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ORANGE COUNTY WATER DISTRICT

ORANGE COUNTY'S GROUNDWATER AUTHORITY

November 20, 2019

Transmitted via Email

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Subject: Comments on Draft Technical Report 598 (TR-598): NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats

Dear Dr. Wolfe:

The Orange County Water District (OCWD) appreciates the opportunity to offer comments on the above-referenced National Toxicology Program (NTP) Draft Report. OCWD's comments can be found in the attached report, which has been prepared by Intertox, our technical consultant. There are several areas where we recommend that NTP provide additional information in order to increase the transparency and clarity of Technical Report 598, including:

- Expand the discussion of the mode of action (MOA) and note any potential limitations in applying this MOA to human exposures.
- Include a discussion of why the selected strain of rat was chosen as the model for human exposures and endpoints.
- Include a discussion of the interpretation of the dose-response relationship and extrapolation to low doses.
- Increase the transparency in what the "below detection" (BD) measurements are.
- Expand the discussion of the epidemiological literature to include data on liver and pancreatic cancers.

Please don't hesitate to contact me via email to [redacted] should NTP have any questions regarding OCWD's comments.

Sincerely,

[redacted]
Jason Dadakis
Executive Director of Water Quality & Technical Resources

Attachment: Intertox Report

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COMMENTS TO NATIONAL TOXICOLOGY PROGRAM ON *NTP TECHNICAL REPORT ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF PERFLUOROOCTANOIC ACID (CAS NO. 335-67-1) ADMINISTERED IN FEED TO SPRAGUE DAWLEY (HSD:SPRAGUE DAWLEY® SD®) RATS*

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1.0 INTRODUCTION

The National Toxicology Program (NTP) has released the Draft Technical Document "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats" (NTP report) and is currently requesting public comments. The study was conducted by NTP, a division of the National Institute of Environmental Health Sciences (NIEHS) that performs testing on chemicals. NTP has standardized approaches to testing and is considered an authoritative body for the evaluation of chemicals.

While the NTP report and data tables are generally well documented, we have identified several areas where we recommend that NTP provide additional information to increase the clarity and transparency of the study report. In this document, we briefly describe the NTP study and provide our comments on the study rationale and design, results and interpretation, and conclusions.

2.0 DESCRIPTION OF THE NTP STUDY

The NTP Report describes the methods, analysis, and results of a feeding study in rats. Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were exposed to 0, 150, or 300 ppm PFOA during the perinatal (gestation and lactation) period, after which the F1 male rats were provided 150 or 300 ppm PFOA (i.e., perinatal/postweaning exposures of 0/0, 0/150, 150/150, 0/300, and 300/300 ppm) in feed and the F1 female rats were provided 300 or 1,000 ppm PFOA (i.e., 0/0, 0/300, 150/300, 0/300, and 300/1,000 ppm) in feed during the postweaning period (n = 50/sex/dose) (Study 1). The impact of perinatal exposure was also compared with postweaning exposure by evaluating perinatally- and postweaning-exposed groups to rats with post weaning exposure only. The authors compared exposed offspring rats to unexposed controls at a 16-week interim sacrifice and at 2 years of age..

Following an interim sacrifice at 16 weeks, the study of male offspring was discontinued due to unanticipated toxicity (it is unclear what the specific health endpoints were). Toxicity was observed in the liver, glandular stomach, kidney, and thyroid gland in males and in the liver, kidney, and thyroid gland in females at the 16-week interim evaluation. The male study was started again with a new cohort of animals (Study 2) with lower post weaning exposure doses of 20, 40, or 80 ppm; dams in the second study were given 0 or 300 ppm as this was tolerated. The study continued for two years.

NTP's primary conclusions were:

- "Under the conditions of these 2-year feed studies, there was clear evidence of carcinogenic activity of PFOA in male Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas. The additional effect of combined perinatal and postweaning exposure was limited to a higher incidence of hepatocellular carcinomas in male rats compared to postweaning exposure alone."
- "There was some evidence of carcinogenic activity of PFOA in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure. The combined perinatal and postweaning exposure was not observed to change the neoplastic or

> nonneoplastic response compared to the postweaning exposure alone in female rats. Perinatal exposure to PFOA in male rats led to higher incidence of liver tumors compared to male rats without perinatal exposure."

3.0 COMMENTS ON THE NTP STUDY

Based on the NTP report, the study was conducted using standard approaches and analyses, although not based on a guideline (e.g., OECD or EPA guideline). Despite the interim finding of unexpected toxicity in male offspring, the rationale and process of restarting the study was effectively described in the report. The following suggestions for NTP with regard to finalizing the report pertain to increasing the clarity and transparency of the report.

