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The Relation Between Perfluorooctanoic Acid Exposure and Cerebrovascular Accidents in a Large Sample of Adults Living Near a Chemical Plant

By

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Master of Science in Public Health

Joint Environmental Health and Epidemiology Program

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Abstract

The Relation Between Perfluorooctanoic Acid Exposure and Cerebrovascular Accidents in a Large Sample of Adults Living Near a Chemical Plant
By Christopher Dumon Simpson

Background: People living near a chemical plant south of Parkersburg, West Virginia were exposed to perfluorooctanoic acid (PFOA) – primarily through drinking water – for more than 50 years, starting in the early 1950s. Some previous studies have reported associations between PFOA exposure and stroke risk factors hypertension and uric acid, but these studies have been cross-sectional, making causal inference difficult.

Objectives: This study examined the relation between estimated PFOA exposure and cerebrovascular accidents (CVAs – strokes and transient ischemic attacks) in community members, including chemical plant workers.

Methods: Study participants completed surveys in 2008–2011 regarding medical history, health-related behaviors, occupational history, and demographics. Cox proportional hazards models were used to compare the hazard of cerebrovascular accident in relation to time-varying lifetime-cumulative PFOA exposure estimates. Retrospective analyses included person-time potentially as early as 1952 through the year of the last survey, first CVA, or death (whichever was earliest). Prospective analyses included person-time after 2005–2006 in a similar fashion, conditional on not having had a CVA up to 2005–2006. All models stratified on birth year and controlled for hypertension, diabetes, gender, education level, smoking, and alcohol consumption.

Results: Of the 32,254 participants in the analysis, 1,596 self-reported CVA, of whom 919 had this self-report validated through a review of medical records. In retrospective analyses of validated CVA, compared with the lowest quintile of lifetime-cumulative exposure, the subsequent quintiles had hazard ratios of 1.39, 1.36, 1.45, and 1.13, respectively. When lifetime-cumulative exposure was modeled as a continuous variable with either log-linear or linear form, there was no suggestion of a positive trend. In prospective analyses, the same hazard ratios were 1.07, 1.07, 1.18, and 0.87, and, again, there was no suggestion of a positive trend.

Conclusions: The intermediate ranges of lifetime-cumulative exposure to PFOA saw a modestly elevated risk of CVA compared with the low and high ranges. The absence of a positive dose-response relationship suggests it is unlikely that PFOA exposure within the ranges observed in this study leads to CVA.

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BACKGROUND

Perfluorooctanoic acid (PFOA), also known as C8, did not exist in appreciable quantities before it was produced in industrial settings after World War II (Steenland et al. 2010a). Yet today, the chemical is detectable in the serum of more than 99% of the US population (Calafat et al. 2007) and in the serum of a wide majority of several other populations throughout the world (Fromme et al. 2009; Kannan et al. 2004; Lau et al. 2007). Even wildlife living in places as remote from PFOA point sources as the Arctic and mid-ocean islands have detectable levels in serum (Houde et al. 2006).

The strength of its carbon–fluorine bonds makes PFOA resistant to metabolism in the body and to being broken down in the environment (Steenland et al. 2010a). Estimates for the half-life of PFOA in the human body range from 2.3 to 3.4 years (Bartell et al. 2010; Brede et al. 2010; Olsen et al. 2007). Elimination in humans is considerably slower than in several non-human animals (including, notably, rodents), limiting the generalizability of non-human animal models of PFOA toxicity (Steenland et al. 2010a). At present, the most important routes of PFOA exposure for people living far from sources of PFOA production are not well understood. However, for people living near these sources, exposure is primarily through drinking water (Emmett et al. 2006).

The present study results from the settlement of a 2005 class action lawsuit filed by members of the community in and around Parkersburg, WV against DuPont, whose Washington Works (WW) plant has produced PFOA since 1951. The class claims to have suffered health damages from PFOA (Jack W. Leach v. E.I. du Pont de Nemours & Co 2002). The C8 Science Panel, consisting of three epidemiologists agreed upon by both the class and DuPont, was commissioned to determine whether or not a “probable link”

exists between exposure to PFOA and a broad spectrum of health outcomes. The present study deals with one such health outcome – cerebrovascular accident (CVA), which includes strokes and transient ischemic attacks (TIAs) – and informs the probable link judgment to be made by the C8 Science Panel on July 31st, 2012.

Exposures to the community started in 1951 and peaked in the early 1990s. As part of the terms of the settlement, DuPont paid for activated carbon filters, which have succeeded in almost completely eliminating new PFOA injections into the water supply since their installation in local wastewater treatment plants in 2007. The settlement also called for a survey conducted during 2005-2006 called the C8 Health Project, in which approximately 69,030 people who lived in one of six water districts in West Virginia and Ohio for at least 12 months between 1951 and 2004 were asked questions regarding demographics, residential history, medical history (including family medical history) and health-related behaviors. In addition, blood draws were collected from participants of the C8 Health Project, yielding information about serum concentrations of PFOA and other relevant chemicals. The C8 Health Project enjoyed a very high participation rate: it is estimated that 81% of adults living in one of the six water districts participated (Frisbee et al. 2009).

A CVA is a disruption of blood flow to brain tissues that results in neurological deficits. TIAs are less serious CVAs in which neurological deficits do not last 24 hours. Highly important risk factors for CVAs include age, hypertension, and previous CVA. Other risk factors include diabetes, smoking, atherosclerosis, and atrial fibrillation (American Stroke Association 2012).

Previous work exploring CVAs in connection with PFOA is limited to two mortality studies among worker populations (Leonard et al. 2008; Lundin et al. 2009). The small number of cases in these studies (35 deaths from stroke in each), together with their mixed results, make them inconclusive on the question of a PFOA–CVA link. The strongest basis in the literature for suspecting such a link comes, instead, from four studies that find positive associations between serum uric acid and serum PFOA, as well as a fifth study that finds a positive association between serum PFOA and hypertension. Hypertension is a tremendously important risk factor for both ischemic and hemorrhagic CVAs, and high uric acid is suspected to predispose one toward hypertension.

