March 31, 2023

Mr. Steve Risotto
Senior Director
700 Second St., NE
Washington, DC 20002

Re: Proposed Amendments to Rules Governing Health Risk Limits for Groundwater, Minnesota Rules, Chapter 4717, Part 7500, Part 7850, and Part 7860; Revisor's ID Number RD4587, OAH Docket No. 5-9000-38941

Dear Steve Risotto:

We thank the American Chemistry Council (ACC) for their written comments on the proposed Health Risk Limits (HRLs) for perfluorobutane sulfonate (PFBS), perfluorohexanoate (PFHxA), and perfluorohexane sulfonate (PFHxS). The Minnesota Department of Health (MDH) presents its written responses below. Text in *italics* is directly quoted from the comments submitted by ACC followed by the response from MDH. For background information on technical topics, please see Attachment A: Risk Assessment Methodology for Health Risk Limits Derivation.

General Comments

1. *For all three substances, the Department inappropriately uses the results of a short-term study as the basis for its proposed subchronic and chronic HRLs even though data from longer-term studies are available.*
    1. The methodology used by MDH for deriving health-protective HRLs was promulgated in the 2008 SONAR[1] and ensures that the derivation process used incorporates the necessary provisions to adequately protect sensitive or highly exposed populations, as required by the 2001 Health Standards Statute (Minnesota Statutes 144.0751)
    2. A key part of the methodology includes careful evaluation of longer-term as well as short-term studies. As recommended by the US Environmental Protection Agency (US EPA)[2], MDH's evaluations carefully consider the relationship between timing, duration, and magnitude of exposure and the subsequent adverse effect(s) in deriving guidance that are protective of sensitive life stages (e.g., development) and short periods of high exposure (e.g., infancy) as well as long-term exposure.
        - MDH has applied this methodology to over 120 chemical reviews; since infants drink large amounts of water for their body weight, it is not unusual for the short-term duration water value to be lower than calculated longer-term water values[3].
    3. By definition, (sub)chronic durations contain exposure periods of short-term duration. Because adverse health effects can result from short-term exposures to fetuses/infants during critical windows of development, relying solely on (sub)chronic studies to derive (sub)chronic HRLs may

underestimate the risk to fetuses and infants, an especially vulnerable population with high water intake rates. To ensure a HRL is health protective for short-term exposures that occur during (sub)chronic durations, the short-term reference dose and/or drinking water guidance value are used for the longer durations if the short-term numbers are lower than those calculated from sub(chronic) studies.

2. *MDH also inappropriately applies a database uncertainty factor (UFD) – 3 in the case of PFBS and 10 in the case of PFHxS and PFHxA.*
    1. Responses to this comment will be located within the respective section for each chemical.

Perfluorobutane Sulfonate

1. *MDH selected the results of a short-term study, however, despite the fact that the biological significance of the Department's critical effect from that study (i.e., decreased T4 in adult euthyroid animals) is unclear in the absence of additional signs of overt thyroid toxicity (e.g., reflex increase in thyroid stimulating hormone and/or alterations in tissue weight or histology). The developmental study by Feng et al. (2017) also reported thyroid effects and is the more appropriate study to use as a basis for the proposed HRL. Feng et al. reported decreased serum total thyroxine (T4) in newborn mice which is considered to be important for normal growth of developing offspring across animal species.*
    1. We are pleased that the commenter agrees about the importance and relevance of thyroid as a critical health endpoint. Thyroxine (T4) is the main hormone produced by the thyroid gland and blood levels of T4 represent a measure of thyroid function. Hypothyroxinemia (low T4 with normal TSH [thyroid stimulating hormone]) has been shown to have adverse effects on human development. Concerns over disruption of thyroid function led to changes in testing guidelines; including measuring blood levels of T4, T3 (triiodothyronine) as well as TSH in response to evidence that changes in serum T4 can produce effects on neurodevelopment without affecting TSH[4] [pages 50-51]. Study testing guidelines recommend using rats as the species for evaluating thyroid related endpoints (e.g., T4, TSH, thyroid weight) and consider hormone level effects observed in these studies to be relevant to human thyroid function[4] [Table 8-1].
    2. MDH selected the National Toxicology Program (NTP) 2019 study[5] as the basis for the proposed HRL to ensure appropriate levels of health protection. The measured decrease in thyroid hormones was much larger in the NTP study conducted in adult rats (~25-75%) compared to the decreases in mice (~10-20%) observed in Feng et al. 2017[6]. While the NTP 2019 study did not directly evaluate pregnant animals or neonatal rats, the significant decrease reported in multiple thyroid hormones surpassed what was seen in pregnant and neonatal animals in Feng et al. This dramatic decline would result in more severe effects on developing fetuses at lower doses than was observed in Feng et al.
    3. MDH's methodology specifies that, in the absence of information to the contrary, identifying and using dose-response information from the most sensitive species is preferred[1] [page 27]. Since Feng et al. demonstrated that maternal PFBS exposures translate to harmful effects in

