May 3, 2021

Lauren Zeise, Ph.D.
Director
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
1001 I Street
Sacramento, California 95814

Re: Notice of intent to list chemical by the Authoritative Bodies mechanism of Health and Safety Code section 25249.8(b) - perfluorooctanoic acid (PFOA)

Dear Dr. Zeise:

The Chemical Products and Technology Division of the American Chemistry Council (ACC/CPTD) submits the following information in response to the notice of intent (NOI) to list perfluorooctanoic acid (PFOA) as a substance known to the state to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) pursuant to the "Authoritative Bodes" listing mechanism of HSC Section 25249.8(b). The enclosed comment provides information on the 2020 National Toxicology Program's technical report on toxicity and carcinogenicity studies that is the basis for the NOI. It also reviews the available epidemiological and animal evidence for carcinogenicity that should be included in the OEHHA consideration of the chemical.

As noted in the enclosed comments, the findings of the NTP report are not supported by the available epidemiology studies, including occupational and large community populations. The findings in the laboratory animals, moreover, are consistent with peroxisome proliferator-activated receptor α (PPAR α) activation which is of uncertain relevance to humans. Consequently, there is not sufficient evidence to conclude that PFOA exposure presents a cancer risk to humans to justify its listing under Proposition 65. This inconsistent evidence has led other authoritative bodies, including the International Agency for Research on Cancer (IARC), the European Food Authority (EFSA) and the Agency for Toxic Substances and Disease Registry (ATSDR), to conclude that the evidence for PFOA carcinogenicity remains suggestive but not conclusive.

Please do not hesitate to contact me if you have questions regarding the enclosed information.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

Enclosure

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Comments of the Chemical Products and Technology Division
of the American Chemistry Council
on the
Notice of Intent to List Perfluorooctanoic Acid
as a Substance Known to the State to Cause Cancer under the
Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65)
March 19, 2021

Summary

The Notice of Intent (NOI) to list perfluorooctanoic acid (PFOA) as a substance known to the state to cause cancer under Proposition 65 is based on the results of a chronic bioassay conducted by the National Toxicology Program (NTP). While NTP reported increased incidence of hepatocellular and pancreatic tumors in male rats exposed to PFOA in their diet, reports of unanticipated toxicity in the study and elevated preneoplastic lesions in the control group raise concerns about the findings.

An association with liver tumors reported by NTP is not supported by the available epidemiological evidence from occupational and general population studies. Human evidence for other tumor types, including pancreatic tumors, is conflicting and a recent comprehensive evaluation of the epidemiology suggests that reported associations are likely the result of chance, confounding, and/or bias. Laboratory studies in rats exposed to PFOA have reported a "tumor triad" – liver, testis, and pancreatic tumors – consistent with evidence for other substances known to activate the peroxisome proliferator-activated receptor α (PPAR α) in rodents with uncertain relevance to human health risk assessment.

NTP Bioassay

The National Toxicology Program (NTP) reported liver adenomas and pancreatic acinar cell (PAC) adenomas in male Sprague-Dawley rats exposed to PFOA in the diet.[1] In the study, male rats were exposed postweaning to 0, 20, 40, and 80 parts per million (ppm), equivalent to 0, 1.0, 2.2, and 4.6 milligrams per kilogram, or mg/kg, per day, while females were exposed to 0, 300, and 1000 ppm (0, 18.2, and 63.4 mg/kg per day).[2] The male rat portion of the study was repeated using significantly lower exposures after "unanticipated toxicity" was observed in male rats exposed to 150 and 300 ppm after 16 weeks. In light of the fact that male SD rats

[1] NTP. Technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid administered in feed to Sprague-Dawley rats. Technical Report 598. Department of Health and Human Services. Research Triangle Park, North Carolina (2019).

[2] The study included groups of animals exposed to PFOA perinatally and postweaning to assess the potential impact of gestational and lactational exposure but reported very few significant differences between the response in animals exposed postweaning only to those with both perinatal and postweaning exposure.

tolerated doses as high as 300 ppm in a previous chronic studies (described below), the reports of unanticipated toxicity at comparable levels in the male rats in the NTP study raise concerns about the overall confidence in the study.[3]

In the NTP study statistically significant increases in hepatocellular adenomas were reported among the male rats exposed to the two highest doses (2.2 and 4.6 mg/kg per day). Hepatocellular carcinomas were increased at the highest dose (4.6 mg/kg per day), but the increase was not statistically significant. The study also reported significant increases in hepatocyte cytoplasmic alteration and hypertrophy in the males in all exposure groups. Significant increases were also observed in hepatocyte single cell death, necrosis, mixed cell foci, inflammation, cystic degeneration, and bile duct hyperplasia.

