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Attention Docket Number EPA-HQ-OW-2014-0138

The 3M Company appreciates the opportunity to submit comments on the draft "Health Effects Document for Perfluorooctanic Acid (PFOA)". Pursuant to USEPA's request for comments by April 29th, 2014, attached please find comments prepared by 3M Medical Department.

Please contact me if you need additional information.

Regards,

Carol Ley

Carol A. Ley, MD, MPH
Vice President & Corporate Medical Director

Attention Docket Number USEPA-HQ-OW-2014-0138

The 3M Company (3M) appreciates the opportunity to review and comment on the draft "Health Effects Document for Perfluorooctanoic Acid (PFOA)".

Due to the limited time period to comment, 3M believes that it is unable to provide detailed commentary on the full document. Of particular concern is the Executive Summary, which, in 3M's opinion, is factually inaccurate, in part, and describes epidemiological studies that show some statistical associations without providing the broader perspective offered by the full body of epidemiological evidence and clinical interpretation of the data. As for toxicological evidence, the USEPA "selected 0.00002 mg/kg/day as the RfD for PFOA based on the consistency of the response and with recognition of the use of liver weight as a common denominator for loss of homeostasis and protection against co-occurring adverse effects." 3M believes that the selection of the RfD based on rodent liver weight is unjustified based on stronger evidence indicating that: 1) an increase in liver weight alone in response to PFOA exposure is an adaptive effect, not an adverse effect; 2) there is an established mode-of-action for the liver hypertrophic effects in rodents from exposure to PFOA that was not considered in the selection of the RfD; 3) the experimental evidence shows the lack of a response, or a markedly reduced response, in human liver as compared to rodent liver; and 4) and the large body of epidemiological evidence demonstrates a lack of evidence of liver toxicity (non-malignant or malignant) from exposure to PFOA. Therefore, the use of increased liver weight by USEPA as a critical effect in the derivation of RfD has led 3M to focus on liver effects in the comments to follow.

3M requests the right to comment on other aspects in anticipation of future drafts resulting from consideration of these comments and contractor managed external peer review. In addition, 3M believes that the full breadth and complexity of the epidemiological, toxicological and pharmacokinetic data related to PFOA exposure should require a formal review of this document by the USEPA Science Advisory Board.

I. COMMENTS SPECIFIC TO THE USE OF RODENT HEPATIC HYPERTROPHY AS THE CRITICAL EFFECT FOR HUMAN RfD DERIVATION

A. The Use Of Increased Rodent Liver Weight As A Critical Effect Is Inappropriate

Increased liver weight without microscopic or clinical evidence of overt liver toxicity is inappropriate as an adverse outcome for human health risk assessment. In addition, a strong case can be made that liver enlargement in rodent models from exposure to PFOA overestimates potential human liver response. Moreover, data to date, in particular from humans that were exposed to relatively high concentrations of PFOA, does not demonstrate liver toxicity. The USEPA needs to consider the following points.

- Based on USEPA guidance documents and the general consensus of the scientific community, the USEPA should not consider PFOA-induced liver weight increase as an

adverse effect in the absence of histological or biochemical evidence of a toxic effect in the liver.

- Moreover, the USEPA should consider the many studies that have examined the mechanism(s) by which PFOA influences the liver. This includes studies performed with human primary hepatocytes and humanized transgenic mice. These studies are important because they have shown the lack of PFOA-induced hepatic responses, or a markedly reduced hepatic response in human models compared to rodent models.

- The USEPA also should consider that, based on results from occupational, community, and general population epidemiological studies, there is a lack of adverse liver effects with PFOA exposure in humans.

- By extension, the USEPA should be cautious in interpreting other organ weight changes as adverse outcomes without consideration of anatomical and clinical pathological findings suggestive of adverse effects.

B. Increased Liver Weight Is Not Adverse

The USEPA generally does not rely on liver enlargement as the sole critical endpoint for risk assessment. In a recent query of the USEPA IRIS database, of 550 chemical substances covered, only 12 (2%) listed liver weight as the sole critical effect forming the basis for the RfD/RfC (http://www.epa.gov/iris/search_keyword.htm (accessed April 16, 2014)). In fact, USEPA internal guidelines in place since 2002 provide a framework for evaluation of hepatocellular hypertrophy as indicative of an adaptive, non-toxic effect as opposed to an adverse, or toxic effect. As noted in the USEPA Office of Pesticide Programs HED [Health Effects Division] Guidance Document # G2002.01 on Hepatocellular Hypertrophy (USEPA, 2002), liver hypertrophy does not necessarily represent liver toxicity, nor is it necessarily a precursor to a particular manifestation of toxicity. Guidance Document # G2002.01 suggests a weight-of-evidence approach that includes evaluation of other findings, such as: 1) type and severity of observed effects; 2) onset, duration, and progression of effects; 3) study method and design; and, 4) other relevant effects and data. This guidance states that liver size or weight changes may be "indicative of adaptation which, by itself, is not necessarily adverse." In the absence of microscopic evidence of liver injury or change, at least two liver-related clinical chemistry parameters should be elevated with clinical significance ("at least 2-fold to 3-fold greater than control levels") before liver weight changes are ascribed to toxicity. The USEPA guidance specifically defines the NOAEL as "a dose which elicits either no response or only adaptive, non-adverse responses (e.g., hepatocellular hypertrophy [liver weight changes] alone)." The LOAEL is defined as a "dose which elicits adverse effects (e.g., hepatocellular hypertrophy in addition to other evidence of liver toxicity)."