Leonard et al. (2008) and Lundin et al. (2009) examined cerebrovascular mortality among workers with different levels of PFOA exposure. Leonard et al. (2008) compared the mortality experience of 6,027 presumably exposed DuPont workers at the WW plant with that of 72,882 presumably non-exposed DuPont workers from 7 nearby states (Ohio, Virginia, Kentucky, Indiana, Pennsylvania, Tennessee, and North Carolina) and found that WW workers died from CVAs less often than expected based on the experience of their out-of-state counterparts (SMR = 0.86; 95% CI, 0.60–1.20; 35 deaths). Lundin et al. (2009) compared the mortality experience of 3,993 differentially exposed workers at a 3M manufacturing facility in Cottage Grove, Minnesota with that of Minnesotans in general and found an upward trend in cerebrovascular mortality across never exposed, ever probably exposed but never definitely exposed, and definitely exposed workers [SMRs of 0.5 (95% CI, 0.3–0.8; 13 deaths), 0.7 (0.4–1.1; 17 deaths), and 1.6 (0.5–3.7; 5 deaths), respectively; no test for trend presented]. When workers were instead categorized by estimated cumulative exposure (using weights of 1, 30, and 100

for time spent in never, probably, and definitely exposed positions, respectively), an internal comparison of the cohort yielded hazard ratios of 1.0 (23 deaths), 0.6 (95% CI, 0.2–2.2; 3 deaths), and 2.1 (95% CI, 1.0–4.6; 9 deaths) for the equivalent of < 1 year, 1–5 years, and > 5 years in a definitely exposed position, respectively.

Both of these studies are limited by the small number of stroke deaths observed. For the purpose of informing the present research, they are also limited by studying only stroke mortality, and not non-fatal strokes or transient ischemic attacks (TIAs).

Another motivation for studying a potential CVA-PFOA link concerns a potential hypertension-PFOA link. Hypertension is a very important risk factor for both ischemic and hemorrhagic strokes. For instance, a recent case-control study spanning 22 countries (O'Donnell et al. 2010) estimated the odds of having hypertension to be 3.89 times greater for stroke cases (either ischemic or hemorrhagic) than for age- and sex-matched controls (99% CI, 3.33–4.54; hypertension defined as self-report or > 160/90 mm Hg). For ischemic strokes, the OR was 2.37 (2.00–2.79), while for hemorrhagic strokes, it was 3.80 (2.96–4.78).

There is some concern that PFOA may be related to hypertension. Min et al. 2012 performed a cross-sectional study using data from 2,208 participants in the 2003-2004 and 2005-2006 NHANES. After adjusting for age, sex, race/ethnicity, income, smoking habits, alcohol use, obesity status, total saturated fatty acid intake, physical activity, serum PFOS concentrations, total cholesterol, and poor kidney function (as measured by eGFR), Min et al. 2012 found that, compared to individuals in the lowest quintile of serum PFOA (< 2.4 μg/L), individuals in the highest quintile of serum PFOA (> 6.1 μg/L) had 2.62 times the odds of being hypertensive (95% CI, 2.09–3.14; hypertension

defined as self-report or systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg). Moreover, using the lowest quartile of serum PFOA as the reference group, there was a monotonic increase in the odds ratios with increasing quartile of serum PFOA [Quartile 2: 1.21 (95% CI, 0.86–1.70), Quartile 3: 1.60 (1.15–2.22), Quartile 4: 1.71 (1.23–2.36)]. When treated as continuous variables, systolic – but not diastolic – blood pressure demonstrated a statistically significant positive association with log-transformed serum PFOA.

Another source of concern about a possible PFOA-hypertension link is the consistent finding (in cross-sectional studies) that serum PFOA levels are positively associated with serum uric acid levels. Uric acid is, in turn, a risk factor for hypertension (Feig et al. 2008; Shankar et al. 2006). Four cross-sectional studies – two of workers (Costa et al. 2009; Sakr et al. 2007), one of a highly-exposed community (Steenland et al. 2010b), and one of the general US population using NHANES data (Shankar et al. 2011) – have found statistically significant positive associations between serum PFOA levels and serum uric acid levels. Costa et al. 2009 included a sub-analysis of repeated measurements in 56 subjects over 7 years, which also revealed a statistically significant positive association between PFOA and uric acid.

The current study provides the best opportunity yet to understand the effect of PFOA on CVAs. Unlike previous studies, the current study follows individuals over time. It also has a very large sample size (in the tens of thousands), covering people with exposure levels ranging from those near the US average to those only seen in occupational settings.

METHODS

Cohort Recruitment

Participants in this study were drawn from two sources: a community cohort and a worker cohort. The community cohort was taken from the subset of the 69,030 participants in the earlier C8 Health Project who were at least 20 years old at the time of participation in that project (yielding ~54,457 individuals) and who additionally consented to having their identifiable information released to the C8 Science Panel. Approximately 74% of these ~54,457 individuals consented, for a total of 40,145 individuals in the target population for the community cohort. The worker cohort was drawn from the one assembled by Leonard et al. (2008), described earlier. The target population for the worker cohort consisted of 6,026 individuals who worked at DuPont between January 1948 and December 2002.

Because workers might have participated in the C8 Health Project, some individuals are in both the community and the worker cohorts. Unless otherwise stated, all analyses presented in this paper are of individuals in the "combined cohort" – i.e., the union of the community cohort and the worker cohort. Figure 1 depicts the creation of the combined cohort.

Survey Administration

All members of the combined cohort were asked to complete a baseline survey during August 2008–April 2010. One important feature of this survey was that, unlike the C8 Health Project, it asked participants *at what age* they were diagnosed with the diseases they claim to have. Knowing the timing of health-related factors makes the data

amenable to survival analysis, since the temporal relations between exposure, disease onset, and the onset of other relevant health-related factors can be established.

During May 2010–May 2011, people who had completed the 2008–2010 baseline survey were asked to complete a follow-up survey that also asked about incident disease. During the same time period, individuals who had not participated in the 2008–2010 survey were given a second opportunity to complete the baseline survey. Individuals who completed a baseline survey (in either time period) were potentially included in the analyses.

Cerebrovascular Accident Ascertainment

The 2008–2010 and 2010–2011 surveys asked, "Have you **ever** been told by a doctor or other health professional that you had a stroke (also called cerebrovascular accident) or ministrokes (also called Transient Ischemic Attack or TIA)?" Those who responded "Yes" were then asked, "How old were you when you were **first** treated for stroke?" as well as, "Were you **ever** hospitalized for treatment of your stroke?" and "Are you currently taking any **prescription** medication as a result of your stroke?" Responses in other parts of the surveys (for instance, in free-text fields for questions about other diseases) that appeared to indicate a CVA were taken into account by a reviewing physician, who reclassified individuals as claiming a CVA where appropriate.

