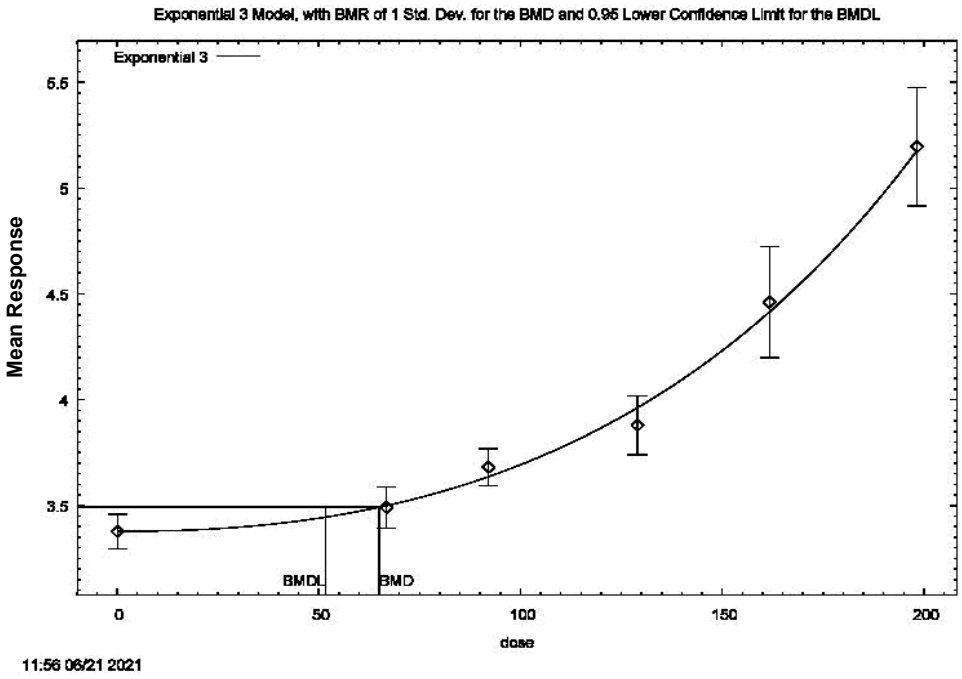
The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

Degrees of freedom for Test 7a are less than or equal to 0.The Chi-Square test for fit is not valid.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is less than .05. Model 5 appears to fit the data better than Model 4.

Figure A2.2. Exponential 3 model output for increased relative liver weight in male rats (NTP, 2019)



Model Run Output for Figure A2.2: Exponential 3 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:
Model 3: Y[dose] = a * exp[sign * (b * dose)^d]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 64.9724
BMDL at the 95% confidence level = **51.6693**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-12.4494	-12.0493
rho	6.6563	6.36543
a	3.37799	3.14393
b	0.00347875	0.00215506
c	--	0*
d	1.59297	1

* Indicates that this parameter has been specified

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
3	0.1022	3.378	0.1138	-0.02758
	66.76	3.499	0.128	-0.2029
	92.08	3.636	0.1454	0.9746
	129	3.962	0.1935	-1.357
	161.7	4.414	0.2773	0.5305
	198.3	5.178	0.4714	0.1219

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
3	68.35174	5	-126.7035

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	155.9	10	< 0.0001
Test 2	29.98	5	< 0.0001
Test 3	2.765	4	0.5978
Test 5a	3.442	3	0.3283

