December 4, 2024

RE: Proposed Amendments to Rules Governing Health Risk Limits for Groundwater, Minnesota Rules, Chapter 4717, Part 7500 and Part 7860; Revisor's ID Number R-4803

OAH Docket No. 22-9000-40331

Submitted electronically to the Minnesota Department of Health website here.

Dear Commissioner Brooke Cunningham:

The American Chemistry Council (ACC) respectfully submits the following comments on behalf of its membership on the proposed amendments to Rules Governing Health Risk Limits for Groundwater.

ACC represents over 190 companies engaged in the business of chemistry—an innovative, $639 billion enterprise that is helping solve the biggest challenges facing our nation and the world. The business of chemistry drives innovations that enable a more sustainable future, creates approximately 555,000 manufacturing and high-tech jobs—plus over four million related jobs—that support families and communities, and enhances safety through the products of chemistry and investment in research.

We offer these comments to further inform the Minnesota Department of Health's evaluation and to strengthen the underlying scientific information for the proposal.

Should you have any questions or would like additional information, please contact me at robert_simon@americanchemistry.com or 202-249-6700.

Sincerely,

Robert J. Simon
Vice President
Chemical Products and Technology

Technical Comments on Proposed Amendments to Rules Governing Health Risk Limits for Groundwater, Minnesota Rules, Chapter 4717, Part 7500 and Part 7860; Revisor's ID Number R-4803

I. Overview

In October 2024, Minnesota Department of Health (MDH) proposed permanent rules on PFOS and PFOA, from advisory health-based values (HBV) into promulgated Health Risk Limits (HRL). For each compound, there are two types of HRL values derived: noncancer-based and cancer-based.

A. For the derivation of noncancer-based HRL (nHRL), similar to the recent actions taken by several regulatory agencies (e.g. US EPA and the European Food Safety Authority) in which human epidemiology studies are being considered over experimental animal data for the purpose of risk assessment, MDH took human epidemiology data to derive the nHRLs for PFOA and PFOS. MDH also utilized its breastmilk model where upper-bound water consumption scenarios were incorporated, but the model has not been validated.

PFOA nHRL

MDH derived a nHRL for PFOA based on decreased H. influenza type B (Hib) antibodies in children (Abraham et al. 2020). The study used for the basis of the nHRL was a small cross-sectional study, which due to the nature of its design, cannot determine causality. To date, there is only one other human epidemiological (a longitudinal cohort) study that evaluated the antibody titer to Hib in relationship to PFOA in children and it did not observe an association (Granum et al. 2013). There are two international expert working groups that have independently expressed their opinions in that human epidemiology studies on antibody titers to Hib vaccines were not adequate for risk evaluations in the regulatory setting (Garvey et al. 2023; Burgoon et al. 2023).

PFOS nHRL

MDH (via EPA) selected the study by Wikstrom et al. (2020) for the observation of lower birth weight on the basis of having the lowest point-of-departure estimate (among all other studies that evaluated birth weight). Even though EPA Scientific Advisory Board (SAB) explicitly questioned the scientific rationale of selecting this study beyond having the lowest point of departure (POD), EPA did not follow SAB's recommendation to provide additional detail and justification in selecting the study by Wikstrom et al. for the assessment of serum PFOS and lower birth weight. Wikstrom et al. reported a statistically significant association between maternal PFOS (obtained during first trimester) and birth weight that was only observed in female infants but not male infants. In addition, Wikstrom et al. acknowledged the uncertainty in data interpretation with regard to gender-based difference. Further, use of lower birthweight as a critical effect for deriving the nHRL for PFOS is not appropriate given strong evidence that observed associations are confounded by physiological factors associated with pregnancy, such as plasma volume expansion and changes in maternal GFR which impact the measured PFOS serum levels.

Breastmilk model and water consumption estimation in nHRL

The proposed nHRLs inherit several assumption-based biases and uncertainties with MDH's breastmilk model. Even though the model has not been fully validated, it has been used to estimate an individual's water consumption for several PFAS compounds throughout the entire life stages instead of the traditional water consumption rates that EPA relied on. Recently, a joint commentary by various agencies (including MDH) acknowledged several major limitations in the breastmilk model, including 1) small sample size which precluded a precise estimation of PFAS distribution in breastmilk relative to serum concentration; 2) over-estimation of breastmilk concentration based on (primarily men's) serum PFAS levels from community studies because few women of reproductive age participated; 3) unknown breastmilk concentrations over time during lactation period; and 4) non-breastmilk source may also contribute to actual PFAS exposure (LaKind et al. 2022). Therefore, these uncertainties and the subsequent breastmilk estimates need to be addressed and validated.