For everyone who was classified as claiming a CVA, an attempt was made to gain consent for the release of their medical records, so that the claim could be validated. Medical records were retrieved and reviewed by a private company. In addition, an in-house process was used to quality-check the work of the company, as well as to adjudicate borderline cases. The toughest borderline cases were reviewed by a physician

for the final validation decision. Both ischemic and hemorrhagic CVAs were counted, although the design of the survey did not allow us to distinguish between these etiologies for the purposes of analysis. The earlier C8 Health Project (2005–2006) had its own medical record validation component, independent of the present one. Because all of the community cohort members and many of the worker cohort members participated in the C8 Health Project, the results of that project's validation efforts were used as a supplementary validation source for the present study.

Yearly Serum PFOA Concentration Estimation

A fate and transport model was used to estimate the concentrations of PFOA present in different environmental media in each year since PFOA production began in 1951. This model utilized information about historic emission rates from the WW plant, the physical and chemical properties of PFOA, and local geologic and meteorological data (Shin et al. 2011a). Individual community members were assigned yearly PFOA exposure estimates using this model in combination with information on residential history, drinking water habits and sources, and public water supply network maps (Shin et al. 2011b). For workers, yearly exposures were estimated using a job history matrix. Finally, an absorption, distribution, metabolism, and excretion model was used to generate yearly serum PFOA concentration estimates based on exposure estimates, demographic information, self-reported body weights, and estimates of the metabolic half-life of PFOA (Shin et al. 2011b). The accuracy of the yearly serum concentration estimates was tested by comparing them to the measured serum PFOA concentrations in 2005-2006 from the C8 Health Project (Shin et al. 2011b).

Data Analysis

Overview

The Cox proportional hazards model was used to compare the hazard of CVA in relation to PFOA exposure. The main analysis included individuals in the combined cohort for whom we had enough information to generate reasonable exposure estimates and who completed at least one survey in the period 2008–2011. Retrospective analyses included person-time from either age 20 or 1952 (whichever was later) to the year of first CVA, the year of the last survey, or death (whichever was earliest). Prospective analyses included person-time from the time of the C8 Health Project (2005–2006) to the year of first CVA, the year of the last survey, or death (whichever was earliest) among individuals who had not yet had a CVA at the time of the C8 Health Project. Age was used as the time variable in all models because the hazard of CVA is strongly related to age. All models stratified on birth year in an attempt to control for secular trends in the hazard of CVA over time (perhaps resulting from, for example, changes in diagnostic practices over time). PFOA exposure was modeled as both a categorical variable (using quintiles) and as a continuous variable (assuming either a log-linear or a linear form). Lifetime-cumulative, lagged lifetime-cumulative, and yearly metrics of estimated serum concentration were considered. Analyses were performed by PROC PHREG in SAS 9.1, using the start-stop method, with age as the time variable, and the EXACT method for handling ties.

Detail

To reduce misclassification of the outcome, the analysis included only cases that were validated through medical record review. Individuals who self-reported a CVA but whose self-report was not validated were excluded from the analysis.

Individuals born before 1920 were found in parallel analyses of thyroid and heart diseases to have unstable baseline hazards for those outcomes, perhaps due to unreliable self-reporting in this age group. This issue was not investigated with respect to CVAs. However, because of the findings for thyroid and heart diseases, and for the sake of uniformity across the different disease investigations in the research group, individuals born before 1920 were excluded from the analysis.

Because CVAs occurring before age 20 are very rare and potentially different from those occurring at later ages, all person-time before age 20 was excluded from the analysis. An individual's estimated cumulative exposure still accrues during the under-age-20 period, but they do not enter the risk set until age 20.

Because the mechanisms by which PFOA might lead to CVAs are not known, several exposure metrics were tested. Each metric reflects, at some level, a different hypothesis (or class of hypotheses) about how PFOA might operate to bring about a CVA. If recent increases in a person's exposure to PFOA are particularly important in bringing about CVAs, then we ought to favor metrics that reflect more recent exposures. To address hypotheses with this general character, we used the yearly serum estimate, as described above. If, alternatively, CVA risk accrues over one's lifetime as a function of all PFOA ever encountered, then we ought to favor metrics that count old exposures as well as newer ones. To address hypotheses with this general character, we used the

lifetime-cumulative serum estimate – a sum of all the yearly serum estimates from birth through a given year. (Cumulative exposure measures are often used when studying chronic diseases.) Finally, an X-year lagged cumulative metric, defined as a person's cumulative serum estimate X years ago was also used. This metric reflects the possibility that a latency period exists between exposure and CVA. 5-year and 10-year lags were considered.

Each of these exposure metrics was modeled in three ways – 1) as a categorical variable (using quintiles), 2) as a continuous variable assuming a log-linear form, and 3) as a continuous variable assuming a linear form. The selection of the main retrospective and main prospective models was made from among these combinations. The Akaike information criterion (AIC), a relative measure of goodness of fit, guided this selection.

For the analyses using the categorical exposure variable, the lowest-exposure quintile ("Q1") was the referent group. The "cut point" values used to define the quintiles were always based on the distribution of the exposure variable *among the cases included in the analysis, at the time of the event (first CVA)*. Defining quintiles in this way has the desirable property that the effect estimates for membership in any non-referent quintile (relative to membership in the referent quintile) all have very nearly the same precision, making results easier to interpret (Steenland and Deddens 2004).

The set of control variables to include in the models was decided *a priori*. Control variables were either known from previous literature to be strongly associated with CVAs (for example, hypertension, smoking, and diabetes), were of social importance (for example, gender and race), or were a combination of both (for example, years of schooling). Consequently, all models adjust for the same set of covariates: 1) time-

varying hypertension (based on self-report of currently taking hypertension medication and the year in which hypertension was first diagnosed), 2) diabetes (non-time-varying, based on self-report), 3) gender, 4) non-time-varying years of schooling (meant to serve as a proxy for socioeconomic status (SES)), 5) race (white vs. non-white), and 6) time-varying current and former smoking and alcohol consumption. Cholesterol was not included as a control variable in part because of the concern that PFOA might raise cholesterol. (This issue is being investigated in a separate part of the project.)

