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

3M Medical Department

3M Center, Building 0220-06-W-08
St. Paul, MN 55144-1000
651 733 0694 Office
651 733 9066 Fax
caley@mmm.com

April 28, 2014

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

The 3M Company appreciates the opportunity to submit comments on the draft "Health Effects Document for Perfluorooctane Sulfonate (PFOS)". 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 Perfluorooctane Sulfonate (PFOS)". 3M encourages USEPA to consider the comments submitted by Butenhoff and Stump on USEPA's choice of the single observation of decreased locomotor habituation in male pups on postnatal day (PND) 17 from a comprehensive developmental neurotoxicity study in rats by Butenhoff et al. (2009) as the critical effect forming the basis of the RfD derivation for PFOS. 3M agrees with Butenhoff and Stump that this choice of a critical effect, without consideration of weight of evidence, is inappropriate and at odds with current guidelines (USEPA, 1991, 1998), OECD guidelines (OECD, 2007), and expert opinion authored by USEPA scientists (Francis *et al.*, 1990) on the interpretation of data from developmental neurotoxicology studies. The comments to follow are related to USEPA's identification of increased liver weight as a co-occurring critical effect in support of the RfD. The use of liver weight increase as a critical effect, with the implication that increased liver weight is adverse, is again at odds with USEPA guidance (USEPA, 2002) as well as the conclusions from the 3rd International European Society of Toxicologic Pathology expert workshop on liver hypertrophy as an adaptive effect (Hall *et al.*, 2012). If USEPA agrees that the reduced habituation in male rats on PND 17 in the study by Butenhoff et al. (2009) is inappropriate as a critical effect for risk assessment in the regulatory context, 3M hopes that USEPA will also consider the following arguments that increased liver weight is also inappropriate as a critical effect for risk assessment.

3M believes that the selection of an RfD based on rodent liver weight will be unjustified based on the following points: 1) increased in liver weight alone in response to PFOS 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 PFOS 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 cells as compared to rodent liver; and 4) the human evidence suggests a lack of adverse liver effects from PFOS exposure.

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 PFOS 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 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 PFOS overestimates potential human liver response. Moreover, data to date, in particular from humans that were exposed to relatively high concentrations of PFOS, 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 PFOS-induced liver weight increase as an adverse effect in the absence of histological or biochemical evidence of an adverse, toxic effect on the liver.

- Moreover, the USEPA should consider the many studies that have examined the mechanism(s) by which PFOS influences the liver. These studies are important because they have shown the lack of PFOS-induced hepatic responses, or a markedly reduced hepatic response in human models compared to rodent models.

- The USEPA also should consider the lack of evidence for adverse liver effects in humans with exposure to PFOS based on occupational and general population epidemiological studies.

- There are data currently under analysis that 3M believes will be relevant to the risk assessment process for PFOS.

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, including: 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 PFOS 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 4 of the 9 (44%) tabulated critical effects in Table 5-1 and 2 of the 6 (33%) tabulated critical effects in Table 5-2. Liver weight was the sole critical effect basis for the LOAEL for 1 of the 6 (or 17%) of the study critical effects tabulated in Table 5-2. Given the findings by Hall and the colleagues, the USEPA should reconsider these latter critical effect listings in light of its own and other current guidance on evaluation of hepatic hypertrophy in the context of liver toxicity.

C. Increased Liver Weight In Rodents Exposed To PFOS 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 and 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 PFOS. As Elcombe et al. (Elcombe *et al.*, 2012a; Elcombe *et al.*, 2012b) point out, the hypertrophic and hyperplastic response of rat liver to PFOS exposure has clearly been demonstrated to be consistent with the criteria used to establish PPARα/CAR/PXR activation as a mode of action. The transcriptional signature (mRNA) for PPARα/CAR/PXR activation was also observed in livers from PND 21 male rat pups exposed via maternal gavage in the developmental neurotoxicology study reported by Butenhoff et al. (2009) (Chang *et al.*, 2009) as well as in adult male wild-type mice (Rosen *et al.*, 2010). In the E3L.CETP mouse transgenic mouse model, dietary PFOS exposure of adult males resulted in transcriptional gene expression profiles and changes in lipid parameters consistent with activation of PPARα and PXR (Bijland *et al.*, 2011). Rosen et al. (2009) observed the same transcriptional signature consistent with activation of PPARα/CAR/PXR in CD-1 mouse fetal liver after maternal exposure to PFOS during gestation.