B. For cancer-based HRL (cHRL), MDH revised its cancer classifications on both compounds to "likely to be carcinogenic to humans," which are in parallel with the recent changes (upgrades) in cancer classifications by the US EPA (2024) and IARC (2023).

PFOA cHRL

MDH derived a cHRL for PFOA based on renal cell carcinomas in humans (Shearer et al. 2021). A major limitation of this study was that it analyzed only a single PFOA blood measurement taken anywhere between 2 – 18 years prior to kidney cancer diagnosis, which calls into question the reliability of blood measurement. Further, these results were inconsistent with the results obtained in a larger and more ethnically diverse cohort that used a similar study design (Rhee et al. 2023), which found no association between PFOA kidney cancer. Burgoon et al. (2023) also evaluated the study by Shearer et al. (2021) and determined that this human cancer epidemiology study was not appropriate for human risk evaluation. MDH did not take full dose response into consideration given that there are also occupationally exposed data available on kidney cancer. The highest exposed group in the study (chosen by MDH) had serum PFOA levels that were substantially lower than the occupational workers during the same timeframe (1993 – 2001), by at least a hundred-fold lower when compared to the geometric means (Raleigh et al. 2014; Steenland and Woskie 2012; Barry et al. 2013). There were two occupational worker studies that reported null findings between PFOA and kidney cancer (Raleigh et al. 2014; Barry et al. 2013) while the third reported a positive association between serum PFOA and kidney cancer, but it did not adjust for a known confounder, tetrafluoroethylene (TFE), that was present in the workplace (Steenland and Woskie 2012). In addition, the study by Shearer et al. did not adequately address the potential of reverse causation.

PFOS cHRL

MDH (via EPA) selected the 2-year bioassay rat data, as reported by Butenhoff et al. (2012), for the derivation of PFOS cHRL. The point of departure was the observation of a statistically significant increase in the incidence of combined hepatocellular adenoma and carcinoma in rats, even though there was only one rat in which hepatocellular carcinoma was found (and the finding was not statistically significant). Even though there is some general agreement in

certain tumor types where carcinomas can potentially develop from adenomas via the adenoma-carcinoma-sequence, specific mode-of-action (MOA) and key events need to be clearly demonstrated in order to apply such inference (EPA 2005). In the current assessment, the weight of evidence on the supporting MOAs and key events lack consistency or concordance, especially when taking human biological relevance into consideration. Using EPA's guidance as well as other studies that focused on species-specific liver tumor MOAs (Corton et al. 2014; Elcombe et al. 2014; Goettel et al. 2024; Haines et al. 2018; Hall et al. 2012), the biological relevance of hepatocellular tumor observed in rodents is called into question given the known (different) mode of actions that exist between rodents and humans.

For further details pertaining to the high-level summary provided above, more in-depth discussions and supporting information for each topic area are included below.

II. Supporting Technical Comments

Analytical Considerations & Implications

Three of the four HRLs are lower than the current US EPA (USEPA 2024) MCLs in drinking water of 4 ppt for PFOS and PFOA (which was set based on analytical feasibility). Per US EPA's Methods 533 ((USEPA 2019) and 537.1 ((USEPA 2020) (the approved analytical methods developed and validated by the US EPA to support the analysis of 29 PFAS in drinking water), the majority of the HRLs set by MDH are lower than the LCMRL (lowest concentration minimum reporting level), which implies that the guidance values proposed by MDH will be difficult to achieve.

	2024 MDH Health Risk Level (HRL) in Drinking Water, ng/L or ppt		LCMRL (lowest concentration minimum reporting level), ng/L or ppt	
	Noncancer-based HRL	Cancer-based HRL	US EPA Method 533	US EPA Method 537.1
PFOA	0.24	0.0079	3.4	0.82
PFOS	2.3	7.6	4.4	2.7