Tests for interaction with exposure were performed for each of the covariates in the main models by adding a simple product term (e.g. the natural log of cumulative exposure times gender) and considering the p-value associated with this term. If necessary, new models were run that stratified on the covariate in question, and the effect estimates across the strata were examined. Finally, the proportional hazards assumption was assessed for the exposure by adding a product term between the exposure and age (which is not explicitly in the model) and following the same process.

Sensitivity Analyses

A number of sensitivity analyses were considered for the retrospective and/or prospective models. First, we considered a model in which background PFOA exposure was subtracted from all exposure estimates. In our principal analysis, background serum levels (i.e., typical levels for the U.S. population in general) were added to everyone's yearly serum estimates. Although the background component was usually much smaller than the non-background component (reflecting the fact that our community was much more highly exposed than the general U.S. population), there was some concern that the

addition of this component was adding noise to the exposure estimates rather than refining them.

A second sensitivity analysis altered the usage of the medical record validation processes by not incorporating cases that were confirmed only through the C8 Health Project's medical record review process. This analysis is meant to investigate a bias that could arise from the usage of both validation sources. Recall that the *only* individuals in the present study who did not necessarily participate in the C8 Health Project were workers (Figure 1), who are highly exposed compared to non-workers. This means that, by using both validation sources, individuals in a highly exposed class of people (i.e., workers who did not participate in the C8 Health Project) are given somewhat less of a chance at validation than the rest of the cohort members, possibly biasing effect estimates toward the null. An additional reason for performing this sensitivity analysis is that the details of the validation process in the C8 Health Project were not known to our research group and may not be comparable to the criteria for validation used in 2010–2012.

A third sensitivity analysis restricted cases to those people who reported being hospitalized for their CVA. The intention here was to create a case group of possibly more serious cases, allowing us to explore, albeit indirectly, whether PFOA perhaps exerts a greater effect on stroke than on the etiologically similar but less severe TIA.

A fourth sensitivity analysis studied only non-workers. The purpose of this analysis was to explore the possibility of a healthy worker effect, which might influence the shape of the dose-response curve, particularly at higher exposure levels.

Finally, a series of retrospective models were run in which the study end time was progressively shortened by 3 year intervals. This allows us to explore the possibility of

effect modification over calendar time. Such an effect modification could occur if, for instance, individuals vary in their susceptibility to the effect of PFOA on CVA. Over time, as the more susceptible individuals get CVAs and therefore fall out of the underlying population at risk, the hazard of CVA in relation to PFOA exposure could decrease, masking the effects of PFOA in the main models.

RESULTS

Cohort Characteristics

Of the 40,145 people in the target population for the community cohort, 32,712 (81.5%) completed at least one survey, making them potentially eligible for analysis. Of the 6,026 people in the target population for the worker cohort, 4,391 (72.9%) completed at least one survey. Finally, some respondents in both groups were excluded from the analyses because reasonable exposure estimates could not be generated for them (see Figure 1). All results are presented for the combined cohort, which had 32,254 members.

Demographic characteristics of the cohorts are presented in Table 1. Workers had higher measured serum PFOA levels in 2005–2006 (median = 25.3 ng/ml) than non-workers (median = 12.3 ng/ml), but both workers and non-workers had higher serum levels than the general U.S. population in 2003–2004 (median = 4.0 ng/ml) (Calafat et al. 2007). The median follow-up time after age 20 was 32.9 years. Approximately a million person-years were included in the retrospective analyses.

The mean estimated serum PFOA concentrations for each calendar year since 1951 among workers, non-workers, and the two groups combined are shown in Figure 2.

Main Models

Of the 32,254 individuals in the combined cohort, 1,596 individuals self-reported CVA, of which 919 individuals (57.6%) had this self-report validated. The commonest reason for non-validation was failure to obtain the participant's consent to have medical records released. Some medical records documented suspicion of TIA (based on, for instance, temporary spells of facial numbness or blurred vision), but not a definitive diagnosis, and thus did not validate the participant's claim. Because TIAs are more likely than strokes to be suspected but not definitively diagnosed, the inclusion of TIAs in the survey question probably resulted in a lower validation rate than would have been had if the survey had asked only about strokes.

After the age-related exclusions described in the previous section, 880 validated cases remained. 55 of these 880 cases (6.25%) were excluded at the time of analysis because of missing data (43 missing in time-varying hypertension, 10 in age at CVA, 1 in alcohol and 1 in smoking), leaving a final total of 825 cases analyzed in the retrospective models.

For the prospective analysis, all person-time before an individual's participation in the C8 Health Project (if the individual participated in that project) or before August 1st, 2006 (if the individual was a worker who did not participate in the C8 Health Project) was excluded. This had the effect of removing 573 of the above 825 cases, for a total of 252 cases analyzed in the prospective models.

Tables 2 and 3 present, respectively, the results of the retrospective and prospective analyses. HR stands for hazard ratio. All cut points are in ng/ml. All confidence intervals are 95% confidence intervals.

For the retrospective analyses, the un-lagged and 5-year-lagged lifetime-cumulative exposure metrics stood out compared with the other metrics as best fitting the data according to AIC. Using either of these metrics, the individual contrasts between quintiles 2–5 and quintile 1 showed somewhat higher hazards in quintiles 2–5 than in quintile 1. In quintiles 2–4, but not in quintile 5, the hazards relative to quintile 1 were statistically significant. Neither of the trend tests showed a significant positive trend across the entire range of exposures.

For the prospective analyses, there were conflicting findings regarding the best-fitting metric, depending on whether exposure was modeled categorically or continuously. No metric, however, showed significantly elevated hazards for the non-referent quintiles. Again, there was no suggestion of positive trend across the entire range of exposures.

To help put the PFOA–CVA results in perspective, Table 4 presents representative hazard ratios for the other covariates in the models. (In particular, the statistics in Table 4 come from the first model presented in Table 2.)

No significant interactions were found between exposure and the other covariates in either the retrospective or the prospective models. However, a significant violation of the proportional hazards assumption was found for the exposure variable in the prospective model. This interaction was explored by partitioning the data into three segments according to age, and running three separate models – one on each partition. The first model analyzed only person-years in which the person's age was in the interval [20, 49], the second in [50, 66], and the third in 67+. These intervals were selected because they provide reasonable balances of person-time-at-risk and events.