An increase in PAC adenomas was statistically significant in male rats in all exposure groups, but not in the female groups.[4] PAC adenocarcinomas were also increased in the males, but the increase was not statistically significant. The study also noted a significant increase in PAC hyperplasia - a potentially preneoplastic lesion - in all the male groups, including the control group in which hyperplasia was reported in 36 percent of the animals. The high background rate for preneoplastic lesions observed in this study is consistent with the historical sensitivity of the Sprague-Dawley rats compared to other rat stains – and more significantly when compared to humans.

Epidemiology

Occupational studies examining cancer mortality have been conducted among workers occupationally exposed to PFOA in Minnesota and West Virginia focusing on kidney, bladder, liver, pancreatic, testicular, prostate, thyroid, and breast cancers. Two studies of communities exposed to PFOA in drinking water also are available. The results from these studies are conflicting and interpretation is limited by the small number of observed deaths and incident cases.

Raleigh et al. (2014) updated a study of cancer mortality among 4,668 PFOA workers in Minnesota followed through 2008.[5] Exposure estimates for inhalation exposures were calculated from work history records and industrial hygiene monitoring data; notably serum levels were not reported. The analysis reported no association between PFOA exposure and mortality from any cancer type. A slight elevation of bladder and pancreatic cancer incidence was reported although the confidence intervals were quite large; no association with kidney

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[3] In addition, survival rates among the female animals were quite low – ranging from 46 percent in the control group to between 46 and 64 percent in the exposure groups.

[4] A non-significant increase of combined PAC adenomas and carcinomas was observed in females at the highest dose. Unlike in the males, acinus hyperplasia was not reported in the females

[5] Raleigh KK *et al.* Mortality and cancer incidence in ammonium perfluorooctanoate production workers. *Occup Environ Med* 71(7):500-506 (2014). http://dx.doi.org/10.1136/oemed-2014-102109

cancer incidence and PFOA exposure was reported.[6] The mean age of the workers was 29 years at the start of employment and 63 years at the end of follow-up.

Steenland and Woskie (2012) updated a cohort mortality study of 5,791 workers in West Virginia who had worked in a manufacturing facility using PFOA for at least 1 year between 1948 and 2002.[7] Mean duration of employment was 19 years. Exposure quartiles were assessed by estimated cumulative annual serum levels based on blood samples taken from 1,308 workers and time spent in various job categories. Referent groups included both nonexposed workers in the same region and the U.S. population. Overall, the mean cumulative exposure among the workers was 7.8 ppm-years and the estimated average annual serum level was 0.35 mgs per liter (mg/L).[8] The authors reported a significant positive trend for kidney cancer incidence among workers in the highest exposure quartile, while no association was reported between PFOA exposure and liver, pancreatic, testicular, or bladder cancer incidence.

Liver cancer mortality was elevated in a small observational study of 642 male employees who had worked at lease 6 months before 2009 for a factory producing PFOA and other chemicals.[9] Confounding factors were not well controlled. Serum levels in 120 workers were used to predict PFOA concentrations of each individual; serum concentrations ranged from 19 to 91,900 nanograms per milliliter (ng/mL). A statistically significant increase for mortality of liver cancer and liver cirrhosis was reported in the highest cumulative internal dose group when compared to the regional populations and workers of a nearby factory

Two studies involving communities in West Virginia and Ohio affected by contaminated drinking water (the C8 Health Project) reported a positive association between blood levels of PFOA and kidney and testicular cancers. Vieira *et al.* (2013) investigated incidences of 18 cancer types among residents supplied by six public water districts in Ohio and West Virginia contaminated with PFOA.[10] The analysis included over 25,000 cancer cases. Exposure levels and serum PFOA concentrations were estimated based on residence at time of diagnosis. Exposures were categorized as very high, high, medium, low, or unexposed based on PFOA serum concentrations.

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6 The authors report that the study had limited power to evaluate exposure response for testicular, bladder, liver, and pancreatic cancers.

7 Steenland K and Woskie S. Cohort mortality study of workers exposed to perfluorooctanoic acid. *Am J Epidemiol* 176(10):909–917 (2012). https://doi.org/10.1093/aje/kws171

8 For comparison, the mean serum level of PFOA in the California Regional Exposure Study, Los Angeles County (CARE-LA) study was 0.001 mg/L.