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 have 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).

As noted above, 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). 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 PFOS 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 PFOS exposure in human as well as Cyp4A1 in rat hepatocytes.

In addition to PPARα, Bjork et al. (2011) characterized the activation of several other hepatic nuclear receptors (PXR, CAR, the liver X receptor α (NR1H3 or LXRα), and the farnesoid X receptor (NR1H4 or FXR) by PFOS in primary rat and human hepatocytes. In rat hepatocytes, they demonstrated multiple nuclear receptors participate in the metabolic response to PFOS 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 PPARA 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 this, the potential activation of human CAR3 isoform and human PXR has been studied. PFOS was not shown to activate directly either human nuclear receptor at concentrations up to 33 µM, with slight activation (much less than for positive control substances) of CAR3 and PXR occurring only at 100 µM (Ehresman *et al.*, 2014).

USEPA's use of rodent liver weight alone as a critical effect to establish a point-of-departure for derivation of a reference dose for PFOS 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 PFOS strongly suggests that rodent liver weight as an endpoint for the human-health risk assessment of PFOS is inappropriate and needlessly conservative.

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

Hepatic Clinical Chemistries

Occupational Populations

Perfluorooctanesulfonyl fluoride (POSF)-based products were manufactured for many years at the 3M Company's Antwerp (Belgium) and Decatur (Alabama) facilities. Medical surveillance of these manufacturing employees was routinely performed which incorporated serum measurements of PFOS between 1994 and the company's phase-out of manufacturing production in 2000-2002. (Note: prior to 1994 analytical capabilities only allowed for the measurement of total organic fluorine.) Medical surveillance included the liver enzymes ALT, AST, GGT, and alkaline phosphatase, and total bilirubin. Olsen et al. (2003a) reported the results of the 2000 voluntary medical surveillance (cross-sectional) program that included 255 Antwerp (206 male, 49 female) and 263 Decatur (215 male, 48 female) employees. The geometric mean for serum PFOS in Antwerp employees was 440 ng/mL (95% CI 380 – 510); whereas, the geometric mean for the Decatur employees was 910 ng/mL (95% CI 820 – 1020). This concentration was similar to a random sample of the Decatur employee population in 1998 (Olsen *et al.*, 2003b). There were different lifestyle risk factors between these two plant populations as the Antwerp employees were considerably less obese but consumed more alcohol than their Decatur counterparts. For the combined plant analyses for male employees (n = 421), the table below provides the means of quartile distributions from Olsen et al. (2003a) (range in parenthesis) of serum PFOS concentrations and unadjusted hepatic clinical chemistry parameters.

Quartile distributions of male subjects from Olsen et al. (2003a) (range in parenthesis) of serum PFOS concentrations and unadjusted hepatic clinical chemistry parameters

	1	2	3	4
PFOS (ng/mL)	270 (40-420)	600 (430-810)	1190 (820-1680)	2690 (1690-10,060)
ALT (IU/L)	26	28	28	33
AST (IU/L)	25	25	24	25
GGT (IU/L	24	29	26	30
AlkPhos (IU/L)	61	67	69	70
Total bil (mg/dL)	1.0	0.9	0.8	0.8

Adjusted odds ratios were calculated for quartiles having values greater than the upper reference range compared to the 1st quarter as a referent. Provided below are these adjusted odds ratios for ALT and GGT.

