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***Probable Link Evaluation of infectious disease***

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 common infections, including influenza, in children or adults.

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 or standardized mortality ratios (SMRs)) was the primary measure of association that we examined. The RR is a marker 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 to 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."

## Background Information on Infectious Disease

### Epidemiologic Studies on Other Populations

There are few epidemiological studies on the effects of PFOA on immune function related to infectious disease. Two studies of childhood infections and maternal PFOA levels during pregnancy found no association between PFOA and the risk of infectious diseases in early childhood and infancy (1, 2). There have been recent reports that suggest low levels of PFOA or PFOS may dampen the anticipated protection provided by vaccines. A small Norwegian study suggested negative association between maternal PFC exposures collectively and rubella vaccination response (3), and a Danish study reported an increased concentration in PFOA and PFOS was associated with a reduced response to childhood diphtheria and tetanus vaccines (4). However, to date there has been no study addressing whether these infectious diseases' incidence may be changed following exposure to PFOA.

### Mechanistic and Toxicologic Evidence

*Evidence for Immunotoxicity of PFOA*

Animal and human studies have suggested PFCs are immunotoxic, affecting both cellular and humoral immunity. Treatment of mice with PFOA has been shown to cause thymic and splenic atrophy which produces a particularly marked effect on the immature CD4+CD8+ (double-positive) cell population (6). Furthermore, oral PFOA treatment in mice has been found to cause severe suppression of the antibody response to horse red blood cells by decreasing antibody levels normally evoked by such immunisation (7-9). Studies in humans also suggest immunotoxic effects from PFOA. In vitro exposure to PFOA inhibits regulatory cytokine production (IL-4, IL-10) in cultured human leukocytes (10).

*In Utero Exposure to PFOA and Immunotoxicity*

The susceptibility of the immune system to environment exposures such as PFOA may be greater during the prenatal period than later in life (12). The potential consequences of this exposure include immunosuppression, immune perturbation leading to autoimmune conditions or immune upregulation causing allergic responses (13). PFOA and perfluorooctane sulfonic acid (PFOS) can cross the placenta in humans, transferring between the mother and the fetus (14, 15). There have been a number of animal studies investigating the developmental toxicity of high dose PFCs (16-21).

The Mid-Ohio Valley Population Studied by the Science Panel

Evidence from the Mid-Ohio Valley comes from three Science Panel studies, an interview of mothers investigating reported infections in their young children, an interview of adults on recent reported infections, and an assessment of impact of PFOA on the effectiveness of flu inoculations and on key clinical markers of the immune system.

The Mid-Ohio population, which has been extensively studied by the C8 Science Panel was formed from people who live or lived in any of six PFOA contaminated water districts and participated in a baseline survey called the C8 Health Project in 2005-2006 (22). The principal route of exposure for this population was via drinking water contaminated with PFOA. In 2005/2006 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 thyroid hormones, cholesterol, uric acid, etc. Most C8 Health Project participants (74% of adults age 20 or above) consented to participate in follow-up studies conducted by the C8 Science Panel, among whom 82% were subsequently interviewed by the C8 Science Panel in 2009-2011. For the study of clinical markers in the population, 50, 680 adults and 10,725 children with measured PFOA levels and hormones in their serum were analysed.

Further blood tests and interviews collecting health information were carried out in a subset of 755 adults who participated during 2010 in the Science Panel Short Term Follow-up Study. This interview included questions regarding the occurrence and frequency of a number of recent (during the last 12 months) common infections, including coughs, colds, flu and other infections. In addition blood samples were collected for studies of immune system biomarkers. A subset of these participants were also offered the current CDC recommended influenza vaccine and asked to consent to pre and post vaccination serum sampling to quantify their response to vaccination. For the purpose of describing the occurrence of infections over the last 12 months in this population, infections were classified into different categories according to severity, site, and probable etiology. Infections were defined as *minor/seasonal* (cold, sinus infection, flu, and sore throat), of *medium severity* (cold sore, bronchitis, ear, tooth, mouth, gastrointestinal, and skin infection), and *severe infections* (pneumonia, shingles, and meningitis). *Respiratory infections* included cold, sinus infection, flu, sore throat, bronchitis and pneumonia; *gastrointestinal infections* included mouth, tooth and gastrointestinal tract infection; *"other sites"* included cold sore, shingles, meningitis, and ear and skin infections. Infections were also classified as of *viral/probable viral aetiology* (cold, sore throat, flu, cold sore, bronchitis, shingles, ear and gastrointestinal infections) and of *bacterial/probable bacterial aetiology* (sinus, mouth, tooth, ear and skin infections).