Additional guidance documents or articles similar to USEPA HED Guidance Document # G2002.01 have existed for many years. More recently, in 2012, the European Society of Toxicologic Pathology (ESTP) published the conclusions from the 3rd International ESTP Expert Workshop. This workshop was convened to "define more clearly when adaptive responses become adverse, and understand the long-term consequences of hepatocellular

hypertrophy in order to guide scientific opinion for risk assessment in man…" (Hall *et al.*, 2012). Hall et al. provide an updated perspective on the consideration of liver hypertrophy as an adaptive versus adverse change which includes a thorough discussion of mechanistic, clinical, microscopic, and epidemiological evidence that allows for more certain interpretation of hepatic hypertrophic changes observed in experimental studies in the context of human health risk assessment.

The conclusion of the expert group with respect to liver hypertrophy as a result of enzyme induction was as follows (Hall *et al.*, 2012):

> *The general opinion of the group was that liver weight increase through hepatocyte enzyme induction, in the absence of histopathologically demonstrated degenerative or necrotic changes and without significant changes in hepatic derived plasma enzymes, would not be considered adverse and would have little relevance to man in terms of risk assessment and the development of liver tumors.*

Further, in considering the histological change caused by an increase in liver weight, the expert group suggested consideration of the following factors "in order to conclude whether the change is adverse or not":

> *1. Is there histological evidence of structural degenerative or necrotic changes such as:*
> - *hepatocyte necrosis, fibrosis, inflammation, and *steatotic vacuolar degeneration*
> - *biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis*
> - *necrosis and degeneration of other resident cells within the liver*
>
> *(*Minimal to mild increases in steatotic macro-vesicular vacuolation without other changes indicating cellular damage should be distinguished from micro-vesicular vacuolation and considered non-adverse since this is a common change induced by feeding high-fat diets.)*
>
> *(Of note, transient increases in proliferative indices together with changes in hepatocyte ploidy, if induced through CAR/PXR/PPARα activation, is likely to be a rodent-specific phenomenon and therefore of little relevance to man even if this results in altered hepatic foci and/or primary liver tumors in chronic studies.)*
>
> *2. In the absence of histological changes, using a weight-of-evidence approach, is there clinical pathology evidence of hepatocyte damage characterized by a dose dependent and biologically significant and consistent increase in at least two liver parameters:*
> - *at least x2 to x3 increase in ALT (EMEA 2010, FDA 2009; HED Guidance Document 2002) or*
> - *a biologically significant change in other biomarkers of hepatobiliary damage (ALP, AST, γGT, GLDH, etc.)*
> - *a biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides etc.).*

> *Clinical pathology changes should corroborate each other, be consistent with the expected species-specific patterns of change resulting from hepatobiliary injury, and take into account target pharmacology that may non-adversely alter one or more of these biomarkers. It should also be noted that statistical significance alone is not a reliable indicator of hepatic toxicity (HED guidance document 2002).*
>
> *If the above mentioned adverse criteria are not observed, then increases in liver organ weight and liver cell hypertrophy due to enzyme induction can be considered as an adaptive response to a xenobiotic and of little relevance to man.*

In considering the critical effects at the LOAEL for oral exposure studies tabulated in Table 5-1 of the draft PFOA document for subchronic and chronic studies (page 5-3) and Table 5-2 for shorter term and developmental studies (page 5-5), liver weight increases, relative and/or absolute, were listed as the sole or co-critical effect at the LOAEL in 6 of the 11 (55%) tabulated critical effects in Table 5-1 and 6 of the 8 (75%) tabulated critical effects in Table 5-2. Liver weight was the sole critical effect basis for the LOAEL for 3 of the 11 (or 27%) study critical effects in Table 5-1 and 1 of the 8 (or 12%) of the study critical effects tabulated in Table 5-2. Given the findings by Hall and colleagues, the USEPA should reconsider these latter critical effect listings in light of its own, and other current guidance, on evaluation of hepatic hypertrophy without corroborating evidence in the context of liver toxicity.