3.1 Comments on NTP's Study Rationale and Design

One of the key issues in the interpretation of the study is its applicability to human exposures as PFOA acts via a peroxisome proliferator activated receptor alpha (PPARα)-activated mechanism of action (MOA). Many different chemicals are PPARα activators and most do not interact directly with the PPARα (a receptor) to cause a downstream effect (Corton *et al*., 2018). There is no dispute that PFOA is a PPARα activator and can induce liver and pancreatic tumors (IARC, 2017; ATSDR, 2018); however, there is concern that this might also lead to tumor formation in humans.

Other animals, notably male rats, express PPARα in higher levels than humans. For example, male rats have more PPAR than female rats (Jalouli *et al*., 2003) and humans (Corton *et al*., 2018). Specific to rats, a "tumor triad" of liver cancer, Leydig cell tumors (not seen in the NTP study), and pancreatic acinar cell tumors has been reported with PPARα activators, including PFOA (Peraza *et al*., 2006). Tumors in these tissues are not reported in hamsters, guinea pigs, and nonhuman primates—animal models that are better human surrogates due to expression and functional similarities than mice and rats for PPARα effects (Corton *et al*., 2018). In addition to differences in expression levels, other functional differences in PPARα may contribute to species differences:

> These include the cellular expression patterns of PPARα and many other co-effector proteins that interact with PPARα, cellular expression patterns of chromatin remodeling proteins, the relative availability of chromatin for PPARα binding sites, and differences in the stoichiometry and relative binding affinities between all of these variables. These differences likely determine, at least in part, the underlying basis for human-rodent differences in PPARα activator biological effects. (Corton *et al*., 2018).

The underlying question is not whether PFOA can activate human PPARα, but rather does exposure to PFOA lead to liver and pancreatic tumors in humans. In addition to the reduced expression and potential functional differences, in *in vitro* studies, activation of human PPARα also does not mediate growth of hepatocytes (Corton *et al*., 2018).

The NTP study measured liver acyl-CoA oxidase activity as evidence of PPARα induction in rats sacrificed at 16-weeks. The NTP report notes:

> At the 16-week interim necropsy in this 2-year study, liver acyl-CoA oxidase enzyme activities were elevated in male and female rats. The hepatocellular hypertrophy and cytoplasmic alteration are likely due to peroxisome proliferation, but may also be mediated through CAR activation or possibly other mechanisms.

The NTP study also evaluated aromatase activity to determine if tumors were hormone related. There was an increase in aromatase activity in Study 2, but not Study 1 with no explanation. The lack of tumors was attributed to lower doses than tested in previous studies and/or strain differences.

The choice of animal model also increased the likelihood of seeing an increase in pancreatic tumors, given that the historical control range for this sex (male) and strain (Hsd:Sprague Dawley® SD® rat) of this type of tumor is up to 28%. NTP states that the incidence of pancreatic adenomas was significantly increased in exposed male animals vs. controls. However, in the current study, the incidence of pancreatic tumors in control animals was 4-6%, which is relatively lower than has been reported in historical controls. Specifically, the NTP report notes:

> Additionally, the Hsd:Sprague Dawley® SD® rat appears to have a higher background incidence of pancreatic acinar neoplasms, up to 28%, historically, in controls, thus they may be more sensitive to these neoplasms compared to the other rat stocks used previously. For example, NTP studies with the Wistar Han and F344/N rats appear to have a lower background incidence for pancreatic acinar cell neoplasms131; 132 and Crl:CD (SD) Sprague Dawley rats are reported to have a background incidence of <1%.

Recent reviews have noted that, while liver and pancreatic tumors are consistently reported in rats exposed to PPARα activators, these endpoints are probably not relevant to humans. For example, IARC (2019) notes:

> An example of this category is the group of peroxisome proliferator-activated receptors (PPARs), which are involved in lipid metabolism but are also activated by xenobiotics with peroxisome proliferating activity; the PPARα subtype has been implicated in the hepatocarcinogenicity in rats of some of these xenobiotics, which **are probably not human hepatocarcinogens**[1] (emphasis added; IARC, 2019).