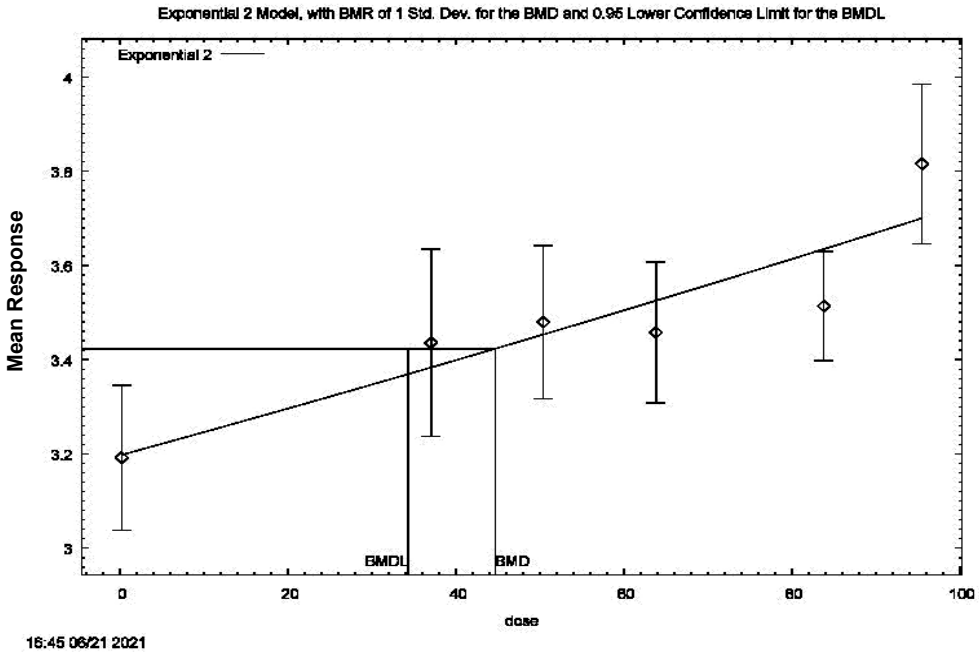
The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

Figure A2.3. Exponential 3 model output for increased relative liver weight in female rats (NTP, 2019)



Model Run Output for Figure A2.3: Exponential 2 Model. (Version: 1.11; Date: 03/14/2017)
The form of the response function:
Model 2: Y[dose] = a * exp[sign * b * dose]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 44.6626
BMDL at the 95% confidence level = **34.3021**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-2.9716	-3.09664
rho	0*	0*
a	3.19712	3.19968
b	0.00153097	0.00151263

* Indicates that this parameter has been specified

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0.1744	3.198	0.2263	-0.08351
	37.03	3.384	0.2263	0.7322
	50.41	3.454	0.2263	0.3685

63.82	3.525	0.2263	-0.9402
83.82	3.635	0.2263	-1.69
95.51	3.701	0.2263	1.614

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
2	59.1649	3	-112.3298

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	36.11	10	< 0.0001
Test 2	3.049	5	0.6924
Test 3	3.049	5	0.6924
Test 4	7.468	4	0.1131

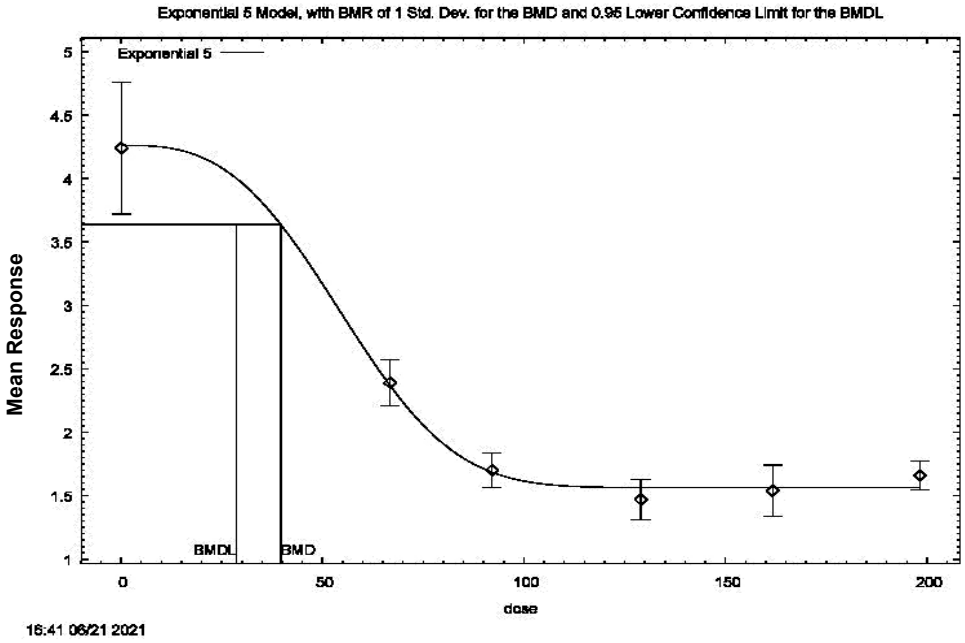
The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