C. Increased Liver Weight In Rodents Exposed To PFOA Is An Adaptive Response Occurring Through Increased Activation of PPARα and CAR/PXR Nuclear Receptors

Adaptive hepatocellular hypertrophy can result from hepatic nuclear receptor activation in response to exposure to endogenous ligands or xenobiotics (Hall *et al.*, 2012; Lake, 2009; Waxman, 1999). Nuclear receptor activation can lead to reversible induction of hepatocellular processes including phase I and phase II metabolic systems and systems that regulate intermediary metabolism (Hall *et al.*, 2012; Lake, 2009; Waxman, 1999). There are three primary xenosensor nuclear receptors that stimulate liver hypertrophy through induction of genes encoding for specific sets of proteins: 1) PPARα (or NR1C1) that regulates proteins associated with lipid metabolism and transport; 2) the constitutive androstane receptor (CAR or NR1I3); and 3) the pregnane X receptor (PXR or NR1I2), the latter two which control the expression of xenobiotic metabolizing enzymes and transporters. Increased activation of these nuclear receptors leads to increases in peroxisomal fractional volume (PPARα) and expansion of the smooth endoplasmic reticulum (PPARα and CAR/PXR) in the liver cell, and this added intracellular mass is reflected in the overall liver weight. Another consequence of PPARα and CAR/PXR activation in rodents is the potential stimulation of cell division (hyperplasia) and a decrease in the normal process of removal of worn out cells (apoptosis). These processes also increase liver mass and can potentially lead to tumor formation in rodents. Rodents are particularly sensitive to the liver hypertrophic and/or hyperplastic effects resulting from activation of these receptors as compared to humans (Corton *et al.*, 2014; Elcombe *et al.*, 2014; Hall *et al.*, 2012; Klaunig *et al.*, 2003; Lake, 2009).

There is a well-established body of experimental evidence for activation of PPARα and CAR/PXR as a major factor in the rodent hepatic response to exposure to PFOA as well as

developmental effects ensuing from gestational exposure in mice (Abbott *et al.*, 2007; Elcombe *et al.*, 2010; Klaunig *et al.*, 2012; Peters and Gonzalez, 2011; Ren *et al.*, 2009; Rosen *et al.*, 2008a; Rosen *et al.*, 2009; Rosen *et al.*, 2008b). As Elcombe et al. (2010) and Klaunig et al. (2012) point out, the hypertrophic and hyperplastic response of rodent liver to PFOA exposure has been demonstrated to be consistent with the criteria used to establish PPARα activation as a mode of action.

As noted above, there are fundamental differences between the responses of human and rodent liver from exposure to agents that increase activation of PPARα and CAR/PXR (Corton *et al.*, 2014; Elcombe *et al.*, 2014). The basis for the fundamental differences between the rodent and human liver response from exposure to agents that activate these receptors has become clearer with development of receptor knock-out and humanized receptor knock-in transgenic mouse models, and the increased availability of human primary hepatocytes. When exposed to PPARα and CAR/PXR agonists, mice that have been genetically modified by removal of the natural mouse receptors and replacement with the natural human forms of the receptors do not exhibit the hyperplastic response observed in wild-type mice (Gonzalez and Shah, 2008; Ross *et al.*, 2010). Key differences between rodent and human hepatocytes, especially the lack of a hyperplastic response in human hepatocytes exposed to PPARα and CAR activators, have also been demonstrated (Elcombe *et al.*, 1996; Goll *et al.*, 1999; Hirose *et al.*, 2009; Parzefall *et al.*, 1991; Perrone *et al.*, 1998).

Human hepatocytes respond to PPARα agonists differently than rodent hepatocytes, and activation of human PPARα does not appear to result in the characteristic hyperplastic response observed in rats and mice (Corton *et al.*, 2014; Gonzalez and Shah, 2008). Nakamura et al. (2009) dosed wild-type mice, PPARα knock-out mice, and humanized PPARα mice with water (vehicle control), 0.1, and 0.3 mg/kg PFOA for two weeks. Protein and mRNA concentrations of PPARα target genes were only increased in the wild-type mouse, indicating that the human PPARα is less responsive to PFOA activation than mouse PPARα. Bjork and Wallace (2009), working with primary rat and human hepatocytes as well as the HepG2 human liver cell line in culture, demonstrated major differences between primary rat hepatocytes and human hepatocytes in response to exposure to PFOA in culture. In comparison to the large increase over control in mRNA for peroxisomal enzymes Cte/Acot1 and Acox, the human hepatocytes showed essentially no increase in transcripts. However, consistent with observations with other peroxisome proliferators, CYP4A11 mRNA was increased by PFOA exposure in human as well as Cyp4A1 in rat hepatocytes. These studies collectively support the notion that human PPARα regulates a different set of gene products and the resulting biological effects as compared to the rodent PPARα. It is also interesting that the developmental effects observed in wild-type mice from gestational exposure to PFOA are mediated by PPARα (Abbott *et al.*, 2007) and are not observed in mice humanized for PPARα (Albrecht *et al.*, 2013).

In addition to PPARα, Bjork et al. (2011) characterized the activation of several other hepatic nuclear receptors (PXR, CAR, the liver X receptor α (LXRα or NR1H3), and the farnesoid X receptor (FXR or NR1H4) by PFOA in primary rat and human hepatocytes. In rat hepatocytes, they demonstrated multiple nuclear receptors participate in the metabolic response to PFOA exposure, resulting in a substantial shift from carbohydrate metabolism to fatty acid oxidation and hepatic triglyceride accumulation. They concluded that, "…while there is some similarity in

the activation of metabolic pathways between rat and humans, particularly in PPARα regulated responses; the changes in primary human cells were more subtle and possibly reflect an adaptive metabolic response rather than an overt metabolic regulation observed in rodents."

