Per- and Polyfluoroalkyl Substance (PFAS) Levels in Drinking Water and Their Association with Cancer in Canines: Implications of Canines as Human Sentinels

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Abstract

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Epidemiology

Per- and polyfluoroalkyl substances (PFAS), known as “forever chemicals” are a range of widely used, human-synthesized chemical compounds applied in numerous capacities across the globe including food packaging, firefighting, and manufacturing products with non-stick or water-resistant properties. PFAS exposure from point-source occupational and industrial contamination has been linked to several types of human cancers including kidney, thyroid, hematopoietic, and reproductive system organs. Because companion canines typically share the same environmental exposure as their human owners or companions and are prone to similar diseases, often with a shorter latency period, they are considered an ideal sentinel for observing environmental health risks to humans. In this cross-sectional study, we examined whether elevated levels of two types of PFAS present in U.S. public water systems--Perfluorooctanoic acid (PFOA) and/or Perfluorooctane sulfonate (PFOS) levels--were associated with lifetime cancer prevalence in companion canines assessed from June 2022 to June 2023. We linked geocoded canine residence information from the 2022 Dog Aging Project (DAP) Curated Data Release with geocoded PFOA and PFOS levels recorded in the U.S. Environmental Protection Agency (EPA) Unregulated Contaminant Monitoring Rule 5 (UCMR 5) dataset. to investigate whether PFOA and/or PFOS exposure exceeding the EPA proposed action level of 4.0 parts per trillion (ppt) was associated

with select cancer outcomes in companion canines that have been linked to human PFAS exposure: kidney, thyroid, and hematopoietic cancer. Due to small case counts for kidney and thyroid cancers in the DAP data, we also analyzed two other prevalent cancer types in the combined dataset: mast cell and muscle and soft tissue cancers (MSTC). Our secondary aim investigated whether canine cancer prevalence was lower in states that elected to implement PFAS maximum contaminant level (MCL) policies prior to the April 2024 EPA federal mandate.

Prevalence ratios (PR) and 95% confidence intervals (CI) estimated from Poisson regression models adjusted for age, canine size, and neighborhood-level socioeconomic disadvantage status did not indicate evidence of positive associations between elevated PFOA and/or PFOS exposure and any of the cancer types investigated: hematopoietic cancer (PR = 0.44, 95% CI: 0.10, 1.86), mast cell cancer (PR = 1.23, 95% CI: 0.70, 2.17), and MSTC (PR = 0.84, 95% CI: 0.45, 1.56). For the secondary aim, PRs and Cis estimated from Poisson regression models adjusted for neighborhood-level particulate matter 2.5 similarly did not find evidence of positive associations between state PFAS MCL policy and the cancer outcomes we investigated: kidney cancer (PR = 0.61, 95% CI: 0.13, 2.94), hematopoietic cancer (PR = 0.54, 95% CI: 0.20, 1.44), mast cell cancer (PR = 1.19, 95% CI: 0.75, 1.90), MSTC (PR = 0.78, 95% CI: 0.47, 1.29). Together, these findings cannot be taken as evidence of clear protective or harmful effects of PFOA and/or PFOS exposure but rather the need for future research into the impacts of these chemicals and PFAS MCL policies on cancer in canine and human populations.

Introduction

Per- and polyfluoroalkyl substances (PFAS), are a vast group of human-synthesized chemical compounds used in numerous capacities across the globe including food packaging, firefighting, and manufacturing products with non-stick or water-resistant properties. Experimental studies in rats provide clear evidence supporting the carcinogenic properties of major PFAS compounds. In a 2-year feed study, exposure to one PFAS, Perfluorooctanoic acid (PFOA), demonstrated increased incidence of hepatocellular and pancreatic neoplasms along with observed toxicity (e.g. liver hypertrophy, necrosis) in the stomach, kidney, liver, and thyroid glands.[1] Research is limited about how exposures to PFAS, coined "forever chemicals" because of their stable chemical properties, affect long term health. Use of these PFAS substances is widespread and community drinking water contamination is common.[2] PFAS has been linked to several types of human cancer including kidney, thyroid, blood, and several reproductive system organ cancers.[3]

Even less research exists about how PFAS affect canine health. Since companion canines are prone to similar diseases, experience a shorter latency period, and reflect their owners and companions' environmental exposures, they are considered an ideal sentinel for observing environmental health risks to humans.[4,5] In this cross-sectional study, we used 2023 Unregulated Contaminant Monitoring Rule (UCMR) 5 United States (US) Environmental Protection Agency (EPA) data and the 2022 Dog Aging Project (DAP)[6] Curated Data Release to determine whether levels of PFAS above the EPA action level of four parts per trillion (ppt) are associated with canine malignancies previously reported in humans to be associated with human PFAS exposure. Secondarily, we also explored this association with the most prevalent cancer types reported in DAP, regardless of their previously reported relationships with human cancers. As a secondary aim, we investigated if lifetime cancer prevalence in companion canines was lower in U.S. states with previously implemented PFAS MCLs in comparison to states that did not implement MCLs prior to the April 2024 federal mandate.