Odds Ratios (95% CI) for Values > Reference Range
PFOS Quartile Distributions – Male Subjects

	1	2	3	4
ALT	1.0	0.6 (0.1-2.8)	1.2 (0.3-4.8)	2.1 (0.7-5.8)
GGT	1.0	1.1 (0.5-2.3)	1.1 (0.4-2.3)	1.6 (0.7-3.3)

Among female workers (n = 97), the quartiles of mean (range in parenthesis) serum PFOS concentrations and unadjusted hepatic enzyme results were the following:

Quartile distributions of female subjects from Olsen et al. (2003a) (range in parenthesis) of serum PFOS concentrations and unadjusted hepatic clinical chemistry parameters

	1	2	3	4
PFOS (ng/mL)	70 (40-100)	130 (100-190)	390 (200-700)	1510 (770-3620)
ALT (IU/L)	13	16	16	19
AST (IU/L)	19	18	19	19
GGT (IU/L	11	13	14	22
AlkPhos (IU/L)	50	44	59	69
Total bilirubin(mg/dL)	0.8	0.8	0.6	0.5

A longitudinal analysis involved a subset of 174 Antwerp and Decatur male employees who participated in the year 2000 medical surveillance analysis and at least one of the two previous programs in 1994/95 and 1997 (Olsen *et al*., 2003a). There were no reported significant PFOS coefficients associated with changes in liver function results during this time period.

Community with Exposure (TO PFOA – <u>NOT PFOS</u>) from an Industrial Source

The C8 Health Project was a cross-sectional medical and exposure assessment of 69,030 persons in 2005-2006 who resided in a mid-Ohio River community (near Parkersburg, West Virginia) whose drinking water contained PFOA from emissions released by a nearby DuPont polytetrafluoroethylene (PTFE) synthesis and polymerization manufacturing facility (Frisbee *et al.*, 2009). Besides PFOA, these investigators measured other perfluoroalkyl concentrations, including PFOS, which was found at concentrations comparable to those measured in the general population. This should not be unexpected because PFOS was neither manufactured or used in the production of consumer products at this DuPont plant, i.e, there was no occupational exposure. According to the NHANES 2005-2006 survey the geometric mean concentration of PFOS was 17.1 ng/mL (95% CI 16 -18.2). The time-comparable geometric mean PFOS concentration in the C8 Health Project was 19.2 ng/mL (SD = 15.6). Gallo et al. (2012) reported ALT (IU/L), GGT (IU/L), and direct bilirubin (ng/mL) measurements among the 47,092 adults from the C8 Health project. According to Gallo et al. the natural log PFOS was associated with natural log ALT in a linear regression model (PFOS coefficient, 0.020; 95% CI 0.014 – 0.026). To better understand, however, the lack of clinical relevance of this statistical association, the mean fitted values of ALT by decile of PFOS can be estimated from Figure 1 in the Gallo et al. paper (see table below).

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

	1	2	3	4	5	6	7	8	9	10
PFOS (ng/ml)	7	12	14	16	20	22	26	30	38	50
ALT (IU/L)	21.3	21.5	21.5	22.0	22.1	22.4	22.3	22.2	22.3	22.3
GGT (IU/L)	22.6	23.0	22.8	23.0	23.2	23.5	23.4	23.2	23.0	23.0

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 PFOS increasing from the estimated mean deciles of 7 to 50 ng/mL. These deciles are approximately >10x lower than those reported in the above table from occupational data (Olsen *et al.*, 2003a).

Gallo et al. (2012) reported adjusted odds ratios for individuals having “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 significant for ALT although odds ratios but plateaued after the 5th decile.