Figure A2.4. Exponential 5 model output for decreased total T4 in male rats (NTP, 2019)



Model Run Output for Figure A2.4: Exponential 5 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:

Model 5: Y[dose] = a * [c-(c-1) * exp(-(b * dose)^d)]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 39.6746
BMDL at the 95% confidence level = **28.6255**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-4.1036	-4.24312
rho	2.18268	2.28535
a	4.26145	4.452
b	0.0159859	0.0185434
c	0.366876	0.314465
d	2.92838	1

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
5	0.1022	4.261	0.6251	-0.1085
	66.76	2.368	0.3292	0.2105

92.08	1.685	0.2271	0.2132
129	1.564	0.2094	-1.421
161.7	1.563	0.2093	-0.3539
198.3	1.563	0.2093	1.459

Likelihoods of interest

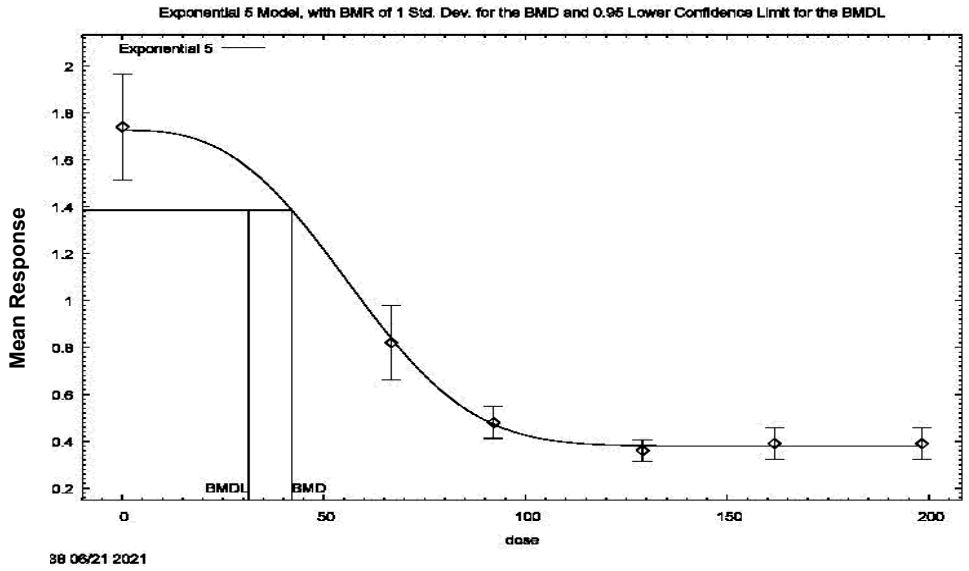
Model	Log(likelihood)	DF	AIC
5	47.55205	6	-83.10411

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	169.5	10	< 0.0001
Test 2	37.17	5	< 0.0001
Test 3	6.956	4	0.1382
Test 7a	3.541	2	0.1702

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.
The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.
The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

Figure A2.5. Exponential 5 model output for decreased free T4 in male rats (NTP, 2019)



Model Run Output for Figure A2.5: Exponential 5 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:

Model 5: Y[dose] = a * [c-(c-1) * exp(-(b * dose)^d)]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 42.0334
BMDL at the 95% confidence level = **31.3294**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-3.17495	-3.12004
rho	1.85918	1.90393
a	1.72543	1.827
b	0.0153767	0.0222241
c	0.22018	0.187661
d	2.83052	1