While PFAS are widespread in the environment, such as in the food and water we consume or the cooking and cleaning products we use, most evidence associating PFAS exposure with human cancer risk

comes from occupationally-exposed populations as in ski waxing, firefighting, and manufacturing.[7,8] Those data as well as results from non-human model systems, led the International Agency for Research on Cancer (IARC) to classify PFOA as a Group 1 ("probable") human carcinogen and PFOS as a Group 2B ("possible") human carcinogen.[9] Both compounds are included in the EPA UCMR 5 dataset as they are monitored in public drinking water systems and will be specifically investigated in this analysis.

This cross-sectional study investigates gaps in knowledge about the extent to which PFAS levels above the recently implemented EPA action level may be associated with selected cancers in companion canines. Studies suggest great potential in using companion animals in human cancer research.[10] Cancer is influenced by both genetic and environmental factors and dogs share approximately 22% of their DNA base pairs with humans along with their living environment.[4,11,12] The latter feature makes them similarly vulnerable to environmental hazards as humans, including exposure from drinking water, which could offer valuable insights into this broad group of risk factors in cancer development.[13]

Under the Safe Drinking Water Act (42 U.S.C. §300f et seq. (1974)), the EPA implemented enforceable PFAS drinking water standards, to begin in 2029.[14] Prior to April 2024, there was no federal regulation on PFAS and drinking water and only 11 states had implemented MCL standards on their own accord.[15] To achieve MCL compliance by 2029, the Federal Infrastructure Investment and Jobs Act (Public Law 117-58) and EPA Water Technical Assistance programs are helping communities by investing in improvements to potable water system infrastructure, developing methods to remove contaminants like PFAS, and providing free guidance in navigating the funding application process.[16] It is important to note the UCMR 5 dataset was not designed to indicate compliance with MCLs but the information it contains offers a valuable snapshot of baseline broad PFAS exposure amongst 29 PFAS subtypes in participating public water systems. Our study will analyze only two of these subtypes: PFOS and PFOA.

Methods

Study Design and Setting:

This is a cross-sectional study conducted at the zip code level, using data from the DAP (including companion canines located across all 50 US states) and the EPA UCMR 5 datasets, which, between June 2022 and June 2023. Together, these two geographically linked datasets provided an opportunity to assess a snapshot of lifetime prevalence of canine cancer in relation to residential PFOA and PFOS exposure levels and provided substantial demographic, environmental, and health-related information collected amongst them. The timeframe was selected to reflect a full year of overlapping data collection between the two sources, as data from the latter part of 2023 were not yet available from DAP at the time the analyses were conducted.

Study Subjects:

The subjects from this study are dogs whose owners have consented to, and filled out, the Health and Life Experiences Survey (HLES) for the DAP in 2022. The HLES questionnaire includes data about dog and owner demographics, environmental factors, activity levels, behavior, and diagnoses of cancer and other health conditions.[6]

Data Sources and Collection

Dog Aging Project (DAP). The first dataset consists of subjects from a longitudinal study of 43,517 companion dogs whose owners have consented to providing substantial demographic, environmental, and health-related information about them through the baseline HLES and annual follow-up surveys.[6] It is an open data initiative accessible to the public through a Google Cloud platform. The platform is managed by the Terra team at the Broad Institute of the Massachusetts Institute of Technology (MIT) and Harvard.[6,11] The specific questionnaires used from the DAP for this analysis included the HLES and Environment Survey.

Unregulated Contaminant Monitoring Rule (UCMR) 5. The second dataset comes from the EPA.[16] These publicly available data were established in March 2021 to provide a better understanding of the frequency and levels at which PFAS exists within U.S. public water systems.[17] Participating public water

systems include all those serve 3,300 people or more, and a nationally representative sample of smaller public water systems that serve fewer than 3,300 people. Samples are sent to laboratories approved through the EPA Laboratory Approval Program and quantified in ug/mL For this analysis, µg/mL were converted to parts per trillion (ppt). Samples taken from participating public water systems were assayed for 29 PFAS starting in 2023 and will be ongoing until the conclusion of 2025.[14] Since this dataset is not yet complete, reporting coverage varied by zip code, i.e., some zip codes had more PFAS data available than others. This inconsistency may have introduced some nondifferential exposure misclassification, potentially obscuring true associations.