PFOA nHRL

Selection of the critical study (Abraham et al. 2020) for PFOA nHRL

MDH derived a nHRL of 0.24 ppt for PFOA based on decreased *H. influenza* type B (Hib) antibodies in children (Abraham et al. 2020). This study was a small, cross-sectional study of 101 1-year old infants living in Germany whose blood was measured for levels of PFOA, PFOS and 7 other PFAS and vaccine antibodies against HiB, tetanus and diphtheria between 1997 and 1999. The mean PFOA serum concentration for breast-fed babies was 16.8 ng/mL and 3.8 ng/mL for formula-fed babies. A significant correlation between adjusted Hib antibody levels and PFOA (r=-0.32, p=0.001) was observed. No significant association was observed between Hib antibody levels and PFOS. Additionally, Abraham et al. 2020 reported no influence of PFOA on infections during the first year of life. Although the authors conclude that "*the study results contribute to the cumulative evidence of a causally related effect of PFASs in humans at relatively low internal exposures,"* the authors also acknowledge that "*...since most studies in this field are cross-sectional, data need to be interpreted with caution. More insight is needed into possible mechanisms of action, dose-response relationships and clinical relevance."*

Only one other epidemiologic study has examined the relationship between PFOA and Hib antibodies in children (Granum et al. 2013). This longitudinal cohort study examined, in a subset (n = 51) of children from the Norwegian Mother and Child Cohort study, the associations between maternal serum concentrations of PFOA (median = 1.1 ng/mL) measured at delivery with serum antibody concentrations in offspring who had followed a routine vaccination program, where vaccines against Hib were administered at ages 3, 5, and 12 months. The authors reported a non-significant association between pre-natal exposure to PFOA and Hib antibodies (β = -0.05, p=0.978). These findings are inconsistent with the findings reported in Abraham et al. 2020.

An international working group of scientific experts collaborated on a project entitled "The Perfluorooctanoate (PFOA) Safe Dose" in 2022 (Burgoon et al. 2023). This project, supported by the Alliance for Risk Assessment, included three independent technical teams with a total of 24 scientists from 8 countries who were tasked with reviewing the relevant information and the positions of various national authorities and other authoritative sources to determine their safe dose ranges. The scientific teams then developed consensus statements on the mode of action, critical effect, and extrapolation method. Regarding the observed associations between PFOA blood concentrations and antibody responses to vaccines, the working group concluded that the existing epidemiological data were not suitable for developing a safe dose since these assessments were based on a secondary immune response (i.e. response to vaccines) rather than a primary immune response. Working group members also questioned the clinical relevance of small decreases in antibody responses to vaccines because of the vast inter- and intra-individual human variability. It was concluded that "this variability precludes any definitive statement in the choice of this endpoint as the critical effect" (Burgoon et al. 2023).

In 2022, a systematic review and meta-analysis was published on epidemiological studies that examined the effects of PFAS on vaccine antibodies in healthy children (Zhang et al. 2022). Authors used the Grading of Recommendations Assessment, Development, and Evaluation system (GRADE) to evaluate the quality of all the results found in each study, which was expressed by four levels of certainty rating (i.e. "high", "moderate", "low", or "very low"). Based on the only two existing studies (Abraham et al. 2020; Granum et al. 2013) that specifically analyzed PFOA and Hib in children, the authors concluded that the overall judgement was "low" in their GRADE assessment for the association between exposure to PFOA and Hib antibody levels.

In 2023, a systematic review and meta-analysis was conducted to determine, in people of all ages, the magnitude of the association between PFAS serum concentration and the difference in antibody concentration following a vaccine (Crawford et al. 2023). The study included 4830 unique participants across 14 reports. Overall, the authors concluded that data on diphtheria, rubella and tetanus were most supportive of an association than for other antibodies (including Hib antibodies); however, the data on any specific antibody were scarce and confounding factors that might account for the relation were not identified.

In sum, the study by Abraham et al. 2020 should not be used as the basis for the nHRL for PFOA given the inherent limitations of the cross-sectional study design, small sample size, potential for confounding, limited and inconsistent evidence of the association between PFOA levels and Hib antibodies, and the lack of human relevance.

PFOS nHRL

Selection of the critical study (Wikstrom et al. 2020) for PFOS nHRL

MDH derived a nHRL of 2.3 ppt for PFOS based on decreased birthweight in infants (Wikstrom et al. 2020). This study measured maternal serum levels of PFOS (and other PFAS) in early pregnancy and birthweight in 1533 infants enrolled in the Swedish Environmental, Longitudinal, Mother and child, Asthma and allergy (SELMA) study. Given that serum sampling later in pregnancy may be related to issues of confounding and reverse causation (a type of bias and occurs when measurement of the physiological outcome has been *moderated by the health outcome itself)*, this study measured

serum PFOS during the first trimester (at a median of 10 weeks gestation) with 96% during the first trimester and the remaining samples collected early during the second trimester.

