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**Probable Link Evaluation for Liver Diseases**

**Conclusion:** On the basis of epidemiological and other data available to the C8 Science Panel, we conclude that there is not a probable link between exposure to C8 (also known as PFOA) and Liver disease.

**Introduction - C8 Science Panel and the Probable Link Reports**

In February 2005, the West Virginia Circuit Court approved a class action Settlement Agreement in a lawsuit about releases of a chemical known as C8, or PFOA, from DuPont's Washington Works facility located in Wood County, West Virginia. The Settlement Agreement had several parts.

One part of the Settlement was the creation of a Science Panel, consisting of three epidemiologists, to conduct research in the community in order to evaluate whether there is a probable link between PFOA exposure and any human disease. A "probable link" in this setting is defined in the Settlement Agreement to mean that given the available scientific evidence, it is more likely than not that among class members a connection exists between PFOA exposure and a particular human disease. The Science Panel recognizes that, given the many diseases we are studying, some may appear to be associated with exposure simply through chance, but we have to judge these associations individually and acknowledge the uncertainty inherent in making these judgments.

Another part of the Settlement established the C8 Health Project, which collected data from Class Members through questionnaires and blood testing. These data represent a portion of what the Science Panel evaluated to answer the question of whether a probable link exists between PFOA and human disease. Evidence comes from Science Panel research that has been published as well as Science Panel research that has not yet been published.

In performing this work, the Science Panel was not limited to consideration of data relating only to Class Members, but examined all scientifically relevant data including, but not limited to, data relating to PFOA exposure among workers, among people in other communities, and other human exposure data, together with relevant animal and toxicological data. The Science Panel has drawn on evidence that has been openly published by other investigators, which means that the detailed evidence used by the Panel to inform its conclusions is available to others.
Criteria used to evaluate the evidence for a probable link included the strength and consistency of reported associations, evidence of a dose-response relationship, the potential for associations to occur as a result of chance or bias, and plausibility based on experiments in laboratory animals. The relative risk (RR – which can include specific measures such as rate ratios, odds ratios, hazards or standardized mortality ratios (SMRs)) was the primary measure of association that we examined. The RR is measure of the risk in exposed compared to the risk in the unexposed or low-exposed. The null value – indicating no association between exposure and outcome – is 1.0. Values above 1.0 are evidence of increased risk with increased exposure. Values from 0.0 to 0.9 are evidence of decreased risk with increased exposure. The RRs discussed below are generally ‘adjusted’ for demographic variables such as age and gender, so that difference in disease risk between exposed and non-exposed are not the result of age and gender differences. We also examined 95% confidence intervals (95% CI) as a measure of the statistical precision of the RR. The 95% CI shows a range of plausible values taking chance into account. Where there are a range of RRs across exposure groups, statistical measures of trend are conducted to determine if RRs are increasing with increasing exposure. These tests of trend generate p-values, which reflect the statistical chance of getting such a result by chance alone. The lower the p-value the more unlikely it is that the observed trend resulted from chance, with many in the scientific community treating p-values less than 0.05 as being “statistically significant.”

Review of Evidence for liver diseases

For the purpose of this report, two classifications of liver disease are presented: all liver diseases (including hepatitis) and a narrower group comprising enlarged liver, fatty liver disease (both alcoholic and non-alcoholic) and cirrhosis. Liver cancer is not included which is covered in the Probable Link report on cancer.

The evidence to evaluate the probable link between PFOA exposure and liver diseases comes from 2 Science Panel studies in the Mid-Ohio Valley: 1) the Science Panel community and worker follow-up study examining the association between PFOA exposure and incidence of reported liver disease; 2) Measured serum PFOA and measured enzymes of liver function during the 2005-06 survey.

In addition, published studies on the associations between liver disease, liver function and exposure to PFOA have been considered alongside toxicological evidence.

Mechanistic and Toxicologic Evidence
In rodents and non-human primates, PFOA has been found in relatively high concentrations in the liver and have been associated with liver enlargement (Son, Kim et al. 2008). In rats, these compounds have been also associated with hepatocellular adenomas (Abdellatif, Al-Tonsy et al. 2003; Lau, Anitole et al. 2007); and a hepatocarcinogenic effect has also been observed in trout (Benninghoff, Orner et al. 2012). In mice, one of the biological effects of PFAAs is the activation of the peroxisome proliferator-activated receptor alpha (PPAR-α), a ligand-activated transcription factor that regulates gene expression, lipid modulation and glucose homeostasis, cell proliferation and inflammation (DeWitt, Shnyra et al. 2009). While some effects in experimental studies are mediated by PPAR-α binding, some other effects occur independently of this receptor (Minata, Harada et al. 2010). In addition, in mice PFOA induced liver enlargement is also associated with significant alterations of the hepatic immune status (Qazi, Abedi et al. 2010).

