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Associations between Historically Modeled Retrospective Serum Perfluorooctanoic Acid Concentrations and Liver Function in a Highly Exposed Community

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Master of Public Health

Epidemiology

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Lyndsey Darrow, Ph.D.

Faculty Thesis Advisor

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By

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Bachelor of Science

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Abstract

Associations between Historically Modeled Retrospective Serum Perfluorooctanoic Acid Concentrations and Liver Function in a Highly Exposed Community

By Alyxandra Christine Groth

Background: People living or working in the Mid-Ohio Valley were exposed to perfluorooctanoic acid (PFOA) released by a chemical plant for over 50 years. Prior research on this population has found associations between PFOA exposure and liver function; however, these analyses have been cross-sectional, making causal inference difficult to assess.

Objectives: We assessed the association between historically modeled retrospective PFOA and liver function, as measured by alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), and direct bilirubin, in a population with elevated PFOA exposure.

Methods: C8 Health Project (C8HP) participants were recruited to enroll in a follow-up study conducted from 2008 to 2011. Of the 40,145 individuals agreeing to participate, a total of 30,723 with retrospective exposure estimates were included in this analysis. Cumulative PFOA exposure was derived from individual estimates of annual PFOA serum concentrations, which were based on residential and work history, plant emissions, and a fate-transport model. Linear regression models were used to estimate associations between PFOA exposure and natural log (ln)-transformed concentrations of ALT, GGT, and direct bilirubin. Logistic regression models were used to examine the relationship between PFOA exposure and abnormal levels of ALT, GGT, and direct bilirubin. PFOA was examined as a continuous log-linear measure and in quintiles.

Results: Estimated cumulative ln-PFOA and estimated 2005/2006 ln-PFOA were associated with ln-ALT levels in linear regression models (cumulative PFOA coefficient: 0.012, 95% CI 0.008, 0.016; 2005/2006 PFOA coefficient: 0.012, 95% CI 0.009, 0.016) and with abnormal ALT levels in logistic regression models (cumulative PFOA: OR 1.04, 95% CI 1.01, 1.07; 2005/2006 PFOA: OR 1.04, 95% CI 1.01, 1.07). Relationships with direct bilirubin were inconsistent across analyses. Associations with GGT were mostly consistent with the null, but there was some indication of a positive association among women only.

Conclusions: These results show a positive association between PFOA serum concentrations and serum ALT levels, which is consistent with findings of a prior C8HP cross-sectional study examining the relationship between PFOA exposure and liver function. Results indicate that an association between PFOA and ALT is not an artifact of reverse causation and that PFOA exposure may have harmful effects on liver function.

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BACKGROUND

Perfluoroalkyl acids (PFAA) are man-made compounds consisting of a 4-14 carbon backbone. PFAAs are contemporary chemicals, only being used in the manufacture of consumer and industrial products since the 1950s. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are two prominent members of the PFAA class, both consisting of 8 carbon backbones. PFOS is best known for its role in manufacturing of 3M products; however, 3M and other manufacturers of PFOS have recently phased out production of the chemical due to findings of its broad distribution and persistence in the environment (Lau et al. 2007). Less is known about the effects and distribution of PFOA, thus it continues to be used in the manufacturing of fluoropolymers such as Teflon. PFOA aids in the polymerization process of fluoropolymers, allowing them to exhibit many valuable properties, including resistance to heat, oil, stains, grease, and water (Steenland et al. 2009).

PFOA is extremely heat stable and does not readily degrade, thus it persists indefinitely in the environment. Manufacturers of fluoropolymers claim that PFOA is not contained in finished consumer products, yet most people in the United States have measurable PFOA in their serum at levels around 4 to 5 ng/mL (Emmett et al. 2006; Steenland et al. 2009). PFOA has also been detected in the tissue of a variety of wildlife species, such as fish, reptiles, birds, and mammals (DeWitt et al. 2013). Zhao et al. (2011) demonstrated the presence of PFOA in several freshwater fish and marine fish collected from local markets in Hong Kong, China and Xiamen, China. Hazard ratios reveal that frequent consumption of contaminated fish does not pose an immediate threat to human health, however, Zhao and colleges point out that fish is not the only source of

human exposure to PFOA and frequent consumption of contaminated fish will contribute to PFOA accumulation in humans. A study by Kannan et al. (2006) reported PFOA concentrations ranging from <5 to 147 ng/g in the liver tissue of sea otters collected from the California coast from 1992 to 2002, with levels increasing significantly over the decade. These findings suggest that PFOA, which was inexistent until the past half century, is now widespread in the environment.

Although exposure sources are still under investigation, likely exposure routes of PFOA to humans include ingestion of contaminated drinking water or food, and inhalation of contaminated air or household dust (Frisbee et al. 2009). Food and beverages may be contaminated either directly or indirectly through packaging, while accumulated household dust is believed to be contaminated through continuous indoor use of products containing PFOA. PFOA released during the manufacturing process of fluoropolymers may also contribute to the environmental contamination through direct emissions from manufacturing facilities to air and water and indirect emissions from landfill leaching to groundwater (Shin et al. 2011). A study by Davis and colleagues (2007) investigated the potential transport pathways of PFOA from a fluoropolymer manufacturing facility in West Virginia near the Ohio River to groundwater, surface water, and soil. They employed air dispersion modeling, and surveyed and sampled water from the Ohio River and other private and public water sources within two miles of the manufacturing facility. Their data suggests that the most likely transport pathway of PFOA from the facility to surrounding areas was through air emissions transported by wind to nearby well fields, deposited onto well field surface soils, and then leached

downward as precipitation, through the aquifer and into the groundwater (Davis et al. 2007).

The elimination half-life of PFOA in adult female rats is estimated at 2-4 hours, while the elimination half-life in male rats is 4-6 days. Gender differences in elimination rates are present in other species; however, rates are not always faster among females (Lau et al. 2007). Animal toxicology studies have found relatively high PFOA concentrations in the livers of rodents and nonhuman primates, in addition to liver enlargement. Liver toxicity and heptaocellular adenomas were also found to be associated with PFOA levels in rats (Lau et al. 2007). The hepatic toxicity and hepatocellular adenomas observed in rodents exposed to PFOA is believed to be attributed primarily to agonism of the peroxisome proliferated-activated receptor-alpha (PPAR-α), which is a major regulator of lipid metabolism in the liver (Bjork et al. 2011). Several studies on rats and mice have shown PFOA and PFOS to be capable of inducing peroxisome proliferation (Lau et al. 2007). However, there are significant species-specific differences in the biological response to PFOA exposure, and consistency in results across other species such as primates and humans is lacking (Bjork et al. 2011).

PFOA is well absorbed by both oral and inhalation routes, and is mainly found in the liver, serum, and kidney (Costa et al. 2009). PFOA is eliminated slowly in humans, with a geometric mean half-life estimated to be 3.5 years (95% CI 3.0-4.1 years) (Olsen et al. 2007). Time trend studies have provided evidence of a positive correlation between duration of PFOA exposure and PFOA levels in the blood, while other research indicates that PFOA levels increase with age (Vierke et al. 2012). Studies on the health effects of

PFOA in humans have generated inconsistent findings. Associations between PFOA exposure and lower birth weight, higher cholesterol, and impaired liver function have been found in several studies, although the correlations tend to be rather weak and modest (Steenland et al. 2009).

Prior to its phase-out in 2002, PFOS production and use greatly overshadowed that of PFOA (3,500 metric tons versus 500 metric tons, respectively). However, the phase-out of PFOS production caused the global PFOA production to skyrocket – reaching an estimated 1,200 metric tons per year by 2004 (Lau et al. 2007). Evidence of negative health effects of PFOA exposure in animal models, along with PFOA's persistence in the environment, long elimination half-life, and presence at low levels in the serum of the general U.S. population, make the continued manufacturing of PFOA a major concern. As a result, the U.S. Environmental Protection Agency (EPA) launched the PFOA Stewardship Program in 2006, with goals to reduce manufacturing emissions and product content of PFOA by 95% by 2010, and further eliminate emissions and product content by 2015 (EPA 2013). Eight major companies in the industry committed to the goals of the program, and the EPA seeks to involve more manufacturing facilities in the future. While the initiation of this program shows promise, many postulate that manufactures will develop new PFAA products to fill the void (Lau et al. 2007). It is also important to note that while the PFOA Stewardship Program may be successful in reducing current and future emissions of PFOA, the health effects potentially associated with prior PFOA exposure still need to be assessed.