Odds Ratios for Values > Reference Range (Gallo et al. 2012)

	PFOS Deciles									
	1	2	3	4	5	6	7	8	9	10
ALT	1.00	1.01	1.06	1.11	1.19	1.19	1.20	1.24	1.18	1.25
GGT	1.00	1.06	0.95	0.93	0.96	1.03	0.97	0.91	0.89	0.94

General Population

Lin et al. (2010) examined liver enzymes and PFOS measurements in the combined 1999-2000 and 2003-2004 US Centers for Disease Control and Prevention's (CDC) Nutrition and Health Examination Survey (NHANES). These were cross-sectional surveys with a total sample size of 2,216. For the two surveys the serum PFOS concentrations were reported as geometric means of 30.4 ng/ml (95% CI 27.1 – 33.9) and 20.7 ng/mL (95% CI 19.2 – 27.3), respectively (Kato *et al.*, 2011). This decline reflects the phase-out of manufacturing PFOS in 2000 – 2002 and the long serum elimination half-life. The 95th percentiles for PFOS for the two cross-sectional NHANES surveys were 75.6 ng/mL and 54.6 ng/mL, respectively. Lin et al. provided a quartile distribution of the study results (see table below).

Quartile Distributions of PFOS and Mean ALT and GGT Values (Lin et al. 2010)

	PFOS Quartiles			
	1	2	3	4
PFOS (ng/mL)	≤15.5	≤ 23.5	≤ 33.8	> 33.8
ALT (IU/L)	23.8	24.6	25.8	27.7
Ln GGT (IU/L)	2.97	3.05	3.03	3.13
(transformed GGT)	19.5	21.1	20.7	22.9

The linear regression adjusted coefficient for ALT with a unit increase in log PFOS concentration was -0.19 (p = 0.77). For log GGT the unit increase in log PFOS coefficient was 0.03 (p = 0.808) and for total bilirubin this coefficient was -0.06 (p = 0.025).

Liver Disease (Malignant)

Occupational population

A retrospective cohort study was designed to assess the mortality experience of 2,083 employees with at least one year of cumulative employment at the 3M Decatur manufacturing facility. This facility had two major plants: a fluorochemical and a film plant. The jobs held by the fluorochemical plant members were assigned to one of three exposure subgroups: high exposed, low, exposed, and non-exposed based on biomonitoring data for PFOS that had been obtained from medical surveillance examinations. There were 145 deaths reported. Workers employed in high exposure jobs had an increased number of deaths from bladder cancer that was further investigated with a cancer incidence study reported elsewhere (Alexander and Olsen, 2007).

Among those who worked in non-exposed PFOS-based jobs, there were no liver cancer deaths. For those workers who were ever employed in low exposure job but never high exposure job, there was 1 liver cancer death (0.25 expected). Among those who were employed in high exposure jobs for one year or more there was 1 liver cancer (expected = 0.39).

Community with Exposure (TO PFOA – NOT PFOS) from Industrial Source
No PFOS-specific analyses were presented in this community related to liver cancer. [Note: Liver cancer has not been associated with PFOA exposure in this community.]

General Population
There has been no published analysis of liver cancer and exposure to PFOS 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 PFOS were measured in blood samples collected at enrollment. Plasma PFOS concentrations were 35.0 ng/mL among 680 men (95^{th} percentile 62.4 ng/mL) and 29.3 ng/mL in 92 women (95^{th} percentile 55.6) who constituted the non-cancer selection group from the cohort. Of the 67 Danish individuals who were diagnosed with liver cancer the median PFOS was 31.0 ng/mL (95^{th} percentile 62.9 ng/mL). Based on quartiles of the PFOS plasma concentration of the liver cancer cases, the incidence rate ratios for liver cancer were 1.00 (referent), 0.62 (95% CI 0.29 – 1.33), 0.72 (95% CI 0.33 – 1.56), and 0.59 (95% CI 0.27 – 1.27). There was no significant trend for liver cancer when plasma PFOS was analyzed as a continuous variable (incidence rate ratio = 0.97 (95% CI 0.79 – 1.19). Eriksen et al. (2009) concluded plasma concentrations of PFOS in this Danish general population (whose levels were similar to those reported in NHANES by Kato et al. (2011) and were not associated with the risk of liver cancer.