Definitions

Outcome

Cancer outcomes were defined using responses from owners who were asked whether their canine had experienced "Cancer or Tumors" by choosing one of the following options: 0 = No disorder(s), 1 = Only congenital disorder(s), 2 = Only non-congenital disorder(s), 3 = Both congenital and non-congenital disorder(s). Owners were then asked to "Please select all areas of the body that were affected by cancer or tumors (select all that apply)" from 34 anatomical locations. Each location was marked either "TRUE" or "FALSE." Canines were classified as cancer cases if the owner selected (2) or (3) on the first question and marked an anatomical location as "TRUE". Canines with a "lipoma" cancer type were excluded from this definition since they are considered benign. Mast cell tumors (MCT) are a cancer type of which some are considered malignant, and others considered benign. Unfortunately, the DAP questions on cancer types did not attempt to elicit the owner's knowledge of the MCT behavior, and thus we were unable to exclude the subset of MCT that were benign. We reviewed accompanying owner comments for MCT cases to identify any evidence indicating that the tumor was benign; of the number of MCTs reported on the Cancer Conditions Survey, we found no evidence that any were benign. In the absence of comments, or if comments did not clearly indicate that the MCT was benign, we assumed that the reported MCT was malignant. We

felt comfortable making this assumption because MCT is the most commonly diagnosed malignant skin tumors in canines, and some studies suggest MCTs accounts for 20.9 - 22.4% of all canine malignancies.[18,19]

Exposure

The main exposures for this study were (1) PFOA and PFOS levels greater than or equal to the four ppt MCL established by the April 2024 federal mandate and (2) whether a U.S. state had implemented its own PFAS MCL policies prior to the federal mandate. PFOA and PFOS levels were measured in the EPA UCMR 5 dataset. For each public water system sample greater than or equal to the MCL, an amount quantified in ppt was reported. Samples that did not meet these criteria did not have a ppt level recorded. For this analysis, PFOA and PFOS results were aggregated by zip code and converted to a binary variable, where PFAS levels greater than or equal to the MCL were marked "YES" and if otherwise, marked "NO." To note, this dataset is not yet completed, and reporting coverage varied by zip code. As a result, some zip codes had more PFOA and PFOS data available than others. The following states implemented their own PFAS MCL policies prior to the April 2024 federal regulatory announcement: ME, MA, MI, NH, NJ, NY, PA, RI, VT, WA, and WI.[15] This exposure was categorized as a binary variable, with "YES" indicating that the dog lived in a state that had implemented its own MCL policy and "NO" to indicate otherwise.

Statistical Analysis:

To estimate the extent to which levels of PFAS above the EPA MCL were associated with lifetime prevalence of selected cancers in companion canines, unadjusted and confounder-adjusted PRs with 95% CIs were calculated using Poisson regression with total number of dogs as the denominator and a robust standard error.[20] Separate models were fit for three grouped cancer types due to small numbers of cases of specific cancer types: hematopoietic (inclusive of leukemia, lymphoma, lymphosarcoma, multiple myeloma, blood, lymph nodes, spleen), MCT, and MSTC. The following potential confounders were identified *a priori* through literature review: age, canine gender, obesity, socioeconomic status and canine breed size. Although obesity was among the *a priori* confounders, we excluded it from the analysis because >10% of the dogs were missing data on this characteristic. Neighborhood-based socioeconomic status (SES) was captured by the disadvantage index, which averages the z-score of the following: percentage of

population below 125% poverty line, percentage of working-age unemployed or not in the labor force, percentage of children living in female-led households with no husband present, percentage of population >25 years with less than a Bachelor's degree, and percentage of households earning under $100,000 in the last twelve months.[6] To determine which confounders to include in each of the grouped cancer type specific models, we compared the PRs for the PFAS exposure of interest between the unadjusted and adjusted models. If the difference was >10% we included the confounder in the adjusted model.[21] Interaction terms were evaluated for effect modification based if a null hypothesis test of heterogeneity yielded a p value <0.05.[22] Although age did not meet confounding criteria in all models, it was included in all adjusted analyses due to its established role as a risk factor for canine cancer.[23] The final terms included in each model are listed in Table 2.