The authors reported a statistically significant association between lower birthweight and maternal PFOS (142-gram lower birthweight in the highest PFOS exposure category of >7.6 ppb relative to the lowest PFOS exposure category); however, this statistical association was only observed in female infants – not male infants, which makes the finding difficult to interpret. The authors acknowledged that the mechanisms behind the influence of PFAS on fetal growth and suggested sex-differences are largely unknown.

In selecting Wikstrom et al. 2020 as the basis for its nHRL for decreased birthweight, MDH did not consider the best available peer-reviewed science which suggests that the observed association between PFOS and lower birthweight is an artifact of pharmacokinetic bias. Specifically, meta-analyses support that the timing of serum measurements during pregnancy (late vs. early) confounds the observed relationship between PFOS and lower birthweight (Dzierlenga et al. 2020; Negri et al. 2017; Verner et al. 2015) and modeling to attempt to control for this confounding results in virtually no effect attributable to PFOS at all (Dzierlenga et al. 2020).

The most recent meta-analysis (Dzierlenga et al. 2020) examining the association between birthweight and PFOS concentrations, included observations from 29 studies. When observations were stratified by the timing of PFOS measurements during pregnancy (i.e. before or early in pregnancy and later in pregnancy), the random effects summary for the early group was -1.35 (95% CI: -2.33, -0.37) and -7.17 (95% CI: -10.93, -3.41) for the latter group. When the authors included a term for timing of blood draw in a meta-regression model, the intercept was essentially zero (0.59 g/ng/ml; 95% CI: -1.94, 3.11) indicating that when blood samples were drawn very early in pregnancy, there was no association between birthweight and PFOS. The authors concluded that "the time of blood draw was a key factor in the association and that there was no significant association present when PFOS is measured at the beginning of pregnancy, which supports the possibility of confounding related to timing of specimen sampling."

The results of the meta-analyses conducted to date indicate that associations between PFOS serum measurements and birthweight are driven almost entirely by physiological aspects of pregnancy, including plasma volume expansion, maternal GFR, and when the maternal PFAS measurement was made during gestation. These are critical points to evaluate.

A new study was published in 2024 that examined pregnancy complications and birth outcomes (including birthweight) following low-level exposure to PFAS (Begum et al. 2024). This study included a racially diverse cohort of 459 pregnant mothers across the U.S. which was weighted towards minority populations (black, 44%, white, 38% and other, 17%). PFOS (and other PFAS) were measured between 32-38 weeks' gestation. The median PFOS serum concentration for the 459 pregnant mothers was 2.7 ng/mL. In the adjusted multivariate linear regression analysis, the study reported a non-significant *increase* in birthweight in relation to PFOS levels ($\beta = 0.04$; 95% CI: -0.20-0.28).

In sum, Wikstrom et al. (2020) should not be selected as the critical study for its PFOS nHRL based on the findings of meta-analyses that indicate that pharmacokinetic bias resulting from the timing for serum measurements during pregnancy explains the observed association between serum

levels of PFOS and lower birthweight. Moreover, the study by Wikstrom et al. (2020) showed sex-differences (i.e. no association observed in male infants) in the relationship between PFOS and lower birthweight and the mechanisms behind the influence of PFAS on fetal growth and sex are not known.

Breastmilk model and water consumption estimation in nHRL

Starting around 2018, MDH began using the breastmilk model to estimate an individual's water consumption in its risk assessment process when developing PFAS water guidance values. To our best knowledge, the MDH breastmilk model has never been validated (against empirical data). When compared to the standard water consumption factors (from EPA's exposure handbook) on which other federal agencies relied, the breastmilk model incorporated excessive water-consumption scenarios for the child-bearing women (pre-, during-, and post-pregnancy) as well as the offsprings (from developing fetuses and continuously into adulthood). These assumption-based scenarios contributed to many uncertainties in the risk assessment process.

In 2022, a joint commentary authored by various entities and agencies, including MDH, acknowledged several major shortcomings of the breastmilk model (LaKind et al. 2022). They include:

1) small sample size (of paired serum and breastmilk samples) which precluded a precise estimation of PFAS distribution in breastmilk relative to serum concentration and subsequently, a reliable estimation of breastmilk: serum partition coefficient for different PFAS compounds;

2) very limited breastmilk PFAS data in the US and Canada do not allow for good estimation of breastmilk PFAS concentration in general; the inferred breastmilk data from community studies were especially vulnerable for over-estimation bias because there were limited participants that were of reproductive age;

3) while the MDH breastmilk model intends to capture one's PFAS exposure via breastmilk consumption throughout the entire lactation period, there has not been a study evaluating the breastmilk PFAS concentrations over time during lactation period; as such, the current MDH breastmilk model may have either over- or underestimated the actual PFAS concentration present in the breastmilk;

4) non-breastmilk source (e.g. infant formula and dietary food source) may also contribute to actual PFAS exposure; these were not taken into account by the MDH breastmilk model.