Supporting the observations of Bjork et al. (2011), the potential activation of human CAR3 isoform and human PXR has been studied *in vitro* (Ehresman *et al.*, 2014). PFOA was not shown to activate directly either human nuclear receptor at concentrations up to 100 µM.

It should be noted that evidence for hepatic mitochondrial biogenesis (proliferation) from exposure to PFOA has been reported in experimental studies with rodents (Cai *et al.*, 1996; Pastoor *et al.*, 1987; Permadi *et al.*, 1993; Sohlenius *et al.*, 1992a; Sohlenius *et al.*, 1992b; Walters *et al.*, 2009) and cynomolgus monkeys (Butenhoff *et al.*, 2002). Thus, the increase in hepatocellular mass observed from PFOA exposure may be, in part, attributable to mitochondrial biogenesis. Walters et al. (2009)have attributed PFOA-induced hepatic mitochondrial biogenesis in Sprague Dawley rats to activation of the peroxisome proliferator-activated receptor γ coactivator-1 α (Pgc-1α) mitochondrial biogenesis pathway. This pathway represents a major adaptation of mitochondria and has been shown to be activated by exercise (Baar, 2004; Hood, 2001), cold (Puigserver *et al.*, 1998), caloric restriction (Nisoli *et al.*, 2005), and early stages of insulin resistance (Mitra *et al.*, 2012). Generally recognized as an important, reversible adaptive response, mitochondrial proliferation should not be viewed as adverse.

The use of rodent liver weight alone by the USEPA as a critical effect to establish a point-of-departure for derivation of a reference dose for PFOA is inconsistent with USEPA guidelines and published expert opinions on the distinction between liver hypertrophy as a non-adverse adaptive change and other endpoints representing liver toxicity. Moreover, the observational human data as well as a significant body of mechanistic experimental data that relates to the liver response to exposure to PFOA strongly suggests that rodent liver weight as an endpoint for the human-health risk assessment of PFOA is inappropriate and needlessly conservative.

D. Review of Human Data for Liver Disease as a Potential Outcome of Exposure to PFOA

The USEPA Health Effects Document for PFOA highlights statistical associations with selected hepatic clinical enzymes reported in studies of occupational a community affected with PFOA from an industrial source, and general populations with exposure to PFOA (see pages 4-10 to 4-12 and Table 4-3). This USEPA document, however, does not consider the amount of testing done (multiple comparisons), the magnitude of effects measured, the clinical relevance of the effects reported in the statistical associations, the potential for residual confounding, and the extent of medically verified diagnoses of malignant or nonmalignant liver diseases in association with exposure to PFOA. It can be concluded that the statistical associations that were described by the USEPA of hepatic clinical enzymes with PFOA in these three types of populations: 1) have very small absolute changes across the wide range of PFOA exposures; 2) lack clinical relevance as the hepatic enzyme results are well-within normal reference ranges; and 3) do not correlate with malignant or nonmalignant liver disease associated with exposure to PFOA because increased risks have not been observed in these populations.

The analysis of the literature presented below focuses on the measured hepatic clinical chemistries and reported (both medically validated and self-reported) liver disease (malignant and nonmalignant) among (1) workers exposed to PFOA (ammonium salt); (2) a community whose drinking water contained PFOA from an industrial source, and (3) the U.S. general population with background exposure to PFOA.

1. Hepatic Clinical Chemistries

Occupational Populations

In Table 4-3, the USEPA Health Effects Document on PFOA highlights, via directional upward arrows, positive associations between PFOA and GGT (Sakr *et al.*, 2007a), AST (Sakr *et al.*, 2007b), and GGT, ALP and ALT (Costa *et al.*, 2009; Olsen and Zobel, 2007). Such a visual display, however, does not distinguish between a statistically significant association and the actual magnitude of effect that was reported. None of these studies reported associations whose mean value, if viewed as individual values, would be considered clinically relevant. As group means, the values represent central tendency distributions within the exposure group analyzed but, nevertheless, the mean values are within normal expected ranges. For example, in the longitudinal analysis (mixed model) by Sakr et al. (2007b) of 454 DuPont workers with 1326 measurements taken between 1979 and 2004, they reported a 1000 ng/mL increase in PFOA was associated with a 0.35 IU/L increase in AST (95% CI 0.10 – 0.60). When they analyzed for ALT (fewer outcome measurements of only 231), Sakr et al. reported a 1000 ng/mL increase in PFOA was associated with a 0.54 IU/L increase in ALT (95% CI -0.46 to 1.54). Given the fact that the normal reference range for ALT, AST, and GGT is generally between 10 and 40 IU/L (may vary slightly by reference laboratory used), such a statistical observation of a minimal change (0.35 IU/L) of AST per 1000 ng/mL PFOA lacks clinical relevance.