The same methods applied when determining whether dogs living in states that implemented MCL policies had lower lifetime prevalence of select cancer outcomes: kidney (inclusive of kidney and transitional cell carcinoma), hematopoietic, MCT, and MSTC. Potential confounders and effect modifiers included those indicated in the prior paragraph as well as ambient particulate matter levels 2.5 (PM2.5) and 10 (PM10). Thresholds from the EPA National Ambient Air Quality Standards (NAAQS) were used to provide meaningful particulate matter levels for this study.[24] The inclusion of PM2.5 and PM10 reflects an observation that states adopting optional MCL regulations may also prioritize reducing general environmental pollution, including these airborne contaminants. Given that exposure to PM2.5 and PM10 has been associated with increased cancer risk in humans, it is biologically plausible that such exposure could also affect canines.[25–27]

All analyses were performed using R version 4.3.1[28]

Ethical approvals

This study was approved by both the DAP Proposals and Publications committee and the University of Washington Institutional Review Board (IRB).

Results

Descriptive Statistics

Table 1 shows the demographic and environmental characteristics of our study population, which included 6,657 canines, stratified by zip-code level PFOA and/or PFOS exposure. Of these, 946 dogs (14%) resided in areas with PFOA and/or PFAS concentrations ≥ 0.04 ppt and 5,698 (86%) in areas with < 0.04 ppt. The median estimated age was 6.4 years (IQR 3.0 – 10.1) and was consistent across exposure groups. Most dogs were either spayed females (44%) or neutered males (44%) and canine size categories were evenly distributed, except for the giant category (6.8%). Socioeconomic status was also evenly distributed between high and low exposure groups. Overall, missing data was minimal; however, our variable on canine history of obesity had >10% missingness and was therefore excluded from our analyses.

Primary Aim

Three types of cancer were pre-identified to investigate an association between canine cancer and PFAS exposure above the PFAS MCL federal mandate based on human cancers associated with PFAS exposure: thyroid, kidney, and all hematopoietic cancer. After generating the count data for our combined dataset, it was determined that the numbers of both thyroid and kidney cancer were too low to permit analysis. Instead, the top two highest prevalence cancer types from the DAP dataset were chosen to investigate instead: MCT and MSTC.

There was a 56% lower prevalence of hematopoietic cancer in dogs exposed to PFOA and/or PFOS levels above the MCL compared to those not exposed (PR = 0.44, 95% CI: 0.10, 1.86). However, the confidence interval included 1 and suggested no evidence of a meaningful association between PFAS exposure above the MCL and lifetime hematopoietic cancer prevalence in canines after adjusting for socioeconomic status and age. Age showed a significant positive association, with each year of age increasing the prevalence of blood cancer by approximately 29% (PR = 1.29, 95% CI: 1.18, 1.33). We found a 23% higher prevalence of MCT in dogs exposed to PFOA and/or PFOS levels above the MCL compared to those not exposed (PR = 1.23, 95% CI: 0.70, 2.17) after adjusting for canine size and age;

again, this result did not reach meaningful significance. The prevalence of MSTC in canines was associated with a non-significant 16% reduction in the prevalence of MSTC (PR = 0.84, 95% CI: 0.45, 1.56) after adjustment for canine size and age.

Secondary Aim

For the secondary aim, each pre-determined potential confounder or effect modifier was evaluated for inclusion into the model based on conditions listed in our Methods section. PM2.5 was determined to be a confounder and was adjusted for in each of the models, otherwise we incorporated the same exclusions as we applied in the primary aim. Due to the lack of companion canines located in zip codes with PM10 above the EPA National Ambient Air Quality Standards (NAAQS) threshold of 150 $\mu g/m^3$, this variable was excluded from our secondary aim analysis (Table 1).[24]

Canines living in states with a PFAS MCL policy prior to 2024 generally had lower lifetime prevalence of cancer compared to those in states without such policies. Adjusted PRs suggested lower prevalence of kidney cancer (PR = 0.61, 95% CI: 0.13, 2.94), hematopoietic cancers (PR = 0.54, 95% CI: 0.20, 1.44), and MSTC (PR = 0.78, 95% CI: 0.47, 1.29) after adjustment for PM2.5. In contrast, the lifetime prevalence of MCT was slightly higher amongst dogs in states with PFAS MCL policies in place (PR = 1.19, 95% CI: 0.75, 1.90). However, none of the associations provided evidence of meaningful significance.