Liver Disease (Nonmalignant)

Occupational population
A retrospective cohort study was designed to assess the mortality experience of 2,083 employees with at least one year of cumulative employment at the 3M Decatur manufacturing facility. This facility had two major plant: a fluorochemical manufacturing facility and a film plant that were separate from each other. See above for further description. Among those who worked in non-exposed PFOS-based jobs, there were 2 deaths from cirrhosis of the liver (expected = 2.4). For those workers who were ever employed in low exposure job but never high exposure job, there was 1 cirrhosis of the liver death (0.93 expected). Among those who were employed in high exposure jobs for one year or more there were 2 cirrhosis of liver (expected = 1.93) deaths.

A questionnaire-based health survey was mailed to the living 1,895 current and former employees at the 3M Decatur manufacturing facility (Grice *et al.*, 2007). This survey was sent to current and former employees who worked one year or longer as defined in an occupational cohort study of the same site (Alexander *et al.*, 2003). A total of 1,400 (74%) questionnaires were returned. Health outcomes reported on the survey were compared to an exposure assessment of having had PFOS-related jobs that was also created for the cohort mortality study. This exposure assessment stratified the analyses by workers who: (1) never worked with PFOS-based materials; (2) “ever” worked with these materials; (3) worked for a minimum in 1 year in

either low or high jobs; and (4) worked for at least one year in high exposure jobs. A total of 92 individuals self reported they had been ever diagnosed with liver disease, including cirrhosis and hepatitis. The odds ratios for self-reported, non-medically verified liver (nonmalignant) disease by this exposure assessment was 1.00 (reference = never), 1.03 (95% CI 0.76 – 1.39), 1.07 (95% CI 0.78 – 1.47), and 1.09 (95% CI 0.78 – 1.54), respectively.

Community with Exposure (TO PFOA – NOT PFOS) from Industrial Source

No PFOS-specific analyses have been presented in this community related to nonmalignant liver disease. [Note: Liver disease (nonmalignant) has not been associated with PFOA exposure in this community.]

General Population

There was one cross-sectional study conducted by Melzer et al. (2010) of the publicly available NHANES database that examined self-reported current liver disease (not specified as to the definition) and PFOS measurements in the combined 1999 - 2000, 2003 - 2004, and 2005 - 2006 surveys. Medians (ng/mL) and 95th percentiles (in parentheses) for PFOS in these three surveys were: 30.2 (75.6); 21.2 (54.6); and 17.5 (47.5), respectively (Kato et al. 2011). 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 PFOS measurements for the combined three survey years were 1.00 (referent), 0.49 (95% CI 0.18 – 1.32), 0.94 (95% CI 0.41 – 2.16), and 0.95 (95% CI 0.39 – 2.29).

II. Other Data Forthcoming that Has Yet to be Published that will be Critically Relevant to this USEPA Health Effects Document on PFOS - A Dose Escalating Study of PFOS by Oral Gavage in the Cynomolgus Monkeys

In 2009, USEPA selected a 6-month subchronic oral capsule dosing study in cynomolgus monkeys with PFOS (Seacat *et al*., 2002) as the critical study for the derivation of a PFOS Provisional Health Advisory (PHA) of 0.2 μg/L (*http://water.epa.gov/action/advisories/drinking/upload/2009_01_15_criteria_drinking_pha-PFOA_PFOS.pdf*). In the PHA for PFOS, changes in HDL cholesterol and thyroid hormones were cited as critical effects. However, because of study design and assay limitations, data interpretation became problematic with these clinical measurements.

To clarify these issues, 3M commenced (in December 2012) an oral dose study of PFOS in *Macaca fasicularis* (cynomolgus monkeys) to robustly determine serum PFOS concentrations that are potentially associated with changes in selected clinical chemistry endpoints from baseline. Of particular interest are potential changes in serum cholesterol and thyroid hormones. The highest serum PFOS concentrations targeted in this study were designed to approximate those achieved in the Seacat *et al.* study. A total of 36 monkeys (N=18/sex) were followed over a period of 400+ days. Data analyses are underway that will examine the repeated clinical measurements (*e.g*., hepatic, renal, lipid, and thyroid panels) and PFOS concentrations throughout the entire study period.