9 Girardi P and Merler E. A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Env Research 179(Part A):108743 (2019). https://doi.org/10.1016/j.envres.2019.108743

10 Vieira VM *et al.* Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. *Environ Health Persp* 121(3):318-323 (2013). https://doi.org/10.1289/ehp.1205829

Among all cancer endpoints, the odds ratio for testicular cancer was elevated in one of the two areas with the highest concentration of PFOA in drinking water. There was no statistically significant increase in the odds ratio for testicular cancer in the total exposed population, however, or in the other districts, or in the other estimated dose-level categories. Kidney cancer incidence was increased significantly in one district with the two highest levels of individual exposure. Despite the large overall sample size, the authors noted that their analysis was limited by small numbers of individual cancers in the high-exposure groups. Moreover, there was little consistency across exposure categories, with no evidence of a dose response.

Barry *et al.* (2012) conducted an analysis of cancer incidence among 32,254 individuals in the same geographic area as Vieira *et al.*, including 3,713 workers with occupational exposure to PFOA.[11] Cumulative PFOA serum concentrations were estimated based on historical regional monitoring data and individual residential histories. Based on measurements taken in 2005-2006, mean serum concentrations were 0.024 mg/L for community residents and 0.113 mg/L for workers. A total of 2,500 cancers were validated through a cancer registry or medical records. The authors reported that PFOA exposure was positively associated with kidney and testicular cancer across the exposure quartiles within the population, although the incidence of either tumor type was not elevated when compared to the US population.

Two additional population studies did not report an association of liver or pancreatic cancer and PFOA exposure. A study of 57,000 individuals with no previous cancer diagnosis enrolled in a prospective cohort during 1993-97 reported no association between liver and pancreatic cancer and elevated levels of PFOA; kidney and testicular cancer information was not presented.[12] PFOA concentrations were based on a single measure of plasma level taken at recruitment. A study of residents exposed to contaminated drinking water near a PFAS manufacturing facility in the Veneto Region of Italy with exposure to multiple PFAS, reported no increase in mortality caused by kidney, pancreatic, liver, or testicular cancer.[13] Some excess kidney cancer mortality was reported among women.

A review of the epidemiological evidence for cancer from 18 studies of occupational and general population exposure to PFOA reported a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population.[14] The authors evaluated the studies based on the study design, subjects,

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[11] Barry V *et al.* Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. *Environ Health Persp* 121(11-12): 1313-1318 (2013). https://doi.org/10.1289/ehp.1306615

[12] Eriksen KT *et al.* Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. *J Natl Cancer Inst* 101:605–609 (2009). https://doi.org/10.1093/jnci/djp041

[13] Mastrantonio M *et al.* Drinking water contamination from perfluoroalkyl substances (PFAS): an ecological mortality study in the Veneto Region. Italy. *Eur J Public Health* 28(1):180–185 (2018). https://doi.org/10.1093/eurpub/ckx066

[14] Chang ET *et al.* A critical review of perfluorooctanoate and prefluorooctanesulfonate exposure and cancer risk in humans. *Crit Rev in Toxicol* 44(51):1–81 (2014). https://doi.org/10.3109/10408444.2014.905767

exposure assessment, outcome assessment, control for confounding, and sources of bias using Bradford Hill guidelines and concluded that the discrepant findings across the study populations were likely due to chance, confounding, and/or bias. A more recent review of the evidence by the epidemiologists involved in the C8 study concluded that the evidence for an association between PFOA exposure and kidney and testicular cancer was suggestive overall, there was little evidence for an association with liver or pancreatic cancer.[15]

The relevance of the liver tumor data from the NTP study is further called into question based on recent clinical data reported by Convertino *et al.* (2018).[16] In a study of a sensitive subpopulation of cancer patients with normal liver function exposed to weekly PFOA doses as high as 1,200 mgs (about 16 mg/kg per day), Convertino *et al.* reported no differences in clinical hepatic measures.[17] Similarly a study of PFOA production workers reported no abnormal liver function, hypolipidemia, or cholestasis.[18]

Animal Bioassays

In addition to the NTP study, two chronic bioassays have been conducted in rats exposed to PFOA through diet. Although the results are not consistent, one or both studies have reported liver, LC, and PAC tumors.[19]

Butenhoff *et al.* (2012), reporting on a previously conducted study of male and female Sprague-Dawley (SD) rats exposed to dietary levels of 30 and 300 parts per million (ppm) of PFOA (approximately 1.5 and 15 mg/kg per day), observed a dose-dependent increase in LC adenomas that was statistically significant at the highest dose.[20] Elevated incidence of hepatic and PAC lesions were also reported in males at 300 ppm, but the authors did not report increases in hepatic or PAC tumors.