A sample of 878 mothers who provided blood samples during or close to the time of their pregnancy were interviewed concerning infectious and other diseases among their children. Information about childhood infections was gathered from the mothers of a child born between 2004 and 2007 and who participated in the C8 Science Panel Study. The interview was focused on the index child in the household only, including questions about infections over the past 12 months, factors affecting immune response, allergies and asthma and factors affecting infection exposure. In total, information was gathered on 878 children. The reported conditions were grouped as for the adults.

## Results of C8 Science Panel Studies

### *Infections in Children*

878 mothers interviewed during 2010 were asked about infections in their children in the previous twelve months. Their patterns of infection were assessed in relation to the serum levels of PFOA in their mothers during pregnancy. When the serum sample was taken after pregnancy, the levels were adjusted for time trends in this population (23), and also took into account how long they breast-fed the child, which can also affect serum levels of PFOA.

There was no evidence of any positive association of increasing risk for any of the categories of infection reported for these children in relation to PFOA during pregnancy among these children. For respiratory infections there was a suggestion of a trend of decreasing risk. Among these children 131 reported an episode of flu and 499 a cold in the last 12 months, and the risk of either cold or flu fell by quartile of PFOA during pregnancy was 1.0, 0.88, 0.67, 0.72; p-value for trend=0.07.

*Infections in Adults*

The primary analysis compared recent PFOA serum levels (measured in 2010) and reported infections in the last 12 months up to the time of interview in 2010 for 755 adults. As for the children, there was no evidence of any positive association of increasing risk for any of the categories of infection reported for these in relation to in PFOA. For respiratory conditions there was a trend of decreasing risk (RRs by quartile 1.0, 1.27, 0.43, 0.27; p-value for trend <0.05. The trend was even steeper for infections grouped as probably bacterial (sinus, oral, ear, and skin) with RRs by quartile being 1, 0.37, 0.73, 0.15; p-value for trend <0.05.

*Cold Sores*

The study of cold sores allowed the reporting of infection to be combined with a more objective measure, the relevant antibodies in serum. During the short-term follow-up study, participants were asked if they had suffered from a cold sore in the previous year, and their anti HSV antibodies (HSV1 and HSV2) were measured. Data were analysed looking at associations between C8 and 1) the presence of both a self-reported cold sore and HSV positivity; 2) the presence of either a self-reported cold sore, or HSV positivity. When using this latter more inclusive classification, a strong inverse association between C8 and cold sore was observed with RRs falling across quartiles 1.0, 0.67, 0.58, 0.59, p-value for trend <0.05, for the narrower definition of both reported cold sore and HSV positive, RRs were all close to 1.0

*Influenza Vaccine Response and Other Blood Tests*

There was evidence of an association between serum PFOA at the time of influenza vaccination and a decreased antibody response to one strain, A/H3N2, of influenza virus in the vaccine, among the three we tested. Thus compared to the lowest quartile there was a poorer vaccine response in the higher PFOA groups (with increasing quartiles the change in antibody titre was 0.52, 0.43 and 0.76 times the baseline, ie a smaller increase following vaccination). The trend of titre in relation to PFOA level gave a p-value of 0.07.

Another way of presenting this is to examine seroprotection (i.e. reaching a 'protective' antibody titre threshold of at least 1:40 following vaccination). The relative risk of reaching seroprotection for A/H3N2 fell with PFOA quartile (RRs 1.0, 0.4, 0.3, 0.4, with a p-value for trend of 0.07). For the other two flu strains (A/H1N1 and influenza B), there were no patterns of increase or decrease of vaccine effectiveness in relation to PFOA. These results suggest that PFOA may weaken the effectiveness of flu vaccination, though this was only evident for one out of the three strains given.

