OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Notification Level Recommendation

Perfluorohexanoic Acid in Drinking Water

September 2024

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

Notification Level Recommendation for Perfluorohexanoic Acid (PFHxA) in Drinking Water

Prepared by

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PREFACE

Notification level (NL) recommendation documents provide information on health effects from contaminants in California drinking water. An NL is a concentration of a contaminant in drinking water that would pose no significant health risk to individuals consuming the water daily over a lifetime. 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 standards 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 NL – the Water Board can recommend 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. OEHHA considers the publicly available studies of health effects in humans and laboratory animals, as well as studies of toxicokinetics and mechanisms of toxicity to develop health-protective concentrations in drinking water. NL recommendations are based on sensitive, well-conducted and scientifically valid studies. The most sensitive health-protective concentration is recommended to the Water Board as the NL.

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SUMMARY

This document presents the notification level (NL) the Office of Environmental Health Hazard Assessment (OEHHA) recommends to the State Water Resources Control Board (Water Board) for perfluorohexanoic acid (PFHxA) in drinking water. OEHHA recommends that the Water Board establish a drinking water NL of 1 part per billion (ppb), equivalent to 1 microgram per liter (µg/L), for PFHxA. The NL recommendation is based on thyroid toxicity in male rats, specifically, decreased total thyroxine levels (NTP, 2019). There were insufficient data to evaluate the potential carcinogenicity of PFHxA.

INTRODUCTION

At the request of the Water Board, OEHHA has developed a recommendation for a drinking water NL for PFHxA. This document reflects OEHHA's focused review of the human and animal toxicity database identified from the open literature. PFHxA (CAS RN 307-24-4) and its related salts, sodium perfluorohexanoate (CAS RN 2923-26-4) and ammonium perfluorohexanoate (CAS RN 21615-47-4), are six-carbon perfluorinated carboxylic acids and are members of a large class of chemicals known as per- and polyfluoroalkyl substances (PFAS). PFAS are anthropogenic chemicals produced since the mid 1900's. Due to the presence of highly stable carbon-fluorine bonds, the PFHxA molecule is very stable and resists degradation, resulting in environmental persistence.

Environmental occurrence

In 2019-2020, the Water Board conducted a California state-wide PFAS monitoring program to sample public water systems (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] PFHxA was detected in 29-38% of samples statewide, with a maximum concentration of 0.3 ppb. Average values ranged from 0.011-0.016 ppb depending on the quarter. PFHxA was not included in US EPA's third Unregulated Contaminant Monitoring Rule (UCMR3), but will be included in UCMR5, which will result in samples being collected between 2023-2025 (US EPA, 2021).

PFAS can provide desirable properties to consumer products, including water, grease, and stain resistance, and have been used historically in a wide variety of products, including textiles, firefighting foams, and various consumer products. Specifically, PFHxA is a metabolite and degradation product of fluorotelomer-based chemicals, including 6:2 fluorotelomer alcohol (FTOH) (Russell et al., 2015), which is used as a starting material for fluoromonomers and fluoropolymers in food contact materials, such as paper tableware, and popcorn bags (Yuan et al., 2016). It is likely that PFHxA has

[1] https://www.waterboards.ca.gov/pfas/drinking_water.html, accessed March 23, 2023

been used in various industrial and consumer products since the 1970s (Anderson et al., 2019).

A major source of human exposure to PFHxA is food. The European Food Safety Authority (EFSA, 2012) compiled data on levels of 27 PFAS in a wide variety of foods from 13 countries, covering a time period from 1998-2012. The frequency of quantified PFHxA in food ranged from 0.1-24%. The highest concentrations of PFHxA were found in edible offal of farmed animals (0.29-3.4 µg/kg) and the liver of game animals (1.4-2.6 µg/kg). Furthermore, PFHxA in food contact materials may migrate into food. For PFAS in general, the degree of migration is dependent on several factors, including properties of the food (fat/moisture/salt content and pH), food contact conditions (temperature and duration) and properties of the chemicals themselves (fluorinated chain length) (Yuan et al., 2016; Ramirez Carnero et al., 2021). For example, increased temperature induced a greater migration of PFHxA from grease-proof paper to the food simulant Tenax, and lyophilized low-fat and high-fat milk, with a migration of approximately 40% at 120°C (Elizalde et al., 2019).

PFHxA is frequently found in industrial products, textiles, and consumer products. PFHxA has been detected in building materials and fabrics, such as awnings and surface treatment products, with detection frequencies ranging between 17-25% of materials tested (Janousek et al., 2019). In Sweden, PFHxA was detected in various consumer goods, including microwave popcorn bags, waterproofing treatments for shoes and textiles, floor polish, shoe wax, and car wax (Borg and Ivarsson, 2017). Borg and Ivarsson (2017) also found PFHxA in 3 out of 8 articles of clothing evaluated. Schellenberger et al. (2022) exposed PFAS-treated functional textiles to environmental stressors (sunlight, wind, precipitation, heat) for 6 months, followed by abrasion and washing. The authors reported that 75-81% of PFHxA was lost following the weathering treatment, due to both microfiber loss and direct PFAS removal from the textiles (the authors indicated that rain was the most likely cause of water soluble PFAS loss). This suggests that with use over time, chemicals from PFAS-treated textiles may be released into the environment.

PFHxA is also commonly found in dust. Anderson et al. (2019) compiled studies reporting PFHxA concentrations in house, office, and vehicle dust in multiple countries, and the frequency of detection in measured samples ranged from 20-100%. Reported mean concentrations ranged from 1.4-33 ng/g. In child care facilities in California, PFHxA was detected in 33.3% of dust samples, with mean and maximum concentrations of 9.5 ng/g and 100 ng/g, respectively (Bradman et al., 2012).

Biomonitoring

PFHxA has been detected in human serum, plasma, and blood across multiple studies in populations in the United States and other countries, as summarized in Gannon et al. (2011) and Anderson et al. (2019). Biomonitoring California[2] evaluated PFHxA levels in

[2] https://biomonitoring.ca.gov/chemicals/perfluorohexanoic-acid-pfhxa, accessed December 19, 2023

the serum of Asian/Pacific Islander communities in 2016 and 2017 and it was detected in ~98% of samples tested. Geometric means of 0.176 ng/ml and 0.189 ng/ml were reported from the 2016 and 2017 studies, respectively (the limit of detection was 0.06 ng/ml).

Calafat et al. (2019) reported that PFHxA was detected in the urine of the 2013-2014 National Health and Nutrition Examination Survey (NHANES) participants, with a detection frequency of 22.6% and a maximum concentration of 7.5 µg/L. A study from South Korea also reported PFHxA concentrations in urine (Kim et al., 2014). Additionally, PFHxA has been detected in breast milk, albeit infrequently (Anderson et al., 2019).

Advisory levels and regulatory standards

Other states and agencies have issued drinking water health advisories or regulatory standards for PFHxA, summarized in Table 1. The Agency for Toxic Substances and Disease Registry assessed the toxicity of PFHxA, but did not derive oral minimal risk levels (ATSDR, 2021).

Table 1. Drinking water health advisories and regulatory standards for PFHxA from other states and agencies

State	Endpoint	Value	Reference
US EPA	Decreased postnatal body weight in rat pups (subchronic and chronic)	Lifetime and subchronic oral reference dose[a] – **0.5 µg/kg-day**	US EPA (2023)
Illinois	Renal tubular degeneration and papillary necrosis of the kidney in female rats (chronic)	Human threshold toxicant advisory concentration – **560 ppb[b]**	Illinois EPA (2021)
Michigan	Renal tubular degeneration and papillary necrosis of the kidney in female rats (chronic)	Maximum contaminant level – **400 ppb**	Michigan SAW (2019)
Minnesota	Decreased total T4[c] (short-term) Nasal epithelium degeneration (subchronic and chronic)	Short-term noncancer health based value – **0.2 ppb** Subchronic and chronic noncancer health based value – **0.2 ppb[d]**	MDH (2021)

[a] This is a value that can be used to derive a drinking water health advisory.
[b] ppb, parts per billion, equivalent to micrograms per liter (µg/L)
[c] T4, thyroxine
[d] Derived subchronic and chronic health-based values were higher than 0.2 ppb, but were set at the short-term noncancer health-based value to be health-protective.

SYSTEMATIC LITERATURE SEARCH

Human Epidemiology Literature Search

PubMed, Embase, related reviews (NTP, 2016a; US EPA, 2016a; US EPA, 2016b; Luz et al., 2019; EFSA, 2020; ATSDR, 2021), and the bibliographies of all identified articles were searched for human epidemiologic studies on the adverse health effects of PFHxA. Keywords in the PubMed and Embase literature searches included PFHxA, perfluorohexanoic acid, perfluorohexanoate, and perfluoro-n-hexanoic acid. All studies published prior to January 1, 2022 were initially included. All human studies involving case-control, prospective or retrospective cohort designs with information on PFHxA exposure and an adverse health outcome were included. Ecologic and cross-sectional studies were also included, although the potential for ecologic fallacy or reverse causation was examined. Case-reports were excluded because of the lack of a comparison group. Studies involving only PFAS mixtures were excluded due to the inability to isolate the specific effects of PFHxA. Abstracts and studies without original data (e.g., editorials) were also excluded. No restrictions were placed on the methods used to evaluate PFHxA exposure although most studies used PFHxA concentrations in serum. Cancer studies that used serum PFHxA measured at or near the time of cancer diagnosis to assess exposure were excluded. This is because of the long latency periods commonly seen for environmentally caused cancers (Marshall et al., 2007; Lacourt et al., 2012; Lipfert and Wyzga, 2019). These studies were also excluded because of the possibility that people with recent cancer diagnoses or pre-diagnosis cancer-related symptoms might change certain behaviors (e.g., diet or household product use) related to their PFAS exposure.

Animal Toxicity Literature Search

For animal toxicity studies, OEHHA conducted a systematic literature search in September 2021 of multiple open literature databases (PubMed, Embase, Scopus, and SciFinder-n) using a search string intended to identify all studies that mention PFHxA 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, OEHHA identified 1,204 individual studies. OEHHA uploaded the identified references into DistillerSR systematic review software (Evidence Partners, Canada) and conducted inclusion/exclusion screening of studies against a PECO (populations, exposures, comparators, and outcomes) statement designed to capture oral animal toxicity and toxicokinetic studies (Appendix 1). Potentially relevant studies, including in vitro and mechanistic studies, studies using non-mammalian models, and studies with non-oral routes of administration were tagged as “potentially relevant supplemental information.”

Two independent reviewers conducted the Tier 1 (title/abstract) reference evaluation against the PECO statement. Tier 1 screening resulted in 24 unique references for PFHxA mammalian oral toxicity and toxicokinetic studies. Of these 24 references, four

were conference abstracts and were excluded. Full text (Tier 2) screening did not exclude any additional studies. Thus, OEHHA identified 20 references (5 animal toxicity studies and 15 toxicokinetic studies) that warranted further evaluation (see Figure A1.3 in Appendix 1). One additional toxicokinetic study was added following Tier 2 screening. This reference was not captured during Tier 1 screening because it evaluated multiple PFAS and did not mention the PFHxA-specific search terms in the title or abstract.

An additional search of PubMed was performed on November 28, 2022 to find studies published after the date of the initial literature search and identified two additional toxicity studies in mammals (Jiang et al., 2021; Weatherly et al., 2023). Thus, a total of 7 animal toxicity and 16 toxicokinetic studies were evaluated.

TOXICOKINETICS

Absorption and Distribution

PFHxA is distributed to multiple tissues in humans. In a study examining autopsy tissues from 20 individuals in Spain, PFHxA was detected frequently in the brain (100% of samples), liver (70% of samples), and lung (89% of samples), and with less frequency in the bone (30% of samples) and kidney (25% of samples) (Perez et al., 2013). Interestingly, of the 21 PFAS evaluated in this study, PFHxA was the predominant chemical detected in the brain and liver, with mean concentrations of 180 ng/g and 115 ng/g, respectively, reported.

PFHxA crosses the placenta and reaches the fetus. Analyses of paired human maternal and cord serum by Gao et al. (2019) and Cai et al. (2020) determined transplacental transfer efficiencies (TTEs) of 110% and 432%, respectively, for PFHxA. A meta-analysis of these data showed that PFHxA had a high TTE compared to other PFAS (Appel et al., 2022).

In pigs, PFHxA was distributed to the liver, kidneys, muscle tissue, fat tissue, and blood plasma after 3 weeks of exposure in food (Numata et al., 2014).

In rats, PFHxA was absorbed and distributed to tissues rapidly following a single dose, with maximum concentrations reached within 1 hour (Gannon et al., 2011; Iwabuchi et al., 2017; Dzierlenga et al., 2020). PFHxA was predominantly found in the serum, liver and kidneys following a single dose and repeated exposures in drinking water (up to 3 months) (Iwabuchi et al., 2017; Dzierlenga et al., 2020). Similarly, absorption in mice was rapid, with maximum plasma concentrations reached in 15-30 minutes (Gannon et al., 2011). Tissue distribution was widespread in pregnant mice with both intravenous and oral exposure to PFHxA (Bartels et al., 2020).

As with humans, PFHxA can cross the placenta when intravenously injected into pregnant mice (Bartels et al., 2020). However, uptake in the mouse placenta and fetus was much lower following administration via oral gavage at the same exposure time of one hour.

Weatherly et al. (2023) demonstrated that PFHxA can be absorbed dermally. The authors administered solutions of PFHxA in acetone (0, 2.5, 5, or 10% volume/volume (v/v)) topically to the ears of female mice (25 µL/ear) for four days, and subsequently reduced the concentrations to 0, 1.25, 2.5 and 5%, respectively, for the rest of the exposure period (a total of 28 days) due to dermal irritation at the application site. The authors do note that oral exposure was possible due to grooming, as mice were caged in groups of five.

OEHHA converted % solution (v/v) to concentration as follows:

$$\%\, Solution\ x\ density\ =\ concentraion,$$

where % solution was the percent of PFHxA in solution, and density was 1.762 g/ml at 20^{o} C (Synquest Laboratories, 2022). The total amount of PFHxA topically applied daily was calculated by multiplying the concentration by the application volume of 0.05 ml. A time-weighted average concentration was determined due to applied concentrations changing partway through the exposure period. The authors measured serum concentration of PFHxA at the end of the study (24 hours after the final application) and presented the data graphically. OEHHA converted the graphical data to numerical values using GetData Graph Digitizer 2.26 software. These data are summarized in Table 2.

Table 2. PFHxA concentrations topically applied to female mice and the resulting serum concentrations after 28 days from Weatherly et al. (2023)

Solution (%)	Concentration (mg/ml)	Total amount topically applied (mg/day)	Time-weighted average concentration over 28 days (mg/ml)	Reported serum concentration (µg/ml) after 28 days
1.25[a]	22	1.1	25.1	1.27
2.5[a]	44	2.2	50.3	2.95
5[a]	88	4.4	100.6	4.33

[a] This refers to the concentration applied on days 5-28. On days 1-4, 2X concentration was applied.

Another recent study examined systemic toxicity from dermal application of a related compound, perfluoroheptanoic acid (PFHpA), a seven-carbon perfluorinated carboxylic acid (Han et al., 2020). In this study, rats were dermally exposed to PFHpA (in saline solution) for 6 hours/day for two weeks. The authors reported a myriad of systemic toxic effects, including hematological changes, and histopathology in the kidneys, liver, spleen, and testes. Dermal ulceration, inflammation, and hyperplasia at the site of application were also observed. These data suggest that PFHpA is dermally absorbed and distributed throughout the body. However, the authors did not report concentrations of PFHpA in the serum or any of the affected organs. Interestingly, an eight-carbon perfluorinated carboxylic acid, perfluorooctanoic acid (PFOA), has shown minimal capacity for dermal absorption. In vitro application of PFOA in aqueous solution over 2 days transferred negligible amounts through human skin (Fasano et al., 2005).

Additionally, application of PFOA dissolved in acetone resulted in approximately 25% absorption of the applied dose at 25 hours in mice, but practically no absorption occurred in the first 5 hours (Franko et al., 2012).

Metabolism

PFHxA was not metabolized in vitro by rat hepatocytes, and metabolites were not observed in either rats or mice following oral administration (Gannon et al., 2011).

Elimination

Elimination via urine was the predominant excretion route in mice (Gannon et al., 2011; Iwai, 2011), rats (Chengelis et al., 2009a; Gannon et al., 2011) and pigs (Numata et al., 2014), followed by elimination in feces. Gannon et al. (2011) reported that >99% of PFHxA was eliminated via urine, with a slightly faster elimination in rats than mice. Similarly, Chengelis et al. (2009a) reported a urinary elimination of approximately 70% in male and female rats 0-6 hours after a single intravenous administration of 10 mg/kg PFHxA. Additionally, sex or dosing pattern (single dose vs. repeated doses) had minimal impact on elimination in rats and mice (Chengelis et al., 2009a; Gannon et al., 2011; Iwai, 2011). Gannon et al. (2011) reported that PFHxA underwent biphasic elimination in mice, with rapid elimination in the first 12 hours, followed by a slower phase when plasma concentration is approximately 0.1% of the maximum concentration.

Half-Life

Russell et al. (2013) evaluated data from a biomonitoring study of male ski wax technicians (Nilsson et al., 2013), where blood from 11 workers exposed via inhalation to fluorinated ski wax was sampled. Exposures occurred for approximately 30 hours/week for 4 months. PFHxA (mean value of 5,300 ng/m^3) and 6:2 FTOH (mean value of 240 ng/m^3) were present in the air where technicians were working. While 6:2 FTOH can be metabolized to PFHxA, the authors noted its contribution to the total PFHxA exposure in this scenario was negligible. Blood concentrations peaked from December to March during ski season, and rapidly declined during the spring and summer months. Whole blood levels of PFHxA from 7 technicians were analyzed using linear regression to determine the elimination rate and half-life. The geometric mean elimination half-life was 32 days, with a range of 14-49 days. The authors also analyzed human half-life data with available animal half-life data using linear regression as a function of body weight and found a reasonable correlation between PFHxA half-life and body weight (R^2 = 0.81).

A recent study by Xu et al. (2020) evaluated PFAS levels in airport employees in Arvidsjaur, Sweden. The airport's drinking water was contaminated with PFAS (including high levels of PFHxA, 330 ng/L), most likely from aqueous firefighting foam. PFAS-free drinking water was provided to the airport, and blood samples were obtained from 26 male and female employees (males and females) within 11-14 days after

discontinuation of exposure to PFAS-contaminated water. A population elimination rate of 0.43 $year^{-1}$ (95% confidence interval (CI): -0.25, 1.1) was determined, which corresponds to a half-life of 1.63 years. The authors speculated that their half-life estimate may be unreliable because PFHxA was measured in serum, as opposed to whole blood, as PFHxA predominantly binds to blood cells (Poothong et al., 2017) and may not be reflective of the body burden of PFHxA.

In monkeys, the serum half-life of PFHxA is 5.3 hours for males and 2.4 hours for females following a single intravenous dose (Chengelis et al., 2009a). These values are higher than the half-lives determined in the single exposure studies in rats, but substantially lower than values in humans and pigs. However, human half-lives were derived from repeated exposure studies, and this type of data are not available in monkeys.

Numata et al. (2014) determined a half-life of 4.1 days for pigs given PFHxA (as part of a PFAS mixture) in feed for 21 days. Of all the PFAS evaluated, PFHxA was the only compound to approach a steady state concentration. Similarly, Guruge et al. (2016) determined a half-life of 2.7 days in microminipigs following a single oral dose. Twenty-one days after compound administration, PFHxA was only found in muscle and spleen in 1 out of 3 animals and absent in all other evaluated tissues.

Chengelis et al. (2009a) reported a serum half-life of 1.0 hour for male rats and 0.42 hours for female rats following a single intravenous dose of PFHxA. Similar results were reported in other rat studies with single oral or intravenous doses (see Table A2.1 in Appendix 2) (Gannon et al., 2011; Dzierlenga et al., 2020). Repeated oral dosing over 25 days increased the serum half-life in male rats to 2.2-2.8 hours and female rats to 2.3-2.7 hours (Chengelis et al., 2009a). Iwabuchi et al. (2017) determined tissue specific half-lives of PFHxA for brain, heart, liver, spleen, kidney, whole blood, and serum in rats from a single dose administered via oral gavage. All the values were comparable and ranged from 2.4-2.9 hours.

Russell et al. (2015) reviewed in vivo and in vitro metabolic data for 6:2 FTOH, the majority of which were found in unpublished reports by DuPont. These studies included: in vitro metabolic studies using hepatocytes from human, mouse and rat; 1-day, 5-day, and 23-day inhalation studies in rats; and a 90-day oral study in rats. The authors developed a one-compartment, multi-species model to quantitatively describe the metabolism of 6:2 FTOH and its metabolites, including PFHxA. The authors derived elimination half-lives for male and female rats based on the 1-day and 23-day inhalation studies (Table A2.1 in Appendix 2). The values derived from the single day inhalation studies are comparable to values derived for rats by Gannon et al. (2011) from a single oral dose, however, a comparison with mice from the Gannon et al. (2011) study is not possible because the biphasic elimination of PFHxA prevented the derivation of half-lives for mice.

Physiologically Based Pharmacokinetic Models

Fabrega et al. (2015) developed a physiologically based pharmacokinetic (PBPK) model designed to estimate tissue concentrations of 11 PFAS, including PFHxA. However, the model was not validated with PFHxA data, and for this reason, OEHHA did not evaluate it for this assessment.

Clearance Rate

PFHxA clearance rates calculated in animal studies are summarized in Table 3. Generally, male animals have lower clearance rates than females of the same species. Also, clearance rates in male rats and monkeys are comparable.

Table 3. PFHxA clearance rates in animals

Species/sex	Exposure	Clearance Rate (L/kg-hr) and Biological Matrix	Reference
Monkey (male)	Single intravenous dose (10 mg/kg)	0.122 (serum)	Chengelis et al. (2009a)
Monkey (female)		0.136 (serum)	
Rat (male)	Single intravenous dose (10 mg/kg)	0.116 (serum)	Chengelis et al. (2009a)
Rat (female)		0.775 (serum)	
Rat (male)	Inhalation of 6:2 FTOH (0.5 or 5 ppm) for 6 hours	0.107 (plasma)	Kabadi et al. (2018)
Rat (female)		0.277 (plasma)	
Rat (male)	Single oral dose (40 mg/kg)	0.103 (plasma)	Dzierlenga et al. (2020)
Rat (female)		0.164 (plasma)	
Rat (male)	Single oral dose (80 mg/kg)	0.153 (plasma)	Dzierlenga et al. (2020)
Rat (female)		0.314 (plasma)	
Rat (male)	Single oral dose (160 mg/kg)	0.147 (plasma)	Dzierlenga et al. (2020)
Rat (female)		0.274 (plasma)	
Rat (male)	Single intravenous dose (40 mg/kg)	0.136 (plasma)	Dzierlenga et al. (2020)
Rat (female)		0.327 (plasma)	

From the 1-day inhalation data of 6:2 FTOH reported in Russell et al. (2015), Kabadi et al. (2018) calculated PFHxA clearance rates of 0.107 L/kg-hr for male rats, and 0.277 L/kg-hr for female rats. The authors subsequently used allometric scaling to calculate a human clearance value of 4.139 L/kg-hr, based on the average rat inhalation clearance value of 0.192 L/kg-hr.

Zhou et al. (2014) determined clearance rates of multiple PFAS in Chinese fishery workers, members of their families, and background controls. The authors reported high serum PFAS concentrations in fishery workers compared to a control reference group

from the same city, and the authors concluded that consumption of contaminated fish was the main contributor of PFAS exposure.