offspring, the NTP 2019 study, which identified effects at lower doses, was selected as the basis for the proposed PFBS HRL because it is the most health protective.

2. *For short-chain PFAS like PFBS, use of the default approach of body-weight scaling to estimate the human equivalent dose is consistent with USEPA guidance and the state of the science. Although the data may not be sufficient to model external dose and clearance in humans, the information available for the substance suggests that it is eliminated relatively rapidly and thus will not accumulate. As a result, body-weight scaling is the most appropriate approach to estimating the human equivalent dose – rather than the serum elimination, half-life adjusted approach used by the Department.*
    1. Consistent with the state of risk assessment science, the United States Environmental Protection Agency (US EPA) did not use the default body weight scaling approach and but rather derived a chemical-specific dosimetric adjustment factor (DAF) for PFBS.
    2. MDH's analysis was consistent with US EPA's. Accordingly, MDH calculated a DAF in a similar manner, in accordance with the 2008 SONAR[1] [pages 30-31].
    3. Moreover, in the US EPA report Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose cited by the commenter[7], US EPA explicitly defines the preferred hierarchy of approaches for extrapolating doses from laboratory animals to humans as [pages ix-xi]:
        a. Physiologically-based toxicokinetic modeling
        b. Use of chemical-specific data
        c. In lieu of useful information about the chemical in question, default to body weight scaling to ¾ power
    4. Body weight scaling is not a preferred approach and is meant to be used as a default only when the other options are not feasible. As evidenced by US EPA and MDH calculating a PFBS-specific DAF, there clearly are chemical-specific data fulfilling the requirements for Option #2. Because data exist to fulfill Option #2, body weight scaling is not appropriate and any discussion of PFBS accumulation in the body is irrelevant.

3. In calculating the toxicity value for PFBS, MDH includes a UFD of 3 based on concerns about developmental and immunotoxicity effects. For PFBS, however, robust data are available on reproductive and developmental effects, including both a prenatal toxicity study and a two-generation reproduction study. Moreover, the developmental effects appear to be "less sensitive than thyroid hormone perturbations in developing mice." Consequently, a toxicity value that protects against effects on thyroid hormones also will protect against developmental effects.

1. The commenter cites "developmental affects appear to be 'less sensitive than thyroid hormone effects'" as appearing in US EPA's *Human Health Toxicity Values for PFBS* assessment. However, the quoted text is from a 2018 draft[8], which explicitly states that it should not be construed to represent Agency policy. Notably, the quoted text does not appear in a later 2020 version, or

the final version released in 2021[9], indicating that US EPA's analysis of the relative sensitivities of developmental and thyroid effects had been refined.

2. In fact, the final *Human Health Toxicity Values for PFBS* report[9] released in 2021 acknowledges – in many places – the remaining uncertainty around developmental and other effects and the need for additional data [e.g., pages 3, 4, 56, 57].
3. Indeed, the US EPA assigned a $UF_D = 3$ for the same reasons that MDH did:
    a. "A UF_D of 3 is applied due to database deficiencies. […] However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, because other health effect domains such as immunotoxicity and mammary gland development are effects of increasing concern across several members of the larger PFAS family (Grandjean, 2018; Liew et al., 2018; White et al., 2007), the lack of studies evaluating these outcomes following PFBS exposure is a limitation in the database." [page 84]

4. The Department provides no explanation for its concern for the potential immunotoxicity of PFBS, moreover. ACC is not aware of available data that would suggest that immunotoxicity is a concern for PFBS, which -- as clearly demonstrated by MDH's analysis -- exhibits dramatically different properties from the PFAS previously evaluated.