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15 Steenland K *et al.* Review: evolution of evidence on PFOA and heath following the assessments of the C8 Science Panel. *Environ Intl* 145: 106125 (2020). https://doi.org/10.1016/j.envint.2020.106125

16 Convertino M *et al.* Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systematic health risk of perfluorooctanoate (PFOA). *Toxicol Sci* 163(1) 293-306 (2018). https://academic.oup.com/toxsci/article/163/1/293/4865972

17 Clinical measurements included triglycerides, urea, glucose, AST, GGT, alkaline phosphatase, total bilirubin, fibrinogen, PTT and aPTT.

18 Olsen GW *et al.* Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. *Drug Chem Toxicol* 23(4):603–20 (2000). https://doi.org/10.1081/DCT-100101973

19 The incidence of testicular (Leydig cell, or LC) adenomas was not reported in the NTP bioassay.

20 Butenhoff JL *et al.* Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicol* 298(1–3): 1–13 (2012). Target doses for the study were 0, 1.3, and 14.2 mg/kg body weight per day in males and 0, 1.6, and 16.1 mg/kg per day in females. https://doi.org/10.1016/j.tox.2012.04.001

A subsequent single-dose, dietary study with male CD rats reported LC adenomas, as well as liver and PAC adenomas and combined pancreatic adenomas and carcinomas at 300 ppm (13.6 mg/kg per day).[21] Increased incidences of LC and PAC hyperplasia were also observed. Hepatic ß-oxidation activity was always significantly elevated, but cell proliferation in the liver was not.

Relevance of the Animal Data

A significant amount of genotoxicity and mechanistic data are available to assist in evaluating the results of the epidemiology and animal bioassay results described above. Multiple *in vivo* and *in vitro* assays provide clear evidence that PFOA is not mutagenic and may only cause genotoxicity at toxic concentrations. Consequently, it is generally agreed that PFOA causes tumors in laboratory animals via a non-genotoxic or epigenetic mechanism.[22]

The tumor types that have been reported consistently in rats exposed to PFOA – liver, LC, and PAC – have been observed with other substances that are PPARα agonists. Because of key toxicodynamic and biological differences in responses between rodents and humans, PPARα activators are considered unlikely to induce tumors in humans. For liver tumors, this conclusion is based on minimal or no effects observed on growth pathways, hepatocellular proliferation and liver tumors in humans and/or species (*e.g.*, hamsters, guinea pigs and *Cynomolgous* monkeys) that are more appropriate animal model surrogates than mice and rats.

Several key studies provide support for the key events in the proposed PPARα-activated mode of action (MOA) for rat liver tumors (Table 1). These data are summarized by Klaunig *et al.* (2012) –

> Analysis of gene expression changes elicited following short-term administration of PFOA demonstrated the up regulation of genes characteristic of PPARα activation, including genes involved in fatty acid homeostasis/peroxisomal proliferation as well as those related to cell cycle. In addition, PFOA has been shown to induce peroxisome proliferation in mouse and rat liver and causes hepatomegaly in mice and rats. While the liver growth caused by PFOA was predominantly attributed to a hypertrophic response, an increase in DNA synthesis following PFOA exposure was observed and predominated in the periportal regions of the liver lobule. Thus, the effect of PFOA on induction of cell cycle gene expression and the increase in DNA synthesis provide evidence in support of both key events 2 and 3 in the proposed MOA for liver tumor induction by PFOA. Empirical evidence also exists in support of the clonal expansion of preneoplastic hepatic lesions by PPARα activators (Step 4). Using an

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[21] Biegel LB *et al.* Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci* 60(1): 44–45 (2001). https://doi.org/10.1093/toxsci/60.1.44

[22] US Environmental Protection Agency (USEPA). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822-R-16-003. Office of Water. Washington, DC. (May 2016).

initiation-promotion protocol for induction of liver tumors in Wistar rats, PFOA was shown to increase the incidence of hepatocellular carcinomas in rat liver (33% in PFOA exposed rats vs. 0% in controls).[23]