Drinking water in the Mid-Ohio Valley has been contaminated with PFOA used in the manufacturing of fluoropolymers at the DuPont Washington Works facilities in

Washington, West Virginia, since 1951. PFOA emissions in this area steadily increased over time, peaking in 1999 and subsequently decreasing due to implementation of control measures (Shin et al. 2011). A class-action lawsuit against DuPont was filed in 2001 by a group of individuals claiming health damage resulting from PFOA-contaminated drinking water. The settlement of the lawsuit mandated for a community study to be conducted to investigate the relationship between PFOA exposure and human disease in the community surrounding the facility. The resulting cross-sectional study, known as the C8 Health Project, includes data collected from 2005-2006 on more than 69,000 persons residing, working, or attending school in any of the six contaminated water districts surrounding the plant. The Project included data on clinical laboratory tests, demographics, validated medical diagnoses, and lifestyle and health behaviors. The Project achieved a high participation rate, with 80.3% of residents in the community participating during the enrollment period (Frisbee et al. 2009).

Several studies have demonstrated the association between high PFOA exposure and liver function damage in animal models (Lau et al. 2007). Evidence also suggests that PFOA exposure in rodents is associated with activation of PPAR-α and cell proliferation in the liver (Bjork et al. 2011). Consequently, it is important to investigate the relationship between PFOA exposure and liver function in the C8 Health Project population. A 2012 study by Gallo et al. examined the cross-sectional association between 2005-2006 PFOA serum concentrations with three liver function biomarkers: direct bilirubin, alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT). Their results revealed a positive association between 2005-2006 PFOA serum concentrations and alanine aminostransferase (ALT) levels, a liver function biomarker indicating

hepatocellular injury (Gallo et al. 2012). However, PFOA serum levels collected in 2005-2006 represent recent or current exposure, and therefore may not adequately indicate PFOA exposure in prior decades. Furthermore, the method of analysis used by Gallo and colleagues yields a cross-sectional study design, as liver function biomarkers and PFOA exposure were measured simultaneously. Cross-sectional studies make causal inference difficult to assess and may introduce reverse causality bias. For example, poor liver function may impair excretion of PFOA leading to higher serum concentrations. Therefore, further research on the association between liver function biomarkers and PFOA exposure using designs that are not vulnerable to reverse causation is needed.

A 2011 study by Shin and colleagues estimated historical year-by-year PFOA exposures based on individual residential history and likely water sources. To calculate these estimates, they designed a sophisticated environmental fate and transport model, modeling PFOA concentrations in air, surface water, and groundwater based on emissions from the DuPont plant. They then assigned exposure concentrations to individuals through linking the environmental fate and transport model to residential histories. Through these methods, they were able to reconstruct individualized retrospective exposure estimates, allowing for annual PFOA serum concentrations for each individual to be analyzed (Shin et al. 2011). This thesis will be a retrospective cohort study investigating the association between the historically modeled retrospective PFOA exposure estimated by Shin and colleagues and liver function biomarkers in participants of the C8 Health Project.

Liver function will be approximated by measurements of three liver enzymes: direct bilirubin, alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT).

These three liver enzymes have been used as proxies of liver damage in numerous epidemiological studies. Bilirubin is a waste product of the normal breakdown of hemoglobin. Direct, or conjugated, bilirubin is water-soluble and travels through the liver to be excreted in the urine. Elevated levels of direct bilirubin usually signal problems with the liver, bile ducts, or gallbladder. ALT enzymes are found in liver parenchymal cells and are elevated during acute liver damage (Lin et al. 2010). Elevation in GGT enzymes occurs earlier and persists longer than that of alkaline phosphatase in cholestatic disorders, conditions in which the flow of bile from the liver is slowed or blocked (Gallo et al. 2012).

Studies in humans have reported inconsistent results regarding the associations between PFOA serum concentrations and liver enzymes. A 2010 study by Lin and colleagues analyzed data on 2,216 adults collected from 1999-2000 and 2003-2004 NHANES, a US population-based cross-sectional survey. They found statistically significant positive associations between PFOA and ALT levels (95% CI 1.24-2.48, *p*-value = 0.005), and PFOA and GGT levels (95% CI 0.05-0.11, *p*-value = 0.019), with stronger associations between PFOA and liver enzymes in obese subjects (Lin et al. 2010). Several occupational studies have also examined the association between PFOA concentration and liver enzymes. Olsen et al. reported increases in total cholesterol/HDL to be associated with increases in PFOA in a worker cohort of 179 3M employees, but found no significant associations between PFOA levels and bilirubin or ALT levels (Olsen et al. 2012). Two occupational studies by Sakr et al. (2007a; 2007b) examined the relationship between serum PFOA levels and liver enzymes among workers at the Washington Works manufacturing site. The first was a longitudinal study on 454

workers, resulting in significant associations between PFOA and total bilirubin (Sakr et al. 2007a). The second was a cross-sectional study of 1,025 active workers, resulting in significant positive relationships between serum PFOA and total cholesterol, low-density lipoprotein, very low-density lipoprotein, and GGT (Sakr et al. 2007b).

Several studies have examined the association between historically modeled PFOA exposure and health effects in C8 Health Project participants, yielding inconsistent conclusions. Savitz et al. (2012) found no associations between estimated PFOA serum concentrations and adverse pregnancy outcomes other than preeclampsia, which had an odds ratio of 1.13 (95% CI 1.00, 1.28). A study by Steenland et al. (2013) investigated the association between reconstructed PFOA exposure and autoimmune diseases in C8 Health Project participants. They found a significant positive association between ulcerative colitis and cumulative PFOA exposure, with adjusted rate ratios by quartile of exposure of 1.00 (referent), 1.76 (95% CI 1.04, 2.99), 2.63 (95% CI 1.56, 4.43), and 2.86 (95% CI 1.65, 4.96), suggesting a positive trend. Watkins et al. (2013) found a significant association between PFOA levels measured in 2005-2006 and reduced kidney function in the C8 Health Project participants; however, they did not find a significant association between reconstructed PFOA exposure and kidney function, as measured by estimated glomerular filtration rate (eGFR). Watkins and colleagues suggest that their findings may provide evidence of the possibility that increased PFOA concentrations may be a consequence, rather than a cause, of decreased kidney function. However, no studies have attempted to examine the association between historically reconstructed PFOA exposure and liver function in the C8 Health Project population. The present study will attempt to address this current gap in the literature by investigating the relationship between

historically modeled PFOA serum levels estimated by Shin and colleagues and liver function, as measured by the liver biomarkers direct bilirubin, ALT, and GGT.

METHODS

Study population

The study population of interest includes residents of a Mid-Ohio Valley community surrounding the DuPont Washington Works facility that participated in the C8 Health Project (C8HP). The C8 Health Project baseline survey enrolled eligible subjects between August 2005 and August 2006, collecting data on 69,030 persons. Individuals were eligible to participate in the C8 Health Project if they were able to document their consumption of PFOA-contaminated water for at least one year between 1950 and December 2004, while living, working, or attending school in one of the six contaminated water districts (Frisbee et al. 2009). The Project achieved a high participation rate, with an estimated 80.3% of residents in the six contaminated water districts participating during the enrollment period (Frisbee et al. 2009). C8HP participants were asked to partake in a follow-up study to examine the association between PFOA exposure and adult chronic diseases. Since the focus was adult chronic diseases, only C8HP participants who were ≥ 20 years of age were eligible to enroll. Of the 69,030 C8HP participants, approximately 54,457 (79%) were ≥ 20 years of age, and of these, about 40,145 (74%) agreed to participate in the follow-up study. We were able to estimate historically modeled PFOA concentrations for 31,057 (77%) individuals who agreed to participate in the follow-up study. Of these, we excluded 175 participants that were missing measurements of ALT, GGT, or direct bilirubin, 108 participants that were missing demographic information, and 51 participants who were not at least 20 years old (Figure 1). After exclusions, the final study population available for analysis was comprised of 30,723 individuals, and included both individuals with no history of

working at the DuPont plant, as well as individuals with a history of working at the plant between 1948 and 2002 (Winquist et al. 2013).