This conclusion was also reached by Olsen and Zobel (2007) in their cross-sectional analysis of PFOA and hepatic clinical measurements of 506 3M manufacturing male employees who did not take cholesterol-lowering medications. Among the three 3M plant sites combined in this study (Antwerp, Cottage Grove, and Decatur), the median PFOA concentrations and respective adjusted (age, BMI, alcohol) mean concentrations of the hepatic clinical parameters for each decile are provided in the table below:

Deciles of Median PFOA (ng/mL) and Adjusted* Mean Liver Measurements
(Olsen and Zobel 2007)

	1	2	3	4	5	6	7	8	9	10
PFOA (ng/mL)	60	190	360	540	910	1250	1630	2180	2960	4940
ALT(IU/L)	29	29	30	28	27	25	31	29	34	34
AST (IU/L)	26	25	26	24	22	22	26	26	26	24
GGT (IU/L)	32	25	27	28	24	21	28	29	33	30
Alk Phos (IU/L)	66	59	64	65	64	66	72	65	70	68
Total bil (mg/dL)	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.9	0.8	0.8

*Adjusted for age, BMI, and alcohol

The upper end of the normal reference range was 40 IU/L for ALT, AST, and GGT. Upper end of the ALP normal reference range was 120 IU/L. None of the adjusted means of the upper three deciles of ALT, AST, GGT, or ALP were significantly higher than any of the lowest three deciles. The highest three deciles of total bilirubin were different than the 3rd decile. There were no statistically significant odds ratios for increasing deciles with hepatic enzymes (AST, ALT, GGT) $\geq$ 40 IU/L. Although the USEPA HED focused on the regression analyses reported in this paper for the 3M Decatur location (Table 4-3), the USEPA analysis did not mention that the variance explained by PFOA in these Decatur regression models was minimal, ranging between < 1 to 3%.

There was one longitudinal study of workers involved with the demolition of perfluoroalkyl (including PFOA) manufacturing facilities (Olsen *et al.*, 2012). For the 120 contract workers who began the demolition project with a distribution of serum PFOA levels that reflected background exposures found in the general population (< 15 ng/mL), their matched-pair mean change in serum PFOA concentrations at the end of the project increased by 44 ng/mL (median 5 ng/mL). This median change of 5 ng/mL approximates the average PFOA reported in the US general population (see Lin et al. 2012 below). For these 120 workers, their matched-pair mean changes in hepatic clinical chemistry parameters were unchanged and were the following: ALT (-0.7 IU/L); AST (0.2 IU/L); alkaline phosphatase (-0.9 IU/L) and total bilirubin (-0.05 mg/dL).

Community with Exposure from Industrial Source

The C8 Health Project was a cross-sectional medical and exposure assessment (conducted in 2005 – 2006) of 69,030 persons who resided in a mid-Ohio River community (near Parkersburg, West Virginia). This community's drinking water contained PFOA from emissions released by a nearby DuPont polytetrafluoroethylene (PTFE) synthesis and polymerization manufacturing facility (Frisbee *et al.*, 2009). PFOA (ammonium salt) was used as a processing aid in the PTFE polymerization process. Gallo et al. (2012) reported the ALT and GGT results from 47,092 adults from this C8 Health Project. The median PFOA serum concentration was 28.0 ng/mL (males 34.3 ng/mL, females 23.1 ng/mL). Decile levels of PFOA were estimated from Figure 1 from the Gallo et al. (2012) paper as well as the fitted adjusted mean values. These estimates are presented in the table below.

Estimates of Median PFOA Deciles (ng/mL) and the Adjusted Mean Liver Measurements
(Estimates from Figure 1 in Gallo et al. 2012)

	1	2	3	4	5	6	7	8	9	10
PFOA (ng/mL)	7	10	15	20	30	38	45	80	150	350
ALT (IU/L)	20.8	21.2	21.5	22.0	22.4	22.2	22.4	22.5	22.4	23.2
GGT (IU/L)	22.0	22.6	23.0	23.0	23.4	23.2	23.5	23.8	23.0	23.8

Neither the magnitude of effect measured (mean values well within normal reference ranges) or the absolute change in ranges observed (approximately +2 IU/L) would suggest the fitted ALT and GGT values indicated clinical relevance with the measured PFOA increasing from the estimated mean deciles of 7 to 350 ng/mL. These deciles are approximately 10x lower than those reported in the above table from occupational data (Olsen and Zobel 2007).

Gallo et al. reported adjusted odds ratios for individuals with "abnormal" high values of ALT or GGT. The definition of "abnormal" values was defined as above the normal reference ranges of 45 IU/L in men and 34 IU/L for women. The trend was signifcant for ALT (trend $p < 0.001$) although odds ratios were essentially not different after the 5th decile (>30 ng/mL PFOA).