1. US EPA (see 3-3 above) and MDH have identified immunotoxicity data as an important data gap for PFBS and have applied a database uncertainty factor of 3. The proposed SONAR and the PFBS Summary Sheet posted on the MDH website provide additional background that immunotoxicity has been consistently observed as a sensitive effect for several other PFAS.
2. The epidemiological data are so strong for more well-studied PFAS that multiple state, federal, and international agencies[10-19] have stated that there is sufficient evidence that immune suppression, especially in infants and children, is associated with PFAS exposure. Immune suppression is among the most sensitive health endpoints observed.
3. The purpose of the UF_D is to account for potential health endpoints that have not been adequately evaluated and which could be sensitive endpoints. For plausible but unstudied/understudied endpoints, it is standard risk assessment practice to assign a UF_D until the data gap is filled. As noted in the 2008 SONAR[1], "[a]pplication of the database uncertainty factor may incorporate an evaluation of how thorough testing is with respect to life stage assessment, endpoint assessment, and duration of exposure." [page 32]

Perfluorohexane Sulfonate and Salts

1. *The data selected by the Health Department to derive the proposed HRL for PFHxS come from the results of a 28-day toxicity study conducted by the federal National Toxicology Program (NTP). The Department's analysis provides no discussion of the available chronic studies conducted by Butenhoff et al (2009) and Chang et el. (2018). While the effects reported by Chang et al. (2018) do not*

represent a significant health effect, the study by Butenhoff et al. (2009) has been used by a number of other states to assess the health effects of PFHxS.

1. The Butenhoff et al. 2009[20] and Chang et al. 2018[21] studies were evaluated by MDH as part of the PFHxS review process. MDH's evaluations carefully consider the relationship between timing, duration, and magnitude of exposure and the subsequent adverse effect(s) in deriving guidance that are protective of sensitive life stages (e.g., development) and short periods of high exposure (e.g., infancy) as well as long-term exposure. The point of departure (POD) – that is, the dose where a toxic effect is first identified – in the NTP 2019 study[5] is based on decreased T4 serum levels and was observed at the lowest dose tested. Thyroid hormone serum levels were not assessed in either Butenhoff et al. 2009 or Chang et al. 2018. Thyroxine (T4) is the main hormone produced by the thyroid gland and is critical for normal human development (see response in PFBS 1-1, above). Additionally, the thyroid hormone decreases observed in NTP 2019 are consistent with several other studies discussed below.
2. Finally, as discussed above in General Comments 1 and in keeping with promulgated MDH methodology[1], if a short-term study results in a lower guidance value than available (sub)chronic studies, the short-term value is used for all durations. Because thyroid hormone decreases were the most sensitive and most consistent across available studies, NTP 2019 was used as the basis for the proposed HRL.

2. The Department's analysis also does not address the suggestion by Butenhoff et al. that thyroid effects (such as those reported in the NTP study) may be related to hepatocellular hypertrophy caused by activation of the peroxisome proliferator-activated receptor alpha (PPARα) leading to hyperplasia of the thyroid that is likely not relevant to human health risk.

1. Notably, Butenhoff et al. 2009 did not directly measure thyroid hormone levels but only examined the thyroid histologically. It is not possible from this study to determine the impact of PFHxS on thyroid hormones or overall thyroid function. Additionally, thyroid weights were not reported from this study, making the proposed significant thyroid hyperplasia ambiguous.
    a. Additionally, PPARα activation alone is not sufficient to determine that a health effect is not relevant to human health risk. Fibrates, a class of cholesterol-reducing pharmaceuticals, have been in use since the 1960s and primarily act through activation of PPARα, demonstrating that PPARα is active and biologically relevant in humans[22; 23].
2. There are studies that do not support Butenhoff et al.'s suggestion of hepatocellular hypertrophy as source of the thyroid effects. Ramhøj et al 2018 observed a significant decrease in T4 levels but no change in liver weight in rats, indicating that hepatocellular hypertrophy is unlikely to occur at PFHxS doses affecting thyroid hormones[24].
3. A subsequent study by Ramhøj also demonstrated that thyroid weights and histology were unchanged after PFHxS exposure, further supporting that thyroid hyperplasia secondary to hepatocellular hypertrophy is unlikely to be a significant factor[25].