Data collection and laboratory methods

Residents scheduled appointments at Project data-collection sites to participate in the C8 Health Project study. If eligible, participants were asked to complete a health survey and provide a blood sample. The health survey collected demographic information, residential and employment history, personal and family medical history, pregnancy history and outcomes for women, and health behavior information. Participants were compensated $150 for completing the health survey, and an additional $250 for providing a blood sample. Participants were not required to fast prior to blood sample collection, although self-reported fasting status was reported. Blood samples were shipped on dry ice daily from data-collection sites to the clinical laboratory for measurement of serum PFAAs (Frisbee et al. 2009).

Measurement of liver function biomarkers and covariates

Serum concentrations of ALT (IU/L), GGT (IU/L), direct bilirubin (mg/dL) were measured to determine liver function. Laboratory analyses were performed at a large, independent, accredited clinical diagnostic laboratory (LabCorp, Inc., Burlington, NC, USA), and the liver function biomarkers were measured using a Roche/Hitachi MODULAR automated analyzer (Frisbee et al. 2009). Above-normal levels of direct bilirubin, ALT, and GGT were defined using the following cut-points: levels of direct bilirubin above 0.3 mg/dL for men and women, levels of ALT above 45 IU/L for men and 34 IU/L for women, and levels of GGT above 55 IU/L for men and 38 IU/L for women (Gallo et al. 2012).

Insulin resistance was represented by the homeostasis model assessment of insulin resistance (HOMA-IR) index, which was calculated as the product of basal glucose and insulin levels divided by 2.25 (Gallo et al. 2012). Although participants were not required to fast before blood samples were collected, fasting status was recorded (fasting for 6 hours prior to blood draw vs. not). Other parameters that were measured included: age, sex, body mass index (BMI; classified as underweight, normal weight, overweight, obese class I/II/III), alcohol consumption (none, < 1 drink/month, < 1 drink/week, a few drinks/week, 1-3 drinks/day, > 3 drinks/day), physical activity level (exercise regularly vs. do not), smoking status (never, former, current < 10 cigarettes/day, current 10-19 cigarettes/day, current 20+ cigarettes/day), education level (< 12 years, high school diploma or GED, some college, bachelor degree or higher), average household income (≤ $10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, > $70,000), history of working at DuPont plant (no vs. yes), and race (white vs. other).

Exposure estimation

Historical annual PFOA serum concentrations from 1951 to 2008 were estimated for each participant using a series of modeling methods described in detail elsewhere (Shin et al. 2011). Briefly, year-by-year PFOA serum levels were generated by estimating individual intake of PFOA-contaminated drinking water and air, which was then linked to an absorption, distribution, metabolism, and excretion (ADME) model. Although the predominant route of exposure was through ingestion of PFOA-contaminated drinking water, another important route contributing to exposure was inhalation of airborne particles. Environmental fate and transport models were created to simulate PFOA levels

in air, surface water, and groundwater. Individual participant information on demographics, residential histories, drinking water source at home and in the workplace, and tap water consumption rates were used in conjunction with the environmental fate and transport models to estimate individual annual potential intake. These individual dose calculations were then coupled with an ADME model to estimate the year-by-year amount of PFOA reaching and remaining in the blood, which was adjusted for historical background serum concentrations.

The observed PFOA serum levels measured in C8HP participants at the time of enrollment (2005 or 2006 depending on the participant) are moderately correlated with the estimated historical serum levels in the year of 2005 or 2006 (Spearman's rank correlation coefficient = 0.67) (Shin et al. 2011). There are two possible explanations for why the measured and estimated PFOA levels are not highly correlated: 1) there could be measurement error in the modeled estimates, 2) measured levels reflect individual variability in pharmacokinetics and metabolism of PFOA in addition to external exposure.

Statistical analysis

The distributions of the continuous variables were examined, and the outcome and exposure measures were found to be non-normally distributed, and were natural log (ln) transformed for analysis. We assessed associations between the liver function biomarkers and estimated cumulative PFOA serum levels, which was calculated by summing the estimated yearly serum concentrations for each individual from 1951 (or birth year if later) through the year of the survey (2005 or 2006). We also assessed

associations between the liver function biomarkers and estimated serum PFOA level in the year of the outcome measurement (2005 or 2006).

First, we assessed the association between ALT, GGT, or direct bilirubin and cumulative PFOA and 2005/2006 estimated PFOA levels using linear regression models adjusted for age and sex alone (Model 1). In a second model (Model 2) we added adjustment for BMI, alcohol consumption, physical activity level, smoking status, insulin resistance, fasting status, race, history of working at DuPont plant, and education level. A third model included control for average household income (Model 3).

We performed additional linear regression analyses considering quintiles of cumulative or 2005/2006 estimated serum concentrations in relation to the liver function biomarkers. Logistic regression was also performed to examine the relationship between PFOA and dichotomized measures of the three liver function biomarkers. Above-normal levels of direct bilirubin, ALT, and GGT were defined using the cut-points described earlier. Sex (male vs. female), age (<50 years old vs. ≥ years old), and history of working at the DuPont plant (no vs. yes) variables were assessed as potential effect modifiers in linear regression models.

Final logistic regression models adjusted for variables that changed the odds ratio (OR) for PFOA exposure by ≥ 10% and were biologically plausible, or variables that were significant predictors of the outcome. Final linear regression models adjusted for variables that changed the beta estimate by ≥ 10% and were biologically plausible, or variables that were significantly associated with ALT, GGT, and direct bilirubin.

RESULTS

Characteristics of the 30,723 participants included in the analysis are shown in Table 1. Participants with estimated 2005/2006 serum PFOA levels in the highest quartile tended to be older, more educated, and to have a higher household income, and were more likely to be male, non-drinkers, regular exercisers, of normal weight/BMI, and previously or currently employed by DuPont plant. The median 2005/2006 estimated serum PFOA concentration was 16.5 ng/mL, with a range of 2.62 – 3,558.8 ng/mL.

A large proportion (9.4%) of participants were missing data on average household income (N=2,882). We assessed whether average household income was a confounder by comparing the beta estimates and odds ratios for the fully adjusted model with those for a model adjusting for all potential confounders except for average household income. As shown in Tables 2 and 3, the additional adjustment for average household income (Model 3) had virtually no effect on the beta estimates and odds ratios. Consequently, we decided to exclude average household income from future analyses.

The final model (Model 2) adjusts for age, sex, BMI, alcohol consumption, physical activity, smoking status, education level, insulin resistance, fasting status, race, and history of working at DuPont plant. Adjusting for these variables still left us with a large dataset (N=28,047). Of the covariates assessed, age, sex, and BMI were the strongest confounders of the liver function biomarker-PFOA relationships.

As shown in Table 2, log-transformed values of ALT were positively associated with cumulative and 2005/2006 PFOA concentrations in linear regression models [per log ng/mL cumulative PFOA: Model 2 coefficient 0.012, 95% CI 0.008, 0.016;

2005/2006 PFOA: Model 2 coefficient 0.012, 95% CI 0.009, 0.016 (Table 2)]. Table 2 also shows that ln-transformed values of direct bilirubin were inversely associated with cumulative and 2005-2006 ln-PFOA concentrations in linear regression models [per log ng/mL cumulative PFOA: Model 2 coefficient -0.005, 95% CI -0.008, -0.002; 2005/2006 PFOA: Model 2 coefficient -0.006, 95% CI -0.009, -0.003]. Ln-transformed values of GGT were not found to be significantly associated with cumulative or 2005/2006 PFOA levels in the final linear regression models (Model 2).

As shown in Table 3, the odds of having abnormally high levels of ALT were significantly associated with a log ng/mL increase in cumulative and 2005/2006 PFOA levels [cumulative PFOA: Model 2 OR 1.04, 95% CI 1.01, 1.07; 2005/2006 PFOA: Model 2 OR 1.04, 95% CI 1.01, 1.07 (Table 3)]. A statistically significant effect for the odds of having abnormally high values of GGT and direct bilirubin for a log ng/mL increase in cumulative and 2005/2006 PFOA levels was not found.