With exposure modeled as the natural log of cumulative exposure, the hazard ratios for the different age groups, youngest to oldest, were 0.85 (0.63–1.15), 0.88 (0.77–0.99), and 1.02 (0.92–1.12). Altogether, these analyses suggest that, while the effect of PFOA on a person's risk for CVA may increase slightly as a person gets older, for no age group does this risk rise significantly above their baseline risk (i.e., their risk had they not been exposed).

Sensitivity Analyses

The sensitivity analysis that modeled only above-background exposures (Table 5A) showed results very similar to those of the main model. The result of the log-linear trend test increased very slightly (1.01 to 1.02), but still did not reach statistical significance (p=0.28). The lack of change in this analysis suggests that bias resulting from the inclusion of the background element in the exposure estimates was minimal.

The sensitivity analysis that excluded the C8 Health Project as a source of validation (Table 5B) resulted in a loss of 74 cases, bringing the total number of cases to 751. Hazard ratios in the non-referent quintiles tended to increase relative to those in the main model, although the hazard ratio for the third quintile decreased. Altogether, the hazard ratios in this analysis fluctuated more from one quintile to the next than they did in the main model, suggesting that the effect estimates in the categorical analysis may be unstable. Again, the result of the log-linear trend test increased very slightly (1.01 to 1.02), but still did not reach statistical significance (p=0.30).

Of the 825 individuals in the final retrospective model, 572 (69.3%) reported hospitalization on at least one survey. The sensitivity analysis restricted to these cases

(Table 5C) showed results very similar to those of the main model, suggesting that effect of PFOA on CVA risk may not vary as a function of CVA severity.

When the analysis was restricted to non-workers to study the possibility of a healthy worker effect (results not shown), the effect estimates across increasing quintiles of exposure showed a pattern similar to that in the main model, indicating that bias due to a healthy worker effect is likely minimal in this study.

Finally, when the end year of the retrospective analysis was progressively decremented (Table 6), the log-linear trend tests showed monotonically increasing hazard ratios. Moreover, the *significance* of these hazard ratios also increased monotonically as the end year was decremented. This finding is especially striking since the number of cases in each analysis drops rather quickly. (A typical 3-year decrement results in a loss of about 25–30% of the cases from the previous model.) The log-linear trend test reached $\alpha = 0.05$ significance for the first time when the analysis was stopped in 1996.

DISCUSSION

On balance, this study provides only modest evidence that PFOA exposure leads to CVAs. Although people falling in the intermediate quintiles of cumulative PFOA exposure had an elevated risk of stroke relative to people in the lowest quintile, people in the highest quintile did not. While a strictly monotonic increase in risk across increasing exposure categories is not necessary to establish a strong suspicion of causation (see, for instance, Stayner et al. 2003), a deviation from monotonic increase as conspicuous as the one in this study casts doubt on a causal link.

It should be stressed, however, that, while the results of this study do not paint an uncomplicated picture supporting a causal link, they also do not justify a hasty dismissal of the issue. A clear-cut null finding would be expected produce results in which the hazard ratios at different exposure levels appear in some sense randomly scattered about the line y = 1. (In particular, it is expected that the likelihood of observing a hazard ratio of X is the same as the likelihood of observing a hazard ratio of 1/X.) By contrast, the effect estimates in the retrospective models in this study are almost uniformly greater than 1. Moreover, the effect estimates in the categorical analyses appear to exhibit something of a shape: they rise moving into Q2, remain roughly steady through Q4, and then dip in Q5. In both of these ways (directionality and shape), the results of the categorical analyses do not conform nicely to the expectations we would have for a totally null relation.

The interpretation of the results of this study depends largely on whether one thinks the retrospective or the prospective analysis is more appropriate. Which analysis is more appropriate depends, in turn, on what biases affect the study the most, since some biases affect the retrospective analysis more than the prospective analysis, and vice-versa.

One possibly important bias results from the fact that, for non-workers, an implicit requirement for eligibility in the study was being alive in 2005–2006 (the time of the C8 Health Project). If PFOA is in fact related to CVAs, then the failure to incorporate the experience of those people who died before 2005–2006 could bias the results of the retrospective analysis toward the null. This bias is a form of selection bias, and it is made worse the longer the period of required survival lasts after the time of exposure. 2005–2006 is a relatively long time after the peak exposures of the early 1990s, increasing

concerns about this bias. Finally, CVAs themselves are often fatal, so the analysis of CVAs might be especially affected by this bias. Insofar as this bias is important in the present study, the prospective model is preferable to the retrospective one, since the prospective model does not incorporate *anyone's* experience prior to 2005–2006.

However, there is another potential bias that would affect the prospective analyses more than the retrospective ones. This bias, concern about which motivated the final sensitivity analysis, results from the possibility that people are differentially susceptible to the effects of PFOA. Imagine, as a stylized example, a fixed population in which certain people (call them the "doomed") are guaranteed to have a CVA upon reaching a certain (sometimes reached) threshold of lifetime PFOA exposure, while other people (call them the "blessed") get CVAs or don't entirely independent of their PFOA exposure. Imagine further that, other than this difference, the doomed and the blessed are comparable to each other in their risk of CVA. If this is so, then PFOA will appear to exert a less potent effect on risk-of-first-CVA in this population as time goes on, since the pool of people who remain at risk for a first CVA loses doomed people faster than it loses blessed ones. The pool is, in other words, depleted of susceptible individuals. In the context of the current study, since peak exposures occurred many years before the start of the follow-up time in the prospective analysis, it is possible that many of the doomed people would have already had their first CVA before 2005–2006, and thus their experience would not be incorporated by the prospective analysis, biasing the hazard ratios of that analysis downward. It has even been established, through simulations, that a purely detrimental exposure can appear protective (i.e., a crossover bias can operate) if

individuals differ greatly enough in their susceptibility to the exposure and follow-up does not begin until after the bulk of exposure ceases (Applebaum et al. 2011).

While the retrospective analysis would also be affected by this bias due to differential susceptibility, it would be not affected as much as the prospective analysis, because it would capture more doomed people's CVAs. As a way to investigate the extent of this bias, we ran the sensitivity analysis that decremented the study end time by 3-year intervals. The results of this analysis indicate that there is clearly effect modification over calendar time. This offers some support for the idea that at earlier points in time, the population at risk in this community may have had greater susceptibility to the effect of PFOA on CVAs than it did in later times.