Therefore, it is important for these uncertainties to be addressed, and the reported breastmilk estimates to be validated.

PFOA cHRL

Selection of the critical study (Shearer et al. 2021) for PFOA cHRL

MDH derived a cHRL of 0.0079 ppt for PFOA based on renal cell carcinomas in humans (Shearer et al. 2021). This case-control study identified 324 cases of renal cell carcinoma (RCC) and 324

matched controls among 75,000 participants of a multi-site study from medical centers in 10 US cities. The subjects had a single blood (serum) measurement taken upon entry into the trial. Archived samples were measured for PFOA and, on average, were collected approximately 8 years prior to the diagnosis of kidney cancer (range 2 – 18 years) which is an important limitation of the study. Shearer et al. states the long half-life of elimination of PFOA indicates that a single serum measurement could be sufficient to provide an accurate and precise measurement of a person's long-term PFOA exposure. This assertion ignores the considerable uncertainty regarding the distribution, calculation, and measurement biases associated with the serum elimination half-lives of PFOA in humans. Shearer et al.'s (2021) conclusion that a single PFOA measurement is sufficient based on PFOA's long-half life in humans contradicts fundamental considerations of the connection between toxicodynamics, toxicokinetics, and time. This highlights the limitations of using serum concentrations measured 2 to 18 years prior to the diagnosis of the disease. This discrepancy limits the accuracy of the reported serum concentrations in Shearer et al. (2021). In a recent study examining the reliability of a single blood sample to represent long-term exposure of PFOA among men, the authors reported that a single baseline serum sample represented "rather well" the mean of repeated samples collected up to 13 years apart (Bartell et al. 2024). However, the study did observe lower correlations over time with strong biases towards the null when using single serum samples further back in time. The authors concluded that "More research is needed to evaluate the reliability of single blood sample for representing long-term exposure for epidemiological studies of PFOA among women and children."

Shearer et al. (2021) reported a statistically significant positive association with RCC risk and a doubling in PFOA serum concentration (adjusted odds ratio, OR = 1.68; 95% CI: 1.07 to 2.63) and a greater than twofold increased risk among those in the highest PFOA exposure group compared with the lowest exposure group (adjusted OR = 2.19; 95% CI: 0.86 to 5.61). It is important to note that the highest exposure group in this study had serum concentrations ranging from 7.3 – 27.2 ppb which was substantially lower than serum concentrations observed in occupational populations during the same timeframe. MDH did not consider any of the three occupational studies that have been published (Barry et al. 2013; Raleigh et al. 2014; Steenland and Woskie 2012), which likely represent the highest exposed individuals based on overall reported biomonitoring data. And of these three studies, only one analysis showed a statistically significant association with kidney cancer (mortality); however, this finding was likely confounded by the authors' decision to not adjust for TFE exposure – a known renal carcinogen in rodents (Steenland and Woskie 2012). For Barry et al. (2013), overall, they did not find an association between kidney cancer and PFOA in occupational workers nor did they observe a significant trend in increasing risk.

Shearer et al. (2021) also did not adequately address reverse causation, which is a type of pharmacokinetic bias and occurs when measurement of the physiological outcome (e.g. estimated glomerular filtration rate, eGFR) has been *moderated by the health outcome itself*. The pharmacokinetic bias occurs when there is a sufficient window of time for the disease state to influence the measured physiological outcome. EPA's IRIS Handbook recommends evaluating epidemiological studies for reverse causality and if reverse causality is a concern in the observed association of the exposure and health outcome, then a study should be labelled as deficient or critically deficient. In Shearer et al. (2021), the lack of an association between eGFR, PFOA, and kidney cancer does not conclusively demonstrate a lack of reverse causation, but it should have

been considered as a factor because the eGFR was measured, on average, 8.8 years *prior to* the diagnosis of kidney cancer. There is the possibility of pre-diagnostic conditions that result in declining renal function, but such a conclusion is highly speculative. Therefore, it is erroneous for Shearer et al. (2021) to suggest the lack of an association between a single eGFR measurement, and the diagnosis of kidney cancer eliminates the concern about this type of pharmacokinetic bias in the association between the exposure to PFOA and kidney cancer.