4. Recent mechanistic studies have suggested that many PFAS, including PFHxS, can disrupt thyroid hormones through non-hepatic interactions, including binding to thyroid hormone transport proteins[26-29].
5. The Agency for Toxic Substances and Disease Registry (part of the Centers for Disease Control) *Toxicological Profile for Perfluoroalkyls*[30], noted that approximately 25% of the gene expression changes caused by PFHxS exposure are independent of any PPARα activity [Table 2-29]; therefore, PFHxS affects cells through mechanisms other than PPARα activation.
6. In total, the evidence for thyroid hyperplasia secondary PPARα-induced liver hypertrophy is woefully insufficient to establish it as the likely mechanism of action, and newer studies suggest that TTR-T4 inhibition may be a major contributor to PFAS-induced thyroid signaling dysfunction.
7. Insufficient thyroid hormone levels during critical periods of development can cause irreversible damage. As noted above, study testing guidelines recommend using rats as the species for evaluating thyroid related endpoints (e.g., T4, TSH, thyroid weight) and consider hormone level effects observed in these studies to be relevant to human thyroid function[4] [Table 8-1]. In the absence of a plausible mechanism of action irrelevant to humans, the severe decrease in thyroid hormones must be assumed to be relevant.

3. Before committing to an onerous HRL based on thyroid effects, the Department should carefully review interspecies differences and human study data on the relevance of thyroid effects and the variability of thyroid hormones across life. A recent French study reports that PFAS levels at birth were not associated with thyroid stimulating hormone (TSH) levels later in life, and similar studies are underway to continue to add to evaluate the potential significance of TSH variance. Previous study data show a lack of strong evidence to suggest that per- and polyfluoroalkyl substances (PFAS) are associated with overall TSH and free T4, and even at the highest levels, any statistical variance in TSH-PFAS concentration correlations does not persist in humans beyond gestational week 10. This would suggest that, even if a potential mechanism of action included possible competition with T4 for binding to transthyretin (a main carrier protein of thyroid hormone in mammals), observational (community epidemiology) studies do not suggest this effect occurs at relevant human exposures, either in the mother or infant.

1. As part of its review process, MDH carefully evaluates all available and relevant human and animal study data. Study design guidelines[4] recommend using rats for evaluating thyroid effects and specifically note "circulating levels of TH [thyroid hormones] can be related to human thyroid function." The cited French study measured only TSH.
2. Epidemiology studies have reported mixed results; some studies have reported associations while others have not. The thyroid is particularly challenging to assess in epidemiology studies, as thyroid hormone levels naturally vary by time of day, fasting state, trimester of pregnancy, and between individuals. Hypothyroxinemia (low T4 <u>but normal TSH levels</u>) have been associated with a higher risk of cognitive delay in early childhood. The cited French study only

measured TSH and did not measure T4 or other thyroid hormones; therefore, hypothyroxinemia or other thyroid hormonal effects were not evaluated.

3. The consistency of observed animal thyroid toxicity without plausible evidence of a mechanism of action irrelevant to humans suggests that the thyroid should be considered a target of PFAS in humans while the epidemiology database continues to grow.
4. In *Human Health Toxicity Values for PFBS*[9], EPA states the following about the human relevance of thyroid as an endpoint [page 57]:
    a. "Overall, based on findings in animal models considered to be informative for evaluating the potential for thyroid effects in humans, the available evidence supports a hazard, and the thyroid is considered a potential target organ for PFBS toxicity in humans."
5. An independent, external peer review report[31] commissioned by EPA for their ongoing PFHxA toxicological review recommended that "EPA conclude that the available evidence indicates that PFHxA exposure is likely to cause thyroid toxicity in humans given relevant exposure circumstances, primarily based on short-term studies in rats reporting a consistent and coherent pattern of effects on thyroid hormones following PFHxA exposure, but also drawing from the consistency of effects when considering evidence from structurally related PFAS." [page 8] and further noted that it is a well-known phenomenon that "some chemicals can reduce serum thyroid hormones without increasing TSH." [page 52]

4. The decision to focus on a short-term study for deriving the proposed MCL reflects the limited amount of toxicity data available for PFHxS. This paucity of data is further amplified by the application of a UF_D of 10 based on unspecified concerns about early life sensitivity and the lack of two-generation and immunotoxicity studies.

1. As explained in MDH's response under General Comments, all available relevant data were considered during MDH's analysis. The use of a short-term study as the basis for the proposed HRL does not directly reflect upon the strengths or weaknesses of the database.
2. As discussed above, the NTP 2019 study[5] was determined to be the most appropriate as the basis of the proposed HRL because it describes the most sensitive effect and ensures that the guidance value is adequately protective of susceptible populations (e.g., developing fetuses and infants).
3. The proposed SONAR and the PFHxS Toxicological Summary Sheet posted on MDH's website provide additional background about the early life concerns regarding decreased T4.
4. MDH does not establish MCLs, which are regulatory values that balance health impacts with cost and feasibility of remediation. HRLs are non-regulatory and are based solely on health effects (Minnesota Statues Chapter 103H).