Ln-transformed values of ALT were found to increase significantly across quintiles of cumulative and 2005/2006 ln-PFOA levels (Table 4). Including the PFOA quintiles as an ordinal variable in the model showed a significant linear trend of ALT levels across quintiles of cumulative and 2005/2006 ln-PFOA levels (p-value <0.0001 for both cumulative and 2005/2006 ln-PFOA). Similar trends between GGT with cumulative and 2005/2006 ln-PFOA levels were not observed. Including the PFOA quintiles as an ordinal variable in the model also showed a significant linear trend of decreasing direct bilirubin by increasing quintiles of cumulative and 2005/2006 ln-PFOA levels (p-value = 0.0029 and 0.0036, respectively); however, only the highest quintile of cumulative or 2005/2006 PFOA had significantly lower direct bilirubin relative to the lowest quintile..

The odds of having abnormally high levels of ALT increased across quintiles of cumulative ln-PFOA concentrations, with the odds possibly leveling off in the fifth quintile (Table 5, Figure 2). A similar effect was found between ALT and quintiles of 2005/2006 ln-PFOA concentrations; however, the confidence intervals for the odds ratios often included 1 (Figure 3). Including the PFOA quintiles as an ordinal variable in the model showed a significant trend of the odds of having abnormally high ALT concentrations across cumulative and 2005/2006 ln-PFOA levels (p-value = 0.0078 and 0.0055, respectively). No significant trend was found between the odds of having abnormally high levels of GGT or direct bilirubin across quintiles of cumulative or 2005/2006 ln-PFOA levels.

A summary of our assessment of sex, age, and history of working at DuPont plant as potential effect modifiers can be seen in Tables 6 and 7. Significant interaction by sex in ALT was found for both exposure metrics (cumulative and 2005/2006), showing stronger associations between PFOA levels and ALT concentrations in women than in men. Significant interaction by sex in GGT was also found for both exposure metrics (cumulative and 2005/2006), showing positive associations between PFOA levels and GGT concentrations among women and negative or null associations among men. There was some evidence that associations with ALT and GGT differed by worker status, although this difference was not consistent across the two PFOA metrics. No interaction between the exposure metrics and age was found for any of the liver function biomarkers.

DISCUSSION

Our study found a positive association between both estimated cumulative PFOA and estimated PFOA in the year of enrollment (2005 or 2006) and ALT serum concentrations, an indicator of liver damage. This positive association was consistently replicated in all linear regression analyses. Additionally, the odds of having abnormally high ALT levels increased with higher serum concentrations of cumulative PFOA and 2005/2006 PFOA. Our results also suggested that this association is stronger among females when compared to males.

The results of our retrospective cohort study analyses based on historically modeled PFOA exposure are consistent with a study by Gallo et al. (2012), which examined the cross-sectional association between measured serum PFOA and liver enzymes in the C8HP study population and found a positive association between PFOA concentrations and ALT serum levels (linear regression coefficient: 0.022, 95% CI 0.018, 0.025). Gallo et al. also reported a steady increase in the odds ratio estimates for abnormal levels of ALT across deciles of PFOA concentrations (p-value for trend < 0.001).

Gallo's ALT findings mirror the results regarding ALT levels in this study, although our effect estimates were slightly smaller than those reported by Gallo et al. However, the cross-sectional design used by Gallo and colleagues raises concerns about reverse causation. In this type of study design, it is impossible to determine whether increasing serum PFOA levels cause, or are the result of, decreased liver function. A notable strength of our study is our use of the historically modeled serum PFOA

concentrations constructed for each individual. Because exposure was modeled based on residential histories rather than measured at the time of the liver function biomarkers, the observed associations in our study are not an artifact of poor liver function causing high serum PFOA levels, thus our analyses are not susceptible to the problem of reverse causation.

Our study also found a weakly negative association between both cumulative and 2005/2006 PFOA levels with direct bilirubin concentrations in linear regression models, however, we did not observe an association in logistic regression analyses. The instability seen in the odds ratios and confidence intervals for having abnormally high levels (> 0.3 mg/dL) of direct bilirubin may have been affected by the small portion of participants having abnormally high levels of direct bilirubin (N=382, 1.24%). A few occupational studies have reported evidence of a negative association between PFOA and total bilirubin (Sakr et al. 2007a; Costa et al. 2009; Olsen & Zobel 2007). Gallo et al. (2012) found suggestion of an inverse U-shaped relationship between PFOA and direct bilirubin, with bilirubin levels increasing up to PFOA levels of 40 ng/mL, followed by a decrease of bilirubin levels after this peak. This might explain some of the differences in association suggested by models including interaction by worker status, as workers were exposed at higher levels than community members.

Overall, we observed little evidence of an association between cumulative PFOA or 2005/2006 PFOA and GGT serum levels; however, there was some indication that PFOA exposure was associated with higher levels of GGT among women, but not among men. These results are similar to the findings of Gallo and colleagues, which reported a significant association between measured PFOA and GGT in their fully-adjusted linear

regression model, but no association in logistic regression models or across deciles of PFOA exposure, leaving the evidence of an association between PFOA and GGT unclear.

The strengths of the present study include the large study population with markedly elevated concentrations of PFOA and the high participation rate achieved by the C8HP, resulting in a representative population with high power to detect associations. Additionally, the C8HP collected data on many covariates through extensive surveys as well as other clinical measures (e.g. insulin resistance).

This study also has several limitations. The first limitation is also one of our key strengths: exposure was classified according to estimated historical serum levels of PFOA based on residential histories, predicted consumption of contaminated drinking water and air, and an absorption and excretion model. Year-by-year exposure estimates were constructed for each individual – thus enabling us to examine the association between cumulative PFOA exposure and liver function. However, these exposure reconstructions are based on modeling methods that are subject to error. Therefore, exposure measurement error is likely due to the modeled exposure in our study. If present, this would be unlikely to be differential by outcome (liver enzymes) and would likely introduce bias toward the null. However, the exposure model reliably, although imperfectly, predicts the measured serum concentrations, with a correlation coefficient of 0.67 (Shin et al. 2011).

Another limitation is the possibility of selection bias, which may have occurred if participation was jointly related to both exposure and disease, for example, if highly exposed people with abnormal liver function were more likely to participate. However, the differences in results observed across liver enzymes and the subclinical nature of the

outcomes diminish concerns about selection bias driving the observed associations in our study.

Other areas of concern include the possibility of residual confounding of the exposure-disease relationship not controlled for in our models, the quality of self-reported measures, and our use of ALT, GGT, and direct bilirubin as outcome measurements, as these are imperfect markers of liver function. However, the large study population size coupled with the vast amount of data collected and adjusted for in our models reduces the possibility of residual confounding. The quality of self-reported measures is of concern in this study, specifically the quality of self-reported residential history, as it was a measure used to construct the estimated historical exposure estimates. However, it is not possible to quantify the error caused by inaccurate self-report, therefore this limitation should be considered when interpreting the results of this study. Finally, the use of ALT, GGT, and direct bilirubin as markers of liver function may also limit our results. While these three liver enzymes are widely used to assess liver damage, they are imperfect measures of liver function and can only serve as a proxy of our desired outcome metric, liver damage.

In conclusion, our retrospective analyses showed an association between predicted historical levels of PFOA and ALT serum concentration, a marker of reduced liver function, in this large population with elevated exposure to PFOA. These results are consistent with a prior cross-sectional study examining liver function in the C8HP participants. Our use of historically modeled retrospective serum PFOA levels diminishes concerns over the issue of reverse causality, thus supporting the hypothesis of a true association between PFOA levels and ALT serum concentrations. However, our study is

the first to attempt examining the relationship between lifetime cumulative PFOA exposure and liver function, and further investigations of this association are warranted.

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TABLES AND FIGURES

Table 1. Participant characteristics, Mid-Ohio Valley, 2005-2006 (N=30,723).