A depletion of susceptibles effect also has the potential to explain some of the "dip" in the hazard ratio when considering the very highest exposure quintile. This is because the population at risk at the highest exposure levels may contain relatively few doomed people. This would occur, for instance, if our hypothetical PFOA exposure threshold for CVA in the doomed people was below the exposure levels in the uppermost quintile.

Ultimately, however, it is difficult to decide for certain whether a strong depletion of susceptibles effect is truly at work, or whether some as-yet unnoticed bias can explain the upward trajectory of the hazard ratios in Table 6 as earlier time periods are considered. And even if we believe such an effect to be important, it is unlikely that it operates strongly enough to explain the entirety of the dip in the dose-response curve at the highest exposure levels.

It should also be pointed out that there are hazards to relying on the results of the categorical analyses, as opposed to the results of the trend tests. For instance, the choice of the referent group drives all the effect estimates in the categorical analyses, so if there is something special about the referent group, all hazard ratios would be thrown off. In the context of the current study, this means that the suggestion of elevated risk in the intermediate exposure quintiles could be an artifact of an underassessment of the risk in the lowest exposure quintile, for reasons unknown.

Another possible bias affecting this study is the healthy worker survivor effect (HWSE). The HWSE refers to the tendency of workers with ill health to drop out of the workforce sooner than healthier workers (Stayner et al. 2003). Dropping earlier from the workforce results in lower cumulative exposure estimates for the more ill workers than for the healthier workers. But the more ill workers, because they are more ill, are at a greater risk for disease than the healthier works. The HWSE is thus a problem of confounding. Unlike the first bias discussed in this section (which is sometimes given the name "survivor bias"), the HWSE does not operate at the level of who enters the analysis. Rather, it operates within the group of people who do enter the analysis. The HWSE can cause otherwise positive dose-response curves to attenuate or even turn negative at high exposure levels. This bias could affect either the retrospective or the prospective analyses.

The importance of the HWSE in the present study is limited by the fact that workers made up only a small fraction of the combined cohort (~11.5%) and a small fraction of the cases (~12.5%). However, *all* of the worker cases fall into the fourth or fifth exposure quintiles (with 21 and 82 workers, respectively). Consequently, the HWSE

has the potential to explain at least some part of decline in the hazard ratio going from the fourth quintile to the fifth.

Finally, it might be objected that the models presented in this paper may be limited in their ability to detect a causal link between PFOA and CVAs because they control for hypertension, since it is plausible that hypertension might lie along a causal pathway from PFOA to CVA (if such a pathway exists). If so, controlling for hypertension would obscure the effect of this pathway. Indeed, as described in the introduction, the (cross-sectional) association between PFOA and hypertension (as well as uric acid, which may lead to hypertension) reported in the literature was a central motivation for studying the PFOA–CVA relation in the first place. This only makes sense as a motivation for the present study if we believe in the plausibility of a PFOA -> hypertension -> CVA pathway.

To address this objection, modified models, not controlling for hypertension in any fashion, were run. These models (results not shown) showed a slight but consistent *decrease* in the hazard ratios, relative to the models that do control for hypertension, indicating that hypertension is not an intermediate variable. Therefore, controlling for hypertension is likely appropriate. A separate investigation by the C8 Science Panel, looking to clarify the PFOA–hypertension relation, is currently underway.

CONCLUSIONS

The absence of a positive dose-response relationship suggests it is unlikely that PFOA exposure within the ranges observed in this study leads to CVA. Although potential biases, such as the depletion of susceptibles effect and the healthy worker

survivor effect might partly explain the finding of lower risk at the highest level of exposure than at intermediate levels of exposure, these biases are unlikely to be of such magnitude as to cause a deviation from a positive dose-response relationship as conspicuous as the one seen in this study.

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Table 1. Cohort demographics by response

		Community Cohort (n=28,541)	Worker Cohort (n=3,713)	Combined Cohort (n=32,254)
Year of Birth	25th percentile	1947	1941	1946
	Median	1958	1951	1957
	75th percentile	1970	1963	1969
Gender	Female	16,602 (58.2%)	758 (20.4%)	17,360 (53.8%)
Race	White, non-Hispanic	27,901 (97.8%)	3,284 (88.5%)	31,185 (96.7%)
	Other	640 (2.2%)	134 (3.6%)	774 (2.4%)
	Missing	0	295 (7.9%)	295 (0.9%)
Education	<High School	3,026 (10.6%)	37 (1.0%)	3,063 (9.5%)
	High School	11,706 (41.0%)	1,265 (34.1%)	12,971 (40.2%)
	Some College	9,441 (33.1%)	1,081 (29.1%)	10,522 (32.6%)
	College or Higher	4,366 (15.3%)	1,328 (35.8%)	5,694 (17.7%)
	Missing	2 (0.01%)	2 (0.1%)	4 (0.01%)
Smoking	Never Smoked	13,527 (47.4%)	1,989 (53.6%)	15,516 (48.1%)
	Smoked and quit	8,899 (31.2%)	1,297 (34.9%)	10,196 (31.6%)
	Smoked, did not quit	6,115 (21.4%)	427 (11.5%)	6,542 (20.3%)
Regular Alcohol Consumption	Never	17,011 (59.6%)	1,683 (45.3%)	18,694 (58.0%)
	Yes and quit	4,105 (14.4%)	535 (14.4%)	4,640 (14.4%)
	Yes, did not quit	7,360 (25.8%)	1,486 (40.0%)	8,846 (27.4%)
	Missing	65 (0.2%)	9 (0.2%)	74 (0.2%)
Measured 2005-2006 Serum PFOA Concentration (ng/ml)	Mean	70.9	324.6*	86.6**
	Standard Deviation	151.2	920.6*	278.9**
	Median	24.2	112.7*	26.1**
Length of follow up after age 20 (years)	Mean	32.1	38.7	32.9
	Standard Deviation	15.6	13.5	15.5
	Median	32.0	39.2	32.9

*Calculated among the 1,890 workers also participating in the C8 Health Project
**Calculated among the 30,431 individuals also participating in the C8 Health Project

Table 2. Results of retrospective survival analysis (825 cases, main model in black)*