5. The lack of a two-generation study would justify the use of a 3-fold uncertainty factor, based on USEPA guidance. Concern about early life sensitivity is addressed by Chang et al. who reported no treatment-related effects on postnatal survival of development in offspring exposed in utero through PND 36.

Although limited, Butenhoff et al. did not find evidence of immunotoxicity in rats exposed to up to 10 mg/kg per day by gavage for up to 56 days.

1. The rationale for the database uncertainty factor of 10 included thyroid hormone effects in early life and immunotoxicity as well as the lack of a 2-generation study. Two-generation studies traditionally do not adequately assess immune development or neurodevelopment. These are critical data gaps because pre- and neonatal immunological and neurological developmental windows are much more susceptible to disruption than in adults.
2. Decrease in circulating antibodies have been identified as a very sensitive measure of immunotoxicity for other PFAS in both animal studies and epidemiology studies. Decreased antibodies in young children is being used as the basis for regulatory guidance of PFAS by the US EPA[11-14] and European Food Safety Authority[18].
3. The studies by Chang et al and Butenhoff et al assessed survival and physical developmental milestones. Neither included evaluation of sensitive immunological (e.g., circulating antibodies) or neurological endpoints in offspring.
4. The 2002 US EPA report *A Review of the Reference Dose and Reference Concentration Processes*[2] lays out recommendations for the application of database uncertainty factors [Section 4, page 44]:
    a. "If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing. […] If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available"
    b. The EPA guidelines are not to be interpreted as rigid rules that constrain a risk assessor from applying uncertainty factors as appropriate. As noted in the last sentence of the excerpt, when significant questions remain about potential sensitive endpoints, they should be fully accounted for in the UF_D.
5. The outstanding questions about the potential for immunotoxicity from PFHxS indicates the need for a $UF_D = 10$ to address multiple critical data gaps. As noted in the 2008 SONAR[1], "[a]pplication of the database uncertainty factor may incorporate an evaluation of how thorough testing is with respect to life stage assessment, endpoint assessment, and duration of exposure." [page 32]

6. ACC's concerns about using the NTP study results, notwithstanding, the calculation on which the Department rely inappropriately uses a benchmark response (BMR) of 20 percent rather than a BMR of one standard deviation directly observed from study results as advised by USEPA's benchmark dose (BMD) modeling guidance. Although the Department indicates that use of a BMR of 20% provides a more reliable result, that analysis has not been made available for review by external scientists and other stakeholders.

1. MDH has never withheld information from the public; MDH followed standard practices during the review and provided standard detail on the PFHxS Summary Sheet. MDH publishes the initiation and completion of every chemical review, as well as other important unit announcements, via a GovDelivery email list that anyone can join. Initiation of the PFHxS review was announced via GovDelivery email and posted to MDH's website on June 22, 2018. Completion of the PFHxS review was announced via GovDelivery email and review documents were posted to MDH's website on April 3, 2019. MDH is always available for inquiries or to provide additional information during and after a chemical review.
2. Similar to the discussion of body weight scaling (PFBS, Question 2), the US EPA's recommendation of one standard deviation (SD) as the benchmark response (BMR) is meant as a default in the absence of additional information. As stated in the 2012 US EPA *Benchmark Dose Technical Guidance*[32], there is a preference hierarchy for the basis of the BMR. The hierarchy range from a minimal level of change that is generally considered to be biologically significant (most preferred) to a default of one standard deviation (SD) (or lower for more severe effects) from control (least preferred). The default is used in the absence of an idea of what level of response to consider adverse.
3. Clinical literature indicates a perceived toxicological significance in decreased T4 concentration. Haddow et al. 1999[33] reported that a 25% decrease in maternal fT4 during the second trimester was associated with neurodevelopmental and cognitive deficits in children. Additionally, Henrichs et al. 2010[34] associated maternal hypothyroxinemia (low T4 but normal TSH levels) with a higher risk of cognitive delay in early childhood.
4. EPA selected a BMR of 20% relative deviation for decreased thyroid hormone in their 2018 public draft *Human Health Toxicity Values for PFBS*[8], noting "[m]ultiple lines of evidence regarding the degree of thyroid hormone disruption and developmental outcomes in pregnant dams or offspring were considered in the identification of this BMR." [page 55]. This was the best available science regarding a level of change of concern at the time of MDH's review.
5. As a matter of practice during our analyses, MDH performs comparisons with a variety of default BMRs (e.g., 10%, 20%, 1 SD). For this study, the POD calculated using a BMR = 1 SD was ~7% lower than the POD calculated using a BMR = 20%, indicating a negligible difference in potential guidance values.