Odds Ratios for an "Abnormal" (> Upper normal reference range)
Hepatic Clinical Measurements by PFOA Deciles

	PFOA Decile									
	1	2	3	4	5	6	7	8	9	10
ALT	1.00	1.09	1.19	1.26	1.40	1.39	1.31	1.42	1.40	1.54
GGT	1.00	1.06	1.07	1.05	1.11	1.10	1.20	1.13	1.06	1.06

In the Gallo et al. study, the linear regression ALT model was statistically significant for exposure to PFOA. The overall model R^2 was between 0.17 and 0.27 (depending upon models). However, the partial R^2 explained by PFOA was inconsequential (ranging between 0.001 and 0.002). Gallo et al. concluded there was a "small but clear linear association between PFOA and ALT" but questioned "it is not clear if this small increase in ALT levels can lead to clinically diagnosable conditions in the future." This question was subsequently addressed by the C8 Science Panel in their community worker study which did not report a probable link with liver disease (malignant or nonmalignant) (see discussion further below).

General Population

A study conducted by Lin et al. (2010) utilized the publicly available database from the US Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination

Survey (NHANES) to examine liver enzymes and PFOA measurements in the combined 1999-2000 and 2003-2004 cross-sectional surveys. The total sample size was 2,216. For the two survey periods, the serum PFOA geometric mean concentrations reported were 5.2 ng/ml (95% CI 4.7 – 5.7) and 4.0 ng/mL (95% CI 3.7 – 4.3), respectively (Kato *et al.*, 2000). The 95th percentiles for the two cross-sectional NHANES surveys were 11.9 ng/mL and 9.8 ng/mL, respectively. Provided below are the quartile levels of PFOA presented by Lin et al. and respective mean ALT and GGT values for all subjects and estimated ALT values for those considered obese (BMI $\geq$ 30 kg/m^2). The PFOA concentrations reported below (in quartiles) would all be within the first quartile of the occupational population (Olsen and Zobel, 2007) and the first few deciles of the exposed community (Gallo *et al.*, 2012).

Quartile Distributions of PFOA and Mean ALT and GGT Values
(Lin *et al.*, 2010)

	PFOA Quartiles			
	1	2	3	4
PFOA (ng/mL)	≤2.90	≤ 4.20	≤ 5.95	> 5.95
ALT (IU/L)				
All subjects	23.5	23.3	28.2	26.5
Obese	25.0	24.5	28.5	29.5
Ln GGT (IU/L)	2.97	2.98	3.07	3.13
(transformed GGT)	19.5	19.7	21.5	22.9

Lin et al. acknowledged that the potential biological significance of the liver enzymes was "small and subclinical." They speculated that there could represent a nonlinear low dose response with PFOA among the least exposed that is not seen in much more highly exposed populations (10 to 1000 times higher exposure based on serum PFOA).

Summary of Hepatic Clinical Chemistries

Across three different populations (occupational, exposed community, general population), the mean hepatic enzyme values reported were within normal reference ranges for individual values. The magnitude of mean changes in hepatic enzymes was small as was noted by all investigators despite orders of magnitude of differences in PFOA concentrations measured in these populations. Although one can suggest, as did Lin et al. (2010), that the hepatic effects may be nonlinear and only seen at low concentrations, such an argument is speculative. Therefore, to further investigate the feasibility of "clinically diagnosable conditions" as suggested by Gallo et al. (2012), actual risk of liver disease (malignant and nonmalignant) in relation to PFOA exposures were determined. These results are described below.

2. Liver Diseases

2a. Liver Cancer (Malignant)

Occupational Populations

Steenland and Woskie (2012) reported the results from a cohort mortality study of 5,791 workers at the DuPont Washington Works plant that had releases of PFOA (ammonium salt) into the nearby community. The estimated average PFOA level was 350 ng/mL. The overall SMR for liver cancer in the cohort (US population as referent) was 0.74 (95% CI 0.35 – 1.47) based on 10 liver cancer deaths. Compared to the referent population of other DuPont workers in the Appalachian region, the SMRs by increasing quartiles of ppm-years exposure of PFOA for liver cancer mortality were 2.39 (95% CI 0.65 – 6.13), 0.00 (95% CI 0.00 – 1.81), 2.01 (95% CI 0.65 – 4.68), and 0.32 (95% CI 0.01 – 1.76), respectively.

An updated analysis of the 3M Cottage Grove PFOA manufacturing facility has been completed that involved a major construction of a task-based job/department exposure matrix for PFOA. The study cohort involved 9,027 employees of which 4,668 were from the Cottage Grove facility and 4,359 from a 3M plant that was not involved with PFOA manufacturing. No exposure response trend was observed for liver cancer with $\mu g/m^3$-years of exposure to PFOA. This study was recently accepted for publication (see Raleigh et al. Occup Environ Med 2014 in press).