Renal clearance rate (CL_{renal}) was calculated by Zhou et al. (2014) with the following equation:

$$CL_{renal}\ (ml/kg-day) = C_{urine}\ x\ V/(C_{serum}\ x\ W)\ ,$$

where C_{urine} is the PFAS concentration in the urine (ng/L), V is the daily urine excretion volume (1.2 L/day for females and 1.4 L/day for males), C_{serum} is the PFAS concentration in the serum (ng/ml), and W is body weight (kg). Using paired serum and urine samples, the authors determined PFHxA clearance rates for each evaluated group. Summary statistics for human renal clearance rates of PFHxA are provided in Table 4. The clearance rate of 23.6 ml/kg-day, determined from fishery employees, was selected as the human clearance rate because it was determined using a larger sample number than the other evaluated groups (Table 4). Thus, there is less uncertainty about the clearance rate for fishery employees compared to the other studied populations. This clearance rate is converted to 9.83×10^{-4} L/kg-hr (using conversion factors of 24 hours per day and 1,000 milliliters per liter).

Table 4. Renal clearance rates of serum PFHxA (ml/kg-day) from Zhou et al. (2014)

	Mean	Median	SD	Min	Max
Background exposed people (n=2 paired samples)	7.97	7.97	5.56	4.03	11.9
Fishery employees (n=16 paired samples)	23.6	17.8	17.0	2.23	53.6
Fishery family (n=1 paired sample)	8.27	8.27		8.27	8.27

In animals, PFHxA is eliminated primarily via urine (see *Elimination* subsection above). Although there are no specific data regarding routes of PFHxA elimination in humans, it is hypothesized that short-chain perfluorocarboxylic acids (<8 carbons) are eliminated predominantly via urine (Zhou et al., 2014). Based on animal elimination data, and in the absence of specific human data, total elimination in humans is estimated from urinary elimination of PFHxA.

For comparison, in its assessment of PFHxA, US EPA (2023) derived a human clearance factor of 1.84×10^{-3} L/kg-hr (equivalent to 44.2 ml/kg-day). This value was calculated using the following equation:

$$CL = V_d\ x\ \ln(2)/T_{1/2}$$

where CL is the human clearance rate, V_d is the volume of distribution of PFHxA in humans, and $T_{1/2}$ is the human half-life of 32 days, determined from Nilsson et al.

(2013). The V_d for PFHxA in humans has not be determined, so US EPA (2023) used the average V_d for male and female monkeys (determined in Chengelis et al. (2009a)). The derived clearance rate is approximately two-fold larger than the clearance value reported by Zhou et al. (2014), so the values are somewhat comparable. Kabadi et al. (2018) derived a clearance factor of 4.139 L/kg-hr (equivalent to 99,336 ml/kg-day) based on allometric scaling of clearance in rats from an inhalation study. This rate is several orders of magnitude larger than the clearance rates determined by Zhou et al. (2014) and US EPA (2022). Furthermore, there is no evidence to suggest that the human clearance rates of PFHxA can be accurately determined through body weight scaling of animal clearance data. Thus, OEHHA does not consider the rate determined by Kabadi et al. (2018) to be reflective of the true human clearance rate of PFHxA.

OEHHA selected the human clearance rate of 23.6 (ml/kg-day) from Zhou et al. (2014) for derivation of a dosimetric adjustment factor because it was determined directly from human data. Deriving human clearance rates from animal data requires various assumptions, which lead to increased uncertainty. Using a reported human clearance estimate constitutes a more direct approach.

TOXICOLOGICAL EFFECTS IN HUMANS

Although studies involving all adverse health outcomes were included in the initial literature searches, the primary focus of OEHHA's more detailed evaluations was on studies of liver biomarkers, thyroid hormone levels, serum lipid concentrations, vaccine response, and cancer. These outcomes were selected because they were the health effects most frequently reported for other PFAS commonly detected in water, like PFOA, PFOS, and perflurorohexane sulfonic acid (PFHxS). Each study meeting the inclusion criteria listed in the *Systematic Literature Search* section above was reviewed for the presence or absence of an association of any health effect(s) with PFHxA exposure. Assessing whether an association was present was based on both statistical significance and effect size using methods described previously (OEHHA, 2021b). Study quality and causal inference were assessed based on factors such as participant selection, outcome and exposure assessment, the likelihood of confounding, statistical power, the range of exposure, multiple comparisons, consistency of findings, co-variance with other PFAS, information on subgroups, dose-response, temporality, and other criteria. These factors were selected based on an updated version of the Hill criteria (Hill, 1965) and the National Toxicology Program's Risk of Bias Tool (NTP, 2019c).

Overall, far fewer human research studies have been published on PFHxA than on other PFAS such as PFOA and PFOS. No human studies of PFHxA and vaccine response were identified. Several studies of PFHxA and liver biomarkers, serum lipid concentrations, or thyroid hormone levels were identified but none reported evidence of associations. Details of these studies, including several criteria related to study quality and causal inference are provided in Table 5. Although certain biases or weaknesses such as the cross-sectional designs, limited information on exposure and outcome, or relatively narrow ranges of exposure may have caused some studies to miss true

effects, the small numbers of studies here make it difficult to make any firm conclusions regarding these outcomes based on human studies.

With regards to cancer, elevated risks of kidney cancer were reported in a retrospective cohort study performed in Ronneby, Sweden, a municipality where drinking water sources were contaminated with PFHxA (Li et al., 2022). However, drinking water concentrations of other PFAS were also elevated in this area (8,000 ng/L PFOS, 1,700 ng/L PFHxS, and 100 ng/L PFOA, compared to 320 ng/L PFHxA). As such, it is unknown whether these increased risks are due to PFHxA or one of these other PFAS. No other human cancer studies meeting the inclusion criteria described above were identified.

Several human studies have examined PFHxA and other health related outcomes. These include (but are not limited to) effects related to cardiovascular disease, asthma, renal disease, and developmental and reproductive effects. Most of these studies have been recently reviewed by ATSDR (2021). Although associations have been reported for a few of these outcomes (e.g., carotid artery intima media thickness, decreased serum testosterone), clear and consistent associations for any given outcome have either yet to be examined or yet to be reported in more than one study population. Overall, ATSDR did not find consistent evidence supporting a causal effect for PFHxA and any health outcome based on the human epidemiologic studies they reviewed.

In summary, because of the relatively small numbers of studies to date and the lack of clear and consistent associations across different study populations, strong conclusions regarding the health effects of PFHxA cannot be made at this time based solely on the current human epidemiologic data.

Table 5. Human epidemiologic studies of PFHxA and liver biomarkers, serum lipids and thyroid hormones

Study (year)	General outcome	Design	Location	Age group	Sex	N	Design notes	Exposure method	Outcome method	Percent detected	Median (ng/ml)[1]	Specific outcomes	Result	Clear results[2]	Co-variates	DR data[3]
Nian et al. (2019)	Liver	Cross-sectional	China: C8 Project area	Adults	All	1,605	Industrial areas with PFAS manufacturing	Serum	Serum	52.1	0.03	ALT, AST, GGT, TB	No association	Yes	Age, sex, career, income, education, alcohol use, smoking, giblet and seafood consumption, exercise, BMI	No
Fu et al. (2014)	Serum lipids	Cross-sectional	China: Henan	All	All	133	Ages 0-88 years; randomly selected from health check-ups	Serum	Serum	76.7	0.03	TC, LDL, HDL, TG	No association	Yes	Age, gender, BMI	No
Zeng et al. (2015)	Serum lipids	Cross-sectional	Taiwan	Adol-escents	All	225	Ages 12-15 years; control group in asthma case-control study	Serum	Serum	94.0	0.2	TC, LDL, HDL, TG	No association	Yes	Age, gender, BMI, parental education level, exercise, ETS	No
Li et al. (2017)	Thyroid	Cross-sectional	China: Southern	All	All	202	Young adults and women oversampled; case-control design of "abnormal thyroid hormones"	Serum	Serum	53.0	0.01	fT3, fT4, TSH	No association	Des-criptive only	Age, sex	No

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DR, dose-response; ETS, environmental tobacco smoke; fT3, free triiodothyronine; fT4, free thyroxine; GGT, gamma-glutamyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein; PFAS, per- and polyfluoroalkyl substances; ref, reference; TB, total bilirubin; TC, total cholesterol; TG, triglycerides; TSH, thyroid stimulating hormone

1. Serum concentrations
2. Coded as "Yes" if clear and consistent quantifiable information on associations are provided. Otherwise coded as "Descriptive only.".
3. Coded as "Yes" if quantifiable information on a dose-response relationship is provided. Otherwise coded "No."

TOXICOLOGICAL EFFECTS IN ANIMALS

OEHHA identified seven animal studies examining the toxicity of orally administered PFHxA in laboratory animals. Serum concentrations were only reported in the NTP (2019a) and Weatherly et al. (2023) studies. These studies are summarized in Table 6.

Table 6. Summary of animal toxicity studies of PFHxA

Sex/Species/ Reference	Exposure/ Duration	Endpoints	NOAEL/ LOAEL
Male and female Crl:CD(SD) rats (10-20/sex/dose) Chengelis et al. (2009b)	0, 10, 50 or 200 mg/kg-day PFHxA (in deionized water) via oral gavage for 90 days	Males: ↓ body weight; ↑ relative liver weight; ↓ total protein; ↑ ALT and ALP; ↓ cholesterol; ↓ calcium; ↑ relative kidney weight; hepatocellular hypertrophy; ↑ peroxisomal beta oxidation Both sexes: altered hematology; ↓ globulin	LOAEL: 10 mg/kg-day for ↑ kidney weight in male rats
Female Crl:CD(SD) rats (number of animals not specified) Loveless et al. (2009)	Single dose of 0, 175, 550, 1,750 or 5,000 mg/kg NaPFHxA (in deionized water)	Mortality at highest dose; systemic toxicity and neurotoxicity (abnormal gait, dehydration, high or low posture, clear oral/nasal discharge, wet/stained fur, salivation, ataxia, lethargy)	Doses at which effects occurred not reported
Male and female Crl:CD(SD) rats (5/sex/dose) Loveless et al. (2009)	0, 20, 100, or 500 mg/kg-day NaPFHxA (in deionized water) for 10 days via oral gavage	↑ peroxisomal beta oxidation in both sexes	NOAEL: 100 mg/kg-day

Sex/Species/ Reference	Exposure/ Duration	Endpoints	NOAEL/ LOAEL
Male and female Crl:CD(SD) rats (10/sex/dose) Loveless et al. (2009)	0, 20, 100, or 500 mg/kg-day NaPFHxA (in deionized water) for 90 days via oral gavage; 30-day and 90-day recovery groups included	Males: ↓ body weight; ↑ relative brain and testes weight; ↑ ALT, AST, and BUN; ↓ globulin; ↓ calcium and sodium; ↑ potassium and chloride; ↓ absolute spleen and thymus weight Females: ↑ absolute thyroid weight (low dose only) Both sexes: ↑ peroxisomal beta oxidation; ↑ relative liver and kidney weight; ↓ bilirubin and creatinine; hematology changes; nasal cavity histopathology; hepatocellular hypertrophy; thyroid hypertrophy; erythroid hypertrophy in bone marrow; extramedullary hematopoiesis in spleen	NOAEL: 20 mg/kg-day
Male and female Crl:CD(SD) rats (20/sex/dose) Loveless et al. (2009)	0, 20, 100, or 500 mg/kg-day NaPFHxA (in deionized water) via oral gavage for 70 days prior to cohabitation; males were dosed for 110 days and females were dosed through gestation and lactation for a total of 126 days F1 pups were not dosed	↓ body weight in males, dams (during gestation) and pups (both sexes combined, up to PND 21)	NOAEL: 20 mg/kg-day
Pregnant Crl:CD(SD) rats (22/dose) Loveless et al. (2009)	0, 20, 100, or 500 mg/kg-day NaPFHxA (in deionized water) via oral gavage on GD 6-20 (separate from experiment above)	↓ body weight in dams	NOAEL: 100 mg/kg-day

Sex/Species/ Reference	Exposure/ Duration	Endpoints	NOAEL/ LOAEL
Pregnant Crl:CD1 mice (20/dose) Iwai and Hoberman (2014)	0, 100, 350, or 500 mg/kg-day via oral gavage on GD 6-18	Dams: ↓ body weight gain during lactation; ↓ live litter size Pups: ↑ mortality and stillbirths; ↓ viability index; ↓ body weight; delayed eye opening; ↓ relative liver weight at termination (PND 41) in males in the highest dose group	NOAEL: 100 mg/kg-day
Pregnant Crl:CD1 mice (20/dose) Iwai and Hoberman (2014)	0, 7, 35, or 175 mg/kg-day via oral gavage on GD 6-18	Pups: ↑ mortality and stillbirths; ↓ body weight	NOAEL: 35 mg/kg-day
Male and female Crl:CD(SD) rats (60-70/sex/dose) Klaunig et al. (2015)	0, 2.5, 15, or 100 mg/kg-day (males) and 0, 5, 30, or 200 mg/kg-day (females) via oral gavage for up to 104 weeks	Males: ↓ triglyceride levels; lung congestion and hemorrhage Females: ↓ survival; altered hematology; changes in LDL + VLDL levels; ↑ triglyceride levels; papillary necrosis in kidney; ↑ lung alveolar macrophages; stomach erosion; hepatocellular necrosis	NOAEL: 15 mg/kg-day for males NOAEL: 30 mg/kg-day for females
Male and female SD rats (10/sex/dose) NTP (2019a)	0, 62.6, 125, 250, 500, or 1,000 mg/kg-day via oral gavage for 28 days	Males: ↓ body weight; ↓ absolute thymus weight; ↓ total cholesterol; ↓ T3, T4, and free T4; ↓ albumin; ↓ sperm count Females: chronic progressive nephropathy Both sexes: ↑ relative kidney and liver weight; altered hematology; ↑ ALT, AST, and ALP; changes in total protein, globulin, and bile salts; hepatocellular hypertrophy and cytoplasmic alteration; nasal olfactory epithelium degeneration, hyperplasia, and inflammation; splenic extramedullary hematopoiesis	LOAEL: 62.6 mg/kg-day for changes in thyroid hormones in male rats

Sex/Species/ Reference	Exposure/ Duration	Endpoints	NOAEL/ LOAEL
Male ICR mice (10/dose) Jiang et al. (2021)	0, 50, or 200 mg/kg-day PFHxA (in deionized water) via oral gavage for two months	↑ hepatosomatic indices (relative liver weight); ↑ hepatic cell infiltration and degeneration; changes in gene and protein expression (with fatty acid pathways frequently altered); hepatic metabolic alterations; ↓ hepatic SOD activity	LOAEL: 50 mg/kg-day for ↑ relative liver weight
Female B6C3F1 mice (5/dose) Weatherly et al. (2022)	0, 2.5, 5, or 10% (v/v) PFHxA (in acetone) on days 1-4. Reduced to 0, 1.25, 2.5 or 5% (v/v) on days 5-28 due to dermal irritation; applied topically to dorsal surface of each ear (25 µL/ear)	↑ relative liver weight; ↑ serum glucose; altered cellularity and/or number and frequency of cell types in draining lymph nodes, skin, and spleen; ↑ activation of MHCII and/or CD86 on lymph node and spleen B-cells; ↓ MHCII activation on lymph node dendritic cells; ↑ centrilobular hepatocellular hypertrophy; ↑ epidermal hyperplasia; ↑ epidermal hyperkeratosis; dermal mixed cell inflammation and focal fibrosis; gene expression changes in liver and skin	NOAEL: 1.25%

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CD, cluster of differentiation; GD, gestation day; LDL, low-density lipoprotein; LOAEL, lowest-observed-adverse-effect level; MHCII, major histocompatibility complex class II; NK, natural killer; NOAEL, no-observed-adverse-effect level; PND, postnatal day; SOD, superoxide dismutase; SD, Sprague Dawley; T3, triiodothyronine; T4, thyroxine; VLDL, very low-density lipoprotein; v/v, volume of solute/volume of solution

Overall, PFHxA adversely impacts multiple biological organs/systems, including the liver, kidney, nasal cavity, thyroid, hematopoietic system, and lipid homeostasis. Additionally, PFHxA can induce developmental and reproductive toxicity. The toxicity profile of PFHxA is comparable to other PFAS (e.g., PFOA, PFOS, perfluorobutane sulfonic acid (PFBS), and PFHxS), with similar organ systems being affected (OEHHA, 2021b; OEHHA, 2021a; OEHHA, 2022). In particular, liver, thyroid, and developmental/reproductive toxicity are commonly observed in human and animal studies of PFAS, and PFHxA is no exception.

PFHxA had no observable impact on locomotor and neurobehavioral parameters in rats (Chengelis et al., 2009b; Loveless et al., 2009; Klaunig et al., 2015). Furthermore, multiple studies reported no observable ophthalmic effects in adult rats (Chengelis et al., 2009b; Loveless et al., 2009; Klaunig et al., 2015).)

Liver Toxicity

Much as with other PFAS, the liver is a major target of PFHxA toxicity. Increased relative liver weight, increased liver enzymes, and hepatocellular hypertrophy were

commonly reported in studies of other PFAS, including PFOA, PFOS, PFHxS, and PFBS (OEHHA, 2021b; OEHHA, 2021a; OEHHA, 2022). Increased relative liver weight, increased serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and hepatocellular hypertrophy were commonly observed in rodents across multiple studies of PFHxA (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2019a; Weatherly et al., 2023).

The hepatosomatic index (relative liver weight) was significantly elevated at doses ≥50 mg/kg-day in mice orally exposed to PFHxA for two months, which the authors attributed to hypertrophy (Jiang et al., 2021). Hepatocellular cytoplasmic alteration was observed only in animals administered the highest dose in the NTP (2019a) 28-day studies.

Klaunig et al. (2015), using data from Kirkpatrick (2010), reported a significant increase in liver necrosis in female rats at the high dose, predominantly in animals that died or were euthanized prior to the scheduled necropsy (2/60 controls; 0/60 at 5 mg/kg-day; 3/60 at 30 mg/kg-day; 12/70 at 200 mg/kg-day). Liver weights were not reported by Klaunig et al. (2015), but incidences of hypertrophy reported in the individual animal data from Kirkpatrick (2010) were minimal.

Statistically significant liver toxicity data are summarized in Tables 7-11.

Table 7. Liver toxicity in male rats following gavage administration of PFHxA for 90 days from Chengelis et al. (2009b)

	0 mg/kg-day	10 mg/kg-day	50 mg/kg-day	100 mg/kg-day
Relative liver weight (g/100g)	2.723 ± 0.1860[a]	2.748 ± 0.1567	2.750 ± 0.2236	3.334 ± 0.1841**
ALP (U/L)	95 ± 19.8	93 ± 15.0	109 ± 23.0	127 ± 31.0*
ALT (U/L)	41 ± 4.6	46 ± 4.9	50 ± 18.3	138 ± 143.1*
AST (U/L)	88 ± 16.0	85 ± 9.2	102 ± 34.3	184 ± 172.1
Hepatocellular hypertrophy	0/10	0/10	0/10	7/10##

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase
[a] mean ± standard deviation
*p<0.05, **p<0.01 using Dunnett's test, calculated by study authors
##p<0.01 using Fisher's exact test, calculated by OEHHA

Table 8. Liver toxicity in male and female rats following gavage administration of PFHxA for 90 days from Loveless et al. (2009)

	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
Relative liver weight (%) (males)	2.687 ± 0.165[a]	2.696 ± 0.255	2.994 ± 0.231	4.378 ± 0.485[b]
Relative liver weight (%) (females)	2.682 ± 0.300	2.650 ± 0.158	2.807 ± 0.147	3.678 ± 0.176[c]
ALT (U/L) (males)	27 ± 5	63 ± 64[b]	39 ± 12[b]	42 ± 9[b]
AST (U/L) (males)	69 ± 12	120 ± 94	86 ± 17[b]	96 ± 26[b]
Hepatocellular hypertrophy (males)	0/10	0/10	4/10	10/10[e]
Hepatocellular hypertrophy (females)	0/10	0/10	0/10	5/10[d]

ALT, alanine aminotransferase; AST, aspartate aminotransferase
[a] mean ± standard deviation
[b] p<0.05 using Dunn's test, calculated by study authors
[c] p<0.05 using Dunnett/Tamhane-Dunnett test, calculated by study authors
[d] p<0.05 using Fisher's exact test, calculated by OEHHA
[e] p<0.001 using Fisher's exact test, calculated by OEHHA

Table 9. Liver toxicity in male and female rats following gavage administration of PFHxA for 28 days from NTP (2019a)

	0 mg/kg-day	62.6 mg/kg-day	125 mg/kg-day	250 mg/kg-day	500 mg/kg-day	1,000 mg/kg-day
Relative liver weight (mg/g) (males)	34.44 ± 0.41[a]	37.26 ± 2.62	36.70 ± 0.50	39.35 ± 0.29**	45.33 ± 0.85**	56.35 ± 1.07**
Relative liver weight (mg/g) (females)	32.18 ± 0.74	32.63 ± 0.67	32.82 ± 0.46	34.37 ± 0.94	37.06 ± 0.73**	47.45 ± 0.99**
ALT (IU/L) (males)	48 ± 1	50 ± 2	50 ± 2	52 ± 2	61 ± 1**	79 ± 3**
ALT (IU/L) (females)	40 ± 1	44 ± 2	46 ± 2	44 ± 3	54 ± 2**	57 ± 3**
AST (IU/L) (males)	57 ± 2	59 ± 2	58 ± 2	61 ± 1	67 ± 2**	78 ± 3**
AST (IU/L) (females)	56 ± 3	55 ± 2	56 ± 1	56 ± 1	62 ± 1**	66 ± 3**
ALP (IU/L) (males)	200 ± 8	191 ± 6	195 ± 6	203 ± 11	245 ± 10**	301 ± 19**
ALP (IU/L) (females)	138 ± 4	149 ± 8	163 ± 7	141 ± 5	147 ± 7	190 ± 10**

	0 mg/kg-day	62.6 mg/kg-day	125 mg/kg-day	250 mg/kg-day	500 mg/kg-day	1,000 mg/kg-day
Hepatocyte cytoplasmic alteration (males)	0/10	0/10	0/10	0/10	2/10	10/10**
Hepatocyte cytoplasmic alteration (females)	0/10	0/10	0/10	0/10	0/10	9/10**
Hepatocellular hypertrophy (males)	0/10	0/10	0/10	0/10	9/10**	10/10**
Hepatocellular hypertrophy (females)	0/10	0/10	0/10	0/10	0/10	9/10**

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase
[a] mean ± standard error
** p<0.01 using Dunn's or Shirley's test, calculated by study authors

Table 10. Liver toxicity in female mice following dermal exposure to PFHxA in acetone for 28 days from Weatherly et al. (2023)

	0%	1.25%	2.5%	5%
	0 µg/mL[a]	1.27 µg/mL	2.95 µg/mL	4.33 µg/mL
Relative liver weight (% body weight)	4.59 ± 0.09[b]	4.77 ± 0.11	4.84 ± 0.15	5.02 ± 0.17**
Hepatocellular hypertrophy (minimal grade)	0/5[c]	2/5	5/5[d]	5/5[d]

[a] Reported serum concentration after 28 days; data were presented graphically and were digitized using GetData Graph Digitizer v2.26
[b] Expressed as mean ± standard error
[c] Expressed as number of incidents/total number examined
[d] p<0.01 using Fisher's exact test, calculated by OEHHA
** p<0.01 using one-way ANOVA followed by Dunnett's post-test, calculated by study authors

Table 11. Hepatosomatic index (relative liver weight) data in male mice following oral gavage exposure to PFHxA for two months from Jiang et al. (2021)

	0 mg/kg-day	50 mg/kg-day	200 mg/kg-day
Hepatosomatic index[a] (%)	4.36 ± 0.35[b]	4.59 ± 0.35*	7.11 ± 0.5***

[a] Data were presented graphically and were digitized using GetData Graph Digitizer v2.26
[b] Expressed as mean ± standard deviation
* p<0.05, *** p<0.001 calculated by authors (statistical methods not provided)

Of the PFHxA studies examining liver toxicity, statistically significant adverse endpoints observed at doses <500 mg/kg-day were evaluated further for candidate critical endpoint determination. Hepatocellular hypertrophy in male rats from (Loveless et al.,

2009) was also considered for critical endpoint determination because of the near-significant increase in incidence at 100 mg/kg-day. Benchmark dose (BMD) modeling was performed on these data sets, and results are presented in Table 24. Dichotomous data sets where toxicity was observed only in highest-dose animals were not modeled because the models would likely not produce useful information in the low dose region of the data set. Effects observed at doses ≥500 mg/kg-day were not included for critical endpoint consideration due to relatively low sensitivity compared to other observed endpoints. Because there are several oral exposure studies of PFHxA that are suitable for critical endpoint determination, Weatherly et al. (2023) was also not evaluated as a candidate critical study. Nonetheless, Weatherly et al. (2023) reported serum levels of PFHxA and several toxicity endpoints, including various liver effects, thus supporting PFHxA toxicity reported in other studies.