7. If MDH does not feel that published reports on PFHxS provide a sufficient basis for developing an MCL, the Department should defer establishing standards until more data on chronic effects are available.

1. MDH is confident in the basis of the proposed HRL for PFHxS. The available toxicity and toxicokinetic data support the use of NTP 2019 data indicating potent thyroid toxicity from PFHxS exposure.
2. As noted above in PFHxS Question 4-4, MDH does not establish MCLs.

Perfluorohexanoate and Salts

1. *Despite the potential for a greater risk of bias and exposure extrapolation error, the Department chose the short-term study instead of one of the available subchronic, chronic, or developmental studies. Of these, the chronic study by Klaunig et al. (2015) evaluated the standard full suite of organs, clinical observations, clinical pathology, reproduction and developmental effects and cancer following PFHxA exposure and is the logical choice for deriving the proposed HRL.*
    1. All available relevant data for PFHxA was included in MDH's analysis, including Klaunig et al. 2015.
    2. There is no greater risk of bias or exposure extrapolation error. As addressed above, short-term durations exist within (sub)chronic durations.
    3. The NTP 2019 28-day study resulted in a lower guidance value than the available (sub)chronic studies. As addressed above and as laid out in our methodology[1], a lower guidance value from a shorter duration will always supersede a higher value from a longer duration – this is necessary to ensure health protection for all populations across all durations.

2. *While the short-term study reported a decrease in thyroid hormones (i.e., total T4), the inconsistency in findings for thyroid endpoints reported across several study designs reduces the strength of the available evidence.*
    1. The major concerns of thyroid toxicity and PFAS exposure have been addressed above.
    2. Specifically, for PFHxA, from US EPA's draft *Toxicological Review of Perfluorohexanoic Acid and Related Salts*[35]:
        a. "A single study evaluated potential PFHxA effects on endocrine function specific to thyroid hormones in rats exposed for 28 days (NTP, 2018). Specifically, males showed statistically significant, dose-dependent decreases in thyroid hormones. These outcomes showed a clear dose dependent pattern of effect with treated animals showing reductions of 25–73% or 20–58% for free or total T4, respectively. Smaller decreases in T3 in males also were observed (18–29%), although the dose-dependence of this effect was less clear." [Section 3, page 77-78]
        b. The NTP study was rated high confidence by US EPA due to measure of thyroid hormones, organ weight, and histopathology.

3. Moreover, the developmental effects (i.e., decreased pup body weight) reported by Loveless et al. (2009) coincided with evidence of maternal toxicity and generally disappeared after weaning. As a result, the authors noted that "NaPFHx [the sodium salt of PFHxA] is therefore concluded not to present a reproductive or developmental hazard." Similarly, Iwai and Hoberman (2014) also reported pup body weight loss only at does resulting in significant maternal toxicity. The decreases in pup weight were not statistically significant at postpartum day 20, moreover, and the authors reported no differences in terminal body weights among the dosage groups.

1. The more complete statement regarding reproductive or developmental hazard from Loveless et al (2009)[36] is: "The maternal and developmental toxicity NOAEL was 100 mg/(kg day), based on maternal and fetal bodyweight effects at 500 mg/(kg day). NaPFHx is therefore concluded not to present a reproductive or developmental hazard."
2. MDH agreed with these designations at these dose levels. However, the POD used as the basis for the RfD was higher than the Loveless NOAEL (5% decrease in pup body weight was observed), but lower than the Loveless LOAEL (statistically significant 17% decrease in pup body weight was observed).
    a. Statistical significance and biological significance are not equivalent. Decreases of more than 5% in body weight in developing animals are considered an adverse effect. Since it is likely that the decrease in pup body weight would be higher than 5% at doses similar to the POD, decreased pup body weight was included as a sensitive endpoint.
3. The US EPA draft *Toxicological Review of Perfluorohexanoic Acid and Related Salts*[35] determined that the evidence for decreased postnatal body weight represented an adverse health effect and was suitably sufficient to use it as the basis of their RfD [Section 5, page 26 and 32].
4. MDH's analysis agrees with US EPA's assessment and developmental is included as an additivity endpoint in our proposed HRL.