Community with Exposure from Industrial Source

There have been two studies of liver cancer among the Mid-Ohio River Valley community. Neither study associated liver cancer with estimated or measured PFOA concentrations in this community (Barry *et al.*, 2013; Vieira *et al.*, 2013). Among a community/worker cohort study of 32,254 participants who participated in subsequent surveys after the C8 Health Project (see Winquist et al. (2013) for study design), Barry et al. (2013) reported a hazard ratio of 0.73 (95% CI 0.43 – 1.23) for liver cancer based on 9 medically validated cases. The hazard ratio was essentially unchanged for liver cancer (RR = 0.74; 95% CI 0.43 – 1.26) when cumulative exposure to PFOA was lagged 10 years. Vieira et al. (2013) examined the relationship between PFOA exposure and cancer diagnosis among residents from 13 counties between 1996 through 2005. A total of 61 liver cancer cases were identified through record linkage with the Ohio and West Virginia statewide cancer reporting systems of which 11 were from the exposed community. Based on the study metrics, individual-level annual PFOA serum exposure, assuming a 10-year residency and latency, Vieira et al. categorized exposures to PFOA as low, medium, high, and very high. The adjusted odds ratios for liver cancer by increasing category of PFOA exposure were 1.1 (95% CI 0.4 – 3.1), 0.9 (95% CI 0.3 – 2.5), 1.0 (95% CI 0.3 – 3.1), and 0.0 (no confidence interval provided), respectively.

General Population

There has been no published analysis of liver cancer and exposure to PFOA from the NHANES study population. However, a case-cohort study was conducted of 57,053 individuals from the Danish general population who enrolled in a diet and health study from 1993 – 1997 with follow-

up for diagnosis of liver cancer through 2006 (Eriksen *et al.*, 2009). Plasma concentrations of PFOA were measured at enrollment. Plasma PFOA concentrations were 6.9 ng/mL among 680 men (95th percentile 13.3 ng/mL) and 5.4 ng/mL (95th percentile 11.6 ng/mL) in 92 women who constituted the non-cancer group from the cohort. Of the 67 Danish individuals who were diagnosed with liver cancer, their median plasma concentration was 5.4 ng/mL (95th percentile was 13.7 ng/mL). Based on a quartile distribution of the plasma concentration of the liver cancer cases, the incidence rate ratios for liver cancer were 1.00 (referent), 1.00 (95% CI 0.44 – 2.23), 0.49 (95% CI 0.22 – 1.09), and 0.60 (95% CI 0.26 – 1.37). There was no significant trend for liver cancer when plasma PFOA was analyzed as a continuous variable (incidence rate ratio = 0.95 (95% CI 0.86 – 1.06)). Eriksen et al. (2009) concluded plasma concentrations of PFOA in this Danish general population (whose levels were similar to those reported in NHANES by Lin et al. (2010)) were not associated with the risk of liver cancer.

2b. Liver Disease (Nonmalignant)

Occupational Populations

As previously mentioned, Steenland and Woskie (2012) reported on the cohort mortality study of 5,791 workers at the DuPont Washington Works plant. The estimated average PFOA level was 350 ng/mL. There were 13 deaths from chronic liver disease (SMR 1.09, 95 % CI 0.54 – 1.95). SMRs by increasing quartile of ppm-years of PFOA were 1.32 (95% CI 0.27 – 3.86), 2.10 (95% CI 0.68 – 4.89), 0.37 (95% CI 0.01 – 2.08), and 0.72 (95% CI 0.09 – 2.61).

Community with Exposure from Industrial Source

The C8 Science Panel's probable link reports for nonmalignant liver disease (http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Liver_29Oct2012.pdf) concluded there was not a probable link with exposure to PFOA. Analyzing the community/worker cohort of 32,254 participants who were interviewed during 2008 - 2010 regarding their medical history of nonmalignant liver disease, the C8 Science Panel medically validated 647 reported liver diseases. Results for all nonmalignant liver disease, or the subcategory defined as fatty liver, enlarged liver, and cirrhosis, were analyzed in relation to cumulative PFOA exposure. According to the C8 Science Panel report, "in no case was there any suggestion of a positive trend." The tables below provide relative risks based on different exposure metrics found in this web-based report.

In the analysis of all validated liver disease the C8 Science Panel reported the following:

	Relative Risk for Liver Disease (Nonmalignant) PFOA Cumulative Exposure Quintile					
	1	2	3	4	5	P trend
All subjects, all follow-up	1.00	1.19	1.08	1.04	0.95	0.32
Restricted to yrs entering study area	1.00	0.98	0.95	0.86	0.89	0.34
PFOA level at year of diagnosis	1.00	0.99	0.94	0.85	0.80	0.09

In the prospective analysis of this study (post-2005, 266 validated cases) the relative risk estimates were similar as above:

	Relative Risk for Liver Disease (Nonmalignant) PFOA Cumulative Exposure Quintile					
	1	2	3	4	5	P trend
All subjects, all follow-up	1.00	0.96	0.96	0.77	0.80	0.21
Serum measurements at baseline	1.00	0.90	0.89	0.76	0.71	0.09

In their evaluation of nonmalignant liver disease the C8 Science Panel concluded there was "no evidence of any increased risk of liver disease in relation to PFOA exposure." Furthermore, they placed the liver enzymes reported into perspective by stating "there are 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 in the overall incidence of diagnosed liver disease."