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
5	0.1022	1.725	0.3395	0.1358
	66.76	0.8382	0.1735	-0.3319
	92.08	0.4725	0.1018	0.2322
	129	0.3812	0.08341	-0.8035
	161.7	0.3799	0.08315	0.3839
	198.3	0.3799	0.08315	0.384

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
5	96.74624	6	-179.4925

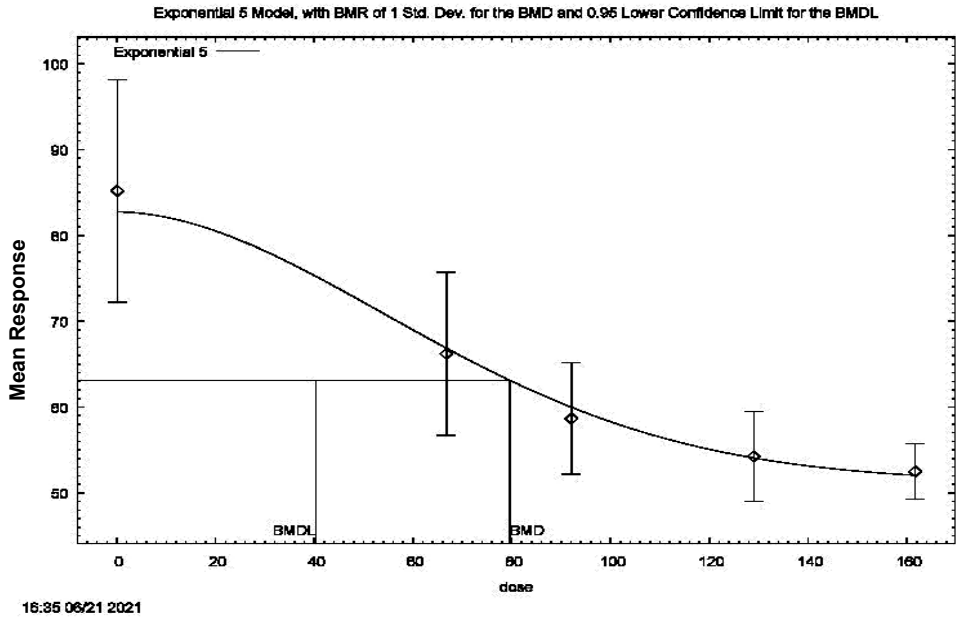
Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	177.3	10	< 0.0001
Test 2	39.32	5	< 0.0001
Test 3	2.839	4	0.5852
Test 7a	1.706	2	0.4261

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.
The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.
The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

Figure A2.6. Exponential 5 model output for decreased total T3 in male rats (NTP, 2019)



Model Run Output for Figure A2.6: Exponential 5 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:
Model 5: Y[dose] = a * [c-(c-1) * exp(-(b * dose)^d)]

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 79.6015
BMDL at the 95% confidence level = **40.2702**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-17.7878	-16.5339
rho	5.37661	5.08287
a	82.7369	89.439
b	0.0124097	0.0167307
c	0.619698	0.55904
d	1.87787	1

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
5	0.1022	82.74	19.62	0.3937
	66.76	66.86	11.07	-0.186
	92.08	59.98	8.265	-0.5012
	129	54.07	6.254	0.09054
	161.7	52.05	5.646	0.2514

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
5	-135.568	6	283.1359

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	59.78	8	< 0.0001
Test 2	20.65	4	0.0003716
Test 3	2.519	3	0.4719
Test 7a	0.2606	1	0.6097