Kidney Toxicity

While Chengelis et al. (2009b) reported statistically significant increases in relative kidney weight in both male and female rats, they did not consider this effect biologically significant because there were no histopathological changes correlating with kidney enlargement. Other studies also reported statistically significant increases in relative kidney weight in male rats, albeit at high doses (≥500 mg/kg-day) (Loveless et al., 2009; NTP, 2019a). As such, dose levels associated with increased kidney weight varied across studies, with only the Chengelis et al. (2009b) study reporting adverse effects at a lower dose level (10 mg/kg-day). Because the low dose increase in relative kidney weight was not supported by any additional studies, OEHHA did not evaluate kidney toxicity from this study for critical endpoint determination.

Kidney histopathology appears to be limited to female rats. Klaunig et al. (2015) reported an increased incidence of papillary necrosis in high dose (200 mg/kg-day) female rats. However, because this effect was only observed in the high dose (no incidences at any other dose), the dose-response data were not modeled. Additionally, NTP (2019a) reported a significant increase in chronic progressive nephropathy in high dose (1,000 mg/kg-day) female rats after 28 days of exposure. Overall, kidney toxicity was not very sensitive compared to endpoints in other organ systems and was not considered for critical endpoint determination.

Developmental and Reproductive Toxicity

A publication by Loveless et al. (2009) reported on two separate experiments relevant to the developmental toxicity of PFHxA. Pregnant rats from the 500 mg/kg-day group showed a statistically significant decrease in gestational weight gain compared to controls. No effects on the weight of the fetuses at gestation day (GD) 21 were identified at any dose in this experiment.

In another experiment reported in the same paper (Loveless et al., 2009), male and female rats were exposed to PFHxA before mating, with females continuing on treatment throughout gestation and lactation. Body weights of gestationally-exposed

pups from the 500 mg/kg-day group were significantly reduced relative to controls on the day of birth (postnatal day (PND) 0), and remained lower than controls with continued exposure throughout lactation, through PND 21 (Table 12). Dams of the 500 mg/kg-day group showed a transitory decrease in weight gain during the first seven days of gestation, which was not reflected at term. However, maternal weight gain was increased in the high-dose group during the lactation period.

Table 12. Mean body weights of F1 rat pups exposed to PFHxA from gestation through PND 21 from Loveless et al. (2009)

Postnatal Day	0 mg/kg-day	20 mg/kg-day	100 mg/kg-day	500 mg/kg-day
0	7.1 ± 0.9[a]	6.8 ± 0.6	6.3 ± 0.4	5.8 ± 0.4*
7	18 ± 2.7	18 ± 2.2	17 ± 1.3	15 ± 1.4*
14	36 ± 3.4	37 ± 3.0	34 ± 2.6	30 ± 2.5*
21	60 ± 5.3	62 ± 5.0	57 ± 5.3	49 ± 4.1*

[a] Values represent mean body weight in grams ± standard deviation
* p<0.05, calculated using analysis of covariance and Dunnett-Hsu, reported by study authors

In a study in two cohorts of mice, Iwai and Hoberman (2014) observed that body weight gain in dams during gestation was unaffected by PFHxA treatment up to 500 mg/kg-day, but was reduced at doses ≥350 mg/kg-day during lactation. Pup body weight per litter on PND 0 was significantly decreased at doses ≥350 mg/kg-day (Table 13).

Table 13. Mouse pup body weights[a] following gestational exposure (GD 6-18) to PFHxA from (Iwai and Hoberman, 2014)[b]

	Cohort 1				Cohort 2			
Dose (mg/kg-day)	**0**	**100**	**350**	**500**	**0**	**7**	**35**	**175**
Litters examined	18-19	15-19	17-19	11-13	16	17	19	20
PND 0	1.6 ± 0.2[c]	1.5 ± 0.1[d]	1.4 ± 0.2**	1.4 ± 0.2**	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.2*
PND 4	3.0 ± 0.4	2.8 ± 0.2	2.2 ± 0.6**	2.4 ± 0.5**	2.8 ± 0.3	2.8 ± 0.3	3.0 ± 0.3	2.7 ± 0.5
PND 7	4.4 ± 0.8	4.1 ± 0.4	3.6 ± 1.0**	3.9 ± 0.8	4.2 ± 0.6	4.2 ± 0.4	4.4 ± 0.4	4.2 ± 0.6
PND 14	7.4 ± 1.9	6.8 ± 0.8	6.4 ± 1.4	6.8 ± 1.1	6.8 ± 1.2	6.7 ± 0.6	7.0 ± 0.7	6.8 ± 0.9
PND 20	11.0 ± 3.0	9.8 ± 1.5	8.8 ± 2.7	9.7 ± 2.0	10.2 ± 1.8	10.0 ± 1.2	10.8 ± 1.3	10.4 ± 1.4

PND, postnatal day
[a] Pup body weight per litter (g)
[b] Average values were determined from 11-20 litters, and means excluded values for litters that had no surviving pups and litters that remained in the study after the dam was found dead.
[c] Data are presented as mean ± standard deviation.

[d] Statistically significant ($p<0.05$) compared to controls, but when covaried with litter size, analysis was not significant.
* $p<0.05$, ** $p<0.01$ compared to controls, calculated by authors.

Iwai and Hoberman (2014) reported several additional developmental and reproductive effects across both cohorts of animals evaluated. These effects included increased pup mortality, decreased litter size, and developmental delays. Additionally, two or three litters in the 175 mg/kg-day dose group had at least one pup with one or more ocular defects. Developmental delays included a significant delay in eye opening at 350 mg/kg-day, and a reduction in percentage of pups with open eyes on postnatal day 14 at doses ≥350 mg/kg-day (Table 14). Preputial separation in pups gestationally exposed to 35 mg/kg-day (administered to dams) was significantly earlier than in control animals. However, this effect was not statistically significant at any other dose across both cohorts.

Table 14. Eye opening of F1-generation mice exposed gestationally (GD 6-18) to PFHxA (Iwai and Hoberman, 2014)[a]

	Cohort 1				Cohort 2			
Dose (mg/kg-day)	**0**	**100**	**350**	**500**	**0**	**7**	**35**	**175**
Litters examined	18	19	17	11	20	16	19	20
Pups with open eyes on day 14 (%)	82.5 ± 24.4[b]	68.6 ± 34.9	42.0 ± 39.5**	50.2 ± 38.0*	85.5 ± 22.7	87.6 ± 24.4	89.3 ± 22.7	78.9 ± 27.4
Average day where 50% of pups have open eyes	13.8 ± 0.7	14.2 ± 0.8	14.9 ± 1.1**	14.5 ± 1.0	13.8 ± 0.7	13.8 ± 0.6	13.4 ± 0.6	14.0 ± 0.8

GD, gestation day
[a] Values for litters with no surviving pups were excluded from means.
[b] Data are presented as mean ± standard deviation.
* $p<0.05$, ** $p<0.01$, compared to controls within specific cohort, calculated by study authors.

Stillbirth and mortality data from Iwai and Hoberman (2014) are presented in Table 15. Iwai et al. (2019) re-evaluated the same data using historical incidence data and a pooled group of control animals from the two experimental cohorts described in Iwai and Hoberman (2014). The authors reported that pooling the controls of the two experiments removed statistical significance from the stillbirth data. Furthermore, the authors argue that the incidences of stillbirth and early postpartum pup mortality falls within the range of historical controls at the laboratory where the experiments were conducted (Charles River Laboratories), and that this effect is not related to treatment with PFHxA. Additionally, the authors argued that ophthalmic effects (microphthalmia and corneal opacity) are spontaneous in nature, are within the range of historical controls, may be attributed to laboratory conditions, and are not rare in this strain of mouse at the evaluated age.

Table 15. Stillbirth and pup mortality data in mice exposed during gestation to PFHxA (Iwai and Hoberman, 2014)[a]

	Cohort 1				Cohort 2			
Dose (mg/kg-day)	**0**	**100**	**350**	**500**	**0**	**7**	**35**	**175**
Litters with ≥1 liveborn pup	19	19	19	16	20	17	19	20
Total pups delivered	221	250	245	177	249	213[a]	232	241
Total pups/litter (mean ± SD)	11.6 ± 4.2	13.2 ± 1.6	12.9 ± 3.8	11.1 ± 2.4	12.4 ± 2.5	12.5 ± 3.0	12.2 ± 1.7	12.0 ± 2.1
Stillborn pups	4	0	5	16	0	0	0	3**
Pups found dead or presumed cannibalized – Day 0	0/217	0/250	3/232	21/150**	0/249	0/211	0/232	4/238**

SD, standard deviation

[a] Two pups in this dose group had unknown vital status

** Statistically significant from controls within specific cohort, p ≤0.01, calculated by study authors

Iwai and Hoberman (2014) reported ophthalmic effects in pups from at least two, possibly three litters (three effects were reported and it is unclear whether the third effect was observed in one of the two litters, or in a third litter), in the high-dose group (175 mg/kg-day), with no incidences in the control, low- and mid-dose groups. Although Iwai et al. (2019) cited a study by Gilger et al. (2018) in which 10% of 5,300 CD-1 mice aged between 1-12 weeks experienced some type of spontaneous ocular lesion, the absence of effects in concurrent controls and low- and mid-dose animals suggests that the effects reported in Iwai and Hoberman (2014) are treatment related.

While historical controls do have utility in certain circumstances, potential differences in environmental lab conditions, animal feed, and laboratory personnel increase the uncertainty that experimental conditions were identical, and generally preclude the use of historical controls when concurrent control data are available (US EPA, 1991). As such, OEHHA considers increases in pup mortality, increased incidences of stillbirth, and developmental ocular toxicity to be biologically significant and relevant to PFHxA exposure.

Developmental and reproductive toxicity have been commonly observed in studies of PFAS. Similar to PFHxA, reduced perinatal body weight and delayed eye opening were reported in mice exposed during gestation to PFBS, PFOA, and PFOS (US EPA, 2016a; US EPA, 2016b; OEHHA, 2021b; OEHHA, 2021a). Additionally, decreased litter sizes and live litters were observed in rodent studies of PFOA, PFOS, and PFHxS (US EPA, 2016a; US EPA, 2016b; OEHHA, 2021b; OEHHA, 2022),

Several developmental/reproductive effects of PFHxA were considered for candidate critical endpoint selection. BMD modeling was performed on data sets with statistically significant effects, including those for decreased pup body weight (Loveless et al., 2009; Iwai and Hoberman, 2014), and delayed eye opening (Iwai and Hoberman, 2014). Modeling results are included in Table 24. Based on BMD modeling results, decreased pup body weight on PND 0 from (Loveless et al., 2009) was selected as a candidate critical endpoint.

Additional Toxicities

Thyroid

Thyroid toxicity was observed in two different studies. Loveless et al. (2009) reported an increase in absolute thyroid weight in female rats, and an increased incidence of thyroid hypertrophy in rats of both sexes. Additionally, NTP (2019a) reported decreased levels of triiodothyronine (T3), thyroxine (T4), and free T4 in male rats exposed to PFHxA for 28 days (Table 16). However, there was no compensatory increase in thyroid stimulating hormone (TSH). Changes in thyroid hormone levels in male rats were the most sensitive endpoints OEHHA identified in the NTP (2019a) studies, with a lowest-observed-adverse-effect level (LOAEL) of 62.6 mg/kg-day.

Table 16. Thyroid hormone levels in male rats exposed to PFHxA for 28 days (NTP, 2019a)

Dose (mg/kg-day)	0	62.6	125	250	500	1,000
T3 (ng/dL)	84.17 ± 5.25[a]	68.88 ± 3.76*	62.16 ± 3.51**	71.59 ± 3.90*	71.03 ± 4.09*	60.07 ± 4.74**
Free T4 (ng/dL)	2.88 ± 0.09	2.16 ± 0.17**	1.78 ± 0.12**	1.74 ± 0.09**	1.30 ± 0.07**	0.77 ± 0.10**
Total T4 (µg/dL)	4.26 ± 0.15	3.40 ± 0.23**	2.93 ± 0.16**	2.90 ± 0.17**	2.37 ± 0.10**	1.77 ± 0.17**

T3, triiodothyronine; T4, thyroxine
[a] mean ± standard error
*p<0.05, **p<0.01 using Dunn's or Shirley's test, calculated by study authors

Reductions in thyroid hormones (hypothyroidism) have been linked to a myriad of adverse effects, including growth retardation, impaired cognitive development, and sometimes impaired hearing in infants and children, anemia and impaired cardiovascular function (bradycardia, increased peripheral resistance, diminished cardiac output), pulmonary function, peristalsis, and renal function in adults (OEHHA, 2015; Gardner and Shoback, 2017). Control of T4 and T3 concentrations in blood is mainly regulated by a negative feedback loop involving three organs: the thyroid gland, which produces thyroid hormones; and the pituitary gland and hypothalamus, which respond to and help maintain optimal levels of thyroid hormones. When levels of thyroid hormone decline, the hypothalamus secretes thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce TSH, which then prompts the thyroid gland to produce T4 and T3. Interestingly, PFHxA-induced reductions in thyroid hormones were

not coupled with compensatory increases in TSH. These results are comparable to results reported in studies with other PFAS, including PFOA, PFOS, and PFHxS (NTP, 2019a; NTP, 2019b; OEHHA, 2022). Nonetheless, subclinical hypothyroidism (where TSH is not elevated) has been correlated with neurodevelopmental and cognitive deficits in children (Haddow et al., 1999; Negro et al., 2011). Although it is not known whether PFHxA reduces thyroid hormone levels during development, the potential effects on human health are severe enough to warrant evaluating these endpoints as candidate critical endpoints for derivation of a health-protective concentration. BMD modeling was performed on these data sets, and results are presented in Table 24.

Respiratory Tract

Nasal cavity toxicity was observed in two different studies. Loveless et al. (2009) reported nasal cavity histopathology in male and female rats, including olfactory epithelium degeneration/atrophy, turbinate adhesion, and respiratory metaplasia. Similarly, NTP (2019a) reported olfactory epithelial degeneration, hyperplasia, and inflammation in male and female rats. These data are summarized in Tables 17 and 18.

PFHxA was administered via oral gavage in both studies, and it is presumed to have low volatility due to its ionic nature (NTP, 2019a; MDH, 2021). Thus, inhalation exposure in these experiments was presumed to be minimal. Loveless et al. (2009) speculated that nasal tissue may be sensitive to the irritating properties of surfactants like PFHxA. It is also possible that gavage reflux (Damsch et al., 2011) of the test compound is contributing to the observed nasal lesions. However, the NTP (2019a) study authors (personal communication) stated that the pattern of nasal pathology observed was not consistent with gavage-related reflux effects. Toxicity was not observed in the esophagus, nor in posterior regions of the nasal cavity, where gavage-related reflux effects are typically observed. Furthermore, a contemporaneous NTP (2016b) evaluation of green tea extract in mice and rats attributed nasal lesions, following chronic administration of the test compound via oral gavage, to gavage reflux and the reported spectrum of effects was quite different from those observed in the NTP (2019a) study. The nasal lesions attributed to the reflux of green tea extract in NTP (2016b) included inflammation, and lesions of the nasopharyngeal duct, lamina propria, olfactory epithelium, and respiratory epithelium. Furthermore, foreign bodies were present in the nasal cavity. The authors speculated that gavage reflux may have been responsible for the nasal lesions and inflammation seen in the green tea extract study. While there may be some overlap in effects, this spectrum of toxicity was not observed in the 28-day PFHxA studies, which suggests that gavage reflux may not be involved in the observed nasal toxicity.

Nasal cavity toxicity was also reported with other PFAS (PFOA, PFNA, PFBS, PFHxS) using the same experimental design (NTP, 2019a; NTP, 2019b), which indicates that various perfluorinated compounds may adversely impact the nasal epithelium. However, not all PFAS tested via gavage (PFDA, PFOS) induced nasal toxicity.

Table 17. Nasal cavity toxicity in rats exposed via gavage to PFHxA for 90 days from Loveless et al. (2009)

Dose (mg/kg-day)	0	20	100	500
Males				
Olfactory degeneration/atrophy	0/10	0/10	4/10	7/10**
Adhesions (turbinates)	0/10	0/10	0/10	3/10
Respiratory metaplasia	0/10	0/10	0/10	4/10
Females				
Olfactory degeneration/atrophy	0/10	0/10	5/11*	4/10
Adhesions (turbinates)	0/10	0/10	0/11	3/10
Respiratory metaplasia	0/10	0/10	1/11	7/10**

* p<0.05, ** p<0.01, calculated by OEHHA using Fisher's exact test

Table 18. Nasal cavity olfactory epithelial toxicity in rats exposed via gavage to PFHxA for 28 days from NTP (2019a)

Dose (mg/kg-day)	0	62.6	125	250	500	1,000
Males						
Degeneration	0/10	0/10	1/10	6/10**	6/10**	6/10**
Hyperplasia	0/10	0/10	1/10	6/10**	5/10*	6/10**
Suppurative inflammation	0/10	0/10	0/10	0/10	3/10	6/10**
Females						
Degeneration	0/10	1/10	3/10	9/10**	9/10**	6/10**
Hyperplasia	0/10	0/10	3/10	4/10*	7/10**	3/10
Suppurative inflammation	0/10	0/10	0/10	1/10	5/10*	8/10**

* p<0.05, ** p<0.01, calculated by study authors using the Fisher's exact test

Because nasal cavity toxicity was observed across multiple studies of PFHxA, and has been reported in studies of other PFAS, OEHHA considers this effect to be treatment related. NTP (2019a) reported statistically significant effects at doses ≥250 mg/kg-day, which is comparable to significant effects reported in other organ systems, such as the liver. As such, endpoints with statistical significance at 250 mg/kg-day were evaluated further for health-protective concentration derivation. Nasal cavity degeneration/atrophy in male and female rats from Loveless et al. (2009) were also evaluated for health-protective concentration derivation. Although statistical significance is only reached at 500 mg/kg-day for males, a near-significant increase was reported at 100 mg/kg-day, and this dose-response relationship was considered to be appropriately sensitive for further analysis. The data for these endpoints were modeled and the results are presented in Table 24.

Lung toxicity was observed in a single study. In animals orally exposed to PFHxA for two years, male rats exhibited lung congestion and hemorrhage, whereas female rats showed an increase in pulmonary alveolar macrophages (Klaunig et al., 2015). Males were given 2-fold lower doses than females. Lung toxicity was graded as either minimal, mild, or moderate, with the majority of incidences classified as minimal. Toxicity data for male animals are presented in Table 19.

Animals were administered PFHxA via oral gavage, thus inhalation exposure is anticipated to be minimal. The authors argue that the pulmonary effects are due to accidental aspiration and the physical properties of PFHxA, including a low pH. The authors also state that reflux injury was observed in some of the incidental premature deaths, and appeared to be due to aspiration of the compound as shown by localized inflammation and/or epithelial necrosis in the larynx or pulmonary airway epithelium. Thus, it is unclear whether the pulmonary effects of PFHxA reported by Klaunig et al. (2015) are compound related or due to gavage-related issues.

No dietary or drinking water studies of PFHxA were identified, so OEHHA is unable to compare lung toxicity profiles using different oral administration procedures.

Table 19. Lung toxicity in male rats administered PFHxA via gavage for two years from Klaunig et al. (2015)

Dose (mg/kg-day)	0	2.5	15	100
Congestion	5/60	9/60	9/60	19/70**
Hemorrhage	1/60	5/60	5/60	10/70*
Alveolar Macrophages	5/60	6/60	13/60	10/70

* p<0.05, ** p<0.01, calculated by OEHHA using Fisher's exact test

Because it is unclear whether pulmonary toxicity is treatment related, these endpoints were not considered for derivation of a health-protective concentration.

Hematologic System

Hematotoxic effects from oral exposure to PFHxA were observed across multiple studies, with decreased hemoglobin, hematocrit, and red blood cell counts commonly reported.

Decreases in hemoglobin levels were reported in male and female rats (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2019a), summarized in Table 20. In a chronic two-year study, Klaunig et al. (2015) reported significantly decreased hemoglobin in female rats administered 200 mg/kg-day PFHxA for 51 weeks, but not for 104 weeks when compared against control values. It should be noted that hemoglobin levels were lower in all animals at 104 weeks compared to 51 weeks. A LOAEL of 62.6 mg/kg-day for decreased hemoglobin in male rats from NTP (2019a) was identified by OEHHA.

Decreased hematocrit was also commonly observed in rats orally exposed to PFHxA (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2019a), summarized in Table 21. Decreased hematocrit was not observed in the chronic two-year study in either sex (Klaunig et al., 2015). A LOAEL of 62.6 mg/kg-day for decreased hematocrit in male rats from NTP (2019a) was identified by OEHHA.

Decreased red blood cell counts in rats exposed to PFHxA were reported in several studies (Chengelis et al., 2009b; Loveless et al., 2009; Klaunig et al., 2015; NTP, 2019a), summarized in Table 22. As with hemoglobin levels, Klaunig et al. (2015) reported significantly decreased red blood cell counts at 51 weeks in female rats, but not at 104 weeks when compared against control values. Similarly, red blood cell counts were lower in all animals at 104 weeks compared to animals at 51 weeks regardless of dose level. A LOAEL of 62.6 mg/kg-day for decreased erythrocyte count in male rats from NTP (2019a) was identified by OEHHA.

Hematological toxicity was observed across multiple studies, the most sensitive of which was the NTP (2019a) 28-day study, with statistically significant effects reported in male animals at 62.6 mg/kg-day. However, Loveless et al. (2009) and Klaunig et al. (2015) reported no significant effects in male animals given 100 mg/kg-day for longer durations. Furthermore, statistically significant effects reported at 51 weeks in Klaunig et al. (2015) were no longer significant at 104 weeks. These data suggest that these endpoints are more prevalent at earlier time points and may resolve as the exposure duration increases. Based on these data, these endpoints are not ideal when considering toxicity over a lifetime of exposure, and as such, hematotoxic effects were not considered for derivation of a health-protective concentration.