Table 1. Selected Characteristics of Canines by Zip Code PFAS status, Dog Aging Project 2022 and EPA UCMR 5* (N = 6,657)

Characteristic, n (%)	Overall (N = 6,657)	Zip Code PFOA or PFOS Level	
		≥ 0.04 ppt (N = 946)	< 0.04 ppt (N = 5,698)
Estimated Age (years)			
Median (IQR)	6.40 (3.00, 10.10)	6.20 (2.90, 10.00)	6.40 (3.00, 10.10)
Unknown	1	0	1
Socioeconomic disadvantage			
No	3,370 (51%)	431 (46%)	2,939 (52%)
Yes or Neutral	3,194 (49%)	502 (54%)	2,692(48%)
Unknown	93 (1.4%)	13 (0.20%)	80 (1.2%)
Particulate Matter 2.5 Exposure			
< 9 μg/m^3	5,113 (83%)	726 (82%)	4,387 (83%)
>= 9 μg/m^3	1,065 (17%)	161 (18%)	904 (17%)
Unknown	479 (7.2%)	59 (0.90%)	420 (6.3%)
History of being Overweight			
Yes	1,543 (23%)	214 (23%)	1,326 (23%)
No	4,378 (66%)	632 (67%)	3,736 (66%)
Unknown	736 (11%)	100 (11%)	636 (11%)
Canine Size			
Giant	452 (6.8%)	56 (5.9%)	395 (6.9%)
Large	1,773 (27%)	250 (26%)	1,519 (27%)
Medium	1,181 (18%)	164 (17%)	1,016 (18%)
Standard	1,889 (28%)	258 (27%)	1,628 (29%)
Toy/Small	1,359 (20%)	217 (23%)	1,138 (20%)
Unknown	3 (<0.1%)	1 (0.1%)	2 (<0.1%)
Canine Gender			
Female, intact	319 (4.8%)	39 (4.1%)	279 (4.9%)
Female, spayed	2,943 (44%)	406 (43%)	2,531 (44%)
Male, intact	457 (6.9%)	77 (8.1%)	380 (6.7%)
Male, neutered	2,938 (44%)	424 (45%)	2,508 (44%)
Unknown	0 (0%)	0 (0%)	0 (0%)

Table 2: Covariates Included as Adjustments in Primary and Secondary Aim Models

Model	Primary Exposure	Adjusted covariates
Primary Aim		
Cancer Type		
Hematopoietic cancer	Zip Code PFAS ≥ 4 ppt	Socioeconomic status, Age
Mast cell cancer	Zip Code PFAS ≥ 4 ppt	Canine size, Age
Muscle or soft tissue cancer	Zip Code PFAS ≥ 4 ppt	Canine size, Age
Secondary Aim		
Cancer Type		
Kidney cancer	State MCL Policy	PM 2.5
Hematopoietic cancer	State MCL Policy	PM 2.5
Mast cell cancer	State MCL Policy	PM 2.5
Muscle or soft tissue cancer	State MCL Policy	PM 2.5

Table 3: Selected Cancer Outcomes, Dog Aging Project 2022 and EPA UCMR 5 (N = 6,657)

Cancer Type, n (%)		**Total** (N= 6657)	**Zip Code PFAS level ≥ 0.04 ppt** (N = 946)	**Zip Code PFAS level < 0.04 ppt** (N = 5,698)	**Missing N (%)**
Kidney Cancer					
	No	6,647 (99.8%)	946 (100%)	5,701 (99.8%)	0 (0%)
	Yes	10 (0.2%)	0 (0%)	10 (0.2%)	0 (0%)
Thyroid Cancer					
	No	6654 (99.9%)	946 (100%)	5708 (99.9%)	0 (0%)
	Yes	3 (<0.1%)	0 (0%)	3 (<0.1%)	0 (0%)
Hematopoietic Cancer					
	No	6626 (99.5%)	943 (99.8%)	5,683 (99.5%)	0 (0%)
	Yes	31 (0.5%)	3 (0.3%)	28 (0.5%)	0 (0%)
Mast Cell Cancer					
	No	6,573 (99%)	932 (99%)	5,641 (99%)	0 (0%)
	Yes	84 (1.3%)	14 (1.5%)	70 (1.2%)	0 (0%)
Muscle or Soft Tissue Cancer					
	No	6,566 (99%)	935 (99%)	5,631 (99%)	0 (0%)
	Yes	91 (1.4%)	11 (1.2%)	80 (1.4%)	0 (0%)

Table 4: Lifetime prevalence of malignant tumors associated with PFAS Exposure Levels (Maximum Contaminant Level [MCL ≥ 4.0 parts per trillion [ppt] vs < 4.0 ppt), adjusted for canine age, socioeconomic status, and size*

Cancer type Zip Code PFAS	Canine Population (N = 6,657)	Malignant Cancer Cases	Prevalence Ratio (95% CI)	
			Unadjusted	Adjusted
Hematopoietic Cancer[a]				
< MCL	5,683	28	1.00 (Ref)	1.00 (Ref)
>= MCL	943	3	0.65 (0.20, 2.13)	0.44 (0.10, 1.86)
Mast Cell Cancer[b]**				
< MCL	5,641	70	1.00 (Ref)	1.00 (Ref)
>= MCL	932	14	1.21 (0.68, 2.13)	1.23 (0.70, 2.17)
Muscle or Soft Tissue Cancer[b]				
< MCL	5,631	80	1.00 (Ref)	1.00 (Ref)
>= MCL	935	11	0.83 (0.44, 1.55)	0.84 (0.45, 1.56)

*[a] Adjusted for socioeconomic status and age.