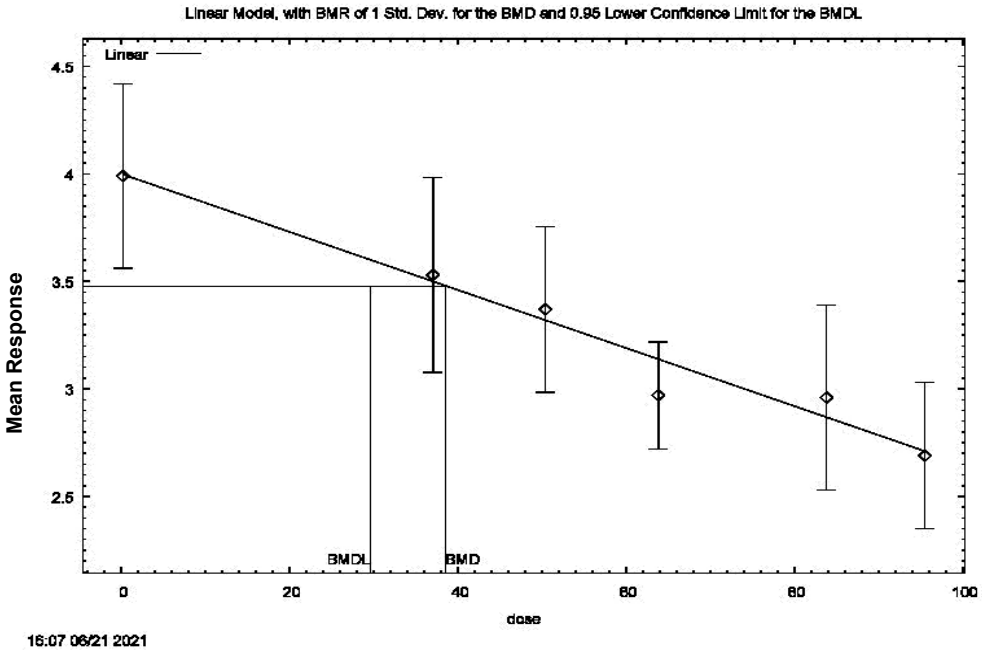
The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

Figure A2.7. Exponential 5 model output for decreased total T4 in female rats (NTP, 2019)



Model Run Output for Figure A2.7: Linear Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Benchmark Dose Computation.

BMR = 1 standard deviation
BMD = 38.4986
BMDL at the 95% confidence level = **29.5734**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
alpha	0.270314	0.292834
rho	-	0 Specified
beta_0	3.99615	3.99615
beta_1	-0.0135049	-0.0135049

Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.1744	10	3.99	3.99	0.601	0.52	-0.0231
37.03	10	3.53	3.5	0.632	0.52	0.206
50.41	10	3.37	3.32	0.538	0.52	0.332
63.82	10	2.97	3.13	0.348	0.52	-0.999

83.82	10	2.96	2.86	0.601	0.52	0.583
95.51	10	2.69	2.71	0.474	0.52	-0.0992

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij) Var[e(ij)] = Sigma^2
Model A2: Yij = Mu(i) + e(ij) Var[e(ij)] = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij) Var[e(ij)] = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user
Model R: Yi = Mu + e(i) Var[e(i)] = Sigma^2

Likelihoods of Interest

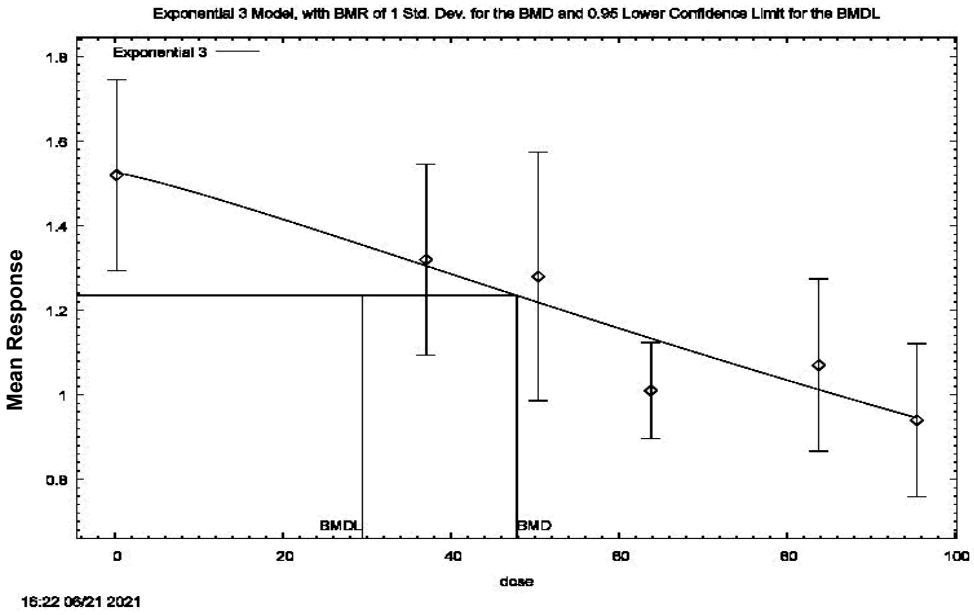
Model	Log(likelihood)	# Param's	AIC
A1	10.005338	7	-6.010675
A2	12.155751	12	-0.311502
A3	10.005338	7	-6.010675
fitted	9.245112	3	-12.490223
R	-6.020146	2	16.040291