Exposure Metric	Modeled Form	HR	LCI	UCI	p-value	AIC
Cumulative	Q2 (>178–319)	1.39	1.11	1.76	**0.005**	8656.90
	Q3 (>319–912)	1.36	1.08	1.71	**0.010**	
	Q4 (>912–4,490)	1.45	1.15	1.82	**0.002**	
	Q5 (>4,490)	1.13	0.90	1.44	0.297	
	Continuous (linear)	1.00	0.99	1.01	0.522	8665.33
	Continuous (log-linear)	1.01	0.97	1.06	0.590	8665.49
Cumulative, 5 year lag	Q2 (>130–217)	1.39	1.09	1.77	**0.009**	8658.88
	Q3 (>217–560)	1.42	1.12	1.81	**0.004**	
	Q4 (>560–3,420)	1.41	1.11	1.80	**0.005**	
	Q5 (>3,420)	1.17	0.91	1.49	0.224	
	Continuous (linear)	1.00	0.99	1.01	0.442	8665.11
	Continuous (log-linear)	1.01	0.96	1.05	0.792	8665.70
Cumulative, 10 year lag	Q2 (>88.2–130)	1.07	0.82	1.40	0.630	8669.59
	Q3 (>130–323)	1.04	0.80	1.36	0.777	
	Q4 (>323–2,330)	1.14	0.88	1.49	0.322	
	Q5 (>2,330)	0.98	0.75	1.29	0.887	
	Continuous (linear)	0.99	0.98	1.01	0.351	8664.74
	Continuous (log-linear)	0.99	0.95	1.04	0.700	8665.60
Yearly	Q2 (>6.29–13.4)	1.19	0.95	1.48	0.130	8664.74
	Q3 (>13.4–38.7)	1.02	0.81	1.27	0.893	
	Q4 (>38.7–131)	1.29	1.03	1.61	**0.029**	
	Q5 (>131)	1.09	0.87	1.36	0.464	
	Continuous (linear)	1.00	1.00	1.00	0.751	8665.68
	Continuous (log-linear)	1.02	0.98	1.07	0.361	8664.95

*Q2–Q5 refer to exposure quintiles two through five. All hazard ratios for quintiles are relative to Q1, the lowest quintile of exposure. Parentheses next to quintiles contain the exposure ranges for those quintiles (in ng/ml).

Table 3. Results of prospective survival analysis (252 cases, main model in black)*

Exposure Metric	Modeled Form	HR	LCI	UCI	p-value	AIC
Cumulative	Q2 (>244–460)	1.07	0.73	1.59	0.724	2612.74
	Q3 (>460–1,240)	1.07	0.72	1.58	0.735	
	Q4 (>1,240–5,500)	1.18	0.79	1.75	0.419	
	Q5 (>5,500)	0.87	0.58	1.30	0.503	
	Continuous (linear)	0.99	0.97	1.01	0.280	2607.74
	Continuous (log-linear)	0.97	0.89	1.05	0.412	2608.50
Cumulative, 5 year lag	Q2 (>189–352)	0.99	0.67	1.47	0.969	2611.36
	Q3 (>352–979)	0.98	0.66	1.45	0.915	
	Q4 (>979–4,360)	1.21	0.81	1.80	0.346	
	Q5 (>4,360)	0.81	0.55	1.22	0.315	
	Continuous (linear)	0.99	0.97	1.01	0.334	2608.02
	Continuous (log-linear)	0.96	0.89	1.05	0.373	2608.38
Cumulative, 10 year lag	Q2 (>127–212)	0.87	0.59	1.31	0.509	2612.73
	Q3 (>212–581)	0.84	0.57	1.25	0.387	
	Q4 (>581–3,630)	1.00	0.67	1.50	0.984	
	Q5 (>3,630)	0.78	0.52	1.16	0.218	
	Continuous (linear)	0.99	0.97	1.01	0.408	2608.33
	Continuous (log-linear)	0.96	0.89	1.04	0.305	2608.11
Yearly	Q2 (>6.28–10.6)	1.31	0.88	1.93	0.183	2612.74
	Q3 (>10.6–23.1)	1.16	0.79	1.73	0.448	
	Q4 (>23.1–68.9)	1.03	0.70	1.53	0.873	
	Q5 (>68.9)	1.03	0.69	1.53	0.900	
	Continuous (linear)	1.00	1.00	1.00	0.136	2606.64
	Continuous (log-linear)	0.97	0.89	1.06	0.542	2608.81

*Q2–Q5 refer to exposure quintiles two through five. All hazard ratios for quintiles are relative to Q1, the lowest quintile of exposure. Parentheses next to quintiles contain the exposure ranges for those quintiles (in ng/ml).

Table 4. Hazard ratios for model covariates

Covariate	HR	LCI	UCI	p-value
Diabetes	1.46	1.26	1.70	**<0.001**
Education: High School*	0.83	0.69	1.01	0.060
Education: Some College*	0.77	0.62	0.96	**0.021**
Education: College or Higher*	0.51	0.38	0.70	**<0.001**
Gender (male)	1.02	0.87	1.19	0.819
Hypertension	2.87	2.44	3.37	**<0.001**
Race (non-white)§	1.17	0.79	1.73	0.441
Regular Alcohol Consumption (current)†	1.01	0.83	1.22	0.939
Regular Alcohol Consumption (former)†	1.23	1.00	1.50	**0.048**
Smoking (current)‡	1.50	1.20	1.88	**<0.001**
Smoking (former)‡	0.94	0.77	1.14	0.514

*Compared to Less Than High School
§Compared to White, non-Hispanic
†Compared to Never Regular Alcohol Consumption
‡Compared to Never Smoking

Table 5A. Results of sensitivity analysis: above-background exposure only (retrospective analysis, 825 cases, exposure metric: cumulative)

	HR (change*)	LCI	UCI	p-value	AIC
Q2 (>44.3–185)	1.35 (-0.04)	1.08	1.69	**0.008**	8657.67
Q3 (>185–784)	1.34 (-0.02)	1.07	1.68	**0.011**	
Q4 (>784–4,460)	1.43 (-0.02)	1.14	1.79	**0.002**	
Q5 (>4,460)	1.12 (-0.01)	0.89	1.42	0.319	
Continuous (linear)	1.00 (0)	0.99	1.01	0.522	8665.34
Continuous (log-linear)	1.02 (+0.01)	0.99	1.05	0.277	8664.60

*change in HR going from main model to sensitivity model

Table 5B. Results of sensitivity analysis: not using C8 Health Project validation (retrospective analysis, 751 cases, exposure metric: cumulative)