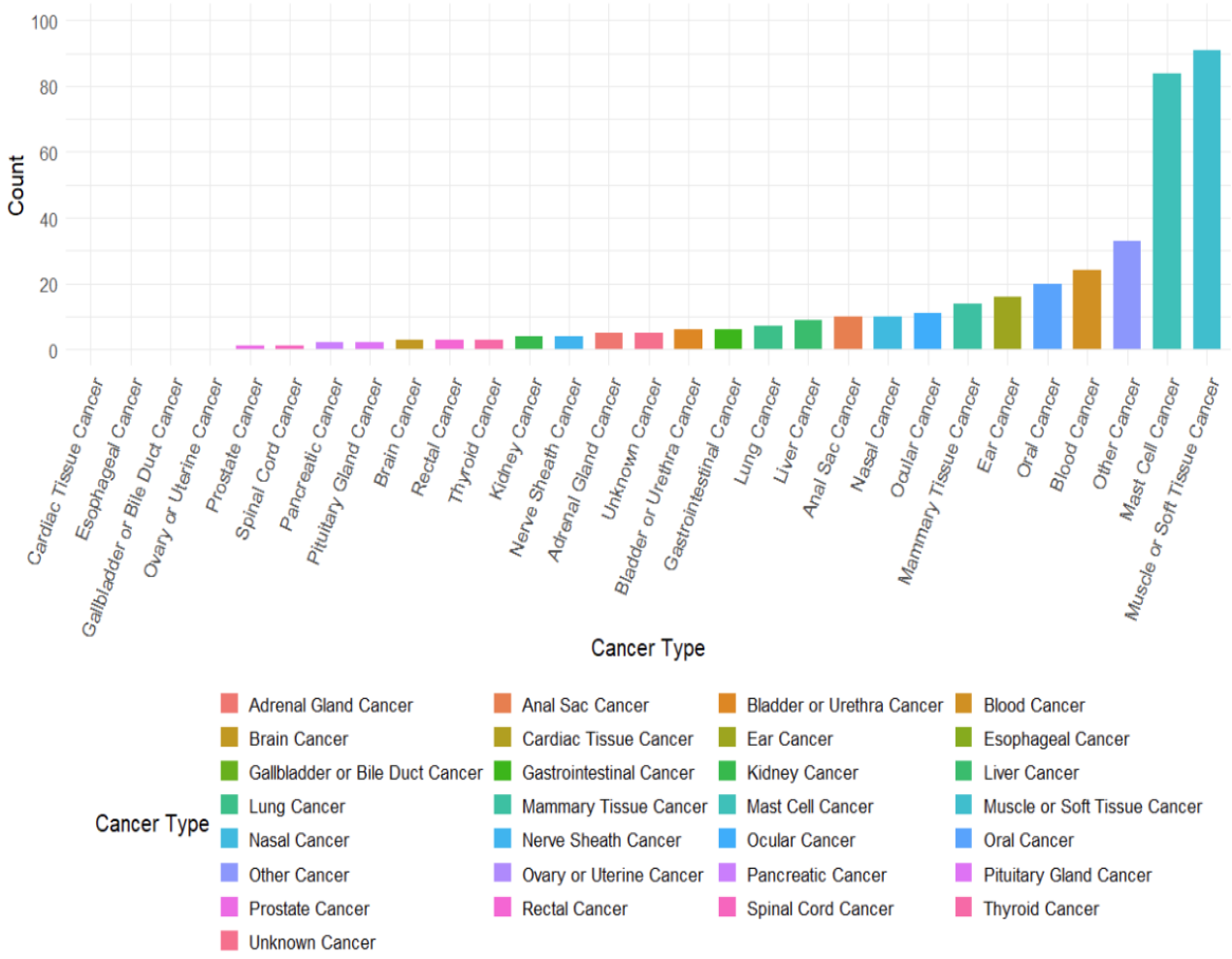
[b] Adjusted for canine size and age.

** Classification of these cases as malignant for Mast Cell Cancer is uncertain, as the source data did not specify whether cases were benign or malignant; they were reported only as "cancer."