**Epidemiologic Studies on Other Populations**

Studies in humans have reported inconsistent associations between PFOA and liver enzymes. PFOA concentrations were positively associated with transaminase levels in three occupational studies (Olsen and Zobel 2007; Sakr, Leonard et al. 2007; Costa, Sartori et al. 2009) and a large population based survey (Lin, Lin et al. 2010), but not in other studies (Emmett, Zhang et al. 2006; Sakr, Kreckmann et al. 2007; Costa, Sartori et al. 2009). PFOA concentrations were positively associated with γ-glutamyl-transferase (GGT) in three studies (Olsen and Zobel 2007; Sakr, Kreckmann et al. 2007; Costa, Sartori et al. 2009; Lin, Lin et al. 2010), but not in others (Emmett, Zhang et al. 2006; Sakr, Leonard et al. 2007; Costa, Sartori et al. 2009). Direct bilirubin was found to be negatively associated with PFOA concentrations in a few studies (Olsen and Zobel 2007; Sakr, Leonard et al. 2007; Costa, Sartori et al. 2009), but no association was observed in others (Emmett, Zhang et al. 2006; Sakr, Kreckmann et al. 2007; Sakr, Leonard et al. 2007; Costa, Sartori et al. 2009).

**Epidemiologic Studies on Mid-Ohio Valley Populations**

**Studies Conducted by the Science Panel**

The Science Panel conducted two studies of the population of the mid-Ohio valley, one of diagnosed liver disease based on interviews in 2008-2011, and one of the cross-sectional associations between liver enzymes and PFOA serum concentrations among adults in 2005-06 (Gallo, 2012).
1) The Science Panel community and worker follow-up study has examined the association between PFOA exposure and incidence of diagnosed liver disease among adult community residents and plant workers.

**Community Residents**

The Mid-Ohio population, which has been extensively studied by the C8 Science Panel, was formed from those who were living or had lived in any of six PFOA contaminated water districts and participated in a baseline survey called the C8 Health Project in 2005-2006 (Frisbee, Brooks et al. 2009). The principal route of exposure for this population was via drinking water contaminated with PFOA. In 2005/2006, most participants in the C8 Health Project (n=69,030) had their PFOA serum levels measured, provided a medical history, and also had a panel of blood measurements, including liver enzymes, cholesterol and uric acid. Most C8 Health Project participants (74% of adults aged 20 or above) consented to participate in follow-up studies conducted by the C8 Science Panel, of whom 82% were subsequently interviewed by the C8 Science Panel in 2008-2011, and in 2010, a sample of 755 provided second blood samples.

Historical serum PFOA estimates for community residents over time were developed by the Science Panel, based on the estimated intake of contaminated drinking water. These estimates of drinking water concentrations, in turn, were based on the amount of PFOA released from the DuPont plant, wind patterns, river flow, groundwater flow and the residential address history provided by study participants (Shin, Vieira et al. 2011a; Shin, Vieira et al. 2011b). Among those interviewed we were able to estimate historical serum concentrations for 28,541 community residents who had never worked at the DuPont plant.

**Workers at the DuPont Plant**

In addition, 4,391 past and current workers at the Washington Works plant were interviewed by the Science Panel. This group is a subset of a cohort of 6,027 Washington Works workers studied by the Science Panel to evaluate their patterns of death.

An estimate of serum levels over time for workers in different jobs in the plant was developed by the C8 Science Panel (Woskie, Gore et al. 2012). These estimates were combined with estimated serum levels from residential exposure to contaminated drinking water. We were able to estimate combined residential and occupational exposure for 3,713 (84%) of the interviewed workers.
**Combined Community and Worker Population**

For the study of diagnosed liver disease, community residents and workers who were interviewed in 2008-2011 were combined to form a final population of 32,254 people for whom we could study the relationship between estimated past PFOA serum levels and disease.

The main statistical approach was a multivariate survival analysis, which modelled disease risk as a function of the estimated serum PFOA levels at the time or a cumulative exposure index at that time (as a sum of yearly modelled serum PFOA concentration estimates), controlling for gender, race, education, body mass index, smoking, alcohol use and birth year.

The main analyses considered all cases through the study period, with most occurring prior to enrolment into the C8 Health Project in 2005-6. We also conducted prospective analyses among the community cohort members restricted to the time and disease development after the date of enrolment into the C8 Health Project. Numbers are thus smaller, but this allowed us to make use of the measured PFOA levels in 2005-6 and assess risk of subsequent disease among those without reported disease at enrolment.