		Quartiles of 2005/2006 estimated serum PFOA concentrations			
Characteristic	Overall	1	2	3	4
Estimated serum PFOA [ng/mL (range)]	2.62 - 3558.80	2.62 -< 7.01	7.01 -< 16.50	16.50 -< 59.72	59.72 -< 3558.8
Estimated serum PFOA [ng/mL (median)]	16.50	4.47	10.55	30.37	168.21
ALT (IU/L)					
Mean ± SD	25.54 ± 19.21	25.56 ± 18.29	25.36 ± 20.45	25.61 ± 20.95	25.63 ± 16.86
≤ 45 (m), ≤ 34 (w) [n(%)]	27,252 (88.70)	6,773 (88.17)	6,831 (88.95)	6,805 (88.60)	6,843 (89.10)
> 45 (m), > 34 (w) [n(%)]	3,471 (11.30)	909 (11.83)	849 (11.05)	876 (11.40)	837 (10.90)
GGT (IU/L)					
Mean ± SD	31.25 ± 44.69	31.13 ± 50.11	31.82 ± 41.86	31.02 ± 42.45	31.05 ± 43.85
≤ 55 (m), ≤ 38 (w) [n(%)]	26,551 (86.42)	6,646 (86.51)	6,558 (85.39)	6,651 (86.59)	6,696 (87.19)
> 55 (m), > 38 (w) [n(%)]	4,172 (13.58)	1,036 (13.49)	1,122 (14.61)	1,030 (13.41)	984 (12.81)
Direct bilirubin (mg/dL)					
Mean ± SD	0.12 ± 0.10	0.12 ± 0.06	0.12 ± 0.06	0.12 ± 0.06	0.12 ± 0.18
≤ 0.3 [n(%)]	30,341 (98.76)	7,590 (98.80)	7,578 (98.67)	7,588 (98.79)	7,585 (98.76)
> 0.3 [n(%)]	382 (1.24)	92 (1.20)	102 (1.33)	93 (1.21)	95 (1.24)
Age [years (n(%))]					
20-24	2,206 (7.18)	725 (9.44)	526 (6.85)	476 (6.20)	479 (6.24)
25-29	2,236 (7.28)	732 (9.53)	593 (7.72)	521 (6.78)	390 (5.08)
30-34	2,392 (7.79)	724 (9.42)	668 (8.70)	581 (7.56)	419 (5.46)
35-39	2,713 (8.83)	800 (10.41)	693 (9.02)	677 (8.81)	543 (7.07)
40-44	3,194 (10.40)	918 (11.95)	798 (10.39)	779 (10.14)	699 (9.10)
45-49	3,571 (11.62)	916 (11.92)	927 (12.07)	854 (11.12)	874 (11.38)
50-54	3,466 (11.28)	814 (10.6)	845 (11.00)	863 (11.24)	944 (12.29)
55-59	3,250 (10.58)	714 (9.29)	747 (9.73)	824 (10.73)	965 (12.57)

60-64	2,693 (8.77)	479 (6.24)	630 (8.2)	663 (8.63)	921 (11.99)
65-69	2,073 (6.75)	354 (4.61)	497 (6.47)	579 (7.54)	643 (8.37)
70-74	1,419 (4.62)	258 (3.36)	343 (4.47)	395 (5.14)	423 (5.51)
75+	1,510 (4.91)	248 (3.23)	413 (5.38)	469 (6.11)	380 (4.95)
Sex [n(%)]					
Male	13,658 (44.46)	3,309 (43.07)	3,405 (44.34)	3,311 (43.11)	3,633 (47.30)
Female	17,065 (55.54)	4,373 (56.93)	4,275 (55.66)	4,370 (56.89)	4,047 (52.70)
BMI [n(%)]					
Underweight (below 18.5)	386 (1.26)	78 (1.02)	79 (1.03)	115 (1.50)	114 (1.48)
Normal weight (18.5-24.9)	8,423 (27.42)	1,886 (24.55)	1,996 (25.99)	2,196 (28.59)	2,345 (30.53)
Overweight (25.0-29.9)	10,730 (34.92)	2,479 (32.27)	2,687 (34.99)	2,718 (35.39)	2,846 (37.06)
Obese, class I (30.0-34.9)	6,377 (20.76)	1,759 (22.9)	1,665 (21.68)	1,511 (19.67)	1,442 (18.78)
Obese, class II (35.0-39.9)	2,681 (8.73)	804 (10.47)	728 (9.48)	642 (8.36)	507 (6.60)
Obese, class III (40+)	1,835 (5.97)	631 (8.21)	479 (6.24)	424 (5.52)	301 (3.92)
Missing	291 (0.95)	45 (0.59)	46 (0.60)	75 (0.98)	125 (1.63)
Alcohol consumption [n(%)]					
None	15,797 (51.42)	4,004 (52.12)	4,204 (54.74)	3,839 (49.98)	3,750 (48.83)
< 1 drink/month	5,147 (16.75)	1,382 (17.99)	1,227 (15.98)	1,296 (16.87)	1,242 (16.17)
< 1 drink/week	3,329 (10.84)	821 (10.69)	732 (9.53)	862 (11.22)	914 (11.90)
Few drinks/week	4,003 (13.03)	988 (12.86)	926 (12.06)	1,012 (13.18)	1,077 (14.02)
1-3 drinks/day	1,007 (3.28)	199 (2.59)	229 (2.98)	281 (3.66)	298 (3.88)
> 3 drinks/day	389 (1.27)	74 (0.96)	100 (1.30)	121 (1.58)	94 (1.22)
Missing	1,051 (3.42)	214 (2.79)	262 (3.41)	270 (3.52)	305 (3.97)
Regular exercise [n(%)]					
Yes	10,017 (32.6)	2,360 (30.72)	2,288 (29.79)	2,570 (33.46)	2,799 (36.45)
No	20,706 (67.4)	5,322 (69.28)	5,392 (70.21)	5,111 (66.54)	4,881 (63.55)
Smoking status [n(%)]					
Never	15,056 (49.01)	3,818 (49.70)	3,566 (46.43)	3,797 (49.43)	3,875 (50.46)
Former	7,912 (25.75)	1,910 (24.86)	2,034 (26.48)	1,921 (25.01)	2,047 (26.65)
Current < 10 cigarettes/day	1,072 (3.49)	291 (3.79)	265 (3.45)	289 (3.76)	227 (2.96)
Current 10-19 cigarettes/day	4,076 (13.27)	1,078 (14.03)	1,124 (14.64)	968 (12.60)	906 (11.80)
Current 20+ cigarettes/day	1,820 (5.92)	429 (5.58)	514 (6.69)	477 (6.21)	400 (5.21)
Missing	787 (2.56)	156 (2.03)	177 (2.30)	229 (2.98)	225 (2.93)