Two more recent epidemiological studies have reported no association or inconsistent associations between PFOA and kidney cancer (Rhee et al. 2023; Winquist et al. 2023). Rhee et al (2023) conducted a case-control study including 428 RCC cases and 428 match controls in a racially and ethnically diverse population. Pre-diagnostic serum concentrations were measured for PFOA and other PFAS compounds. Overall, PFOA was not associated with RCC risk (OR = 0.89, 95% CI: 0.67-1.18). Among White participants, a positive but non-statistically significant association was observed for PFOA and RCC risk (OR = 2.12, 95% CI: 0.87-5.18). No associations were observed between PFOA and risk of RCC in other racial and ethnic groups. Moreover, PFOS was statistically significantly associated with a *decreased* risk of RCC among African Americans (OR=0.40, 95%CI: 0.20-0.79) and Whites (OR = 0.36, 95% CI: 0.13-0.95).

In a case-cohort study, within the American Cancer Society's prospective Cancer Prevention Study II, Winquist et al. (2023) observed no association between PFOA and risk of kidney cancer (n=158 kidney cancer cases). However, in a sex-specific analyses, they reported an elevated, but non-statistically significant association between PFOA and kidney cancer (HR = 1.33, 95% CI:0.97-1.83) among women (though there was a statistically significant association in females between PFOA and renal cell carcinoma). No associations between PFOA serum concentrations and kidney cancer were observed among men.

In 2022, an international working group of scientific experts collaborated on a project entitled "The Perfluorooctanoate (PFOA) Safe Dose" (Burgoon et al. 2023). This project, supported by the Alliance for Risk Assessment, included three independent technical teams with a total of 24 scientists from 8 countries who were tasked with reviewing the relevant information and the positions of various national authorities and other authoritative sources to determine their safe dose ranges. The scientific teams then developed consensus statements on the mode of action, critical effect, and extrapolation method. Regarding the Shearer et al. 2021 study, the working group discussed that "While Shearer et al. (2021) adjusted their results for estimated glomerular filtration rate (eGFR), adjusting for eGFR alone would not adequately control for this potential confounding due to the extensive role of renal transporters in the clearance of PFOA." Further, the working group concluded that the available epidemiologic data could not be used as a reliable basis for a PFOA safe-dose assessment considering the lack of information regarding the mode of action (Burgoon et al. 2023).

Given the important limitations of Shearer et al. (2021) including the use of a single serum measurement, potential for confounding and reverse causation, and the findings of inconsistent or no associations reported in recent studies, a ccHRL for PFOA should not be derived based on renal cell carcinomas in humans.

PFOS cHRL

Selection of the critical study (Butenhoff et al. 2012) for PFOS cHRL

MDH proposed to adopt a cHRL of 7.6 ppt for PFOS based on the final assessment done by the US EPA in the derivation of MCLG. The critical study used to determine the upgrade of PFOS cancer classification was based on a 2-year bioassay data in Sprague Dawley rats, in which dietary potassium PFOS was given to rats for up to 20 ppm for two years. The entire dataset was available to the regulatory agencies for risk assessment evaluation since the completion of its final report in 2002 (Thomford 2002). Even though the key data were later published as Butenhoff et al. (2012) in a scientific journal, there has not been any additional data appended to the original dataset. Given the numerous risk assessment evaluations that both EPA and MDH have formally conducted over the last two decades on PFOS, the classification on PFOS had always been "suggestive" or "possibly." In MDH's most recent classification on carcinogenicity potential (via US EPA's MCLG assessment), however, PFOS was upgraded to "likely to be carcinogenic to humans" solely based on a different statistical analysis and not any new data. However, there are compelling scientific data and evidence why the current cancer classification by MDH for PFOS is mis-classified.

First, it is important to note that PFOS treatment did not affect the survival in rats in the 2-year cancer bioassay. In fact, the PFOS-treated rats had higher survival than the control rats. This observation is in direct contrast to other known carcinogens, such as benzene, in which decreased survivals are observed in rodents (IARC 2012).

Second, in the (only) 2-year cancer bioassay data available to date (Butenhoff et al. 2012; Thomford 2002), the only notable neoplastic observation in rats due to potassium PFOS treatment was a statistically significant increase in benign hepatocellular adenomas in both male and female rats when potassium PFOS was administered at the highest dietary dose (20 ppm), see Table 1A (*vide infra*). While there was only one hepatocellular carcinoma observed which was a 20 ppm dose group female rat, the study authors did not consider this single isolated observation of hepatocellular carcinoma in and of itself significant.