For each analysis, overall trend of risk with increasing exposure was assessed and, to explore the pattern of risk with exposure, the risk by increasing exposure quintiles (compared to the lowest exposure group) was calculated. Because the exposure prediction model is more uncertain at the lower exposure levels, we are especially interested in the presence or absence of trends of risk across the whole range of exposure categories. Various additional analyses were done, for example specific subcategories of reported disease; applying a lag which focuses on exposures estimated prior to 5 or 10 years before year of diagnosis; investigating patterns in women and men separately; separating out worker and community sub-cohorts; including or excluding the time before study participants met the entry criteria of started employment in the plant or moving into the study area. Where any of these additional analyses reveal informative contrasts these are highlighted.

2) The Science Panel studied liver enzymes in the population of 47,092 liver disease-free adults who participated in the 2005-2006 C8 Health Panel survey. These subjects had measured PFOA levels and liver enzymes (conjugated bilirubin - also known as “direct”, alanine aminotransferase - ALT, and gamma-glutamyl-transefere - GGT) in their serum. The primary analysis was a regression of liver enzymes in relation to log transformed PFOA to assess overall trend and deciles of distribution to investigate the
shape of the relationship, in each model potential confounders were included (age, sex, physical activity, fasting status, etc). More detailed analyses distinguished the associations between and within water districts.

Results of Science Panel Studies

1) The Science Panel community and worker follow up study (described above) examined the association between PFOA exposure and incidence of reported diagnoses of liver disease among adult community residents and plant workers who were interviewed by the Science Panel.

All subjects were interviewed during 2008-2010 regarding their medical history, including non-malignant liver disease. Participants reporting liver disease were asked to classify their liver disease by type and reported the age at diagnosis and whether they had received medication for the disease. The Science Panel sought medical records to confirm these cases to validate the diagnoses, and the analysis addresses a total of 647 validated reported liver disease.

Results for all liver disease or the fatty liver, enlarged liver and cirrhosis were analysed in relation to PFOA categories and in no case was there any suggestion of a positive trend. In the analysis looking at all validated liver disease in relation to all years of follow up, the pattern of risk by cumulative PFOA exposure quintile was 1.0, 1.19, 1.08, 1.04, 0.95 with p-value for trend of 0.32. For the analysis restricted to years since entering the study area there was similarly no evidence of trend: for 1.0, 0.98, 0.95, 0.86, 0.89, p-value for trend=0.34. When the analysis was repeated looking at the serum PFOA level at the year of diagnosis the association was similar (RRs 1.0, 0.99, 0.94, 0.85, 0.80, p-value for trend 0.09. Neither more restrictive subcategories of disease nor males or females suggested any PFOA related pattern of risk.

Prospective analyses (including 266 cases) applied the same method following the baseline survey to estimate RRs for disease onset after the time of the C8 Health Project and excluded people who had developed liver disease before that time. The overall pattern by cumulative serum PFOA category remained similar (RRs 1.0, 0.96, 0.96, 0.77, and 0.80, p-value for trend 0.21); and the risks looking at the serum measurements at baseline showed some non-significant decreasing trend (RRs 1.0, 0.90, 0.89, 0.76, 0.71, p-value for trend=0.09).

Overall, there was no evidence of a positive association between liver disease and estimated PFOA exposure.
2) In the cross-sectional analysis of serum PFOA and PFOS concentrations with markers of liver function in adults (Gallo, 2012), In-PFOA was associated with In-ALT in linear regression models and with raised ALT in logistic regression models (with a steady increase in the Odds Ratio (OR) estimates across deciles of PFOA, the RRS being 1.0, 1.09, 1.19, 1.26, 1.40, 1.39, 1.31, 1.42, 1.40, 1.54, \( P\)-trend <0.001).

There was less consistent evidence of an association of PFOA and GGT or bilirubin. The relationship with bilirubin appears to rise at low levels of PFOA and to fall again at higher levels. These results show a positive association between PFOA concentrations and a marker of hepatocellular damage, as measured by ALT serum levels.

**Evaluation**

From our studies of patterns of diagnosed liver disease there is no evidence of any increased risk of liver disease in relation to PFOA exposure. Based on our studies of liver enzymes and inconsistent findings in reported literature there is some evidence of small shifts in liver function, mainly within the normal physiologic range, being associated with increasing PFOA exposure. It is uncertain if PFOA is the cause of the association, but if so there is no evidence that this is reflected in any increase the overall incidence of diagnosed liver disease. Therefore, the Science Panel does not find a probable link between exposure to C8 (also known as PFOA) and liver disease.

**References**