Education [n(%)]					
< 12 years	3,138 (10.21)	786 (10.23)	967 (12.59)	832 (10.83)	553 (7.20)
High school diploma or GED	12,590 (40.98)	3,111 (40.50)	3,191 (41.55)	3,069 (39.96)	3,219 (41.91)
Some college	10,100 (32.87)	2,545 (33.13)	2,416 (31.46)	2,461 (32.04)	2,678 (34.87)
Bachelor degree +	4,748 (15.45)	1,205 (15.69)	1,060 (13.80)	1,283 (16.70)	1,200 (15.63)
Missing	147 (0.48)	35 (0.46)	46 (0.60)	36 (0.47)	30 (0.39)
Household income, US$/year [n(%)]					
≤ 10,000	2,455 (7.99)	706 (9.19)	738 (9.61)	627 (8.16)	384 (5.00)
10,001-20,000	4,097 (13.34)	1,067 (13.89)	1,175 (15.30)	1,014 (13.20)	841 (10.95)
20,001-30,000	4,415 (14.37)	1,171 (15.24)	1,133 (14.75)	1,122 (14.61)	989 (12.88)
30,001-40,000	3,997 (13.01)	956 (12.44)	1,031 (13.42)	1,062 (13.83)	948 (12.34)
40,001-50,000	3,301 (10.74)	853 (11.10)	807 (10.51)	814 (10.60)	827 (10.77)
50,001-60,000	2,786 (9.07)	688 (8.96)	642 (8.36)	692 (9.01)	764 (9.95)
60,001-70,000	2,207 (7.18)	512 (6.66)	468 (6.09)	529 (6.89)	698 (9.09)
> 70,000	4,583 (14.92)	1,040 (13.54)	931 (12.12)	1,111 (14.46)	1,501 (19.54)
Missing	2,882 (9.38)	689 (8.97)	755 (9.83)	710 (9.24)	728 (9.48)
Insulin resistance (HOMA-IR) [mean±SD]	1,116.56 ± 1,894.33	1,149.37 ± 1,901.57	1,220.22 ± 2,114.06	1,095.22 ± 1,843.27	1,001.46 ± 1,687.37
Fasting status [n(%)]					
Fasting before exam	13,087 (42.6)	3,254 (42.36)	3,337 (43.45)	3,208 (41.77)	3,288 (42.81)
Not fasting before exam	17,121 (55.73)	4,234 (55.12)	4,241 (55.22)	4,349 (56.62)	4,297 (55.95)
Missing	515 (1.68)	194 (2.53)	102 (1.33)	124 (1.61)	95 (1.24)
Worker at plant [n(%)]					
Ever	1,892 (6.16)	49 (0.64)	100 (1.30)	229 (2.98)	1,514 (19.71)
Never	28,831 (93.84)	7,633 (99.36)	7,580 (98.70)	7,452 (97.02)	6,166 (80.29)
Race [n(%)]					
White	29,767 (96.55)	7,458 (97.08)	7,447 (96.97)	7,446 (96.94)	7,416 (96.56)
Other	786 (2.55)	193 (2.51)	182 (2.37)	198 (2.58)	213 (2.77)
Missing	278 (0.90)	31 (0.40)	51 (0.66)	37 (0.48)	51 (0.66)

Abbreviations: m, men; w, women.

Table 2. Linear regression coefficients for ln-transformed liver function biomarkers per ln ng/mL increase in cumulative PFOA and 2005/2006 PFOA concentrations.

	Cumulative ln-PFOA				2005/2006 ln-PFOA			
Liver function biomarker	No.	Coefficient (95% CI)	R^2	*p*-value	No.	Coefficient (95% CI)	R^2	*p*-value
ln-ALT								
Model 1[a]	30,723	0.003 (-0.000, 0.007)	0.150	0.0598	30,723	0.004 (0.000, 0.007)	0.150	0.0487*
Model 2[b]	28,047	0.012 (0.008, 0.016)	0.232	<0.0001**	28,047	0.012 (0.009, 0.016)	0.233	<0.0001**
Model 3[c]	25,561	0.011 (0.007, 0.015)	0.235	<0.0001**	25,561	0.011 (0.007, 0.015)	0.235	<0.0001**
ln-GGT								
Model 1[a]	30,723	-0.014 (-0.018, -0.009)	0.120	<0.0001**	30,723	-0.011 (-0.015, -0.006)	0.120	<0.0001**
Model 2[b]	28,047	0.003 (-0.003, 0.008)	0.207	0.3078	28,047	0.003 (-0.002, 0.008)	0.207	0.3080
Model 3[c]	25,561	0.004 (-0.002, 0.009)	0.208	0.1789	25,561	0.004 (-0.002, 0.009)	0.208	0.1832
ln-Direct bilirubin								
Model 1[a]	30,723	0.002 (-0.001, 0.004)	0.096	0.3162	30,723	-0.001 (-0.004, 0.002)	0.096	0.4453
Model 2[b]	28,047	-0.005 (-0.008, -0.002)	0.150	0.0042*	28,047	-0.006 (-0.009, -0.003)	0.150	0.0004**
Model 3[c]	25,561	-0.005 (-0.009, -0.002)	0.151	0.0026*	25,561	-0.006 (-0.010, -0.003)	0.151	<0.0001**

[a]Adjusted for age and sex. [b]Adjusted for BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race in addition to adjustment in model 1. [c]Adjusted for average household income in addition to adjustment in model 2. *$p < 0.05$. **$p < 0.001$.

Table 3. Logistic regression OR (95% CI) of having abnormally high values of ALT, GGT, or direct bilirubin per ln ng/mL increase in cumulative PFOA and 2005/2006 PFOA.

	Cumulative ln-PFOA			2005/2006 ln-PFOA		
Liver function biomarker	No.	OR (95% CI)	*p*-value	No.	OR (95% CI)	*p*-value
ln-ALT						
Model 1[a]	30,723	1.00 (0.98, 1.03)	0.9398	30,723	1.00 (0.98, 1.03)	0.9644
Model 2[b]	28,047	1.04 (1.01, 1.07)	0.0062*	28,047	1.04 (1.01, 1.07)	0.0065*
Model 3[c]	25,561	1.04 (1.01, 1.07)	0.0162*	25,561	1.04 (1.00, 1.06)	0.0239*
ln-GGT						
Model 1[a]	30,723	0.95 (0.93, 0.97)	<0.0001**	30,723	0.97 (0.95, 0.99)	0.0040*
Model 2[b]	28,047	0.99 (0.96, 1.01)	0.3562	28,047	0.99 (0.97, 1.02)	0.5051
Model 3[c]	25,561	0.99 (0.97, 1.02)	0.6792	25,561	1.00 (0.97, 1.02)	0.8766
ln-Direct bilirubin						
Model 1[a]	30,723	1.00 (0.93, 1.06)	0.9231	30,723	1.00 (0.93, 1.07)	0.8986
Model 2[b]	28,047	0.97 (0.90, 1.05)	0.4311	28,047	0.97 (0.90, 1.05)	0.4874
Model 3[c]	25,561	0.96 (0.88, 1.04)	0.3109	25,561	0.95 (0.88, 1.03)	0.2508

[a]Adjusted for age and sex. [b]Adjusted for BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race in addition to adjustment in model 1. [c]Adjusted for average household income in addition to adjustment in model 2.
*$p < 0.05$. **$p < 0.001$.

Table 4. Linear regression coefficients[a] (95% CI) for ln-transformed liver function biomarkers across quintiles of cumulative ln-PFOA and 2005/2006 ln-PFOA.

	Cumulative ln-PFOA			2005/2006 ln-PFOA		
Quintile	ALT	GGT	Direct Bilirubin	ALT	GGT	Direct Bilirubin
Quintile 1	Reference	Reference	Reference	Reference	Reference	Reference
Quintile 2	0.023 (0.006, 0.040)	0.009 (-0.014, 0.031)	0.012 (-0.002, 0.026)	0.001 (-0.016, 0.018)	0.004 (-0.018, 0.026)	0.006 (-0.008, 0.019)
Quintile 3	0.035 (0.018, 0.052)	0.025 (0.003, 0.047)	-0.003 (-0.017, 0.011)	0.023 (0.007, 0.040)	0.014 (-0.008, 0.036)	0.003 (-0.011, 0.017)
Quintile 4	0.039 (0.022, 0.056)	0.011 (-0.011, 0.033)	-0.007 (-0.021, 0.007)	0.036 (0.019, 0.053)	0.015 (-0.007, 0.038)	-0.008 (-0.022, 0.006)
Quintile 5	0.058 (0.040, 0.076)	0.020 (-0.004, 0.044)	-0.017 (-0.032, -0.001)	0.048 (0.031, 0.066)	0.013 (-0.010, 0.036)	-0.018 (-0.033, -0.004)
Trend[b]	<.0001	0.1021	0.0029	<.0001	0.1552	0.0036

[a]Adjusted for age, sex, BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race. [b]*p*-value for trend across quintiles.

Table 5. Logistic regression OR[a] (95% CI) of having abnormally high values of ALT, GGT, or direct bilirubin across quintiles of cumulative ln-PFOA and 2005/2006 ln-PFOA.