Third, while the distinct histological feature and presentation have served as the key anchoring points by which risk assessment and decision processes can differentiate a benign tumor (i.e. adenoma) from a malignant tumor (i.e. carcinoma), in the latest EPA MCLG assessment for PFOS, it combined both hepatocellular adenoma and carcinoma data together. It is not surprising that the statistical significance observed in adenoma data alone can and did contribute to the statistical significance of the combined adenoma/carcinoma incidence (Table 1A, *vide infra*).

Fourth, as a standard and conventional method of calculating liver tumor incidence shown on Table 1A for female rats, the total tumor incidence rate calculated by Butenhoff et al. 2012 was based on the total number of the tissues examined per specific dose group upon study termination at the end of two years. The US EPA, on the other hand, calculated the tumor incidence rate for female rats based on the number of animals alive at the time when the tumor first occurred (Table 1B), which excluded a subset of rats from control (n=10) and the highest dose group (n=10) that were sacrificed at week 52. The latter method done by the US EPA inflated the % incidence even though the dataset remained unchanged, and this difference in statistical analyses contributed to PFOS

being associated with increased incidence in hepatocellular adenoma/carcinoma combined, albeit the statistical association was primarily due to adenoma, not carcinoma.

Table 1A

	From Butenhoff et al. 2012				
	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
Adenoma (% incidence)	0/60 (0%)	1/50 (2%)	1/49 (2%)	1/50 (2%)	5/60* (8%)
Carcinoma (% incidence)	0/60 (0%)	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/60 (2%)
Combined adenoma/carcinoma, (% incidence)	0/60 (0%)	1/50 (2%)	1/49 (2%)	1/50 (2%)	6/60* (10%)

Table 1B

	From US EPA, 2023				
	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
Adenoma (% incidence)	0/28 (0%)	1/26 (4%)	1/15 (7%)	1/28 (4%)	5/31* (16%)
Carcinoma (% incidence)	0/28 (0%)	0/29 (0%)	0/16 (0%)	0/31 (0%)	1/32 (3%)
Combined adenoma/carcinoma, (% incidence)	0/28 (0%)	1/29 (3%)	1/16 (6%)	1/31 (3%)	6/32* (19%)

*statistically significant p <0.05 relative to control

Fifth, it should be noted that the key event data used by EPA to support the relevant MOA lacks consistency. While the nuclear receptor PPARα and its role in liver tumor development has been largely accepted as a rodent-specific event (Corton et al. 2014), in US EPA's MCLG document, it stated the following with regards to the mode of action for hepatic tumors: "Specifically, the available studies provide varying levels of support for the role of several plausible MOAs: nuclear receptor (PPARα and CAR activation), HNF4α suppression, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression".

> *MOA & nuclear receptors:* on the nuclear receptor, the weight of evidence consideration on the key events showed inconsistency and a lack of dose response (see an example of "Table 3.23" below, excerpted from EPA's final MCLG document). Albeit each of the MOA evidence tables was constructed with escalating doses (presumably to show a dose response), the doses listed in the table actually were from several different studies, each with different study design as well as different life stages of the animals (i.e. pups at weaning, young adult rats, and aged geriatric rats), and the latter certainly plays an important role in many of the cell growth-related parameters such as cell signaling (and not surprisingly, accompanying enzyme changes).

"Table 3-23", excerpted from EPA Final MCLG toxicity assessment for PFOS

Table 3-23. Evidence of Key Events Associated With the PPARα Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	PPARα Activation[c]	Altered Cell Growth Signaling	Hepatic Cell Proliferation	Apoptosis	Preneoplastic Clonal Expansion	Hepatic Tumors
0.024	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.098	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.242	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.312	↑ (4w)	NR	NR	– (4w)	NR	NR
0.625	↑ (4w)	NR	NR	– (4w)	NR	NR
0.984	↑ (4w) – (14w)	↑ (4w)	↑ (4w) – (14, 53w)	↓ (103w) – (14, 53w)	NR	↑ (103w)
1	↑ (F1 PND 21)	NR	NR	NR	NR	NR
1.25	↑ (4w)	NR	NR	– (4w)	NR	NR
1.33/1.51	– (4, 14w)	NR	– (4w)	NR	NR	NR
1.66	↑ (28d) – (1, 7d)	NR	↑ (7d) – (1, 28d)	↑ (7d) – (1, 28d)	NR	NR
1.93	– (7d)	NR	↑ (7d)	↓ (7d)	NR	NR

Table 2 shown below details the source of the studies where the doses in "Table 3-23" originated from. It is clear that the MOA assessment did not take all these intrinsic factors into account when integrating for the evidence of key events.