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	36.3518	10	<.0001
Test 2	4.30083	5	0.507
Test 3	4.30083	5	0.507
Test 4	1.52045	4	0.823

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels.
It seems appropriate to model the data.
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.
The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.
The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data.

Figure A2.8. Exponential 5 model output for decreased free T4 in female rats (NTP, 2019)



Model Run Output for Figure A2.8: Exponential 3 Model. (Version: 1.11; Date: 03/14/2017)

The form of the response function:
Model 3: Y[dose] = a * exp[sign * (b * dose)^d]

Benchmark Dose Computation

BMR = 1 standard deviation
BMD = 47.8634
BMDL at the 95% confidence level = **29.4554**

Parameter Estimates

Variable	Estimate	Default Initial Parameter Estimate
lnalpha	-2.46956	-2.51516
rho	0*	0*
a	1.52576	1.0592
b	0.00561846	1.17973e-005
c	--	0*
d	1.18276	2

-- Indicates that this parameter does not appear in model

* Indicates that this parameter has been specified

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
3	0.1744	1.525	0.2909	-0.05807
	37.03	1.305	0.2909	0.1611
	50.41	1.218	0.2909	0.669
	63.82	1.133	0.2909	-1.341
	83.82	1.012	0.2909	0.6286
	95.51	0.9451	0.2909	-0.05588

Likelihoods of interest

Model	Log(likelihood)	DF	AIC
3	44.08691	4	-80.17383

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	33.13	10	0.0002586
Test 2	8.753	5	0.1193
Test 3	8.753	5	0.1193
Test 5a	2.736	3	0.4342

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.
The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.
The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

APPENDIX 3. DEFAULT UNCERTAINTY FACTORS FOR PUBLIC HEALTH GOAL (PHG) DERIVATION

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving PHGs. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results. Table A1 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table A3.1. Default uncertainty factors for PHG derivation, adapted from OEHHA (2008)

Uncertainty Factor	Value
Interspecies uncertainty factor (UF_A)	
Combined interspecies uncertainty factor (UF_A):	1 human observation √10 animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 where animal and human PBPK models are used to describe interspecies differences √10 non-primate studies with no chemical- or species-specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 where animal and human mechanistic data fully describe interspecies differences. (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 non-primate studies with no data on toxicodynamic interspecies differences
Intraspecies uncertainty factor (UF_H)	
Toxicokinetic component (UF_{H-k}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured inter-individual variability √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data
	1 human study including sensitive subpopulations (e.g., infants and children)

Uncertainty Factor	Value
Toxicodynamic component (UF_{H-d}) of UF_H:	√10 studies including human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)
LOAEL uncertainty factor (UF_L)	
Values used:	10 LOAEL, any effect 1 NOAEL or BMDL used
Subchronic uncertainty factor (UF_S)[1]	
Values used:	1 study duration >12% of estimated lifetime √10 study duration 8-12% of estimated lifetime 10 study duration <8% of estimated lifetime
Database deficiency factor (UF_D)	
Values used:	1 no substantial data gaps √10 substantial data gaps including, but not limited to, developmental toxicity

[1]Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008)