4. As with PFHxS, the Department inappropriately applies a UF_D of 10. In the case of PFHxA, MDH points to concerns about developmental, thyroid, and immunotoxicity. As noted however, the available evidence does not provide support for developmental effects and, while limited, the evidence for thyroid effects is inconsistent. With the exception of changes in thymus weights, the available animal evidence does not show a clear pattern of immune effects across studies.

1. As noted above, the purpose of the UF_D is to account for potential health endpoints that have not experimentally been determined to be irrelevant. For plausible but unstudied/understudied endpoints, it is appropriate to assign a UF_D until the data gap is filled.
2. In both MDH's and US EPA's analyses, developmental was identified as one of the most sensitive health endpoints. Because of PFHxA's effects on developing animals at low doses, there are several outstanding data gaps in the PFHxA developmental database that need to be addressed, including: a 2-generation study, developmental neurotoxicity study and developmental immunotoxicity study. As noted above in the PFHxS response [Question 5], these represent critical data gaps because neonatal immunological and neurological developmental windows are much more susceptible to disruption than in adults.
3. These multiple data gaps warrant the application of a $UF_D = 10$ to ensure an adequate margin of safety for vulnerable populations.

Conclusions

We again thank ACC for their comments on the proposed HRLs for PFBS, PFHxA, and PFHxS. After careful consideration of each comment, and in keeping with our methodology and in accordance with our obligation and authority under Minnesota Statutes 114.0751 and 103H.201, MDH maintains its proposed HRLs for PFBS, PFHxA, and PFHxS in order to "adequately protect the health of infants, children, and adults."

Sincerely,

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Enclosure: Attachment A, Risk Assessment Methodology for Health Risk Limits Derivation, Summarized from 2023 SONAR

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ATTACHMENT A "Risk 101"

Risk Assessment Methodology for Health Risk Limits Derivation, Summarized from 2023 SONAR[1]

The Minnesota Department of Health (MDH) derives Health Risk Limits (HRLs) based on United States Environmental Protection Agency (EPA) risk assessment methods and guidelines. Risk assessment methods require that MDH determine: the health effects associated with a chemical and the lowest dose at which an adverse effect may arise; an evaluation of human exposure; and an integration of these and other considerations that may contribute to human health risk. The following is a brief step-wise description of the approach MDH's scientists use to calculate the HRLs.

An MDH-derived HRL is the concentration of a chemical in drinking water that is likely to pose little or no health risk to humans, including vulnerable subpopulations, based on current levels of scientific understanding. Vulnerable populations vary depending on the chemical of interest, but may include: fetuses, infants, pregnant women, prepubescent childrenn, and others. The HRL concentration is a function of how toxic a chemical is (that is, the minimum quantity that will cause health effects), the duration of exposure, and the amount of water individuals drink during the exposure period. In addition, a HRL value incorporates several adjustment factors to account for uncertainty in our understanding of a chemical's health risks.

1) Toxicity Evaluation – Noncancer Effects

Rather than wait until health effects are evident in humans, the accepted method for assessing potential toxicity to humans is through controlled laboratory studies using mammals (the term "animal" shall be used throughout to describe mammalian species). In toxicity testing, animals are divided into groups and each group is administered one of several doses of a chemical, usually daily, over a set period of time. Testing has two goals: (1.) to identify the hazard or toxic effects caused by the chemical, and; (2.) to evaluate the relationship between the dose and the animal's response. The dose-response relationship may vary depending on when (e.g., the life stage) during the life stage and for how long (duration) the exposure occurred.

In evaluating the dose and the response for noncancer health effects, researchers seek to determine the lowest dose where adverse effects related to dosing are observed (the "lowest observed adverse effect level," or LOAEL) and the highest dose where no adverse effects related to dosing are observed (the "no observed adverse effect level," or NOAEL). By definition, LOAELs and NOAELs can only be a dose used in the study of interest. A newer analysis method, benchmark dose (BMD) modeling, uses statistical modeling to evaluate a dose-response dataset using a pre-determined effect level. Modeling assesses the shape of the dose response relationship and allows scientists to calculate a dose where a given response level (e.g., 10% change in organ weight) is expected to be seen. While not all datasets are compatible with BMD modeling, when feasible, it is preferable to a NOAEL/LOAEL approach because it considers the entire dose-response curve rather than relying on discrete dose points. BMD modeling is now a standard risk assessment practice that is used by many state, federal, and international regulatory agencies; indeed, the US EPA developed and maintains a free-to-use BMD modeling software that is employed by MDH and other states to evaluate appropriate datasets.