The Science Panel has also looked at other tests in blood samples of markers for immune dysfunction or altered inflammatory responses. In analyses of the C8 Health Project data (over 50,000) people we found that serum IgA thought to be involved in resistance to respiratory infections and C reactive protein (CRP) a marker of inflammatory responses were both reduced in relation to increasing PFOA levels. In the short-term follow up study, specific types of immune cells (immunophenotypes) were looked at among 755 adult participants, and we have found the numbers of some specific CD4/CD8 subtypes involved in the immune response being negatively associated with PFOA. Also, changes in CRP levels from 2006 to 2010 were correlated with degree of fall in PFOA: the more the PFOA fell, the more the CRP rose, consistent in direction with the cross sectional analysis. In a smaller subset of 50 adults serum was tested for a panel of inflammatory cytokines, and the pattern of results suggested some reduction of inflammatory response. Taken together these results provide supportive evidence that PFOA is associated with some immune dysfunction and the direction is suppression.

Evaluation

We have found some evidence that PFOA may reduce vaccine efficiency against the common flu strain A/H3N2, and that PFOA is associated with decreases in a number of clinical markers of the immune system. However despite the evidence on clinical markers of the immune system and vaccination responses, we have not found any evidence that the frequency of common infections in childhood or adulthood, including flu and common colds, are increased related to contrasts of exposure to PFOA. Further our data are limited because they address self-reported colds and influenza in one season only. Taken together, the C8 Science Panel concludes that there is not a probable link between exposure to C8 (also known as PFOA) and common infections, including influenza, in children or adults.

**References:**

1. Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 2010;110(8):773-7. Epub 2010/08/31.

2. Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, et al. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res. 2012;112:118-25. Epub 2011/10/28.

3. Nygaard U, Haug L, Stolevik S, Namork E, Thomsen C, van Loveren H, et al. Perfluorinated compounds and immune health outcomes in 3-year-old children. Abstract of Conference Poster Presentation Society of Toxicology, 2012.

4. Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012;307(4):391-7. Epub 2012/01/26.

6. Yang Q, Xie Y, Depierre JW. Effects of peroxisome proliferators on the thymus and spleen of mice. Clin Exp Immunol. 2000;122(2):219-26. Epub 2000/11/25.

7. Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Moller G, Nelson BD, et al. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. Int Immunopharmacol. 2002;2(2-3):389-97. Epub 2002/01/29.

8. Dewitt JC, Copeland CB, Strynar MJ, Luebke RW. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ Health Perspect. 2008;116(5):644-50. Epub 2008/05/13.

9. Peden-Adams MM, EuDaly JG, Dabra S, EuDaly A, Heesemann L, Smythe J, et al. Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. J Toxicol Environ Health A. 2007;70(13):1130-41. Epub 2007/06/15.

10. Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, et al. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). Toxicol Appl Pharmacol. 2011;250(2):108-16. Epub 2010/11/16.

12. Luster MI, Dean JH, Germolec DR. Consensus workshop on methods to evaluate developmental immunotoxicity. Environ Health Perspect. 2003;111(4):579-83. Epub 2003/04/05.

13. Holsapple MP, Paustenbach DJ, Charnley G, West LJ, Luster MI, Dietert RR, et al. Symposium summary: children's health risk--what's so special about the developing immune system? Toxicol Appl Pharmacol. 2004;199(1):61-70. Epub 2004/08/04.

14. Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. International archives of occupational and environmental health. 2007;80(7):643-8. Epub 2007/01/16.

15. Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, et al. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res. 2008;108(1):56-62. Epub 2008/07/25.

16. Butenhoff JL, Kennedy GL, Jr., Frame SR, O'Connor JC, York RG. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology. 2004;196(1-2):95-116. Epub 2004/03/24.

17. Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol Appl Pharmacol. 2004;198(2):231-41. Epub 2004/07/09.

18. Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, et al. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-8. Epub 2006/01/18.

19. Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci. 2003;74(2):382-92. Epub 2003/05/30.

20. Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. Toxicol Sci. 2003;74(2):369-81. Epub 2003/05/30.

21. Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology. 2005;215(1-2):149-69. Epub 2005/09/01.

22. Frisbee, S. J., Brooks, A. P., jr., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S. S., Pollard, C., Halverson, J. A., Vieira, V. M., Jin, C., Leyden, K. M. & Ducatman, A. M. 2009. The C8 health project: design, methods, and participants. *Environ Health Perspect,* 117**,** 1873-82.

23. Shin HM, Vieira VM, Ryan PB, Steenland K, Bartell SM. 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environ Health Perspect; doi:10.1289/ehp.1103729.