Table 5: Lifetime prevalence of malignant tumors associated with PFAS Maximum Contaminant Level (MCL) policies prior to the April 2024 federal mandate adjusted for particulate matter (PM2.5) levels

Cancer Type State MCL Policy	Canine Population (N = 6,657)	Malignant Cancer Cases	Prevalence Ratio (95% CI)	
			Unadjusted	Adjusted
Kidney Cancer				
Yes	2,137	8	0.53 (0.11, 2.49)	0.61 (0.13, 2.94)
No	4,510	2	1.00 (Ref)	1.00 (Ref)
Hematopoietic Cancer				
Yes	2,134	5	0.41 (0.16, 1.06)	0.54 (0.20, 1.43)
No	4,492	26	1.00 (Ref)	1.00 (Ref)
Mast Cell Tumor				
Yes	2,107	32	1.30 (0.84, 2.01)	1.19 (0.75, 1.90)
No	4,466	52	1.00 (Ref)	1.00 (Ref)
Muscle or Soft Tissue Cancer				
Yes	2,110	29	0.99 (0.64, 1.53)	0.78 (0.48, 1.29)
No	4,456	62	1.00 (Ref)	1.00 (Ref)

Figure 1: Distribution of Cancer Types of DAP Canines with PFAS Testing Results from UCMR 5



Discussion

This cross-sectional study was the first, to our knowledge, to examine the potential impacts of PFAS MCL policy on lifetime cancer prevalence in companion canines. Our primary aim was to assess the extent to which PFAS levels exceeding the EPA action level are associated with selected cancers in those animals. We would then consider how the results could inform the use of companion dogs as sentinels for the impact of a vast array of "forever chemicals" on human health.

Due to the small number of cancers in our dataset, we modified our planned analysis to explore two cancer types with the highest prevalence: MCT and MSTC. In the adjusted models, controlling for characteristics such as age, socioeconomic status, and canine size, we did not observe a strong indication of associations between canine residence in zip codes with PFAS levels ≥ 4.0 ppt and the lifetime prevalence of any of the cancer types evaluated. The estimated PRs (Table 4) were not consistent with a positive association between PFOA and/or PFOS exposure and lifetime cancer prevalence. However, the wide CIs indicate substantial uncertainty and do not rule out the existence of such association given our small sample size. Some PRs were below 1.0, suggesting an inverse association of cancer prevalence with PFAS ≥ 4.0 ppt, an unexpected finding that may have been attributed to the small case numbers as well as unmeasured confounding or differences in access to veterinary care. Although we adjusted for socioeconomic status in models where it was indicated, which captured aspects of barriers in accessing to veterinary care (i.e. poverty, unemployment, education), other factors like differences in veterinary care-seeking behavior in zip codes with high PFAS levels may have influenced exposure or outcome classification.[6,29] Our socioeconomic status variable also did not fully capture geographic barriers to care, particularly in rural areas where longer travel to veterinary clinics could have led to underdiagnosis and the negative PRs we discovered.[30] Additionally, owners who choose to participate in the DAP may be systematically different between high and low PFAS zip codes, which could have also contributed to the negative PRs we determined. With these results, we conclude that PFAS exposure above the MCL does not appear to substantially affect lifetime cancer prevalence in canines.

Our results contrast with results from previous similar studies in dogs and humans.[10,31,32] Many organizations like the National Cancer Institute, select PFAS manufacturers, and various academic institutions identify PFAS as toxic substances or potential risk factors in cancer development.[3,31,33] Only recently, after long court battles with PFAS manufacturers such as 3M and DuPont, have human and animal effects of PFAS been directly measured.[10,13,31,34] Our analyses did not reveal any substantial associations, so the direction and magnitude of the relationship between zip code level PFOA and/or PFOS and cancer in companion dogs warrants further investigation with larger numbers of cancer diagnoses. Given that PFAS exposure was aggregated at the zip code level using the EPA PWS sampling data, it is possible that nondifferential misclassification of exposure occurred, biasing some of the PR estimates towards the null. Additional non-differential misclassification may have occurred in the MCT analysis, as we were unable to distinguish between benign and malignant cases.

For the second part of the analysis, we focused on investigating the impacts of state PFAS MCL legislation. Our findings offer preliminary insights into how these policy efforts may correlate with cancer patterns in regions with varying levels of PFAS regulation. The direction of the adjusted prevalence ratios for kidney, hematopoietic, and MSTC suggest the possibility of a lower lifetime cancer prevalence in states with PFAS MCL policies in place. For example, hematopoietic cancer showed a 46% lower lifetime prevalence, but the corresponding 95% confidence interval crossed the null and was very wide. In contrast, MCT showed a slightly elevated prevalence with the same policies (PR = 1.19), which may justify further investigations. Similarly to the primary aim analysis, the generally non-significant associations may be partly attributed to nondifferential misclassification of exposure, both from aggregating PFAS levels from public water systems to the zip code level as well as uncertainty of the effectiveness of the implemented PFAS MCL policies. It is unclear whether these regulations resulted in any meaningful reductions in exposure during the study period, which may have biased the PR estimations towards the null.