	Cumulative ln-PFOA			2005/2006 ln-PFOA		
Quintile	ALT	GGT	Direct Bilirubin	ALT	GGT	Direct Bilirubin
Quintile 1	Reference	Reference	Reference	Reference	Reference	Reference
Quintile 2	1.12 (1.00, 1.27)	1.16 (1.04, 1.29)	0.99 (0.70, 1.38)	0.94 (0.84, 1.06)	1.11 (1.00, 1.24)	0.92 (0.66, 1.29)
Quintile 3	1.14 (1.01, 1.29)	1.12 (1.01, 1.26)	0.99 (0.70, 1.39)	1.06 (0.94, 1.20)	1.04 (0.93, 1.16)	1.07 (0.77, 1.49)
Quintile 4	1.20 (1.06, 1.35)	1.09 (0.97, 1.22)	0.83 (0.59, 1.18)	1.16 (1.03, 1.31)	1.08 (0.97, 1.21)	0.81 (0.57, 1.15)
Quintile 5	1.16 (1.02, 1.33)	0.96 (0.85, 1.09)	0.95 (0.66, 1.37)	1.10 (0.97, 1.24)	0.99 (0.88, 1.12)	0.94 (0.66, 1.33)
Trend[b]	0.0078	0.4991	0.5000	0.0055	0.7940	0.5361

[a]Adjusted for age, sex, BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race. [b]*p*-value for trend across quintiles.

Table 6. Linear regression coefficients[a] (95% CI) for ln-transformed liver function biomarkers per ln ng/mL increase in cumulative PFOA concentrations by sex (male vs. female), age (< 50 years old vs. ≥ 50 years old), and history of working at DuPont plant (yes vs. no).

	Sex		Age		Worker	
	Male (N=13,658)	Female (N=17,065)	< 50 years (N=16,312)	≥ 50 years (N=14,411)	No (N=28,831)	Yes (N=1,892)
ln-ALT	0.005 (-0.001, 0.011)	0.017 (0.017, 0.021)	0.010 (0.005, 0.015)	0.009 (0.003, 0.014)	0.013 (0.009, 0.017)	-0.020 (-0.045, 0.004)
	p-value[b] = 0.0025		*p*-value[b] = 0.7243		*p*-value[b] = 0.0093	
ln-GGT	-0.009 (-0.017, -0.001)	0.011 (0.004, 0.017)	0.006 (-0.001, 0.013)	-0.001 (-0.008, 0.006)	0.003 (-0.002, 0.008)	-0.019 (-0.051, 0.014)
	p-value[b] < 0.0001		*p*-value[b] = 0.1778		*p*-value[b] = 0.1899	
ln-Direct Bilirubin	-0.003 (-0.008, 0.002)	-0.006 (-0.010, -0.002)	-0.004 (-0.009, 0.000)	-0.002 (-0.007, 0.002)	-0.005 (-0.008, -0.002)	0.007 (-0.013, 0.028)
	p-value[b] = 0.4400		*p*-value[b] = 0.5107		*p*-value[b] = 0.2377	

[a]Adjusted for age, sex, BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race. [b]*p*-value for significance of interaction term.

Table 7. Linear regression coefficients[a] (95% CI) for ln-transformed liver function biomarkers per ln ng/mL increase in 2005/2006 PFOA concentrations by sex (male vs. female), age (< 50 years old vs. ≥ 50 years old), and history of working at DuPont plant (yes vs. no).

	Sex		Age		Worker	
	Male (N=13,658)	Female (N=17,065)	< 50 years (N=16,312)	≥ 50 years (N=14,411)	No (N=28,831)	Yes (N=1,892)
ln-ALT	0.006 (0.001, 0.012)	0.017 (0.012, 0.022)	0.010 (0.005, 0.015)	0.014 (0.008, 0.019)	0.012 (0.008, 0.016)	0.019 (0.001, 0.038)
	p-value[b] = 0.0054		*p*-value[b] = 0.3903		*p*-value[b] = 0.4496	
ln-GGT	-0.004 (-0.012, 0.003)	0.008 (0.001, 0.014)	0.004 (-0.003, 0.010)	0.004 (-0.003, 0.011)	0.001 (-0.004, 0.006)	0.033 (0.009, 0.057)
	p-value[b] = 0.0155		*p*-value[b] = 0.8782		*p*-value[b] = 0.0125	
ln-Direct Bilirubin	-0.006 (-0.011, -0.002)	-0.005 (-0.009, -0.001)	-0.005 (-0.009, -0.001)	-0.006 (-0.010, -0.001)	-0.006 (-0.009, -0.003)	-0.004 (-0.019, 0.012)
	p-value[b] = 0.7409		*p*-value[b] = 0.9064		*p*-value[b] = 0.7902	

[a]Adjusted for age, sex, BMI, alcohol consumption, physical activity, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, and race. [b]*p*-value for significance of interaction term.

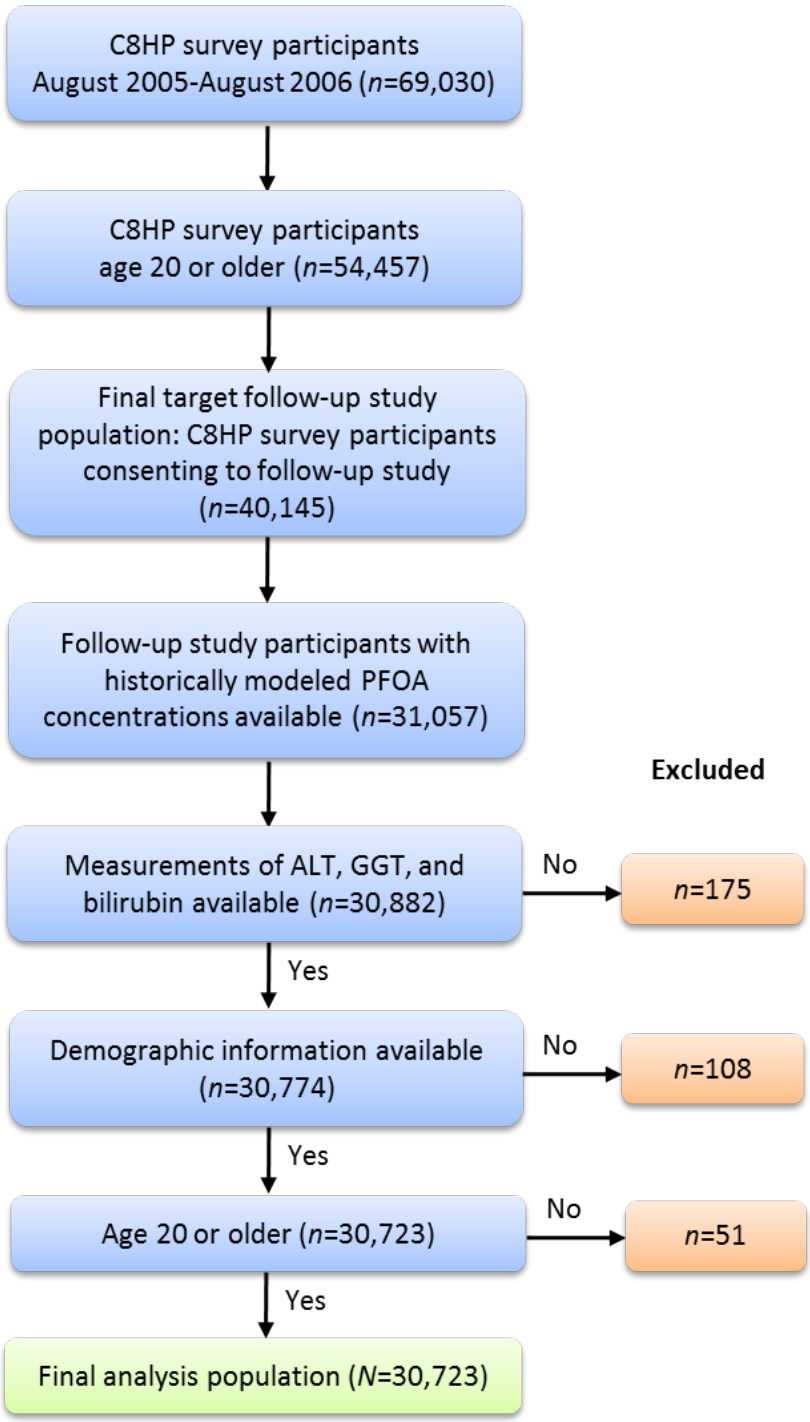

Figure 1. Population flow chart algorithm.