In a review of PPARα-dependent liver tumor response in rodents, Corton *et al*. (2018) notes:

> The two aforementioned reviews [Klaunig *et al*., 2003; Corton *et al*., 2014 as cited in Corton *et al*., 2018] on the role of PPARα in liver cancer were the consensus of lengthy literature synthesis and debate among many stakeholders including those from industry, academia, and regulatory agencies. The analysis of the MOA included assessment of the associations between the KEs and liver tumor formation with respect to: (1) strength, consistency and specificity, (2) temporal relationships between the KEs and the liver tumors, (3) the dose–response aspects of the KEs, biological plausibility and coherence of the KEs, and (4) evaluation of possible alternative MOAs. The participants in these efforts uniformly agreed that there was enough information to conclude that there is an established MOA for rodent liver tumor induction by PPARα activators, and that **the MOA is either "not relevant" or "not likely to be relevant" to humans**[1] (emphasis added; Corton *et al*., 2018).

Thus, the findings in the NTP study of increases in pancreatic and liver cancers in rats with sufficient PFOA dose and exposure are consistent with previous studies. The NTP report also supports that this occurs through a PPARα MOA. As the relevance of these types of cancer is questionable in humans, and the results of this study may be used as a Point of Departure (POD) in human health risk assessment, NTP should increase the discussion of this important caveat. We also request that NTP include a discussion of why this strain of rat was chosen as the model for human exposures and endpoints.

[1] In text references removed for readability. Please see original text for references.

3.2 Comments on NTP's Results and Interpretation

Regarding the results, there are two points for which NTP could provide additional information to clarify the results reported.

First, the dose-response curve is very flat for pancreatic tumors. For example, in Study 2, the overall rates for acinar cell adenoma or carcinoma in 2-year old males were 58%, 52%, and 64% for the 20, 40, and 80 ppm dose groups, respectively. The increase in tumor incidence relative to controls (6%) was significant at all doses. However, because there was little difference in response across the three doses, there is uncertainty in the extrapolation to lower doses. An explanation of what occurred andif it is related to the MOA or strain of rat would be useful.

Second, it appears that some of the control animals had detectable PFOA in plasma. The NTP report lists all control data as "BD" or "Below Detection," with BD defined as "BD = below detection; group did not have over 20% of its values above the limit of quantification. In these cases, no statistical analyses were performed." However, when the individual animal tables are examined,[2] the concentrations are sometimes listed as "None Detected" and sometimes as "Below Limit of Quantitation." Based on the variation in the description, it appears that there is a detectable PFOA, but it is listed as "Below Limit of Quantitation." If NTP has detectable values for these points, it would be more transparent to report these values and note they are below the limit of quantitation. It would be useful to understand what could be the source.

3.3 Comments on NTP's Conclusions and Discussion

We recognize that NTP does not perform risk assessments or provide regulatory or guidance values based on the study data. However, because NTP is considered to be an authoritative body with regard to toxicological testing, NTP data are often used as the "gold standard" for toxicological risk assessment. Therefore, it would be useful for the NTP report to explicitly state some of the potential caveats in using these data as a POD in risk assessment. NTP is clear that they do not extrapolate the results of this study to human exposures and that the results are applicable "under the conditions of these 2-year feed studies."

The report only provides a brief description of human health effects that have been attributed to PFOA. It would be useful for NTP to also report that for the cancer endpoints in which there was "clear evidence of carcinogenic activity" in rats, the human epidemiological literature does not report the same information. For example, the epidemiological literature has not reported any association between PFOA exposure and pancreatic and liver tumors in humans: the C8 Science Panel (2012) evaluated liver and pancreatic cancer and concluded, "There were no suggestions of positive findings for other cancers of interest, including liver, pancreas, or breast." In its exhaustive review, ATSDR also reported the same conclusion:

> The occupational exposure studies have consistently found no increases in the risk of pancreatic, liver, or respiratory tract cancers or deaths from these cancers; a general population case:control study also found no associations between serum PFOA and pancreas or liver cancer[3] (ATSDR, 2019).

[2] https://tools.niehs.nih.gov/cebs3/views/?action=main.dataReview&bin_id=13658
[3] In text references removed for readability. Please see original text for references.

4.0 SUMMARY AND CONCLUSIONS

We appreciate the opportunity to request clarity in the report. The transparency of the NTP report would be improved with the following additions or expansions of the pertinent sections of the report:

- Expand the discussion of the MOA and note any potential limitations in applying this to human exposures.
- Include a discussion of why this strain of rat was chosen as the model for human exposures and endpoints.
- Include a discussion of the interpretation of the dose-response relationship and extrapolation to low doses.
- Increase the transparency in what the "below detection" measurements are.
- Expand the discussion of the concordance of cancer endpoints with the epidemiological literature.

5.0 REFERENCES

ATSDR, 2018. Toxicological profile for perfluoroalkyls: Draft for public comment. Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry. Atlanta, GA. Accessed November 18, 2019 at https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=1117&tid=237.

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