Table 20. Hemoglobin levels (g/dL) in Sprague Dawley rats orally exposed to PFHxA

Reference	Dose (mg/kg-day)														
Males	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	15.6 ± 0.51[a]			15.4 ± 0.58				15.4 ± 0.65				14.3 ± 1.08**			
Loveless et al. (2009)	15.4 ± 0.5					15.5 ± 0.41				4.5 ± 0.7[b]				9.9 ± 2.8*	
Klaunig et al. (2015) (week 51)	15.9 ± 0.63	15.0 ± 2.47			15.1 ± 1.72					15.4 ± 1.02					
Klaunig et al. (2015) (week 104)	13.5 ± 2.14	12.3 ± 2.14			12.9 ± 1.98					12.3 ± 2.06					
NTP (2019a)	15.5 ± 0.63								15.0 ± 0.63*		14.7 ± 0.6**		14.6 ± 0.63**	12.6 ± 0.95**	9.3 ± 1.90**
Females	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	15.6 ± 0.46			15.8 ± 1.40				15.2 ± 0.85				14.6 ± 0.83			
Loveless et al. (2009)	15.6 ± 0.7					15.8 ± 0.8				15.6 ± 0.4				13.3 ± 0.9*	
Klaunig et al. (2015) (week 51)	15.5 ± 0.97		15.5 ± 0.73				15.5 ± 0.79					14.7 ± 0.91*			
Klaunig et al. (2015) (week 104)	12.9 ± 1.41		12.9 ± 1.30				12.9 ± 2.31					12.8 ± 1.12			
NTP (2019a)	14.0 ± 0.63								14.0 ± 0.32		13.9 ± 0.32		13.1 ± 0.32**	12.9 ± 0.63**	11.3 ± 1.26**

* p<0.05, ** p<0.01, calculated by study authors; blank cells indicate dose group was not part of the study

[a] Mean ± standard deviation; for NTP (2019a), standard error was converted to standard deviation by OEHHA

[b] Value reported as 4.5 ± 0.7 g/dL in the publication, without statistical significance

Table 21. Hematocrit (%) in Sprague Dawley rats orally exposed to PFHxA

Reference	Dose (mg/kg-day)														
Males	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	51.2 ± 2.13[a]			49.9 ± 1.56				49.9 ± 2.37				46.9 ± 2.98**			
Loveless et al. (2009)	49.0 ± 1.4					49.2 ± 1.7				46.2 ± 1.8				34.0 ± 7.8*	
Klaunig et al. (2015) (week 51)	46.6 ± 2.03	44.2 ± 7.16			44.8 ± 4.36					45.4 ± 2.90					
Klaunig et al. (2015) (week 104)	41.4 ± 5.80	37.7 ± 5.85			39.5 ± 5.17					38.0 ± 5.51					
NTP (2019a)	51.9 ± 1.58								49.6 ± 2.21*		48.6 ± 2.10**		48.7 ± 1.90**	43.3 ± 2.85**	34.2 ± 5.69**
Females	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	50.9 ± 2.32			51.1 ± 5.13				48.6 ± 2.88				47.7 ± 2.54			
Loveless et al. (2009)	47.2 ± 2.3					47.7 ± 2.5				47.1 ± 1.4				41.2 ± 2.4*	
Klaunig et al. (2015) (week 51)	44.0 ± 2.95		44.5 ± 2.52				44.2 ± 2.28					42.3 ± 2.76			
Klaunig et al. (2015) (week 104)	39.2 ± 4.08		39.3 ± 3.43				39.0 ± 6.22					39.5 ± 2.84			
NTP (2019a)	45.1 ± 1.58								44.8 ± 0.95		45.1 ± 1.26		42.1 ± 0.95**	41.7 ± 2.21**	37.6 ± 3.79**

* p<0.05, ** p<0.01, calculated by study authors; blank cells indicate dose group was not part of the study

[a] Mean ± standard deviation; for NTP (2019a), standard error was converted to standard deviation by OEHHA

Table 22. Red blood cell counts (10^6/µL) in Sprague Dawley rats exposed to PFHxA

Reference			Dose (mg/kg-day)												
Males	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	8.89 ± 0.320[a]			8.84 ± 0.281				8.88 ± 0.690				8.17 ± 0.593**			
Loveless et al. (2009)	8.89 ± 0.36					8.95 ± 0.34				8.46 ± 0.41				6.09 ± 1.27*	
Klaunig et al. (2015) (week 51)	9.20 ± 0.17	8.80 ± 1.52			8.66 ± 0.92					8.80 ± 1.00					
Klaunig et al. (2015) (week 104)	7.55 ± 1.33	7.02 ± 1.05			7.46 ± 0.92					6.93 ± 1.00					
NTP (2019a)	8.69 ± 0.25								8.28 ± 0.35*		8.24 ± 0.36**		7.91 ± 0.32**	6.68 ± 0.70**	4.56 ± 1.01**
Females	**0**	**2.5**	**5**	**10**	**15**	**20**	**30**	**50**	**62.6**	**100**	**125**	**200**	**250**	**500**	**1,000**
Chengelis et al. (2009b)	8.62 ± 0.338			8.53 ± 0.696				8.32 ± 0.491				7.03 ± 0.430*			
Loveless et al. (2009)	8.34 ± 0.43					8.53 ± 0.52				8.32 ± 0.27				6.85 ± 0.63*	
Klaunig et al. (2015) (week 51)	8.14 ± 0.52		8.23 ± 0.58				8.12 ± 0.37					7.48 ± 0.68*			
Klaunig et al. (2015) (week 104)	6.80 ± 0.76		6.71 ± 0.89				6.6 9± 1.22					6.86 ± 0.96			
NTP (2019a)	7.76 ± 0.28								7.61 ± 0.22		7.78 ± 0.35		7.10 ± 0.28**	6.88 ± 0.47**	5.64 ± 0.57**

* p<0.05, ** p<0.01, calculated by study authors; blank cells indicate dose group was not part of the study

[a] Mean ± standard deviation; for NTP (2019a), standard error was converted to standard deviation by OEHHA

Immune System

Weatherly et al. (2023) evaluated immune system phenotypic changes in the draining lymph node, skin, and spleen of female mice dermally exposed to PFHxA in acetone for 28 days. Statistically significant phenotypic changes are presented in Table 23. The multitude of phenotypic changes in various immune cell types in the draining lymph node, skin, and spleen suggest that PFHxA impacts the immune system. Additionally, hepatic gene expression of the pro-inflammatory cytokines interleukin-6 (IL-6*)* and tumor necrosis factor alpha (TNFα) were upregulated in response to PFHxA exposure (Jiang et al., 2021). However, there were no studies that examined PFHxA-induced deficits in functional immune responses, which is a significant data gap considering that structurally similar PFAS (e.g., PFOA and PFOS) are immunotoxic in humans and laboratory animals (NTP, 2016a; OEHHA, 2021b).

Table 23. Phenotypic changes in immune cells in female mice from dermal exposure to PFHxA for 28 days, from Weatherly et al. (2023)

Cell Type	Draining Lymph Node				Skin				Spleen			
	0%	1.25%	2.5%	5%	0%	1.25%	2.5%	5%	0%	1.25%	2.5%	5%
Total cellularity[b]	13.1 ± 1.52[a]	15.0 ± 1.92	20.3 ± 1.81*	21.7 ± 1.26**	0.81 ± 0.09	0.83 ± 0.09	0.91 ± 0.05	0.99 ± 0.11	1.01 ± 0.13	1.03 ± 0.16	1.12 ± 0.19	0.99 ± 0.67
CD45+ ($\times 10^4$)					5.93 ± 0.78	7.21 ± 1.13	9.82 ± 0.79	11.5 ± 2.03*				
CD45+ (%)					7.34 ± 0.67	8.51 ± 0.85	10.9 ± 1.00*	11.2 ± 1.00*				
CD4+[c]	5.41 ± 0.61	5.93 ± 0.59	8.16 ± 0.58*	8.99 ± 0.49**	1.63 ± 0.21	2.40 ± 0.36	5.01 ± 0.60**	6.11 ± 1.17***	0.88 ± 0.12	0.92 ± 0.15	0.99 ± 0.17	0.86 ± 0.06
CD4+ (%)	41.3 ± 1.08	40.2 ± 1.70	40.6 ± 1.03	41.6 ± 0.62	2.75 ± 0.11	3.34 ± 0.09	5.05 ± 0.25***	5.29 ± 0.32***	20.3 ± 0.42	19.9 ± 0.28	20.5 ± 0.54	20.0 ± 0.41
CD8+[d]	37.3 ± 4.13	42.5 ± 4.15	56.8 ± 5.72*	58.4 ± 4.46*	0.66 ± 0.16	3.38 ± 2.57	1.91 ± 0.23*	2.15 ± 0.46**	1.28 ± 0.13	1.21 ± 0.18	1.33 ± 0.24	1.16 ± 0.05
B-cells (%)	18.1 ± 1.00	18.1 ± 2.12	15.9 ± 0.38	17.4 ± 0.94					42.4 ± 1.01	44.8 ± 0.62	42.8 ± 0.74	43.7 ± 0.91
NK[e]	8.45 ± 1.04	11.6 ± 0.64	19.5 ± 2.26**	17.7 ± 2.11**	3.72 ± 0.47	4.76 ± 0.96	6.45 ± 0.60	9.94 ± 2.21**	2.63 ± 0.30	2.65 ± 0.40	3.58 ± 0.42	3.20 ± 0.26
NK (%)	0.65 ± 0.06	0.82 ± 0.08	0.95 ± 0.03*	0.80 ± 0.06	0.63 ± 0.01	0.66 ± 0.07	0.66 ± 0.04	0.85 ± 0.09*	2.61 ± 0.06	2.62 ± 0.20	3.35 ± 0.25*	3.27 ± 0.20
Eosinophils[f]	75.1 ± 13.2	96.7 ± 17.6	140.0 ± 17.3	142.0 ± 22.9	1.87 ± 0.30	2.77 ± 0.56	7.42 ± 0.74**	9.87 ± 1.78***	8.07 ± 0.85	7.24 ± 1.73	9.15 ± 0.09	8.16 ± 1.40
Eosinophils (%)[g]	5.70 ± 0.62	6.40 ± 0.64	6.94 ± 0.71	6.40 ± 0.84	3.09 ± 0.13	3.82 ± 0.34	7.59 ± 0.65***	8.60 ± 0.38***	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Neutrophils[h]	20.6 ± 3.18	18.8 ± 5.25	21.5 ± 1.28	16.7 ± 2.99	0.85 ± 0.14	1.18 ± 0.22	4.94 ± 1.24	13.0 ± 3.65**	9.44 ± 1.20	8.48 ± 1.60	9.25 ± 1.33	7.46 ± 0.50
Neutrophils (%)[i]	1.59 ± 0.22	1.19 ± 0.23	1.08 ± 0.07	0.76 ± 0.12*	0.14 ± 0.01	0.17 ± 0.02	0.51 ± 0.13	1.13 ± 0.23***	0.93 ± 0.01	0.81 ± 0.05	0.85 ± 0.05	0.76 ± 0.04
Dendritic cells[j]	13.3 ± 1.43	29.0 ± 2.99	47.0 ± 5.39***	35.4 ± 5.48**	5.22 ± 0.67	4.99 ± 0.68	9.09 ± 0.72	10.1 ± 1.77*	1.23 ± 0.22	1.41 ± 0.17	1.30 ± 0.18	1.25 ± 0.12
Dendritic cells (%)	1.04 ± 0.12	2.01 ± 0.22**	2.32 ± 0.14***	1.60 ± 0.19	9.15 ± 1.15	7.09 ± 0.33	9.30 ± 0.47	8.88 ± 0.39	1.19 ± 0.11	1.41 ± 0.10	1.19 ± 0.07	1.26 ± 0.05
CD11b+[k]	11.1 ± 2.61	16.2 ± 3.71	25.3 ± 2.29*	26.0 ± 3.54*					1.51 ± 0.21	1.38 ± 0.23	1.48 ± 0.24	1.32 ± 0.13

Cell Type	Draining Lymph Node				Skin				Spleen			
	0%	1.25%	2.5%	5%	0%	1.25%	2.5%	5%	0%	1.25%	2.5%	5%
CD11b+ (%)	0.82 ± 0.09	1.04 ± 0.12	1.25 ± 0.04*	1.18 ± 0.11					1.49 ± 0.09	1.34 ± 0.05	1.34 ± 0.03	1.33 ± 0.06
CD11b- F4/80+ DC (%)					1.24 ± 0.28	0.87 ± 0.14	0.67 ± 0.05	0.39 ± 0.03**				
CD11b+ F4/80+ DC ($\times 10^2$)					19.2 ± 2.86	22.3 ± 3.03	35.8 ± 2.66	48.3 ± 7.74**				
CD11b- F4/80- DC (%)					3.05 ± 0.33	2.05 ± 0.05**	2.89 ± 0.19	2.51 ± 0.14				
MHCII B-cells[l]	5.84 ± 0.61	7.07 ± 0.37	7.16 ± 0.11	8.02 ± 0.64*					461.6 ± 14.8	461.6 ± 13.0	496.6 ± 12.7	460.0 ± 21.1
MHCII DC[l]	45.3 ± 3.36	57.3 ± 2.79	63.5 ± 5.71*	46.3 ± 2.97					1,285 ± 11.8	1,241 ± 11.3	1,242 ± 10.5	1,257 ± 21.7
CD86 B-cells (MFI)	80.6 ± 5.92	98.7 ± 6.64	93.5 ± 1.71	101.5 ± 3.64					76.3 ± 0.66	78.6 ± 1.42	89.7 ± 1.93***	86.6 ± 1.56**

DC, dendritic cells; DLN, draining lymph node; MFI, mean fluorescence intensity; MHCII, major histocompatibility complex class II; NK, natural killer

[a] Data presented as mean ± standard error

[b] Values × 10^6 for DLN and skin, and × 10^8 for spleen

[c] Values × 10^6 for DLN, × 10^3 for skin, and ×10^7 for spleen

[d] Values × 10^5 for DLN, × 10^2 for skin, and × 10^7 for spleen

[e] Values × 10^4 for DLN, × 10^2 for skin, and × 10^7 for spleen

[f] Values × 10^2 for DLN and spleen, and × 10^3 for skin

[g] Values × 10^{-2} for DLN and spleen, and × 1 for skin

[h] Values × 10^2 for DLN and skin, and × 10^5 for spleen

[I] Values × 10^{-2} for DLN, and × 1 for skin and spleen

[j] Values × 10^4 for DLN, × 10^3 for skin, and × 10^6 for spleen

[k] Values × 10^4 for DLN, and × 10^6 for spleen

[l] Values are MFI × 10^2 for DLN, and MFI × 1 for spleen

* p<0.05, ** p<0.01, ***p<0.001

Cancer

No significant increases in neoplasms in male and female rats were reported in the single chronic bioassay of PFHxA (Klaunig et al., 2015). Additionally, no increases in micronucleated reticulocytes were observed in the peripheral blood of male and female rats exposed to PFHxA for 28 days (NTP, 2019a). In vitro genotoxicity assays, including bacterial mutagenicity assays and a chromosome aberration assay in human peripheral blood lymphocytes, were negative (Loveless et al., 2009; NTP, 2019a).

EVALUATION OF CANDIDATE CRITICAL ENDPOINTS AND CALCULATION OF HEALTH-PROTECTIVE CONCENTRATIONS

Analysis of Candidate Critical Endpoints

OEHHA develops health-protective concentrations (HPCs) that are not anticipated to cause or contribute to adverse health effects from exposure over a lifetime. For noncancer effects, HPC derivation starts with the point of departure (POD) derived from the most sensitive animal or human study of sufficient quality, i.e., the study that observed health effects at the lowest dose. This dose is converted to an acceptable daily dose (ADD), which is then back-calculated to the HPC in tap water. The single chronic study of PFHxA (Klaunig et al., 2015) reported no increases of neoplasms related to treatment. Thus, 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. The candidate critical endpoints for HPC derivation included effects on the liver, developmental/reproductive system, respiratory tract, and thyroid following oral exposure to PFHxA. OEHHA determines the POD from toxicity studies by fitting a dose-response model to the data using the US EPA Benchmark Dose Software[3] (BMDS version 3.2) when possible. 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 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). However, a BMR of 5% relative deviation (RD) was used for decreased pup body weight from gestational PFHxA exposure due to the occurrence of this effect during a sensitive life stage (US EPA, 2023). BMD analysis was performed on selected data sets and the results are summarized in Table 24. Models were selected based on criteria outlined in Davis et al. (2011), namely goodness-of-fit, magnitude of scaled residuals, model parsimony, Akaike information criterion (AIC) values, and visual inspection of the curve. For data sets that did not produce models meeting the selection criteria, OEHHA determined a no-observed-adverse-effect level (NOAEL) or LOAEL to use as the POD.

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

The list of candidate PODs for HPC derivation is presented in Table 24.

Table 24. Points of departure (PODs) for PFHxA candidate critical endpoints

Study Sex/Species/ (N) Duration	Dose (mg/kg-day)	Critical Effect	Critical Effect Value	NOAEL or LOAEL (mg/kg-day)	BMR	Goodness of fit p-value	BMD/BMDL (mg/kg-day) Model
Chengelis et al. (2009b) Male rats (10) 90 days	0 10 50 200	↑ relative liver weight (g/100 g)	2.723 ± 0.1860 2.748 ± 0.1567 2.750 ± 0.2236 3.334 ± 0.1841**	NOAEL: 50	1 SD	0.96	129.4/61.6 Polynomial 3
Chengelis et al. (2009b) Male rats (10) 90 days	0 10 50 200	↑ alkaline phosphatase (U/L)	95 ± 19.8 93 ± 15.0 109 ± 23.0 127 ± 31.0*	NOAEL: 50	1 SD	0.61	134.2/91.1 Polynomial 2
Chengelis et al. (2009b) Male rats (10) 90 days	0 10 50 200	↑ alanine aminotrans-ferase (U/L)	41 ± 4.6 46 ± 4.9 50 ± 18.3 138 ± 143.1*	NOAEL: 50	1 SD	0.70	16.5/9.46 Polynomial 3
Chengelis et al. (2009b) Male rats (10) 90 days	0 10 50 200	↑ hepatocellular hypertrophy	0/10 0/10 0/10 7/10**	NOAEL: 50	5% ER	0.85	43.8/16.6 Multistage 2
Loveless et al. (2009) Male rats (10) 90 days	0 20 100 500	↑ hepatocellular hypertrophy	0/10 0/10 4/10 10/10**	NOAEL: 100	5% ER	0.99	61.5/23.3 Probit
Loveless et al. (2009) Male rats (10) 90 days	0 20 100 500	↑ alanine aminotrans-ferase (U/L)	27 ± 5 63 ± 64* 39 ±12* 42 ± 9	LOAEL: 20	NA	NA	NA (poor model fit)
Loveless et al. (2009) Male rats (10) 90 days	0 20 100 500	↑ aspartate aminotrans-ferase (U/L)	69 ± 12 120 ± 94 86 ± 17* 96 ± 26*	NOAEL: 20	NA	NA	NA (poor model fit)
Loveless et al. (2009) Male rats (10) 90 days	0 20 100 500	↑ nasal cavity olfactory degeneration/ atrophy	0/10 0/10 4/10 7/10*	NOAEL: 100	5% ER	0.57	17.9/11.0 Gamma[a]
Loveless et al. (2009) Female rats (10) 90 days	0 20 100 500	↑ nasal cavity olfactory degeneration/ atrophy	0/10 0/10 5/11* 4/10	NOAEL: 20	5% ER	0.96	41.4/11.0 Dichotomous Hill
Loveless et al. (2009) Female rats (10) 90 days	0 20 100 500	↑ nasal cavity respiratory metaplasia	0/10 0/10 1/11 7/10*	NOAEL: 100	5% ER	0.75	27.1/15.6 Multistage 1
Loveless et al. (2009)	0 20 100	↓ pup body weight on PND 0[b] (g)	7.1 ± 0.9 6.8 ± 0.6 6.3 ± 0.4	NOAEL: 100	5% RD	0.72	20.4/10.6 Hill

Study Sex/Species/ (N) Duration	Dose (mg/kg-day)	Critical Effect	Critical Effect Value	NOAEL or LOAEL (mg/kg-day)	BMR	Good-ness of fit p-value	BMD/BMDL (mg/kg-day) Model
Pregnant female rats (10) 126 days	500		5.8 ± 0.4*				
Iwai and Hoberman (2014) Mouse pups (11-19 litters) GD 6-18	0 100 350 500	↓ pup body weight per litter on PND 0 (g)	1.6 ± 0.2 1.5 ± 0.1 1.4 ± 0.2** 1.4 ± 0.2**	NOAEL: 100	5% RD	NA	NA (poor model fit, failed variance test)
Iwai and Hoberman (2014) Mouse pups (16-20 litters) GD 6-18	0 7 35 175	↓ pup body weight per litter on PND 0 (g)	1.6 ± 0.1 1.6 ± 0.1 1.6 ± 0.1 1.4 ± 0.2**	NOAEL: 35	5% RD	0.99	128.8/59.8 Polynomial 3
Iwai and Hoberman (2014) Mouse pups (11-19 litters) GD 6-18	0 100 350 500	↓ pup body weight per litter on PND 4 (g)	3.0 ± 0.4 2.8 ± 0.2 2.2 ± 0.6** 2.4 ± 0.5**	NOAEL: 100	5% RD	NA	NA (poor model fit, failed variance test)
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	↑ relative liver weight (mg/g body weight)	34.44 ± 1.30[b] 37.26 ± 8.29 36.70 ± 1.58 39.35 ± 0.92** 45.33 ± 2.69** 56.35 ± 3.38**	NOAEL: 125	NA	NA	NA (poor model fit)
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	Nasal olfactory epithelium degeneration	0/10 0/10 1/10 6/10** 6/10** 6/10**	NOAEL: 125	5% ER	0.99	115.0/60.9 Dichotomous Hill
NTP (2019a) Female rats (10) 28 days	0 62.6 125 250 500 1,000	Nasal olfactory epithelium degeneration	0/10 1/10 3/10 9/10** 9/10** 6/10**	NOAEL: 125	5% ER	0.41	48.8/19.5 Log-Logistic (highest dose dropped)
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	Nasal olfactory epithelium hyperplasia	0/10 0/10 0/10 6/10** 5/10* 6/10**	NOAEL: 125	5% ER	0.98	156.1/101.8 Dichotomous Hill

Study Sex/Species/ (N) Duration	Dose (mg/kg-day)	Critical Effect	Critical Effect Value	NOAEL or LOAEL (mg/kg-day)	BMR	Goodness of fit p-value	BMD/BMDL (mg/kg-day) Model
NTP (2019a) Female rats (10) 28 days	0 62.6 125 250 500 1,000	Nasal olfactory epithelium hyperplasia	0/10 0/10 3/10 4/10* 7/10** 3/10	NOAEL: 125	5% ER	0.66	53.4/13.4 Log-Logistic (high dose dropped)
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	↓ free T4 (ng/dL)	2.88 ± 0.28[c] 2.16 ± 0.54** 1.78 ± 0.36** 1.74 ± 0.28** 1.30 ± 0.22** 0.77 ± 0.32**	LOAEL: 62.6	NA	NA	NA (poor model fit)
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	↓ total T4 (µg/dL)	4.26 ± 0.47[c] 3.40 ± 0.73** 2.93 ± 0.51** 2.90 ± 0.54* 2.37 ± 0.32** 1.77 ± 0.54**	LOAEL: 62.6	1 SD	0.12	46.4/25.8 Hill
NTP (2019a) Male rats (10) 28 days	0 62.6 125 250 500 1,000	↓ T3 (ng/dL)	84.17 ± 16.6[c] 68.88 ± 11.89* 62.16 ± 10.53** 71.59 ± 12.33* 71.03 ± 12.93* 60.07 ± 14.99**	LOAEL: 62.6	NA	NA	NA (poor model fit)
Jiang et al. (2021) Male mice (10) 2 months	0 50 200	↑ hepato-somatic index (%)	4.36 ± 0.35 4.59 ± 0.35* 7.11 ± 0.50***	LOAEL: 50	1 SD	No p-value given	52.8/39.8 Hill

* p<0.05, ** p<0.01, ***p<0.001

[a] Multiple models produced the same result

[b] Only data for PND 0 are presented here because it was the most sensitive timepoint

[c] Reported standard error was converted to standard deviation by OEHHA

BMR, benchmark response; ER, extra risk; GD, gestation day; LOAEL, lowest-observed-adverse-effect level; NA, data not amenable for Benchmark dose modeling (i.e., poor model fit); NOAEL, no observed-adverse-effect level; PND, postnatal day; RD, relative deviation; SD, standard deviation; T3, triiodothyronine; T4, thyroxine

Of the candidate critical endpoints presented in Table 24, increased alanine aminotransferase (ALT) in male rats from Chengelis et al. (2009b) generated the lowest BMDL of 9.46 mg/kg-day. Reduced pup body weight on PND 0 and nasal cavity degeneration/atrophy in male and female rats from Loveless et al. (2009) generated comparable BMDLs of 10.6 and 11.0 mg/kg-day, respectively. Nasal olfactory epithelium hyperplasia in female rats also generated a comparable BMDL of 13.4 mg/kg-day (NTP, 2019a). Decreased total T4 in male rats produced a BMDL of 25.8 mg/kg-day. Although the POD for this endpoint is higher than for some of the other endpoints, reduction in thyroid hormones is considered for HPC derivation because thyroid hormone homeostasis is critical for proper development, and disruption during

critical windows, such as pregnancy and early childhood, can lead to severe adverse effects.