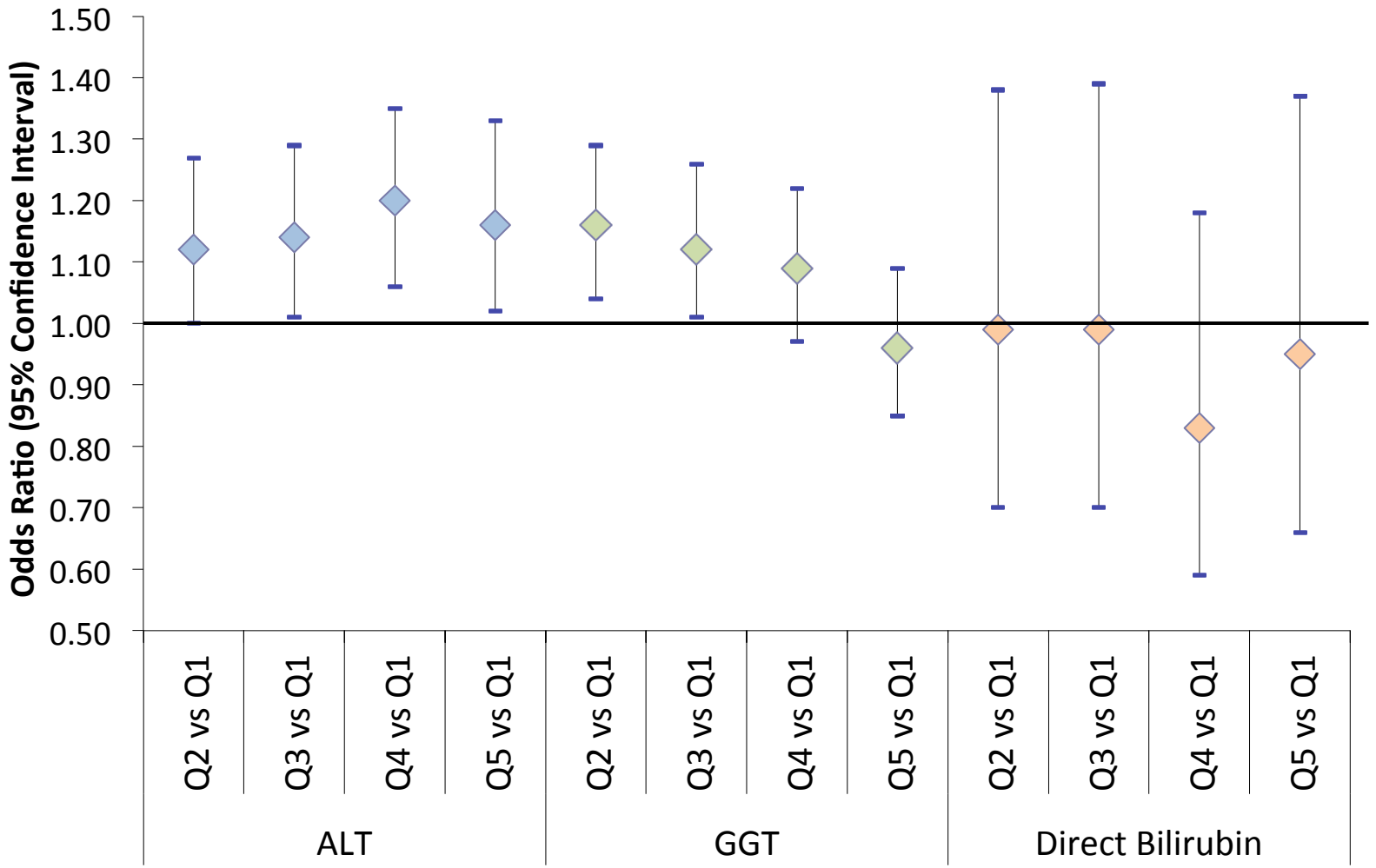

Figure 2. Odds ratios and 95% confidence intervals of having abnormally high values of ALT, GGT, or direct bilirubin by quintile of cumulative PFOA (relative to the lowest quintile).

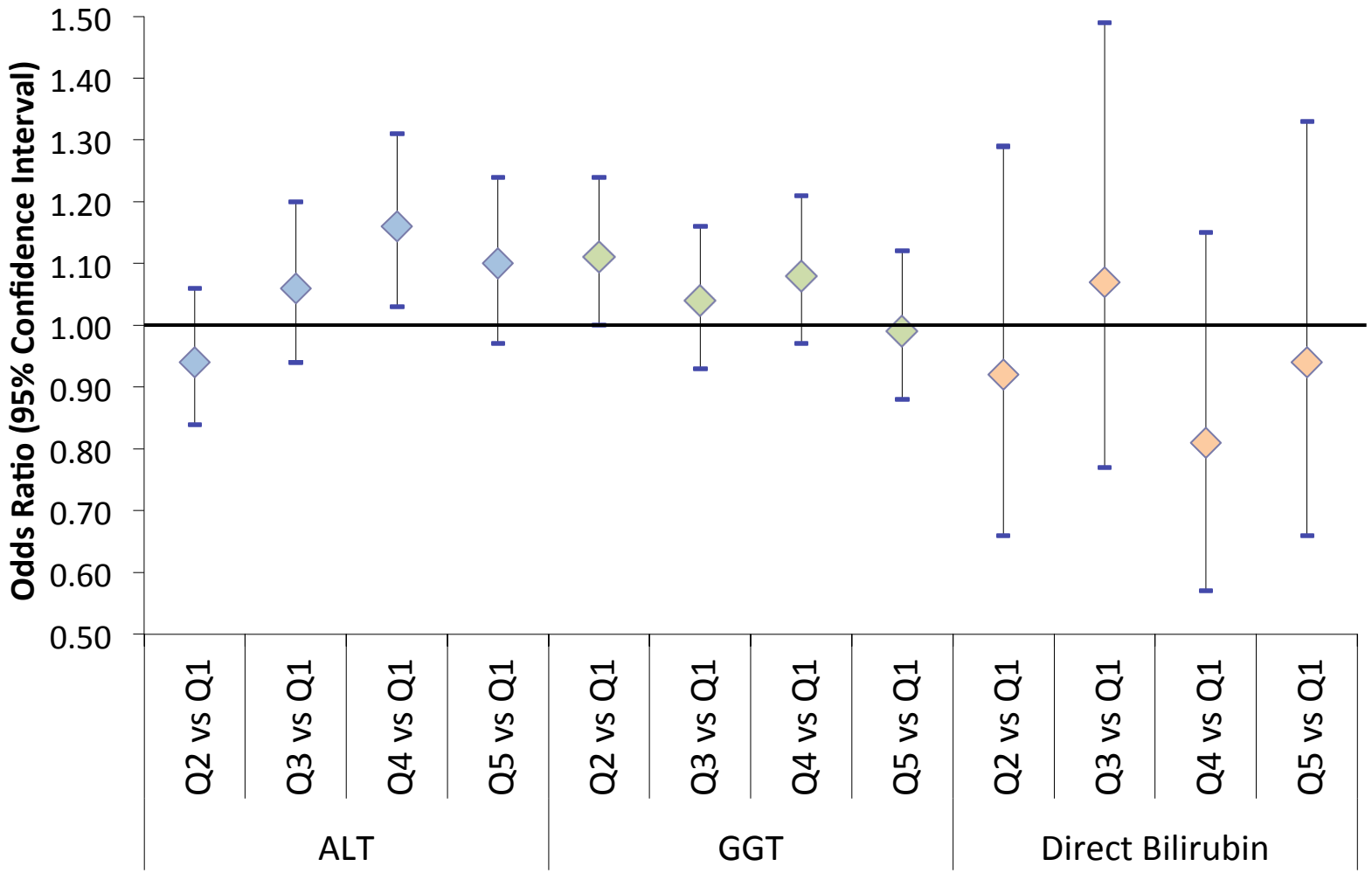

Figure 3. Odds ratios and 95% confidence intervals of having abnormally high values of ALT, GGT, or direct bilirubin by quintile of 2005/2006 PFOA (relative to the lowest quintile).