	HR (change)	LCI	UCI	p-value	AIC
Q2 (>186–334)	1.44 (+0.05)	1.13	1.84	**0.003**	7908.78
Q3 (>334–1,120)	1.26 (-0.10)	0.99	1.60	0.064	
Q4 (>1,120–4,830)	1.62 (+0.17)	1.28	2.06	**<0.001**	
Q5 (>4,830)	1.20 (+0.07)	0.94	1.53	0.149	
Continuous (linear)	1.00 (0)	0.99	1.01	0.758	7921.42
Continuous (log-linear)	1.02 (+0.01)	0.98	1.07	0.300	7920.45

Table 5C. Results of sensitivity analysis: hospitalized cases only (retrospective analysis, 572 cases, exposure metric: cumulative)

	HR (change)	LCI	UCI	p-Value	AIC
Q2 (>177–319)	1.30 (-0.09)	0.98	1.72	**0.068**	6062.36
Q3 (>319–912)	1.36 (0)	1.03	1.79	**0.032**	
Q4 (>912–4,491)	1.41 (-0.04)	1.07	1.86	**0.014**	
Q5 (>4,491)	1.15 (+0.02)	0.87	1.53	0.322	
Continuous (linear)	1.00 (0)	0.99	1.01	0.650	6064.16
Continuous (log-linear)	1.02 (+0.01)	0.97	1.07	0.484	6063.90

Table 6. Results of retrospective analyses with varying study end times (exposure metric: cumulative, continuous (log-linear)*)

	End Time (Year of Last Observation)							
	Present	**2008**	**2005**	**2002**	**1999**	**1996**	**1993**	**1990**
HR	1.01	1.02	1.03	1.05	1.07	1.11	1.18	1.22
CI	0.97–1.06	0.97–1.06	0.98–1.08	0.99–1.12	1.00–1.15	1.01–1.21	1.07–1.30	1.08–1.38
p-value	0.590	0.529	0.295	0.096	0.059	**0.023**	**0.001**	**0.001**
# cases	825	758	566	411	270	174	125	83

*log-linear form is presented instead of linear form because, for most end times, log-linear fit better according to AIC. The linear form fit better for end times 'Present' and 2008, while the log-linear form fit better for all earlier end times.

Figure 1. Participant recruitment

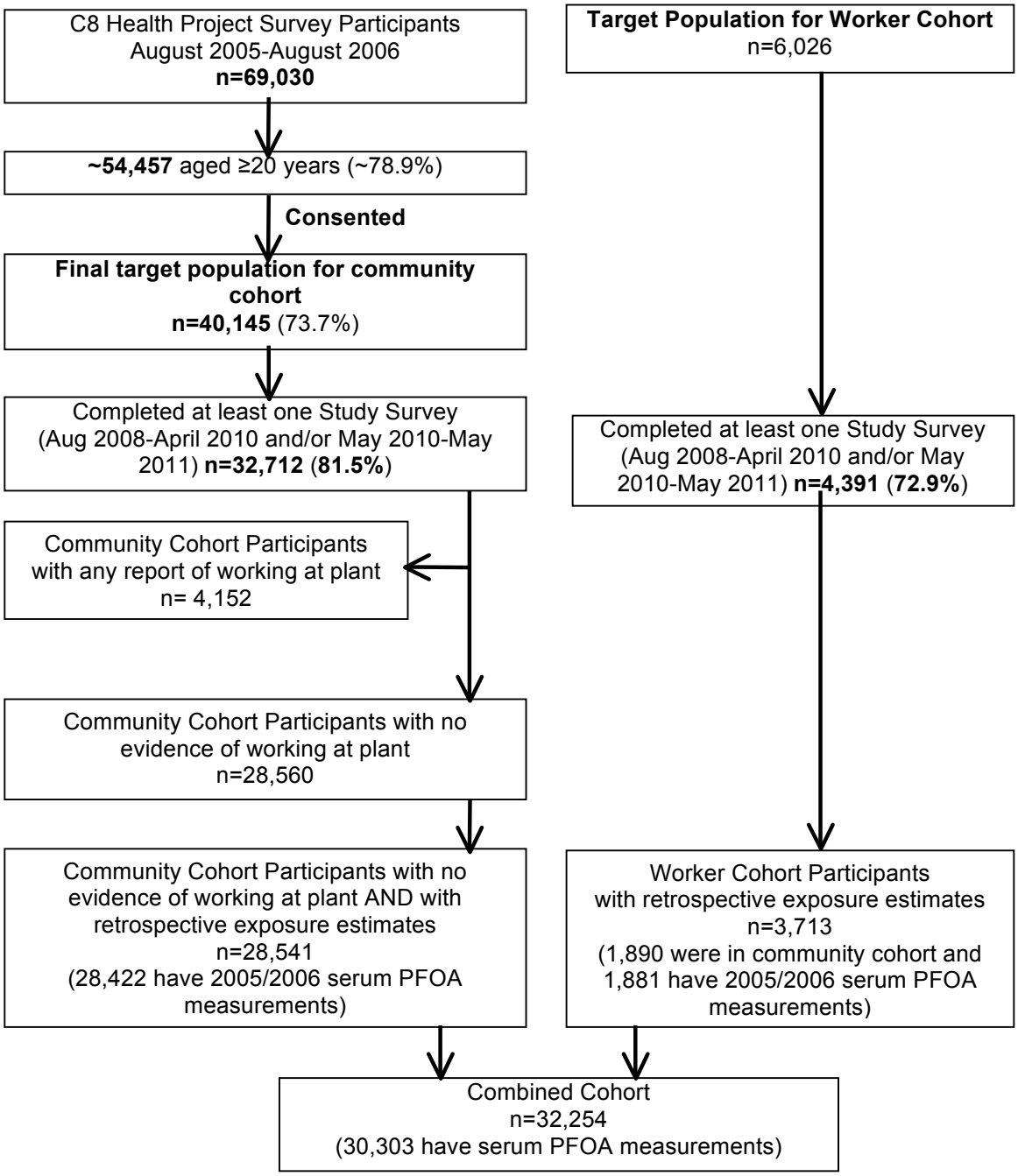

Figure 2. Mean retrospective serum PFOA concentration estimates among survey respondents for the community cohort (bottom line), worker cohort (top line), and combined cohort (middle line).