Klaunig *et al.* also note that the key events in Table 1 appear in a temporal sequence and demonstrate dose-related effects further strengthening the evidence for the PPARα-agonist MOA. Although there are indications that PFOA may also act through PPARα-independent mechanisms[24] in rodents, differences in binding affinity between the rodent and human receptors suggest that it is also unlikely that PFOA induces cancers in humans through the other mechanisms that have been suggested.[25] In evaluating their results, Convertino *et al.* concluded that the disparity between animal and human liver endpoint studies, emphasizing a lack of risk of hepatomegaly, fatty liver, or cirrhosis, are likely due to MOA differences. Increased liver weight due to hepatocellular hypertrophy can often be an adaptive (protective) response in animals due to up-regulation of detoxification enzymes, leading toxicologists to revisit the relevance key liver endpoint studies in animals.[26]

Table 1. PPARα Mode of Action for PFOA-Induced Liver Tumors in Rats (from Klaunig *et al.* 2012)

	Key Event	Support	Key Reference[27]
1	Activation of the PPARα receptor	✓	Maloney & Waxman 1999; Vanden Heuvel *et al.* 2006
2	Induction of cell growth gene expression in the liver	✓	Martin *et al.* 2007; Kennedy *et al.* 2004
3	Cell proliferation	✓	Biegel *et al.* 2001; Martin *et al.* 2007; Thottassery *et al.* 1992
4	Selective clonal expansion of preneoplastic hepatic foci	✓	Abdellatif *et al.* 1990
5	Liver neoplasms	✓	Biegel *et al.* 2001

[23] Klaunig JE *et al.* Mode of action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and human relevance. *Reprod Toxicol* 33:410-418 (2012). https://doi.org/10.1016/j.reprotox.2011.10.014

[24] Activation of the constitutive activated receptor (CAR) and pregnane X receptor (PXR) by PFOA have been suggested in animal studies.

[25] Hall AP *et al.* Liver Hypertrophy: A Review of Adaptive (Adverse and Non-Adverse) Changes-Conclusions from the 3rd International ESTP Expert Workshop. *Toxicol Pathol* 40:971-994 (2012). https://doi.org/10.1177%2F0192623312448935

[26] See for example: Bjork JA *et al.* Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. *Toxicol* 288: 8-17 (2011). https://doi.org/10.1016/j.tox.2011.06.012

[27] Complete citations are provided in Klaunig *et al.* 2012.

For the induction of rat PAC tumors by PFOA, the available mechanistic data are less robust, but also point to the importance of PPARα activation in the liver. Several factors may contribute to the development of PAC hypertrophy, hyperplasia, and adenomas in the rat, such as testosterone and estradiol levels, growth factor expression (cholecystokinin, or CCK), growth factor receptor overexpression (CCKA receptor),and high fat diet (Klaunig *et al.*).[28] Studies with the compound Wyeth 14,643, a well-studied and potent peroxisome proliferator in rodents, suggest that peroxisome proliferation induces PAC tumors by an indirect mechanism. In this study PPARα activation in the liver caused by exposure to Wyeth triggered reduced bile flow and/or changes in bile composition that produced an increase in CCK levels secondary to hepatic cholestasis.[29] As CCK has been shown to act as a growth factor for PACs in rats, a sustained increase in CCK levels would explain the increase in PAC proliferation observed following PFOA exposure and is likely therefore a preneoplastic lesion.

Expression of CCK receptors in humans is much lower as compared to rodents, and the available non-human primate and human data suggest that the CCK pathway is not relevant to human cancer risk. A study with Cynomolgus monkeys exposed to PFOA did not demonstrate an effect on CCK levels or evidence of hepatic cholestasis.[30] Olsen et al reported a statistically significant negative (inverse) association between mean CCK levels and serum PFOA levels among PFOA production workers, even after adjusting for potential confounders.[31]

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[28] Differences in the diets used in the Butenhoff *et al.* and Biegel *et al.* studies have been suggested as the likely reason for the quantitative difference in the PAC lesions observed in the two studies (USEPA 2016).

[29] Obourn JD *et al.* Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferatorWyeth-14,643. *Toxicol Appl Pharmacol* 145:425–36 (1997). https://doi.org/10.1006/taap.1997.8210

[30] Butenhoff J *et al.* Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci* 69(1):244–57 (2002). https://doi.org/10.1093/toxsci/69.1.244

[31] Olsen *et al.* 2000.