Limitations

First, while there are 43,517 dogs in the 2022 DAP Curated Data Release, only 6,657 (approximately 15%) lived at residences that matched with the EPA UCMR 5 dataset based on zip code.

This subset may not be representative of the overall canine population with respect to PFAS exposure or the relationship between that exposure and cancer prevalence due to selection bias. DAP study participation is voluntary, and those who elect to enroll likely represent a small portion of dog owners who are particularly engaged in their canine's health and medical care. This selection bias could influence factors such as access to veterinary care, socioeconomic status, and environmental awareness. Second, many canines are spayed or neutered to reduce reproductive health issues or as a standardized requirement after purchasing from a breeder.[35] Although PFAS has been associated with reproductive cancer risk in humans, this analysis did not include reproductive cancers due to limited available data and likelihood they would not provide meaningful insights within the scope of this study.[3] Third, the DAP environmental survey variable representing socioeconomic disadvantage contained data drawn from multiple years, which varied across individual canines. To address this, the values were averaged for simplicity of analysis. However, it is important to note these values do not precisely reflect conditions during the exact timeframe of this study. It is also important to note that some zip codes had more available PFAS data than others, which may have introduced bias related to differential exposure classification or underestimation of PFAS levels in zip codes with fewer PWS sample results. Additionally, the aggregation of these data from PWS to the zip code level may have led to nondifferential misclassification of exposure, potentially biasing our estimates toward the null.

Furthermore, certain dog breeds may be genetically predisposed to cancer development, while others may be exposed to environmental carcinogens other than PFAS. And some dogs may carry pre-existing comorbidities that could contribute to a higher prevalence of cancer.[36] The small number of cancer cases observed for the selected cancer types severely limited our statistical power to detect association, contributing to the wide confidence intervals. Therefore, these results should be interpreted with great caution. Lastly, since this is a cross-sectional study, the results from this analysis cannot establish a temporal relationship between PFAS exposure and cancer and cannot be used to determine causality.

Conclusion

Although this study did not find evidence of a positive association between either PFAS levels exceeding the EPA action level or lack of state PFAS MCL policies and lifetime prevalence of selected cancers in companion canines, further investigation in studies with larger sample sizes and incidence outcomes are warranted. The final UCMR 5 dataset, which will conclude data collection at the end of 2025, will help provide a more robust foundation for analysis and PFAS exposure characterization for future studies.

Overall, these findings cannot be taken as evidence of clear protective or harmful effects of PFAS exposure but rather are a reminder of the need for ongoing, statistically well-powered, research on the impacts of these forever chemicals, state PFAS MCL policies, and planning future longitudinal analysis to understand the connection between environmental regulation and cancer risk. The widespread nature of PFAS necessitates a better understanding of its toxicologic processes, direct and indirect exposure sources, and how they relate to both human and animal health.[1,37] Further research into the effects of PFAS on canine health should focus on outcomes such as soft tissue and mast cell cancers, and potentially other health concerns linked to these chemicals such as endocrine disruption.[13] Previous studies have identified elevated risk of soft tissue cancers in humans associated PFAS exposure.[38] While the DAP database does not distinguish between soft tissue cancers and muscle cancers in its dataset, the potential relevance of soft tissue cancers may warrant more precise classification in future canine health research.[31] Furthermore, although there is no current evidence of associations between MCT and PFAS exposure, our study suggests an elevated prevalence and further investigation could be beneficial. These future studies could also benefit from including measures to better capture geographical access to care (clinic density, rural-urban commuting area codes) that are not accounted for by the DAP disadvantage index alone.[30,39]

In conclusion, this study contributes to the growing body of knowledge supporting the use of companion canines as human sentinels for monitoring PFAS exposure in U.S. drinking water and the impacts of state PFAS MCL policies on select cancer outcomes. In addition to addressing evidence gaps in this area, this study also highlights how the results could inform the use of companion dogs as sentinels for

the impact of a vast array of “forever chemicals” on human health. Finally, it emphasizes the need for ongoing, rigorously-designed research into associations between PFAS exposure and cancer, and how we can use federal regulatory policy measures to protect the health of humans and their companion canines.

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