Hepatocellular hypertrophy in male rats, as reported by Chengelis et al. (2009b), produced a BMDL of 16.6 mg/kg-day. Hepatocellular hypertrophy in rodents was observed in other studies (Loveless et al., 2009; NTP, 2019a; Weatherly et al., 2023), but was not observed to an appreciable extent in the two-year chronic studies by Klaunig et al. (2015). Strain differences do not explain why hepatocellular hypertrophy was not observed in the chronic study, as Sprague Dawley rats were used in both the Chengelis et al. (2009b) and NTP (2019a) studies as well as the Klaunig et al. (2015) study. The highest dose in the Klaunig et al. (2015) study was 100 mg/kg-day, which was around the mid or low dose for some of the other studies (Loveless et al., 2009; NTP, 2019a), so it is possible that the high dose was too low to induce these effects. Nonetheless, there is substantial evidence from other studies that indicates the liver is a major target of PFHxA.

Calculation of Health-Protective Concentrations

Calculation of an HPC starts with derivation of an acceptable daily dose (ADD), which is the estimated maximum daily dose of a chemical (in mg/kg-day) that can be consumed by a human 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 ($\sqrt{10}$ for toxicokinetics and $\sqrt{10}$ for toxicodynamics) and 30 for intraspecies variability (10 for toxicokinetics and $\sqrt{10}$ for toxicodynamics) (OEHHA, 2008). A detailed description of these factors is presented in Appendix 4.

When calculating the ADD for PFHxA, OEHHA applied an interspecies UF of $\sqrt{10}$ to account for potential toxicodynamic differences when extrapolating data from animal studies to humans. Because PFHxA is not known to be metabolized in animals or humans (Gannon et al., 2011), and because a toxicokinetic adjustment is being applied to the animal data to derive a human equivalent dose, the toxicokinetic component of the interspecies UF is reduced from $\sqrt{10}$ to 1 and the toxicokinetic component of the intraspecies UF was reduced from 10 to $\sqrt{10}$, leaving a factor of 30 to account for potential differences in toxicodynamics between animals and humans, and for variability between humans.

A subchronic-to-chronic UF of √10 was applied to the nasal, liver, and thyroid endpoints because it is uncertain whether increased durations would exacerbate toxicity. A subchronic-to-chronic UF of 1 was applied for reduced pup body weight because exposure occurred during gestation, which is a critical window of susceptibility, and a longer exposure duration would not increase the severity of toxicity. A database deficiency UF of √10 was included due to the lack of functional immunotoxicity studies, and the absence of a 2-generation toxicity study.

As described in the *Toxicokinetics* section above, a human clearance factor of 23.6 ml/day-kg (9.83×10^{-4} L/kg-hr) was derived by Zhou et al. (2014). To account for interspecies differences in clearance, a dosimetric adjustment factor (DAF) is calculated by taking the ratio of the two clearance values (CL_{human}/CL_{animal}). A summary of animal clearance factors is presented in Table 3. For reduced pup body weight, a female rat clearance value of 0.164 L/kg-hr was selected. For the other endpoints, a male rat clearance value of 0.103 L/kg-hr was selected. These values were derived from a single oral dose of 40 mg/kg-day in each respective sex (Dzierlenga et al., 2020), and were chosen because the route of administration was the same as in the candidate critical studies, and the administered dose of 40 mg/kg-day was closest to the calculated BMDL values. Using these clearance factors, DAFs of 0.006 for females and 0.010 for males were determined.

The acceptable daily dose (ADD) is calculated using the following equation:

$$ADD = (POD \times DAF) \div UF,$$

where POD is the point of departure, DAF is the dosimetric adjustment factor, and UF is the composite uncertainty factor.

Loveless et al. (2009) administered PFHxA as the sodium salt (NaPFHxA), so the ADDs derived from this study need to be adjusted to account for weight differences between the salt and the free acid. This is done by taking the ratio of their molecular weights

$$MW_{free}/MW_{Na} = 314/336 = 0.935,$$

where MW_{free} is the molecular weight of the free acid, and MW_{Na} is the molecular weight of the sodium salt. Since at the physiological pH, both the free acid PFHxA and its sodium salt are expected to exist in the similar ionized form, no difference in toxicity would be expected between the two on molar bases.

ADD calculations are summarized in Table 25.

Table 25. Acceptable daily doses and uncertainty factors for candidate critical endpoints

Endpoint/Reference	POD (mg/kg-day)	UF_A	UF_H	UF_S	UF_D	DAF	MW adjust-ment	ADD (mg/kg-day)
Increased ALT in male rats (Chengelis et al., 2009b)	9.46	√10	10	√10	√10	0.010	NA	0.0003
Hepatocellular hyper-trophy in male rats Chengelis et al., 2009b)	16.6	√10	10	√10	√10	0.010	NA	0.0006
Reduced pup body weight in rats (both sexes) (Loveless et al., 2009)	10.6	√10	10	1	√10	0.006	0.935	0.0006
Nasal cavity degen-eration/atrophy in rats[a] (Loveless et al., 2009)	11.0	√10	10	√10	√10	0.006	0.935	0.0002
Hepatocellular hyper-trophy in male rats (Loveless et al., 2009)	23.3	√10	10	√10	√10	0.010	0.935	0.0007
Nasal olfactory epithelium degeneration in female rats (NTP, 2019a)	19.5	√10	10	√10	√10	0.006	NA	0.0004
Reduced total T4 in male rats (NTP, 2019a)	25.8	√10	10	√10	√10	0.010	NA	0.0009

[a] POD is the same for male and female rats.
ADD, acceptable daily dose; ALT, alanine aminotransferase; DAF, dosimetric adjustment factor; MW, molecular weight; NA, not applicable; POD, point of departure; UF_A, interspecies extrapolation uncertainty factor; UF_D, database deficiency uncertainty factor; UF_H, intraspecies variability uncertainty factor; UF_S, subchronic-to-chronic extrapolation uncertainty factor

Relative Source Contribution

In estimating HPCs of chemicals in drinking water for noncancer endpoints, OEHHA considers the relative source contribution (RSC), which is the proportion of chemical exposures attributed to 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). This approach takes into account the availability of exposure data, including the levels and relevant sources of exposure, and any other non-water regulatory standards for the chemical. A chemical-specific RSC value can be calculated when adequate data are available for all sources of exposure, including exposures from drinking water. If data are not adequate for determining the contribution of one or more exposure sources, then default values may be used (US EPA, 2000). The default estimates include a low

estimate of 20% and a high estimate of 80%, used when data are not otherwise available to better characterize sources of exposure.

PFHxA is a ubiquitous environmental contaminant with multiple potential exposure sources. Several studies have examined exposure pathways of PFHxA and found that diet and indoor dust can account for 42-91% of the total exposure (De Silva et al., 2021). Interestingly, these studies also estimated that inhalation exposure from air accounted for 2-8% of the total exposure, (Gebbink et al., 2015; Poothong et al., 2020; De Silva et al., 2021). Thus, drinking water does not appear to be the primary source of PFHxA exposure. Due to insufficient human data to assess specific PFHxA exposure pathways from all sources other than tap water, a default RSC of 20% was selected, consistent with the US EPA (2000) guidance.

Drinking Water Intake

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.

PFHxA is not expected to volatilize from water due to its pKA of -0.16. At this pKA, PFHxA will be entirely in the anion form at pH 5-9 (PubChem, 2022). Thus, inhalation expsosure from typical uses of tap water is not anticipated. Dermal absorption was shown to occur in mice exposed to PFHxA in acetone over 28 days (Weatherly et al., 2023). However, there were no data about the rate of absorption, or how much was absorbed during each exposure period. There was also potential for oral exposure from grooming, as animals were caged together for the duration of the experiment. Thus, it is difficult to ascertain the true extent of dermal absorption of PFHxA from this experiment. Dermal toxicity studies with similar chemicals (PFHpA and PFOA) indicate that absorption can occur with prolonged exposure durations (Franko et al., 2012; Han et al., 2020). However, it is unknown if typical uses of tap water (bathing, showering) would lead to similar exposure durations that demonstrated substantial absorption. Furthermore, it is unknown whether PFHxA would be significantly absorbed following routine uses of tap water, particularly since animals in the Weatherly et al. (2023) study were exposed to PFHxA in acetone, and not water. As such, OEHHA anticipates dermal absoprtion to be minimal compared to the oral route.

Ingestion is the primary route of exposure to PFHxA in drinking water. 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 from gestational exposure, the drinking water intake (DWI) is that of the pregnant woman, 0.047 L/kg-

day. For general, non-developmental toxicity endpoints reflecting lifetime exposure, the DWI is the lifetime weighted average over different life stages and equals 0.053 L/kg-day. Because infants have been identified as a sensitive group for the effects of decreased 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.

The health-protective concentration (HPC) is calculated as follows:

HPC = ADD × RSC ÷ DWI, where:

ADD = acceptable daily dose,
RSC = relative source contribution of 0.2, and
DWI = daily water intake rate.

Table 26. Health-protective concentrations for the candidate critical endpoints.

Endpoint	ADD (mg/kg-day)	RSC	DWI (L/kg-day)	HPC (mg/L)
Increased ALT in male rats (Chengelis et al., 2009b)	0.0003	0.2	0.053	0.0011
Hepatocellular hypertrophy in male rats (Chengelis et al., 2009b)	0.0006	0.2	0.053	0.0022
Reduced pup body weight in rats (Loveless et al., 2009)	0.0006	0.2	0.047	0.0026
Nasal cavity degeneration/ atrophy in female rats (Loveless et al., 2009)	0.0002	0.2	0.053	0.0008
Hepatocellular hypertrophy in male rats (Loveless et al., 2009)	0.0007	0.2	0.053	0.0026
Nasal olfactory epithelium degeneration in female rats (NTP, 2019a)	0.0004	0.2	0.053	0.0015
Reduced total T4 in male rats (NTP, 2019a)	0.0009	0.2	0.237	0.0008

ALT, alanine aminotransferase

The lowest HPCs (Table 26) were for reduced total T4 in male rats (NTP, 2019a) and nasal cavity degeneration/atrophy in female rats (Loveless et al., 2009), both with a value of 0.0008 mg/L (or 0.8 µg/L, equivalent to 0.8 parts per billion (ppb)). This value is comparable to the HPCs calculated for increased ALT in male rats (from Chengelis et al., 2009b) and nasal olfactory epithelium degeneration in female rats (NTP, 2019a), and should be health-protective for all other adverse effects. Thus, OEHHA recommends that the Water Board set the NL for PFHxA in drinking water at 1 ppb (rounded).

REFERENCES

Anderson JK, Luz AL, Goodrum P and Durda J (2019). Perfluorohexanoic acid toxicity, part II: Application of human health toxicity value for risk characterization. *Regul Toxicol Pharmacol* 103: 10-20.

Appel M, Forsthuber M, Ramos R, et al. (2022). The transplacental transfer efficiency of per- and polyfluoroalkyl substances (PFAS): a first meta-analysis. *J Toxicol Environ Health B Crit Rev* 25(1): 23-42.

ATSDR (2021). Toxicological Profile for Perfluoroalkyls. Atlanta, GA, Agency for Toxic Substances and Disease Registry.

Bartels JL, Fernandez SR, Aweda TA, et al. (2020). Comparative Uptake and Biological Distribution of [18F]-Labeled C6 and C8 Perfluorinated Alkyl Substances in Pregnant Mice via Different Routes of Administration. *Environ Sci Technol Lett* 7(9): 665-671.

Borg D and Ivarsson J (2017). Analysis of PFASs and TOF in Products. N. C. o. Ministers.

Bradman A, Gaspar F, Castorina R, Tong-Lin E, McKone T and Maddalena R (2012). Environmental Exposures in Early Childhood Education Environments, California Air Resources Board, California Environmental Protection Agency.

Cai D, Li QQ, Chu C, et al. (2020). High trans-placental transfer of perfluoroalkyl substances alternatives in the matched maternal-cord blood serum: Evidence from a birth cohort study. *Sci Total Environ* 705: 135885.

Calafat AM, Kato K, Hubbard K, Jia T, Botelho JC and Wong LY (2019). Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013-2014 National Health and Nutrition Examination Survey. *Environ Int* 131: 105048.

Chengelis CP, Kirkpatrick JB, Myers NR, Shinohara M, Stetson PL and Sved DW (2009a). Comparison of the toxicokinetic behavior of perfluorohexanoic acid (PFHxA) and nonafluorobutane-1-sulfonic acid (PFBS) in cynomolgus monkeys and rats. *Reprod Toxicol* 27(3-4): 400-406.

Chengelis CP, Kirkpatrick JB, Radovsky A and Shinohara M (2009b). A 90-day repeated dose oral (gavage) toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and motor activity determinations). *Reprod Toxicol* 27(3-4): 342-351.

Damsch S, Eichenbaum G, Tonelli A, et al. (2011). Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). *Toxicol Pathol* 39(2): 348-360.

Davis JA, Gift JS and Zhao QJ (2011). Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. *Toxicol Appl Pharmacol* 254(2): 181-191.

De Silva AO, Armitage JM, Bruton TA, et al. (2021). PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environ Toxicol Chem* 40(3): 631-657.

Dzierlenga AL, Robinson VG, Waidyanatha S, et al. (2020). Toxicokinetics of perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) in male and female Hsd:Sprague dawley SD rats following intravenous or gavage administration. *Xenobiotica* 50(6): 722-732.

EFSA (2012). Perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA Journal* 10(6:2743): 1-55.

EFSA (2020). Risk To Human Health Related To The Presence Of Perfluoroalkyl Substances In Food. *EFSA Journal* 18(9).

Elizalde MP, Gomez-Lavin S and Urtiaga A (2019). Migration of perfluorinated compounds from paperbag to Tenax® and lyophilised milk at different temperatures. *Int J Environ Anal Chem* 98(15): 1423-1433.

Fabrega F, Kumar V, Benfenati E, Schuhmacher M, Domingo JL and Nadal M (2015). Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. *Toxicol. Environ. Chem.* 97(6): 814-827.

Fasano WJ, Kennedy GL, Szostek B, et al. (2005). Penetration of ammonium perfluorooctanoate through rat and human skin in vitro. *Drug Chem Toxicol* 28(1): 79-90.

Franko J, Meade BJ, Frasch HF, Barbero AM and Anderson SE (2012). Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. *J Toxicol Environ Health A* 75(1): 50-62.

Fu Y, Wang T, Fu Q, Wang P and Lu Y (2014). Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. *Ecotoxicol Environ Saf* 106: 246-252.

Gannon SA, Johnson T, Nabb DL, Serex TL, Buck RC and Loveless SE (2011). Absorption, distribution, metabolism, and excretion of 1-^{14}C -perfluorohexanoate (^{14}C -PFHx) in rats and mice. *Toxicology* 283(1): 55-62.

Gao K, Zhuang T, Liu X, et al. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. *Environ Sci Technol* 53(11): 6529-6538.

Gardner DG and Shoback DM (2017). Greenspan's basic and clinical endocrinology, McGraw-Hill Education.

Gebbink WA, Berger U and Cousins IT (2015). Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. *Environ Int* 74: 160-169.

Gilger BC, Brown MH, Munger RJ and Bartoe JT (2018). Spontaneous incidence of ocular abnormalities in laboratory animals. Standards for Ocular Toxicology and Inflammation. B. C. Gilger, C. S. Cook and M. H. Brown. Switzerland, Springer Nature.

Guruge KS, Noguchi M, Yoshioka K, et al. (2016). Microminipigs as a new experimental animal model for toxicological studies: comparative pharmacokinetics of perfluoroalkyl acids. *J. Appl. Toxicol.* 36(1): 68-75.

Haddow JE, Palomaki GE, Allan WC, et al. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med* 341(8): 549-555.

Han JS, Jang S, Son HY, et al. (2020). Subacute dermal toxicity of perfluoroalkyl carboxylic acids: comparison with different carbon-chain lengths in human skin equivalents and systemic effects of perfluoroheptanoic acid in Sprague Dawley rats. *Arch Toxicol* 94(2): 523-539.

Hill AB (1965). The Environment and Disease: Association or Causation? *Proc R Soc Med* 58: 295-300.

Illinois EPA (2021). Health Advisory for Perfluorohexanoic Acid (Pfhxa) Chemical Abstract Services Registry Number (CASRN) 307-24-4. Springfield, IL, Illnois Environmental Protection Agency.

Iwabuchi K, Senzaki N, Mazawa D, et al. (2017). Tissue toxicokinetics of perfluoro compounds with single and chronic low doses in male rats. *J Toxicol Sci* 42(3): 301-317.

Iwai H (2011). Toxicokinetics of ammonium perfluorohexanoate. *Drug Chem Toxicol* 34(4): 341-346.

Iwai H and Hoberman AM (2014). Oral (Gavage) Combined Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Ammonium Salt of Perfluorinated Hexanoic Acid in Mice. *Int J Toxicol* 33(3): 219-237.

Iwai H, Hoberman AM, Goodrum PE, Mendelsohn E and Anderson JK (2019). Addendum to Iwai and Hoberman (2014)-Reassessment of Developmental Toxicity of PFHxA in Mice. *Int J Toxicol* 38(3): 183-191.

Janousek RM, Lebertz S and Knepper TP (2019). Previously unidentified sources of perfluoroalkyl and polyfluoroalkyl substances from building materials and industrial fabrics. *Environ Sci Process Impacts* 21(11): 1936-1945.

Jiang L, Hong Y, Xie G, Zhang J, Zhang H and Cai Z (2021). Comprehensive multi-omics approaches reveal the hepatotoxic mechanism of perfluorohexanoic acid (PFHxA) in mice. *Sci Total Environ* 790: 148160.

Kabadi SV, Fisher J, Aungst J and Rice P (2018). Internal exposure-based pharmacokinetic evaluation of potential for biopersistence of 6:2 fluorotelomer alcohol (FTOH) and its metabolites. *Food Chem Toxicol* 112: 375-382.

Kim DH, Lee MY and Oh JE (2014). Perfluorinated compounds in serum and urine samples from children aged 5-13 years in South Korea. *Environ Pollut* 192: 171-178.

Kirkpatrick JB (2010). A 24-Month Oral (Gavage) Combined Chronic Toxicity/Carcinogenicity Study of Perfluorohexanoic Acid (Pfhxa) in Rats. Ashland, OH, WIL Research Laboratories.

Klaunig JE, Shinohara M, Iwai H, et al. (2015). Evaluation of the chronic toxicity and carcinogenicity of perfluorohexanoic acid (PFHxA) in Sprague-Dawley rats. *Toxicol Pathol* 43(2): 209-220.

Lacourt A, Leffondre K, Gramond C, et al. (2012). Temporal patterns of occupational asbestos exposure and risk of pleural mesothelioma. *Eur Respir J* 39(6): 1304-1312.

Li H, Hammarstrand S, Midberg B, et al. (2022). Cancer incidence in a Swedish cohort with high exposure to perfluoroalkyl substances in drinking water. *Environ Res* 204(Pt C): 112217.

Li Y, Cheng Y, Xie Z and Zeng F (2017). Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. *Sci Rep* 7: 43380.

Lipfert FW and Wyzga RE (2019). Longitudinal relationships between lung cancer mortality rates, smoking, and ambient air quality: a comprehensive review and analysis. *Crit Rev Toxicol* 49(9): 790-818.

Loveless SE, Slezak B, Serex T, et al. (2009). Toxicological evaluation of sodium perfluorohexanoate. *Toxicology* 264(1-2): 32-44.

Luz AL, Anderson JK, Goodrum P and Durda J (2019). Perfluorohexanoic acid toxicity, part I: Development of a chronic human health toxicity value for use in risk assessment. *Regul Toxicol Pharmacol* 103: 41-55.

Marshall G, Ferreccio C, Yuan Y, et al. (2007). Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. *J Natl Cancer Inst* 99(12): 920-928.

MDH (2021). Toxicological Summary for: Perfluorohexanoate. Minnesota, USA, Minnesota Department of Health.

Michigan SAW (2019). Health-Based Drinking Water Value Recommendations For PFAS in Michigan. Lansing, MI, Michigan Science Advisory Workgroup.

Negro R, Soldin OP, Obregon MJ and Stagnaro-Green A (2011). Hypothyroxinemia and pregnancy. *Endocr Pract* 17(3): 422-429.

Nian M, Li QQ, Bloom M, et al. (2019). Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. *Environ Res* 172: 81-88.

Nilsson H, Kaerrman A, Rotander A, van Bavel B, Lindstroem G and Westberg H (2013). Biotransformation of fluorotelomer compound to perfluorocarboxylates in humans. *Environ. Int.* 51: 8-12.

NTP (2016a). Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate. National Toxicology Program. Research Triange Park, NC, National Toxicology Program.

NTP (2016b). NTP Technical Report on the Toxicology Studies of Green Tea Extract in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Green Tea Extract in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies). Research Triangle Park, NC, National Toxicology Program.

NTP (2019a). NTP Technical Report on the Toxicity Studies of Perfluoroalkyl Carboxylates (Perfluorohexanoic Acid, Perfluorooctanoic Acid, Perfluorononanoic Acid, and Perfluorodecanoic Acid) Administered by Gavage to Sprague Dawley (Hsd:Sprague Dawley SD) Rats. Research Triangle Park, NC, National Toxicology Program. **Tox 97**.