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

Investigations comparing hepatocellular responses of rat and human primary hepatocytes to PFOS exposure and the current state of our knowledge regarding the mode of action for liver effects in rodents and humans support a reduced risk of humans to liver effects from PFOS exposure. All of these factors argue strongly that human health risk assessment for exposure to PFOS should not be based on increased rodent liver weight and/or hepatocellular hypertrophy, and the relative lack of human evidence for PFOS-induced liver toxicity should be carefully considered by USEPA.

Citations in the text:

Alexander, B.H., Olsen, G.W., Burris, J.M., Mandel, J.H., Mandel, J.S., 2003. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occup Environ Med 60, 722-729.

Alexander, B.H., Olsen, G.W., 2007. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Ann Epidemiol 17, 471-478.

Bijland, S., Rensen, P.C.N., Pieterman, E.J., Maas, A.C.E., van der Hoorn, J.W., Van Erk, M.J., Havekes, L.M., van Dijk, K.W., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., Princen, H.M.G., 2011. Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. Toxicol Sci 123, 290-303.

Bjork, J.A., Butenhoff, J.L., Wallace, K.B., 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology 288, 8-17.

Butenhoff, J.L., Ehresman, D.J., Chang, S.C., Parker, G.A., Stump, D.G., 2009. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K^+PFOS) in rats: developmental neurotoxicity. Reprod Toxicol 27, 319-330.

Chang, S.C., Ehresman, D.J., Bjork, J.A., Wallace, K., Parker, G.A., Stump, D.G., Butenhoff, J.L., 2009 Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression. Reprod Toxicol 27, 387-399.

Corton, J.C., Cunningham, M.L., Hummer, B.T., Lau, C., Meek, B., Peters, J.M., Popp, J.A., Rhomberg, L., Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study. Crit Rev Toxicol 44, 1-49.

Ehresman, D.J., Webb, P., Ayers, S.D., Vanden Heuvel, J., Olsen, G.W., Chang, S.C., Butenhoff, J.L., 2014. Effects of perfluoroalkyls on the activation of human CAR3, PXR, and TR receptors in vitro (Abstract 1135 occurring in The Toxicologist, supplement to Toxicological Sciences). Toxicol Sci 138, 302.

Elcombe, C.R., Bell, D.R., Elias, E., Hasmall, S.C., Plant, N.J., 1996. Peroxisome proliferators: species differences in response of primary hepatocyte cultures. Ann N Y Acad Sci 804, 628-635.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., 2012a. Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. Toxicology 293, 16-29.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Noker, P.E., Butenhoff, J.L., 2012b. Evaluation of hepatic and thyroid responses in male Sprague Dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. Toxicology 293, 30-40.

Elcombe, C.R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., Lake, B.G., 2014. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol 44, 64-82.

Eriksen, K.T., Sorensen, M., McLaughlin, J.K., Lipworth, L., Tjonneland, A., Overvad, K., Raaschou-Nielsen, O., 2009. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general danish population. J Natl Cancer Inst 101, 605-609.

Francis, E.Z., Kimmel, C.A., Rees, D.C., 1990. Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity: summary and implications. Neurotoxicol Teratol 12, 285-292.

Frisbee, S., Brooks Jr., A.P., 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., 2009. The C8 Health Project: Design, methods, and participants. Environ Health Perspect 117, 1873-1882.

Gallo, V., Leonardi, G., Genser, B., Lopez-Espinosa, M.-J., Frisbee, S.J., Karlsson, L., Ducatman, A.M., Fletcher, J., 2012. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environ Health Perspect 120, 655-660.