General Population

There was one cross-sectional study conducted by Melzer et al. (2010) of the publicly available US Centers for Disease Control and Prevention (CDC) NHANES database that examined self-reported current liver disease (not specified as to the definition) and PFOA measurements in the combined 1999 - 2000, 2003 - 2004, and 2005 - 2006 surveys. Medians (ng/mL) and 95th percentiles (in parentheses) for PFOA in these three surveys were: 5.1 (11.9); 4.1 (9.8); and 4.2 (11.3), respectively. A total of 57 individuals identified themselves as having current liver disease among 3,942 individuals in these 3 NHANES surveys. The odds ratios by increasing quartiles of serum PFOA measurements for the combined three survey years were 1.00 (referent), 0.66 (95% CI 0.25 – 1.74), 1.93 (95% CI 0.96 – 3.88), and 0.61 (95% CI 0.21 – 1.78). The analyses from Melzer et al. (2001) are difficult to interpret because the liver disease was neither specific nor medically validated.

2.3 Final Conclusion on the Association of Liver Disease in Humans with PFOA Exposure

Although statistically significant positive associations between hepatic enzymes and exposure to PFOA have been reported, the mean serum levels measured of these hepatic enzymes were: (1) invariably well-within normal reference ranges; (2) the magnitude of changes observed was minimal across the targeted population (occupational, community, and general population) that had orders of magnitudes differences in exposure to PFOA; and (3) none of these populations had increased risk for malignant or nonmalignant liver disease associated with PFOA exposure.

II. Other, Non-Liver Organ Weight Changes Are Not Necessarily Adverse

A Society of Toxicologic Pathology position paper on organ weight recommendations for toxicology studies (Sellers *et al.*, 2007) provides this general guidance on the evaluation of organ weight data from toxicological studies:

> *The proper evaluation of absolute organ weights and organ-to-body weight or organ-to-brain weight ratios should include examination of both individual animal values and group means. While organ weights provide useful signals indicating test article-related effects, organ weight data must be interpreted in an integrated fashion with gross pathology, clinical pathology, and histopathology findings. Detectable weight changes in and of themselves may not necessarily be treatment-related or adverse. Organ weight changes without macroscopic or microscopic correlation should be interpreted with caution.*

Organ-weight data other than liver weight were identified as a sole critical effect for 2 of the 11 critical effects in Table 5-1 (increased relative pituitary weight in male rhesus monkeys and decreased absolute and relative heart weight in female rhesus monkeys). The study consisted of groups of 2 male and 2 female rhesus monkeys, a factor that calls into question the relevance of statistical comparisons to the control. Sellers et al. (2007) also comment on interpretation of statistical findings for organ weights:

> *Reliance on statistical significance (or the lack thereof) alone in the evaluation of organ weight changes is not satisfactory, particularly in studies with a small sample size. It is important to note that organ weight alterations may be test article-related but not statistically different from controls, or conversely, statistically different from controls but not related to treatment (Gad et al., 2002). When organ weight changes are statistically significant from control values or in any way outstanding, interpretations should clearly distinguish treatment-related findings from incidental findings and provide perspective on the reasons for these distinctions.*

It is surprising that these pituitary and heart weight changes are even listed as effects. In considering statistically-significant organ-weight changes as compared to controls, the study authors noted (Goldenthal, 1978):

> *Statistically significant variations in sex group mean weights of a few organs occurred between the control and experimental groups. These variations were of unknown biological significance and were not accompanied by morphologic alterations.*

In addition to the monkey pituitary and heart weights discussed above, increased F0-generation male rat kidney weight was listed in Table 5-2 as a co-critical effect along with increased absolute liver weight. Both liver and kidney weight can increase in male rats from activation of the xenosensor nuclear receptor, PPARα (Gibson, 1989; Green, 1995; Hardwick *et al.*, 1987; Ishizuka *et al.*, 2003; Sundseth and Waxman, 1992), and the adaptive responses that follow activation of PPARα are not necessarily adverse (Hall *et al.*, 2012). As discussed above, the activation of PPARα from exposure to PFOA in rats and mice has been thoroughly established. These factors support the argument that the USEPA should also not treat kidney weight change in male rats from exposure to PFOA as an adverse effect when no clinical or histological evidence of kidney injury is present.

III. Final Conclusion on the Risk of Liver Toxicity in Humans from Exposure to PFOA

Investigations comparing hepatocellular responses of rat and human primary hepatocytes to PFOA exposure, studies conducted using transgenic mice that included mice humanized for PPARα, and the current state of our knowledge regarding the mode of action for liver effects in rodents exposed to PFOA and human relevance of these modes of action further support a reduced risk of humans to liver effects from PFOA exposure. As discussed above, exposure to PFOA has not been associated with liver disease (malignant or nonmalignant) in humans. All of these factors argue strongly that human health risk assessment for exposure to PFOA should not be based on increased rodent liver weight and/or hepatocellular hypertrophy, and that the lack of human evidence for PFOA-induced liver toxicity should be carefully considered by USEPA.

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