NTP (2019b). NTP Technical Report on the Toxicity Studies of Perfluoroalkyl Sulfonates (Perfluorobutane Sulfonic Acid, Perfluorohexane Sulfonate Potassium Salt, and Perfluorooctane Sulfonic Acid) Administered by Gavage to Sprague Dawley (Hsd:Sprague Dawley SD) Rats. Research Triangle Park, NC, National Toxicology Program. **Tox 96**.

NTP (2019c). Risk of Bias Tool. Research Triangle Park, NC, National Toxicology Program.

Numata J, Kowalczyk J, Adolphs J, et al. (2014). Toxicokinetics of seven perfluoroalkyl sulfonic and carboxylic acids in pigs fed a contaminated diet. *J Agric Food Chem* 62(28): 6861-6870.

OEHHA (2008). Air Toxics Hot Spots Risk Assessment Guidelines: Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. Oakland, CA, Office of Environmental Health Hazard Assessment.

OEHHA (2012). Air Toxics Hot Spots Risk Assessment Guidelines: Technical Support Document for Exposure Assessment and Stochastic Analysis. Sacramento, CA, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA (2015). Perchlorate in Drinking Water: Public Health Goal. Sacramento, CA, Office of Environmental Health Hazard Assessment.

OEHHA (2021a). Perfluorobutane Sulfonic Acid in Drinking Water. Sacramento, CA, Office of Environmental Health Hazard Assessment.

OEHHA (2021b). Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water: Public Health Goal (Draft). Sacramento, CA, Office of Environmental Health Hazard Assessment.

OEHHA (2022). Perfluorohexane Sulfonic Acid in Drinking Water. Sacramento, CA, Office of Environmental Health Hazard Assessment.

Perez F, Nadal M, Navarro-Ortega A, et al. (2013). Accumulation of perfluoroalkyl substances in human tissues. *Environ Int* 59: 354-362.

Poothong S, Papadopoulou E, Padilla-Sanchez JA, Thomsen C and Haug LS (2020). Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. *Environ Int* 134: 105244.

Poothong S, Thomsen C, Padilla-Sanchez JA, Papadopoulou E and Haug LS (2017). Distribution of Novel and Well-Known Poly- and Perfluoroalkyl Substances (PFASs) in Human Serum, Plasma, and Whole Blood. *Environ Sci Technol* 51(22): 13388-13396.

PubChem. (2022). "Perfluorohexanoic Acid." Retrieved March 29, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Perfluorohexanoic-acid.

Ramirez Carnero A, Lestido-Cardama A, Vazquez Loureiro P, Barbosa-Pereira L, Rodriguez Bernaldo de Quiros A and Sendon R (2021). Presence of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials (FCM) and Its Migration to Food. *Foods* 10(7).

Russell MH, Himmelstein MW and Buck RC (2015). Inhalation and oral toxicokinetics of 6:2 FTOH and its metabolites in mammals. *Chemosphere* 120: 328-335.

Russell MH, Nilsson H and Buck RC (2013). Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. *Chemosphere* 93(10): 2419-2425.

Schellenberger S, Liagkouridis I, Awad R, et al. (2022). An Outdoor Aging Study to Investigate the Release of Per- And Polyfluoroalkyl Substances (PFAS) from Functional Textiles. *Environ Sci Technol* 56(6): 3471-3479.

Synquest Laboratories. (2022). "Perfluorohexanoic Acid." from https://www.synquestlabs.com/Home/ProductDetail?SearchText=Perfluorohexanoic%20acid.

US EPA (1991). Guidelines for Developmental Toxicity Risk Assessment. Washington DC, United States Environmental Protection Agency.

US EPA (2000). Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. Washington, DC, United States Environmental Protection Agency.

US EPA (2016a). Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). Washington DC, United States Environmental Protection Agency.

US EPA (2016b). Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). Washington DC, United State Environmental Protection Agency.

US EPA (2021). The Fifth Unregulated Contaminant Monitoring Rule (UCRM5) - Program Overview Fact Sheet, United States Environmental Protection Agency, Office of Water.

US EPA (2023). Toxicological Review of Perfluorohexanoic Acid [CASRN 307244] and Related Salts . Washington DC, United States Environmental Protection Agency, Integrated Risk Information System.

Weatherly LM, Shane HL, Lukomska E, Baur R and Anderson SE (2023). Systemic toxicity induced by topical application of perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA) in a murine model. *Food Chem Toxicol* 171: 113515.

Xu Y, Fletcher T, Pineda D, et al. (2020). Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. *Environ Health Perspect* 128(7): 77004.

Yuan G, Peng H, Huang C and Hu J (2016). Ubiquitous Occurrence of Fluorotelomer Alcohols in Eco-Friendly Paper-Made Food-Contact Materials and Their Implication for Human Exposure. *Environ Sci Technol* 50(2): 942-950.

Zeng XW, Qian Z, Emo B, et al. (2015). Association of polyfluoroalkyl chemical exposure with serum lipids in children. *Sci Total Environ* 512-513: 364-370.

Zhou Z, Shi Y, Vestergren R, Wang T, Liang Y and Cai Y (2014). Highly elevated serum concentrations of perfluoroalkyl substances in fishery employees from Tangxun Lake, China. *Environ Sci Technol* 48(7): 3864-3874.

APPENDIX 1. LITERATURE SEARCH STRATEGY

ANIMAL EVIDENCE

The literature search for PFHxA was done on September 15, 2021, using multiple synonyms for the chemical name. Four databases were searched: PubMed, Embase, Scopus and SciFinder-n. Below is the search strategy used in PubMed. This search syntax was translated as appropriate for use in each of the other databases, except SciFinder-n, where only the CAS RN was used as the search term. A total of 1,204 references were identified.

Figure A1.1. PubMed – Search executed

Search Terms
("perfluorohexanoic acid"[Supplementary Concept] OR "Perfluorohexanoic acid"[tiab] OR "PFHxA"[tiab] OR "307-24-4"[rn] OR "Undecafluorohexanoic acid"[tiab] OR "Hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-"[tiab] OR "Hexanoic acid, undecafluoro-"[tiab] OR "2,2,3,3,4,4,5,5,6,6,6-Undecafluorohexanoic acid"[tiab] OR "2,2,3,3,4,4,5,5,6,6,6-undecafluoro-hexanoic acid"[tiab] OR "undecafluoro-1-hexanoic acid"[tiab] OR "Perfluoro-1-pentanecarboxylic acid"[tiab] OR "Undecafluorocaproic acid"[tiab] OR "perfluorocaproic acid"[tiab] OR "Undecafluorohexanoic acid"[tiab] OR "Perfluoro-n-hexanoic acid"[tiab] OR "undecafluorocaproic acid"[tiab] OR "undecafluorohexanoic acid"[tiab] OR "perfluorohexanoate"[tiab] OR (c6 [tiab] AND perfluorinated [tiab]) OR "C6 PFCA"[tiab])

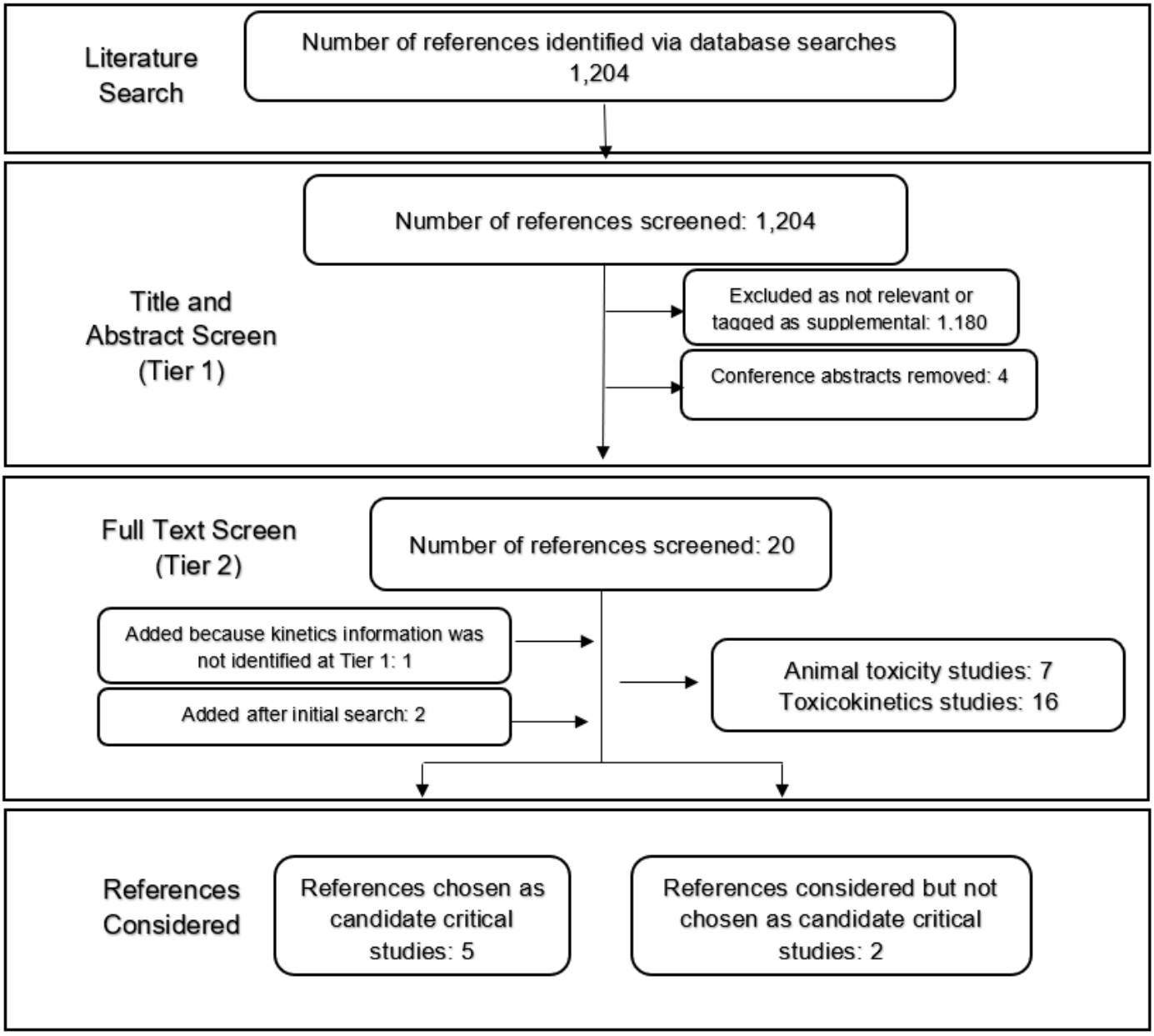
Figure A1.2. PECO statement used for Tier 1 literature screening

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."

PECO element	Evidence
Exposures	Perfluorohexanoic acid (any salt) (CAS 307-24-4) and any synonyms. If uncertain about chemical identity, please look it up. **Human:** Any exposure to PFHxA via any route. **Animal:** Any exposure to PFHxA 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 PFHxA 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) of PFHxA, or exposure to PFHxA 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 PFHxA will be included. Studies describing toxicokinetic data and ADME will also be included.

ADME, absorption, distribution, metabolism, excretion; PBPK, physiologically based pharmacokinetic

Figure A1.3. Flowchart of literature screen (animal toxicity studies)



APPENDIX 2. PFHxA HALF-LIVES

A summary of half-life data is presented in Table A2.1.

Table A2.1. Serum/plasma/whole blood half-life values for PFHxA in multiple species

Species/sex	Exposure	Half-life	Reference
Human (n=7) Male ski wax technicians (blood)	Occupational inhalation exposure to PFAS mixture, including 6:2 FTOH and PFHxA (mean, 5,300 ng/m^3), 30 hours/week, 4 months/year	Geometric mean: 32 days Range: 14-49 days	Russell et al. (2013)
Human, both males and females (n=26, controls=58) Airport employees (serum)	PFAS contaminated drinking water (330 ng/L of PFHxA), Arvidsjaur, Sweden	1.63 years	Xu et al. (2020)
Monkeys (male) (serum)	Single intravenous injection	5.3 hours	Chengelis et al. (2009a)
Monkeys (female) (serum)		2.4 hours	
Pigs (male and female) (plasma)	PFAS contaminated feed for 21 days	4.1 days (geometric mean)	Numata et al. (2014)
Microminipigs (female) (blood)	Single oral dose	2.7 days	Guruge et al. (2016)
Rat (male) (serum)	Single intravenous dose	1.0 hours	Chengelis et al. (2009a)
Rat (female) (serum)		0.42 hours	
Rat (male) (serum)	Repeated oral doses for 25 days	2.2-2.8 hours	Chengelis et al. (2009a)
Rat (female) (serum)		2.3-2.7 hours	
Rat (male) (plasma)	Single oral dose	1.5-1.7 hours	Gannon et al. (2011)
Rat (female) (plasma)		0.5-0.7 hours	
Rat (male) (plasma)	1-day inhalation exposure (6 hours)	1.3 hours	Russell et al. (2015)
Rat (female) (plasma)		0.5 hours	

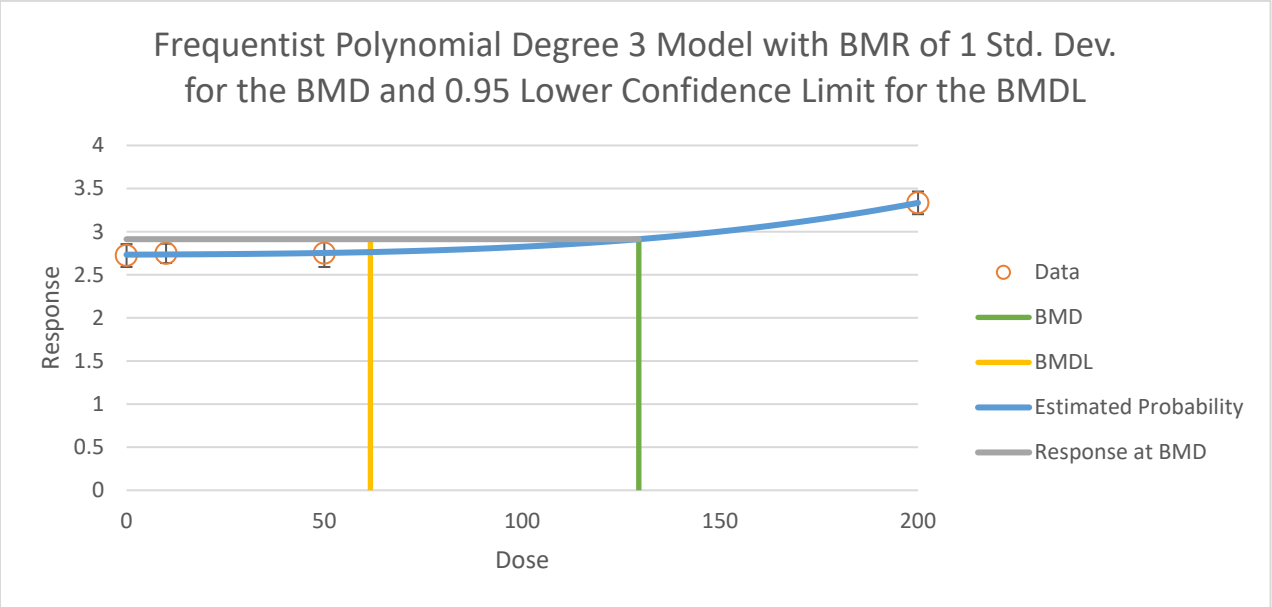
Species/sex	Exposure	Half-life	Reference
Rat (male) (plasma)	23-day inhalation exposure (6 hours/day)	3.8 hours	Russell et al. (2015)
Rat (male) (serum)	Single oral dose	2.6 hours	Iwabuchi et al. (2017)
Rat (male) (whole blood)		2.9 hours	
Rat (male) (plasma)	Single intravenous dose	0.81 hours	Dzierlenga et al. (2020)
Rat (female) (plasma)		0.35 hours	
Rat (male) (plasma)	Single oral dose	1.6-2.9 hours	Dzierlenga et al. (2020)
Rat (female) (plasma)		1.1-1.4 hours	
Mouse (female, pregnant) (serum)	Repeated oral doses for 12 days (GD 6- 18)	0.889-1.24 hours	Iwai and Hoberman (2014)

APPENDIX 3. BENCHMARK DOSE MODELING

This appendix provides the BMD modeling outputs for PFHxA toxicity data that were amenable to dose-response modeling. All models were run with default parameters and a benchmark response of 5% extra risk for dichotomous data or one standard deviation from the control mean for continuous data unless otherwise noted. Model selection criteria when comparing outputs of different models for the same endpoint/dataset were: scaled residual ≤ the absolute value of two, goodness of fit p-value ≥ 0.05,[4] 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, is selected as the POD.

[4] 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.

Figure A3.1 Polynomial 3 output for increased relative liver weight in male rats (Chengelis et al., 2009b)



Benchmark Dose	
BMD	129.4446945
BMDL	61.62213687
BMDU	147.7994586
AIC	-17.85254904
Test 4 P-value	0.959053175
D.O.F.	2

Model Parameters	
# of Parameters	5
Variable	Estimate
g	2.73301194
beta1	0.00022064
beta2	Bounded
beta3	Bounded
alpha	0.032229707

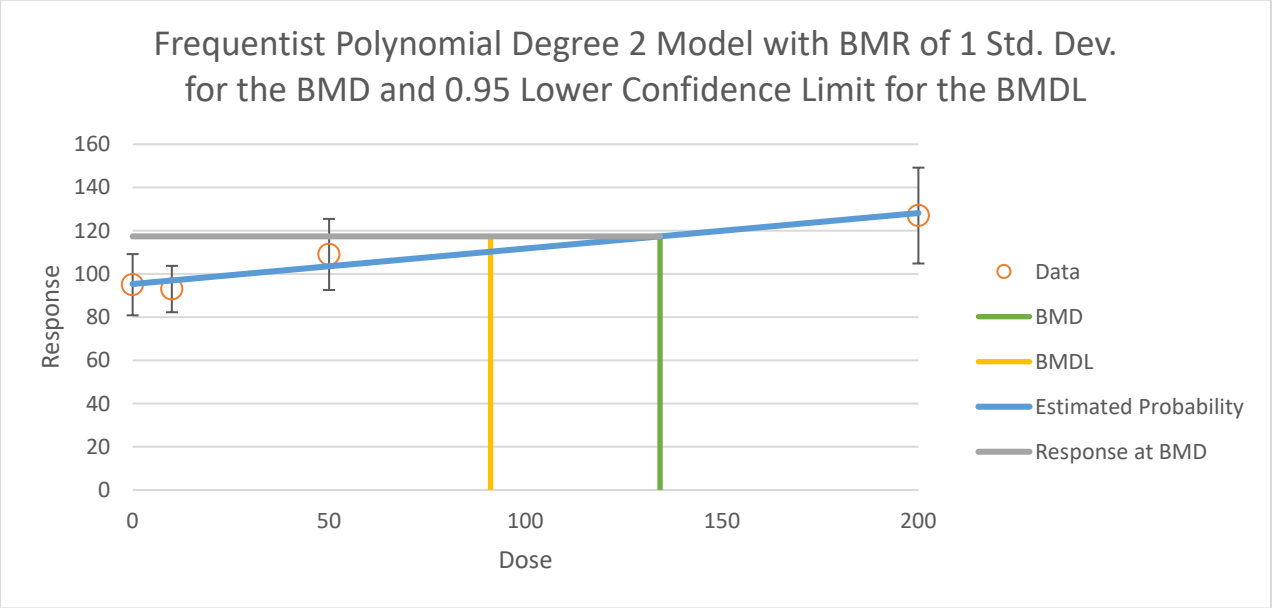
Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	2.73301194	2.723	2.723	0.17952634	0.186	0.186	-0.176355928
10	10	2.735287939	2.748	2.748	0.17952634	0.1567	0.1567	0.223917382
50	10	2.752744261	2.75	2.75	0.17952634	0.2236	0.2236	-0.048338953
200	10	3.333961748	3.334	3.334	0.17952634	0.1841	0.1841	0.000673801

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	11.96808328	5	-13.936167
A2	12.6057036	8	-9.2114072
A3	11.96808328	5	-13.936167
fitted	11.92627452	3	-17.852549
R	-10.37915304	2	24.7583061

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	45.96971328	6	<0.0001
2	1.275240656	3	0.73502065
3	1.275240656	3	0.73502065
4	0.083617516	2	0.95905317

Figure A3.2 Polynomial 2 model output for increased alkaline phosphatase in male rats (Chengelis et al., 2009b)



Benchmark Dose	
BMD	134.236908
BMDL	91.08925124
BMDU	254.20237
AIC	366.9511242
Test 4 P-value	0.612424037
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
g	95.32684814
beta1	0.164202336
beta2	Bounded
alpha	485.8505407

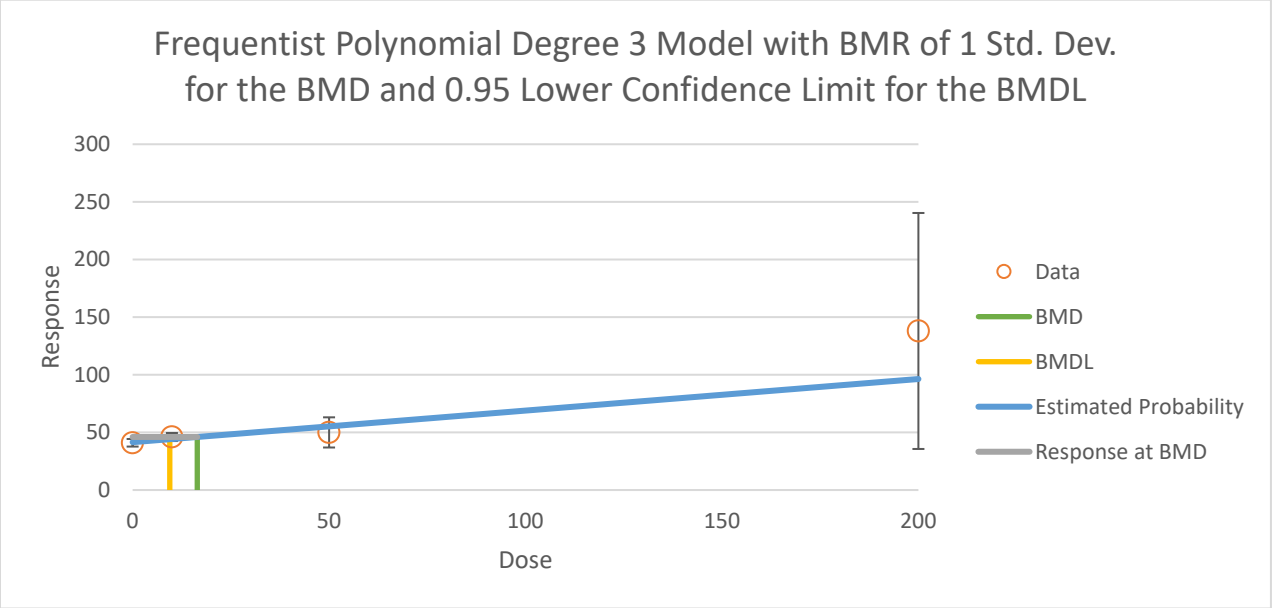
Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	95.32684814	95	95	22.0420176	19.8	19.8	-0.046891559
10	10	96.9688715	93	93	22.0420176	15	15	-0.56939768
50	10	103.536965	109	109	22.0420176	23	23	0.783759182
200	10	128.1673154	127	127	22.0420176	31	31	-0.16746994

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-179.9852318	5	369.970464
A2	-177.2824666	8	370.564933
A3	-179.9852318	5	369.970464
fitted	-180.4755621	3	366.951124
R	-186.5745612	2	377.149122