The dose resulting from dose-response evaluation (also referred to as a point of departure (POD) dose) serves as the starting point for deriving health-protective concentrations for environmental media.

The dose level selected from the dose-response evaluation of the animal study(s) is identified as a point of departure dose (POD). The dose to the laboratory animal is converted to a human equivalent dose (HED) by adjusting for differences in how these species handle the chemical in the body. An HED represents the dose to humans that would result in the same internal dose as the dose administered to the laboratory animal species, assuming that the toxic response is similar in the two species.

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[1] MDH. 2023 Statement of Need and Reasonableness (SONAR), as cited in MDH 2023 SONAR. (https://www.health.state.mn.us/communities/environment/risk/docs/rules/hrlsonar23full.pdf).

The HED is then reduced by variability and uncertainty factors (UFs) to account for what is not known about a chemical's toxicity to a human population. The factors account for:

- UF_A - uncertainty in extrapolating from animal data to humans (e.g., it may not be known whether humans are more or less sensitive than the test animal);
- UF_H - variation in sensitivity among human individuals (e.g., variability in internal dose levels or sensitivity to the toxicological effects);
- UF_S - uncertainty in extrapolating from effects observed in a short-term study to potential effects from a longer exposure;
- UF_L - uncertainty associated with using a study in which health effects were found at all doses tested (lowest dose was a LOAEL and no NOAEL was identified); and
- UF_{DB} - deficiencies (data gaps) in available data.

In the absence of chemical-specific information, each of the five factors is typically assigned a value between 1 and 10. Values of 1, $10^{0.5}$ and 10 are most common. Values assigned to all factors are multiplied to determine the overall uncertainty factor. By convention, half-power values (e.g., $10^{0.5}$) are factored as whole numbers when they occur singly but as powers or logs when they occur in tandem. For example, individual UFs of 3 and 10 would be expressed as 30 (3×10^1), whereas individual UFs of 3 and 3 would be expressed as 10 ($10^{0.5} \times 10^{0.5} = 10^1$).

The HED is divided by the product of the uncertainty and variability factors to calculate a reference dose (RfD). An RfD is expressed in milligrams of chemical per kilogram of body weight per day (mg/kg-day) and is defined as an estimate of a dose level that is likely to be without an appreciable risk of adverse effects.

2) Exposure

HRLs must be protective against adverse health effects from short-term as well as long-term exposures to contaminants in drinking water. MDH considers sensitive life stages and subpopulations as well as the magnitude and duration of exposure necessary to elicit a toxic effect. Intake rate is expressed as the quantity of water consumed per kilogram of body weight per day (L/kg-day). Studies of water consumption indicate that infants and young children drink more water for their body weight than do adults. Newborns derive all, or nearly all, their nutrition from liquid. Intake rates fall rapidly with age; by age seven, intake rates are nearly the same as those of adults.

MDH uses water intake rates that are recommended by US EPA Exposures Factor Handbook (EPA 2019). These rates are based on data collected from individuals across the US as part of the US Department of Agriculture's Continuing Survey of Food Intake by Individuals (CSFII) survey.

3) Risk Characterization

An RfD incorporates information about the toxicity of a single chemical associated with a given dose. Exposure to a chemical may result from multiple sources. The Groundwater Protection Act requires that MDH use a "relative source contribution" (RSC) factor when deriving HRLs for noncancer effects. The RSC allocates only a portion of the RfD to exposure from ingestion of water, and reserves the remainder of the RfD for other water-related exposures (e.g., inhalation of volatilized chemicals, dermal absorption) as well as exposures via other contaminated media such as food, air, and soil. MDH has relied upon EPA's Exposure Decision Tree approach (EPA 2000) to facilitate determining appropriate default RSC values.

MDH combines the above information into an equation for noncancer health effects:

$$\text{Noncancer HRL } (\mu g/L) = \frac{\text{RfD (mg/kg-d) x RSC x 1,000 } \mu g/mg}{\text{Intake Rate (L/kg-d)}}$$

References:

Minnesota Department of Health 2023. Statement of Need and Reasonableness in the Matter of Proposed Rules Relating to Health Risk Limits for Groundwater. Available online: https://www.health.state.mn.us/communities/environment/risk/docs/rules/hrlsonar23full.pdf