Goll, V., Alexandre, E., Viollon-Abadie, C., Nicod, L., Jaeck, D., Richert, L., 1999. Comparison of the effects of various peroxisome proliferators on peroxisomal enzyme activities, DNA synthesis, and apoptosis in rat and human hepatocyte cultures. Toxicol Appl Pharmacol 160, 21-32.

Gonzalez, F.J., Shah, Y.M., 2008. PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. Toxicology 246, 2-8.

Grice, M.M., Alexander, B.H., Hoffbeck, R., Kampa, D.M., 2007. Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. J Occup Environ Med 49, 722-729.

Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., Knippel, A., Kuttler, K., Malarkey, D.E., Maronpot, R.R., Nishikawa, A., Nolte, T., Schulte, A., Strauss, V., York, M.J., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes - conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol 40, 971-994.

Hirose, Y., Nagahori, H., Yamada, T., Deguchi, Y., Tomigahara, Y., Nishioka, K., Uwagawa, S., Kawamura, S., Isobe, N., Lake, B.G., Okuno, Y., 2009. Comparison of the effects of the synthetic pyrethroid Metofluthrin and phenobarbital on CYP2B form induction and replicative DNA synthesis in cultured rat and human hepatocytes. Toxicology 258, 64-69.

Kato, K., Wong, L.-Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. Environ Sci Technol 45, 8037-8045.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 33, 655-780.

Lake, B.G., 2009. Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596.

Lin, C.Y., Lin, L.Y., Chiang, C.K., Wang, W.J., Su, Y.N., Hung, K.Y., Chen, P.C., 2010. Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. Am J Gastroenterol 105, 1354-1363.

Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the NHANES study. Environ Health Perspect 118, 686-692.

OECD, 2007. OECD GUIDELINE FOR THE TESTING OF CHEMICALS: Guideline 426, Developmental Neurotoxicity Study, adopted October 16, 2007.

Olsen, G.W., Burris, J.M., Burlew, M.M., Mandel, J.H., 2003a. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J Occup Environ Med 45, 260-270.

Olsen, G.W., Logan, P.W., Hansen, K.J., Simpson, C.A., Burris, J.M., Burlew, M.M., Vorarath, P.P., Venkateswarlu, P., Schumpert, J.C., Mandel, J.H., 2003b. An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. AIHA J (Fairfax, Va) 64, 651-659.

Parzefall, W., Erber, E., Sedivy, R., Schulte-Hermann, R., 1991. Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. Cancer Res 51, 1143-1147.

Perrone, C.E., Shao, L., Williams, G.M., 1998. Effect of rodent hepatocarcinogenic peroxisome proliferators on fatty acyl-CoA oxidase, DNA synthesis, and apoptosis in cultured human and rat hepatocytes. Toxicol Appl Pharmacol 150, 277-286.

Rosen, M.B., Schmid, J.E., Das, K.P., Wood, C.R., Zehr, R.D., Lau, C., 2009. Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid. Reprod Toxicol 27, 278-288.

Rosen, M.B., Schmid, J.E., Corton, J.C., Zehr, R.D., Das, K.P., Lau, C., 2010. Gene expression profiling in wild-type and PPARα-null mice exposed to perfluorooctane sulfonate reveals PPARα–independent effects. PPAR Research 2010, 1-23.

Ross, J., Plummer, S.M., Rode, A., Scheer, N., Bower, C.C., Vogel, O., Henderson, C.J., Wolf, C.R., Elcombe, C.R., 2010. Human constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongentoxic hepatocarcinogens phenobarbital and chlordane *in vivo*. Toxicol Sci 116, 452466.

USEPA, 1991. Guidelines for developmental toxicity risk assessment. Federal Register 56, 63798-63826.

USEPA, 1998. Guidelines for neurotoxicity risk assessment. Federal Register 63, 26926-26954.

USEPA, 2002. Hepatocellular hypertrophy. HED ToxSAC 2002 HED Guidance Doc G2002.01.

Waxman, D.J., 1999. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys 369, 11-23.