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	18.58418912	6	0.00492666
2	5.405530279	3	0.14439941
3	5.405530279	3	0.14439941
4	0.980660732	2	0.61242404

Figure A3.3. Polynomial 3 model output for increased alanine aminotransferase in male rats (Chengelis et al., 2009b)



Benchmark Dose	
BMD	16.45743847
BMDL	9.460550272
BMDU	39.84493713
AIC	341.770089
Test 4 P-value	0.70433951
D.O.F.	2

Model Parameters

# of Parameters	6
Variable	Estimate
g	41.52763076
beta1	0.2739763
beta2	Bounded
beta3	Bounded
rho	8.381774368
alpha	-28.22137264

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	41.52763076	41	41	4.50894809	4.6	4.6	-0.370045279
10	10	44.26739376	46	46	5.89330531	4.9	4.9	0.92969594
50	10	55.22644576	50	50	14.8918525	18.3	18.3	-1.109833227
200	10	96.32289076	138	138	153.247489	143.1	143.1	0.860011427

Likelihoods of Interest

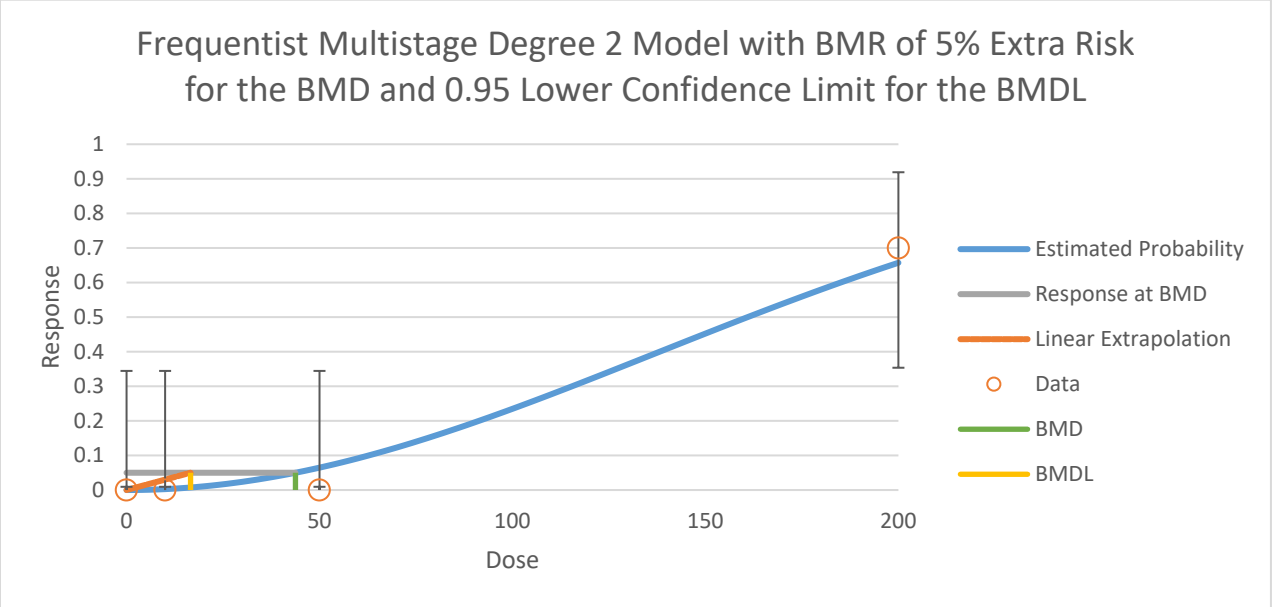
Model	Log Likelihood*	# of Parameters	AIC
A1	-225.8339843	5	461.667969
A2	-164.5076936	8	345.015387
A3	-166.5345497	6	345.069099

fitted	-166.8850445	4	341.770089
R	-231.728919	2	467.457838

* Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	134.4424509	6	<0.0001
2	122.6525815	3	<0.0001
3	4.053712332	2	0.13174907
4	0.700989562	2	0.70433951

Figure A3.4. Multistage 2 model output for hepatocellular hypertrophy in male rats (Chengelis et al., 2009b)



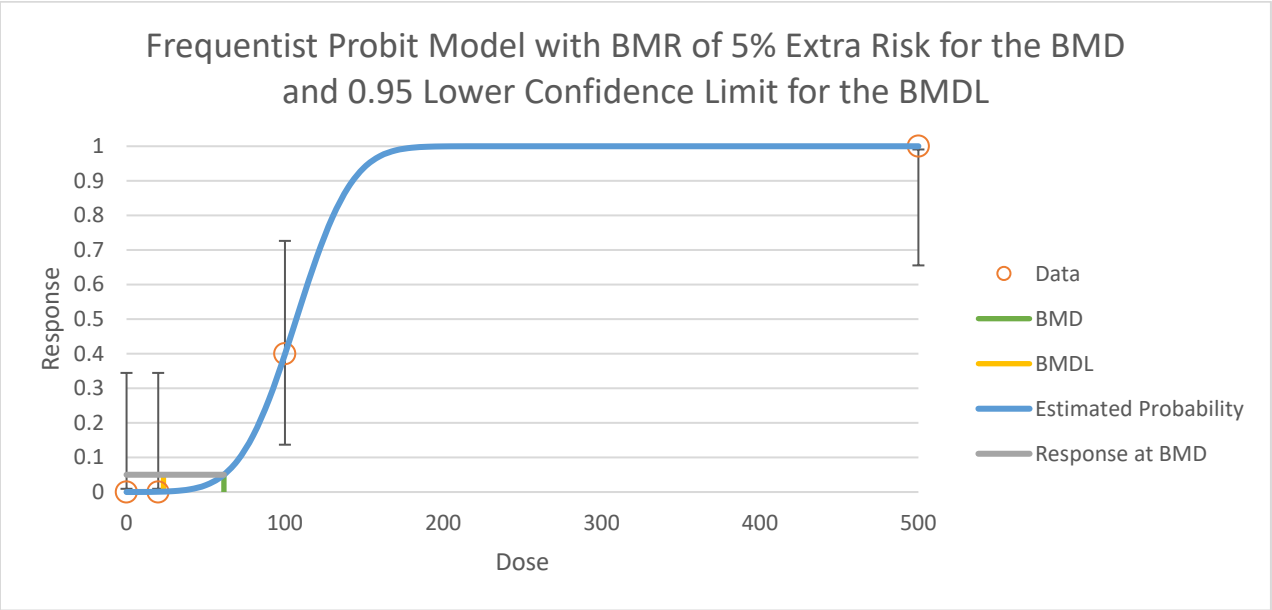
Benchmark Dose	
BMD	43.77231002
BMDL	16.59071911
BMDU	62.65347784
AIC	15.69217619
P-value	0.849464821
D.O.F.	3
Chi^2	0.800009251
Slope Factor	0.003013733

Model Parameters	
# of Parameters	3
Variable	Estimate
g	Bounded
b1	Bounded
b2	2.67708E-05

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.0003903
10	0.002673517	0.026735168	0	10	-0.1637279
50	0.064736582	0.647365822	0	10	-0.8319705
200	0.657276993	6.572769928	7	10	0.2846531

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-6.108643021	4	-	-	NA
Fitted Model	-6.846088093	1	1.47489015	3	0.6880777
Reduced Model	-18.54905759	1	24.8808291	3	<0.0001

Figure A3.5. Probit model output for hepatocellular hypertrophy in male rats (Loveless et al., 2009)



Benchmark Dose	
BMD	61.501661
BMDL	23.32823636
BMDU	80.04576141
AIC	15.47852795
P-value	0.999764809
D.O.F.	3
Chi^2	0.009230021

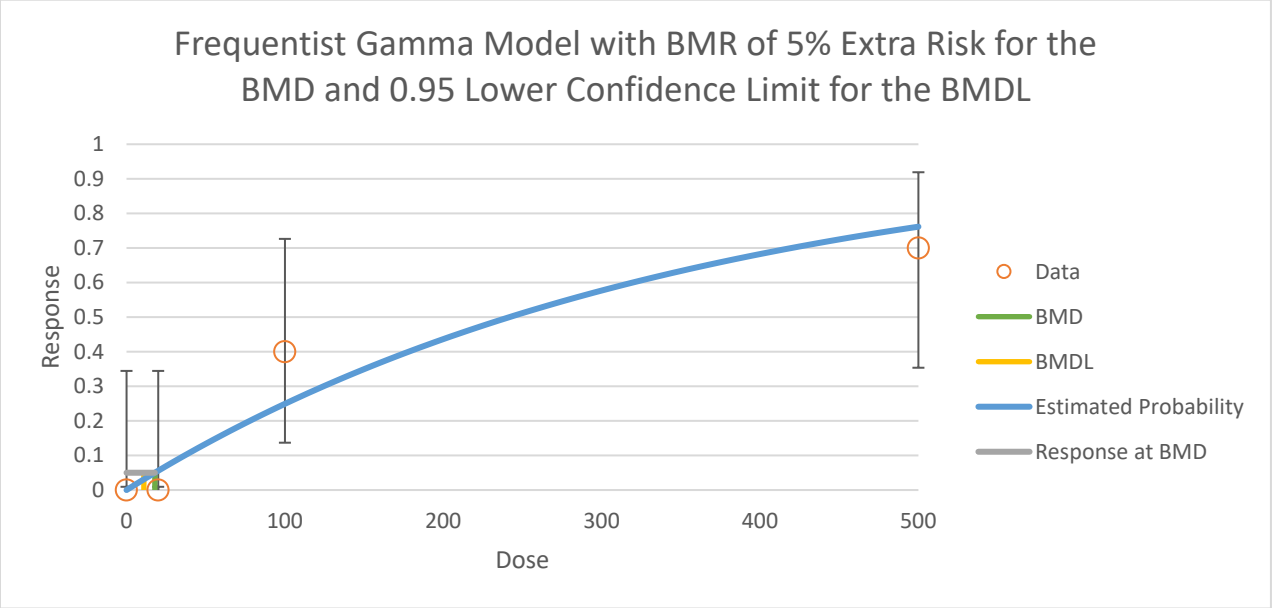
Model Parameters

# of Parameters	2
Variable	Estimate
a	-3.858387981
b	Bounded

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	5.70687E-05	0.000570687	0	10	-0.02389
20	0.000849399	0.008493993	0	10	-0.092202
100	0.398053746	3.980537459	4	10	0.0125733
500	1	10	10	10	0

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-6.73011667	4	-	-	NA
Fitted Model	-6.739263976	1	0.01829461	3	0.9993455
Reduced Model	-25.89786556	1	38.3354978	3	<0.0001

Figure A3.6. Gamma model output for nasal cavity degeneration/atrophy in male rats (Loveless et al., 2009)



Benchmark Dose	
BMD	17.88699879
BMDL	10.95782939
BMDU	55.66216077
AIC	30.11618253
P-value	0.569796967
D.O.F.	3
Chi^2	2.012593638

Model Parameters

# of Parameters	3
Variable	Estimate
g	Bounded
a	Bounded
b	0.00286763

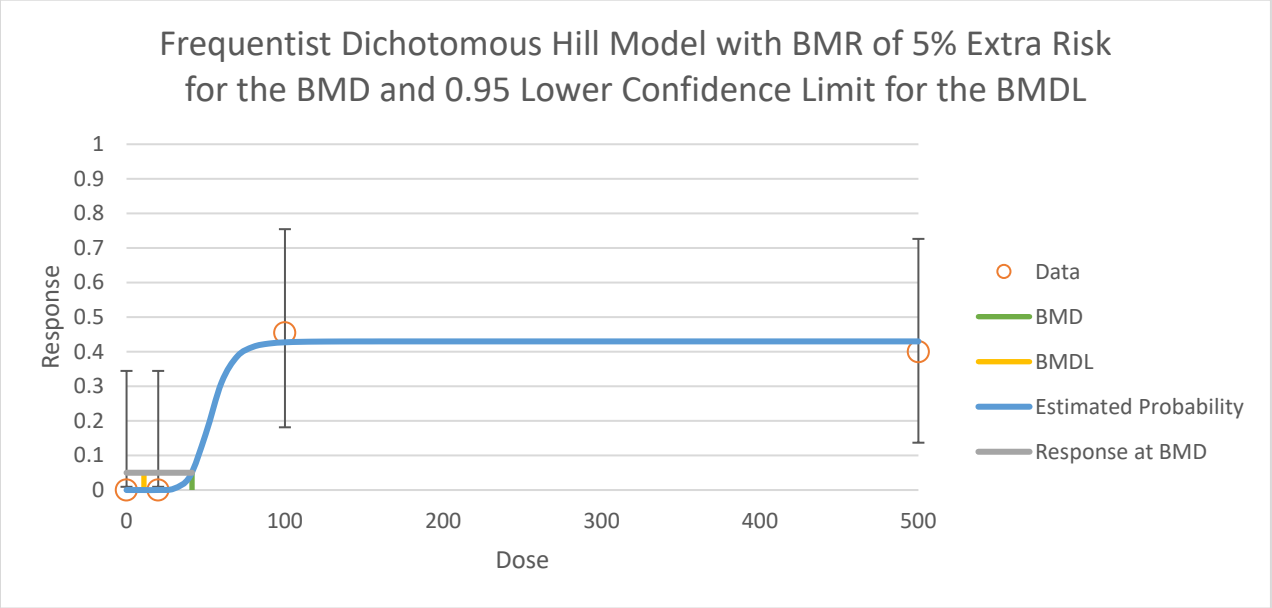
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
20	0.05573895	0.557389502	0	10	-0.768304
100	0.24931039	2.493103904	4	10	1.101496
500	0.761602306	7.61602306	7	10	-0.457174

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-12.83875969	4	-	-	NA
Fitted Model	-14.05809127	1	2.43866315	3	0.4864781
Reduced Model	-23.52675109	1	21.3759828	3	<0.0001

Figure A3.7. Dichotomous-Hill model output for nasal cavity degeneration/atrophy in female rats (Loveless et al., 2009)



Benchmark Dose	
BMD	41.35978205
BMDL	11.00023589
BMDU	82.76404122
AIC	32.69187472
P-value	0.964773882
D.O.F.	2
Chi^2	0.071723048

Model Parameters

# of Parameters	4
Variable	Estimate
g	Bounded
v	0.429871142
a	Bounded
b	8.035876908

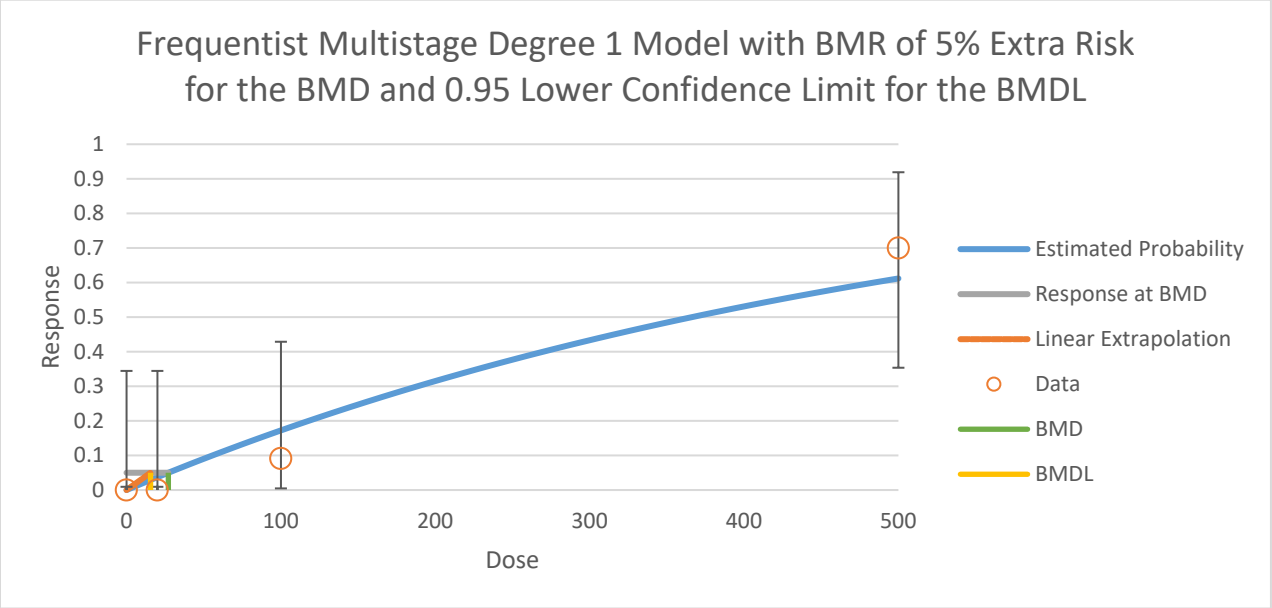
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
20	0.000164755	0.001647551	0	10	-0.040593
100	0.427178712	4.698965828	5	11	0.1834869
500	0.429871144	4.298711439	4	10	-0.190808

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-14.30921829	4	-	-	NA
Fitted Model	-14.34593736	2	0.07343813	2	0.9639469
Reduced Model	-21.57788465	1	14.5373327	3	0.0022579

Figure A3.8. Multistage 1 model output for nasal cavity respiratory metaplasia in female rats (Loveless et al., 2009)



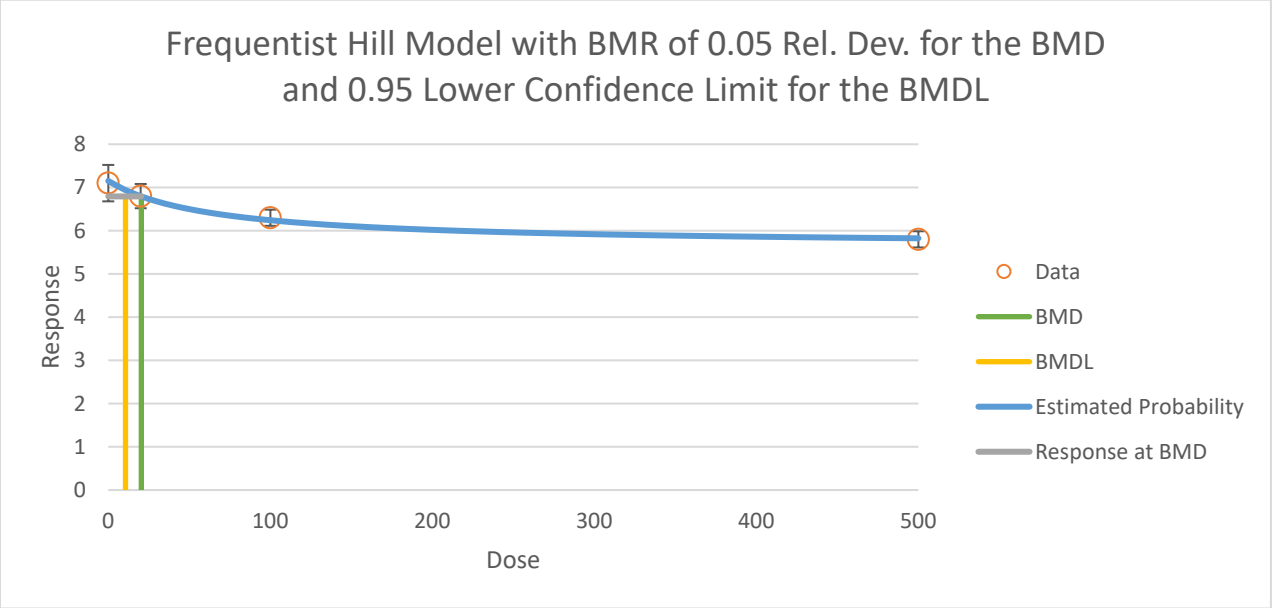
Benchmark Dose	
BMD	27.09835768
BMDL	15.5785492
BMDU	52.39645632
AIC	22.61390063
P-value	0.746935722
D.O.F.	3
Chi^2	1.225330164
Slope Factor	0.003209541

Model Parameters	
# of Parameters	2
Variable	Estimate
g	Bounded
b1	0.001892856

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
20	0.037149516	0.371495159	0	10	-0.621151
100	0.172449907	1.896948977	1	11	-0.715884
500	0.611875118	6.118751179	7	10	0.5718491

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-9.459640091	4	-	-	NA
Fitted Model	-10.30695031	1	1.69462044	3	0.6381305
Reduced Model	-20.23617287	1	21.5530656	3	<0.0001

Figure A3.9. Hill model output for reduced body weight on day 0 in rat pups, 5% relative deviation (Loveless et al., 2009)



Benchmark Dose	
BMD	20.37685658
BMDL	10.63002603
BMDU	48.11769118
AIC	137.8146234
Test 4 P-value	0.721892528
D.O.F.	1

Model Parameters

# of Parameters	6
Variable	Estimate
g	7.150597322
v	-1.499168499
k	65.06591165
n	Bounded
rho	7.905717747
alpha	-16.01832972

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	20	7.150597322	7.1	7.1	0.79206081	0.9	0.9	-0.285682743
20	20	6.798125111	6.8	6.8	0.64860568	0.6	0.6	0.012927357
100	20	6.242373157	6.3	6.3	0.46298735	0.4	0.4	0.55663525
500	20	5.824054288	5.8	5.8	0.35195891	0.4	0.4	-0.305643758

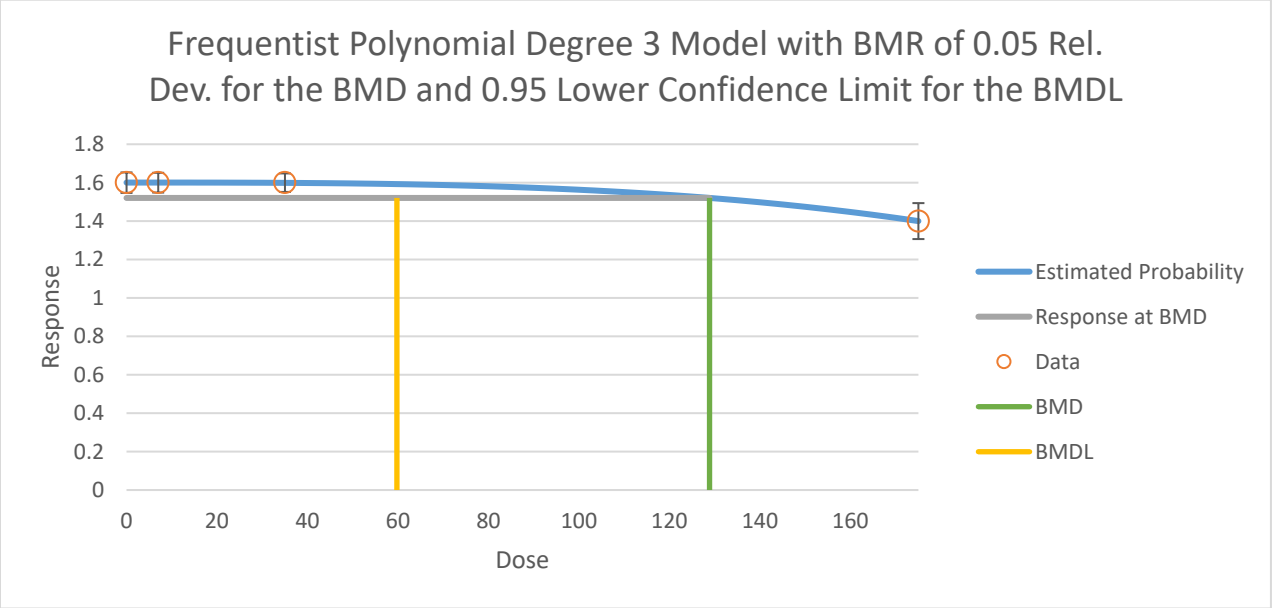
Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-71.96262124	5	153.925242
A2	-62.48799882	8	140.975998
A3	-63.84396841	6	139.687937
fitted	-63.90731171	5	137.814623
R	-93.00698731	2	190.013975