Table 2

Doses (mg/kg/day)	Study Duration	Dosing Route	Reference
0.024	2 years	Dietary	Butenhoff et al. 2012
0.098			
0.242			
0.312	28 days	Oral gavage	NTP 2019
0.625			
0.984	2 years	Dietary	Butenhoff et al. 2012
1	21 days	Lactational	Chang et al. 2009
1.25	28 days	Oral gavage	NTP 2019
1.31 / 1.51	4- and 14-weeks	Dietary	Seacat et al. 2003
1.66	1-, 7-, and 28-days	Dietary	Elcombe et al. 2012
1.93			

MOA & HNF4α suppression: in addition to the nuclear receptors, EPA MCLG also cited HNF4α suppression as a plausible MOA for eliciting liver carcinogenicity. Liver HNF4α, a

transcription factor, controls various facets of liver pathways. While it is continued to be studied for its exact role(s), EPA MCLG cited a single study that showed PFOS can lead to HNF4α suppression which corresponded to a downregulation in its target gene CYP7A1 under via both in vitro and in vivo conditions – a finding that was not consistently observed by other published toxicology studies (which some had reported PFOS was associated with increased CYP7A1 levels (Chang et al. 2009; Rosen et al. 2010)).

MOA & genotoxicity, cytotoxicity, and oxidative stress: while there were studies reporting positive findings in genotoxicity and oxidative stress with PFOS, many of these studies were conducted *in vitro* and typically at high and cytotoxic concentrations which reflected the likely consequence of cytotoxic disruption of normal cellular processes and not a specific genotoxic or oxidative stress effect. Under a battery of guideline-driven genotoxicity and mutagenicity tests, PFOS has not been shown to pose a direct mutagenic or genotoxic risk (see USEPA 2024).

MOA & immunosuppression: Albeit there were studies reporting on the potential effect of PFOS and immunotoxicity in mice and a few of them had been used by the regulatory agencies for their risk assessment (Dong et al. 2011; Dong et al. 2009), none of these studies evaluated immune functions in a thorough and comprehensive matter, which is the fundamental principle because immunology is a rather complex process. Using the most up-to-date techniques with an emphasis on the dynamic (non-static) response of immune functions (versus the single measurement other studies had reported), the multi-discipline analyses of both primary[1] and secondary[2] immune marker analyses did not reveal evidence of immune suppression in the mice with PFOS even after 28 daily doses (Pierpont et al. 2023; Torres et al. 2021). The study conclusion was further solidified with a concurrent comparison to mice that were treated with a positive control compound, cyclophosphamide, which is a well- known immune suppressant in mice and has been used widely in tissue transplant medicine in humans. Cyclophosphamide-treated mice exhibited a wide array of biological response such as decreased body weight, reduced overall immune cell populations in thymus, bone marrow, and spleen, as well as reduced serum immunoglobulins.

In sum, MDH's classification on PFOS carcinogenicity potential (via US EPA's assessment) was based on a different statistical analysis and no new data from a 2-year bioassay in rats that has been available for years and repeatedly analyzed previously. There was no excess incidence of hepatocellular carcinoma (only an isolated single hepatocellular carcinoma in one female rat); only benign hepatocellular adenoma was observed with statistical significance (the latter has been well-documented to be a likely rodent-specific response). Furthermore, as documented by EPA's own guidance (*vide supra*) as well as other studies that focused on liver MOA (Corton et al. 2014;

[1] Primary immune markers include fundamental metabolic endpoints such as body weight, hematology data, organ weights, immune cell populations (on thymus, spleen, bone marrow, lymph node, blood, and liver), gross pathology, and histopathology.

[2] Secondary immune markers evaluate the functional aspect of immune cells which include cell-based assays (e.g. NK cell activity or neutralize antibody activity), imunoassays (e.g. antibody levels or cytokine levels), and flow cytometry assays (e.g. receptor binding or surface and cytoplasmic immunophenotyping).

Elcombe et al. 2014; Goettel et al. 2024; Haines et al. 2018; Hall et al. 2012), the biological relevance of nuclear receptor-mediated hepatocellular tumor observed in rodents is further called into question given the known mode of action differences that exist between rodents and humans.

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