***** Includes additive constant of -36.75754. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	61.03797697	6	<0.0001
2	18.94924484	3	0.00028009
3	2.711939174	2	0.25769731
4	0.126686602	1	0.72189253

Figure A3.10. Polynomial 3 model output for reduced body weight on day 0 in rat pups (cohort 2), 5% relative deviation (Iwai and Hoberman, 2014)



Benchmark Dose	
BMD	128.8382292
BMDL	59.75214056
BMDU	151.402896
AIC	-97.63225313
Test 4 P-value	0.999957841
D.O.F.	3

Model Parameters

# of Parameters	6
Variable	Estimate
g	1.600583622
beta1	Bounded
beta2	Bounded
beta3	Bounded
rho	-10.44423951
alpha	0.244249282

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	16	1.600583622	1.6	1.6	0.09688844	0.1	0.1	-0.024094605
7	17	1.600570787	1.6	1.6	0.0968925	0.1	0.1	-0.024288922
35	19	1.5989792	1.6	1.6	0.0973972	0.1	0.1	0.045684716
175	20	1.40003087	1.4	1.4	0.19493506	0.2	0.2	-0.000708213

Likelihoods of Interest

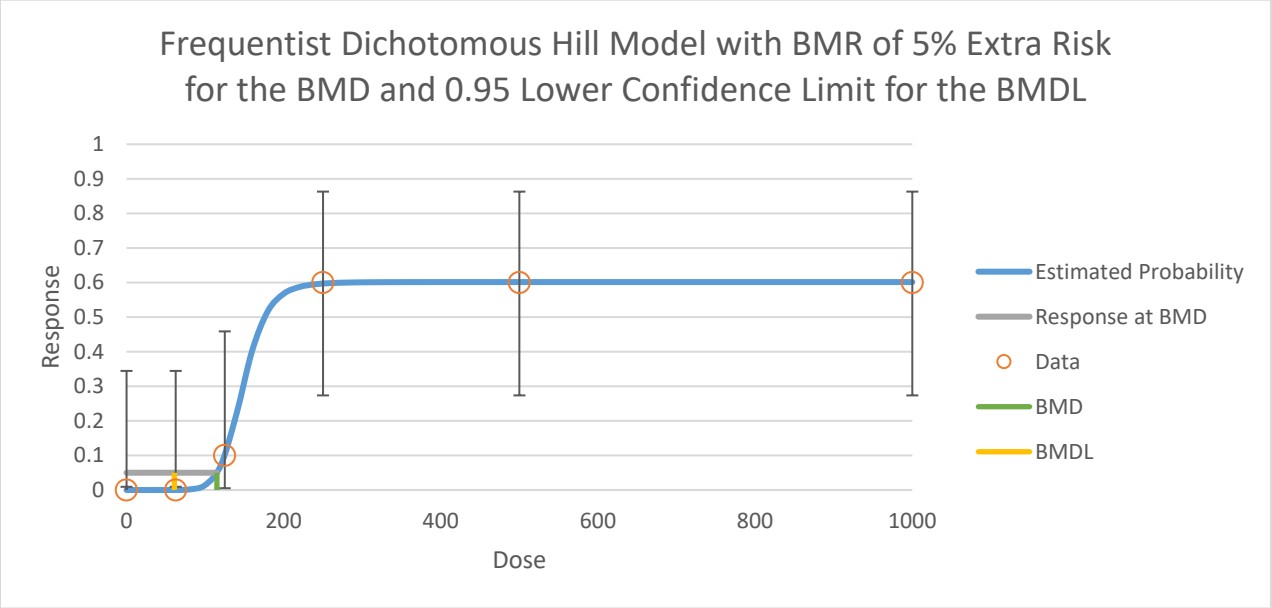
Model	Log Likelihood*	# of Parameters	AIC
A1	43.76323804	5	-77.5264761
A2	51.81779745	8	-87.6355949

A3	51.8175919	6	-91.6351838
fitted	51.81612657	3	-97.6322531
R	30.0847735	2	-56.169547

* Includes additive constant of -66.16357. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	43.46604791	6	<0.0001
2	16.10911883	3	0.00107704
3	0.000411113	2	0.99979446
4	0.002930663	3	0.99995784

Figure A3.11. Dichotomous Hill model output for nasal olfactory epithelium degeneration in male rats (NTP, 2019a)



Benchmark Dose	
BMD	115.0118438
BMDL	60.90805042
BMDU	149.3486399
AIC	52.88640207
P-value	0.999970962
D.O.F.	3
Chi^2	0.002285417

Model Parameters

# of Parameters	4
Variable	Estimate
g	1.52425E-08
v	0.601450826
a	Bounded
b	9.432810581

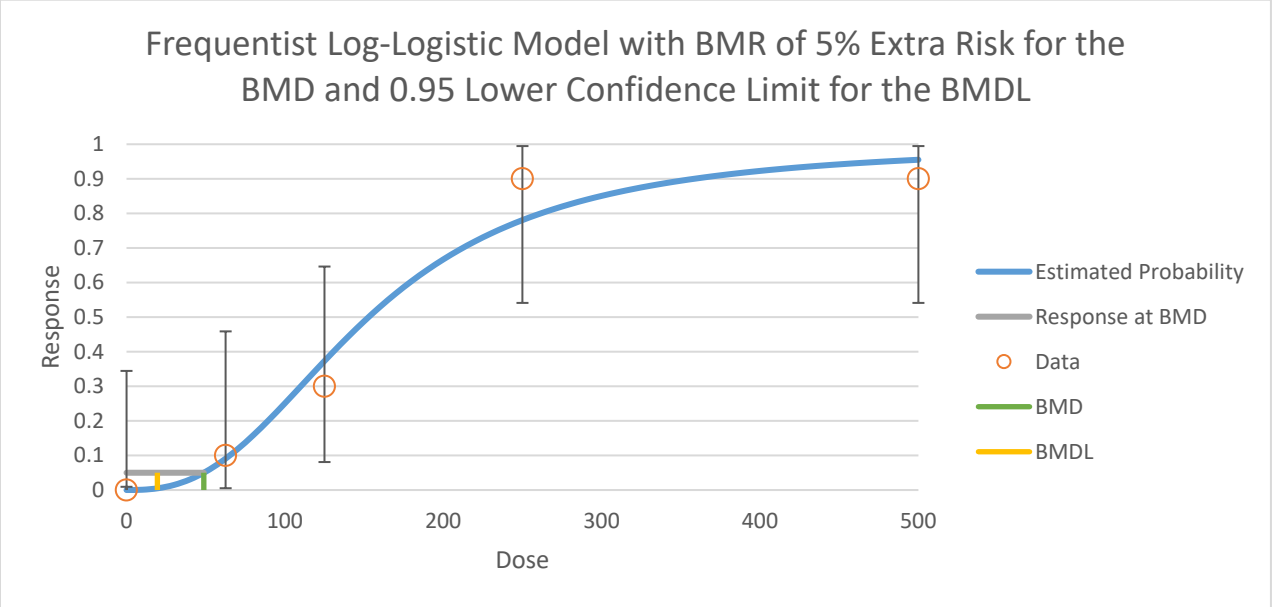
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.52425E-08	1.52425E-07	0	10	-0.0003904
62.6	0.000175681	0.001756812	0	10	-0.041918
125	0.099779867	0.997798673	1	10	0.0023227
250	0.597107037	5.971070369	6	10	0.0186519
500	0.601444502	6.014445016	6	10	-0.0093299
1000	0.601450823	6.014508232	6	10	-0.0093707

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-23.44117974	6	-	-	NA
Fitted Model	-23.44320103	3	0.00404258	3	0.9999317
Reduced Model	-37.45987839	1	28.0373973	5	<0.0001

Figure A3.12. Log-Logistic model output for nasal olfactory epithelium degeneration in female rats (high dose dropped) (NTP, 2019a)



Benchmark Dose	
BMD	48.80621542
BMDL	19.49155542
BMDU	79.32995398
AIC	39.49004847
P-value	0.410784743
D.O.F.	2
Chi^2	1.779371882

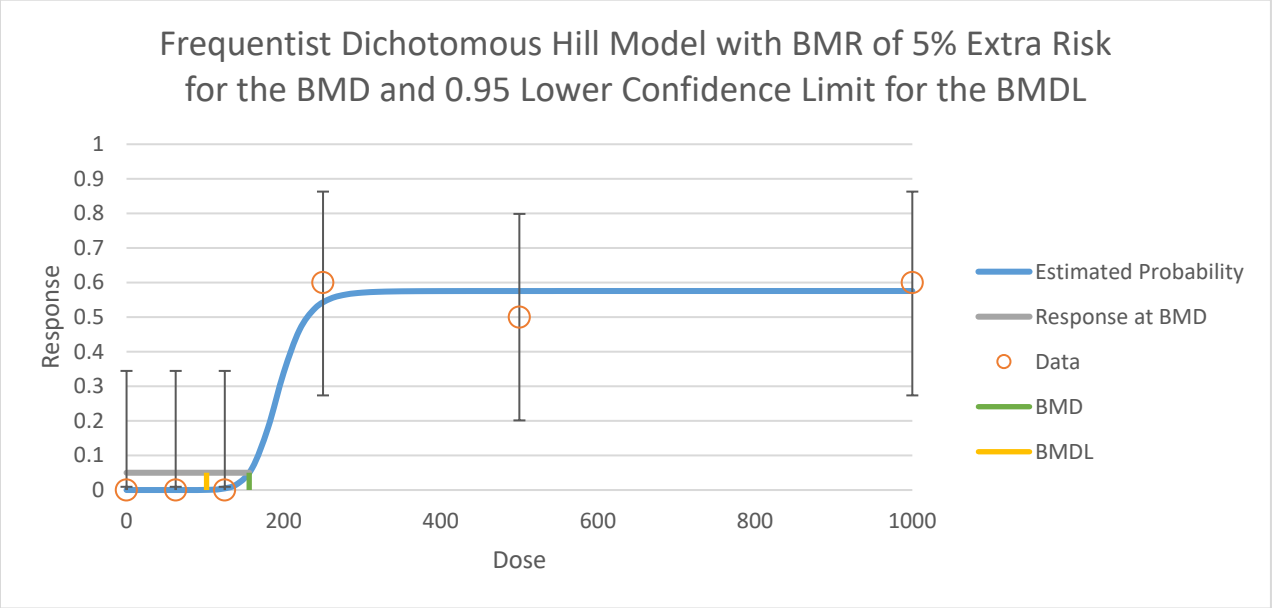
Model Parameters

# of Parameters	3
Variable	Estimate
g	2.36074E-08
a	-12.97398001
b	2.579708901

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	2.36074E-08	2.36074E-07	0	10	-0.0004859
62.6	0.090930213	0.909302126	1	10	0.0997572
125	0.373241408	3.732414082	3	10	-0.4788634
250	0.780705409	7.807054088	9	10	0.9117236
500	0.955122362	9.551223623	9	10	-0.8419442

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-15.86113222	5	-	-	NA
Fitted Model	-16.74502423	3	1.76778402	2	0.4131717
Reduced Model	-34.29649001	1	36.8707156	4	<0.0001

Figure A3.13. Figure A3.13 Dichotomous Hill model output for nasal olfactory epithelium hyperplasia in male rats (NTP, 2019a)



Benchmark Dose	
BMD	156.106743
BMDL	101.845545
BMDU	202.7263835
AIC	45.26721802
P-value	0.979284073
D.O.F.	4
Chi^2	0.437603124

Model Parameters	
# of Parameters	4
Variable	Estimate
g	Bounded
v	0.575544714
a	Bounded
b	10.95856401

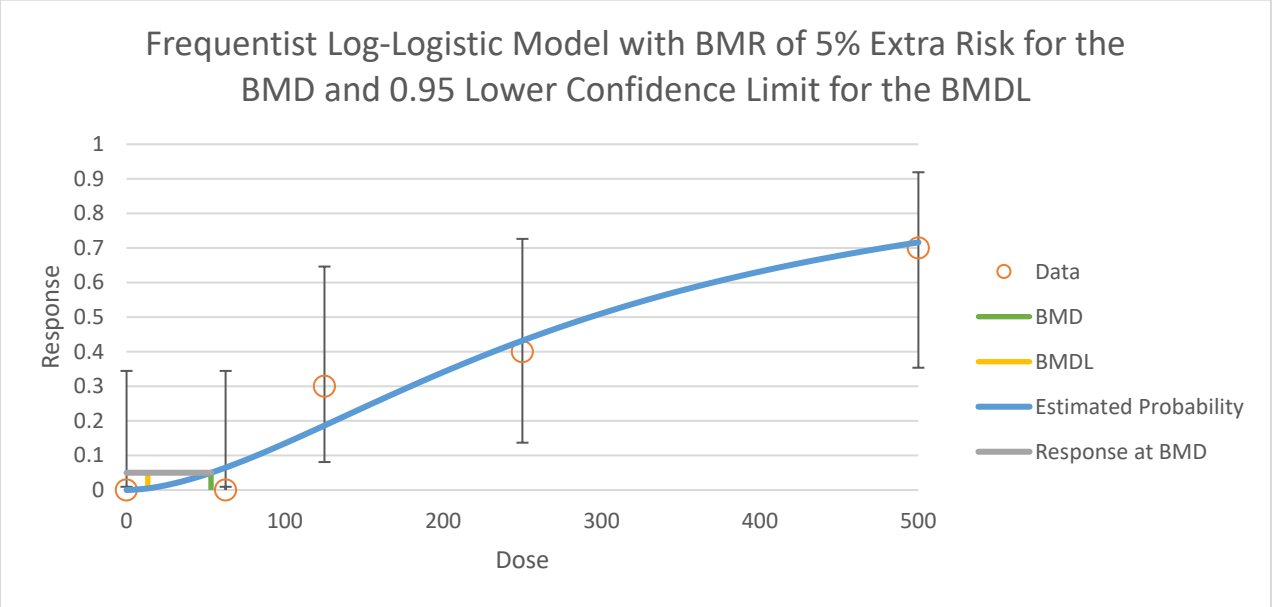
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.0003903
62.6	2.46746E-06	2.46746E-05	0	10	-0.0049674
125	0.004755633	0.047556326	0	10	-0.2185945
250	0.542806341	5.428063406	6	10	0.3630574
500	0.575527277	5.755272773	5	10	-0.4832212
1000	0.575544712	5.755447115	6	10	0.156465

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-20.39170515	6	-	-	NA
Fitted Model	-20.63360901	2	0.48380772	4	0.975058
Reduced Model	-35.76444191	1	30.7454735	5	<0.0001

Figure A3.14. Log-Logistic model output for nasal olfactory epithelium hyperplasia in female rats (high dose dropped) (NTP, 2019a)



Benchmark Dose	
BMD	53.35675659
BMDL	13.44321167
BMDU	104.3228116
AIC	44.03792593
P-value	0.660451822
D.O.F.	3
Chi^2	1.595318331

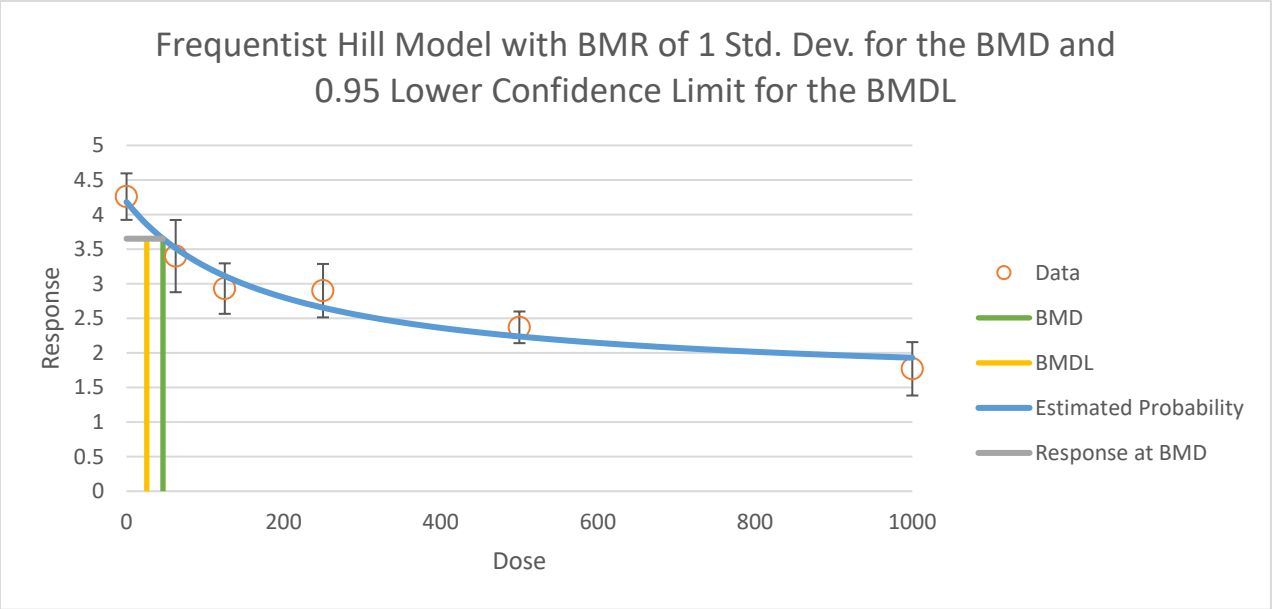
Model Parameters

# of Parameters	3
Variable	Estimate
g	Bounded
a	-9.823617862
b	1.729740462

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.0003903
62.6	0.064882919	0.648829189	0	10	-0.8329754
125	0.186655795	1.866557946	3	10	0.9199016
250	0.432189529	4.32189529	4	10	-0.205483
500	0.71627145	7.162714495	7	10	-0.1141395

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-18.94740271	5	-	-	NA
Fitted Model	-20.01896297	2	2.14312051	3	0.5432386
Reduced Model	-29.64766587	1	21.4005263	4	0.0002637

Figure A3.15. Hill model output for decreased total thyroxine (T4) in male rats (NTP, 2019a)



Benchmark Dose	
BMD	46.40182487
BMDL	25.79306473
BMDU	91.93440408
AIC	102.0873677
Test 4 P-value	0.120604237
D.O.F.	3

Model Parameters	
# of Parameters	5

Variable	Estimate
g	4.181647428
v	-2.673639635
k	187.6766988
n	Bounded
alpha	0.280900637

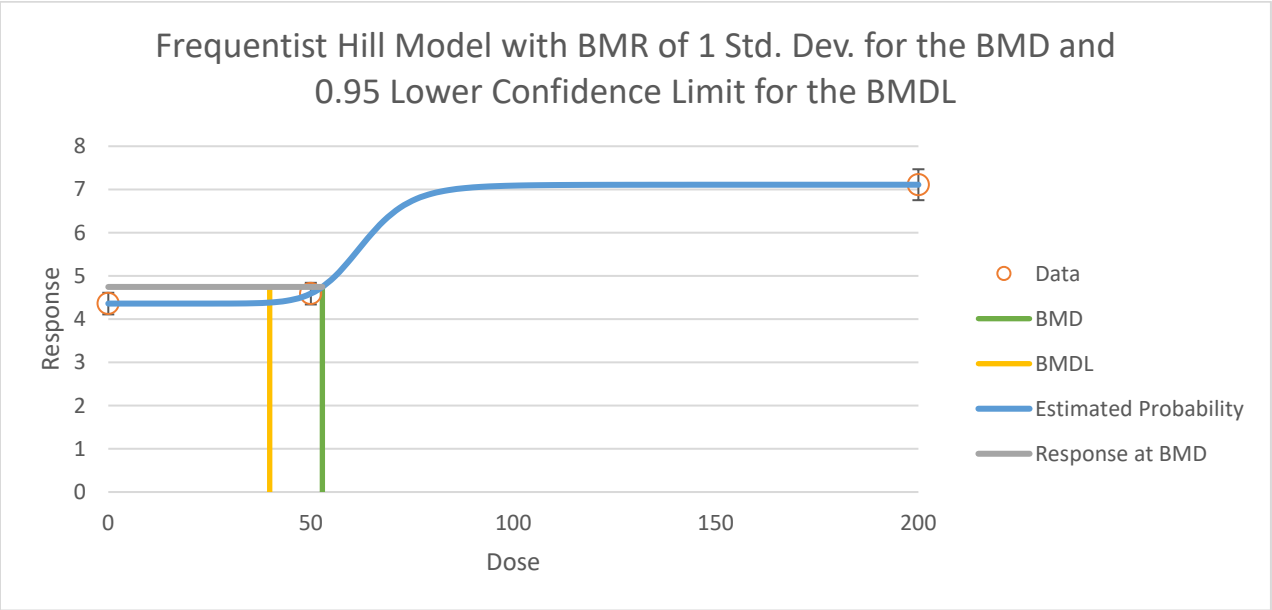
Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	4.181647428	4.26	4.26	0.5300006	0.47	0.47	0.467494917
62.6	10	3.512908221	3.4	3.4	0.5300006	0.73	0.73	-0.673673095
125	10	3.112795941	2.93	2.93	0.5300006	0.51	0.51	-1.090662013
250	10	2.654470152	2.9	2.9	0.5300006	0.54	0.54	1.464967309
500	10	2.23768187	2.37	2.37	0.5300006	0.32	0.32	0.789483381
1000	10	1.930496389	1.77	1.77	0.5300006	0.54	0.54	-0.957610508

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-44.13273515	7	102.26547
A2	-40.82665209	12	105.653304
A3	-44.13273515	7	102.26547
fitted	-47.04368387	4	102.087368
R	-80.72208063	2	165.444161

* Includes additive constant of -55.13631. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	79.79085707	10	<0.0001
2	6.612166115	5	0.25111805
3	6.612166115	5	0.25111805
4	5.821897433	3	0.12060424

Figure A3.16 Hill model output for increased hepatosomatic index in male mice (Jiang et al. 2021)



Benchmark Dose	
BMD	52.84511132
BMDL	39.84663586
BMDU	180.9486703
AIC	37.92120238
Test 4 P-value	NA
D.O.F.	-1

Model Parameters	
# of Parameters	5

Variable	Estimate
g	4.359999608
v	2.750015354
k	62.8375439
n	10.47538802
alpha	0.148503126

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	10	4.359999608	4.36	4.36	0.38536103	0.35	0.35	3.2137E-06
50	10	4.590000336	4.59	4.59	0.38536103	0.35	0.35	-2.75332E-06
200	10	7.110000096	7.11	7.11	0.38536103	0.5	0.5	-7.8837E-07

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-13.96060119	4	35.9212024
A2	-13.05983397	6	38.1196679
A3	-13.96060119	4	35.9212024
fitted	-13.96060119	5	37.9212024
R	-50.52983449	2	105.059669

* Includes additive constant of -27.56816. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value

1	74.94000106	4	<0.0001
2	1.801534439	2	0.40625785
3	1.801534439	2	0.40625785
4	6.66429E-09	-1	NA

APPENDIX 4. DEFAULT UNCERTAINTY FACTORS FOR NOTIFICATION LEVEL (NL) DERIVATION

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving notification level recommendations. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results. Table A4.1 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table A4.1. Default uncertainty factors for NL 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)