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March 9, 2005

Dr. Suhair Shallal
Designated Federal Officer
U.S. Environmental Protection Agency, Science Advisory Board (1400F)
Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

Re: PFOA Risk Assessment Science Advisory Board

Dear Dr. Shallal:

On behalf of E. I. DuPont de Nemours and Company (DuPont), I want to thank you for the opportunity to provide additional comments to the PFOA Risk Assessment Science Advisory Board (SAB). A number of issues and recommendations were discussed in the February 22-23rd SAB review meetings, in particular, the need for additional data. With this submission, DuPont is informing the SAB of significant additional data that will be available very soon and providing clarification and perspective on a number of issues that were discussed during the SAB review.

The January 2005 EPA Draft Risk Assessment on PFOA reviewed data available through June 2004. EPA acknowledged that additional data were available and would be incorporated in future drafts. Although human health data were reviewed, the Draft Risk Assessment was based on results from laboratory studies and therefore, there were no specific charge questions for the SAB relative to the significance of human health data. The SAB clearly expressed a need for more human data and a desire to consider the relevance of human health data to the risk assessment. We concur and believe that health data on workers, the highest exposed population, are the most relevant data and deserve greater consideration in the final risk assessment.

Average serum levels in workers are 100–3000 times greater than the average serum level in the general population, and employee health data from five plant sites have been reported. DuPont has recently begun a comprehensive two-phase employee health study on PFOA at its Washington Works site in West Virginia. A report of the Phase 1 cross-sectional study of over 1000 employees (781 males; 243 females) is scheduled for May 2005, and results of the Phase 2 retrospective mortality study is expected by mid-year. In addition, investigators at the University of Pennsylvania are also conducting an independent, NIEHS-sponsored community exposure/health study. This study will examine a population living in Ohio around the Washington Works plant. The results of this study are expected later this year.

The SAB also suggested that studies to assess PFOA selectivity across the PPAR receptor class would be additive. In April of 2005, DuPont expects to report the results of a study investigating PFOA activation of PPARs and other nuclear receptors in a mouse-based cell line transfected

2

Dr. Suhair Shallal
March 9, 2005

with human, rat and mouse receptors. PFOA activity was compared with dietary fatty acids and relevant positive controls. Preliminary results of this study confirm that PFOA is a PPAR- α agonist. Based on the EC₅₀ in these assays relative to serum levels in the general population, PFOA is unlikely to elicit PPAR- α -mediated changes in the general population. Furthermore, PFOA showed weak or no activity against other human nuclear receptors known to play a role in lipid metabolism.

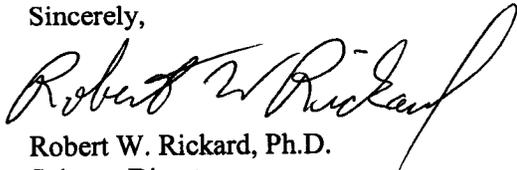
To date, no human health effects are known to be caused by PFOA exposure. The only potentially relevant association is a modest increase in some, but not all, serum lipid parameters in some of the highest exposed workers from the Phase I study noted above. It is unclear if this association is caused by PFOA exposure or is related to some other variable. DuPont is committed to investigate this issue further.

A more detailed description of the employee health study and the receptor study is provided in Attachment I. Full reports of both studies will be provided as soon as they are issued. Attachments I and II also provide additional clarification and perspectives on many of the issues discussed by the SAB.

In conclusion, DuPont believes that the weight-of-evidence indicates an overall lack of biological response to PFOA at exposure levels observed in the general population. Therefore, we believe that PFOA does not pose a risk to this population. The additional data from employee and community studies will provide important information to more fully assess the potential health effects of PFOA in higher exposed populations. Given the importance of this risk assessment and the near-term availability of these highly relevant data, it is strongly recommended that the SAB incorporate these studies into its review prior to finalizing any conclusions or recommendations.

DuPont recognizes that the presence of PFOA in human blood raises questions that should be addressed and is fully supportive of the EPA risk assessment process. DuPont is committed to objective and transparent research and looks forward to sharing the results of its studies as soon as available.

Sincerely,



Robert W. Rickard, Ph.D.
Science Director

RWR:jhh

Attachment I (17 pages)
Attachment II (23 pages)

CC: Charles M. Auer
Oscar Hernandez
Jennifer G. Seed

ATTACHMENT I

Comments on behalf of E. I. DuPont de Nemours and Company to the PFOA Science Advisory Board

This document provides additional comment to the PFOA Science Advisory Board (SAB) on the following subjects:

1. Human Health Studies
2. Activation of Nuclear Receptors by PFOA and Naturally Occurring Fatty Acids
3. Mammary Gland Tumors in the 2-Year PFOA Study (Riker)
4. Pituitary Weight Changes in F1-Females in the Two-Generation Reproduction Study
5. Evaluation of PFOA for Immunotoxicity
6. Evaluation of PFOA for Neurotoxicity

Each subject is addressed in detail in the following discussion.

Gerald L. Kennedy, Jr., B.S.

Robin C. Leonard, Ph.D.

Nancy E. Everds, D.V.M.

Steven R. Frame, D.V.M., Ph.D.

Peter J. Gillies, Ph.D.

Scott E. Loveless, Ph.D.

1. Human Health Studies

No known health effects have been observed in an occupational setting due to exposure to PFOA. Several studies by the DuPont Company and 3M that have examined a range of outcomes including liver enzyme measurements, cancer incidence, all-cause mortality, and incidence of care frequency from health insurance data, have not indicated any health issues occurring as a result of exposure to PFOA (Fayerweather, 1981; Gilliland and Mandel, 1996; Olsen et al., 1998; Olsen et al., 2000; Olsen et al., 2003a; Olsen et al., 2003b).

a. DuPont Epidemiology Program

Data from the DuPont Epidemiology Program's most recent Washington Works, West Virginia plant site cancer incidence and mortality surveillance (DuPont, 2003a; DuPont 2003b), referenced in the risk assessment, have been misinterpreted in some discussions as representing PFOA-specific findings. It is important to note that the epidemiology surveillance program is a screening program for comparing mortality and cancer incidence at each DuPont manufacturing site with the mortality and cancer incidence of U.S. DuPont employees. These data cannot be used to make any conclusion as to PFOA causality for the following reasons:

- Results are plant-site specific, and not chemical specific
- They compare data from the plant employees to the US Dupont employee population, and not to the regional community
- Only about 25% of the Washington Works employees work with PFOA, thus precluding any conclusions about PFOA specifically

If potentially significant increases in any cause of death, or any incidence of cancer, are noted from a routine surveillance, further steps are taken to collect work and medical histories. It is not unusual to observe occasional increases in some parameters which require further follow-up. For example, a 1981 surveillance of the Washington Work site identified an increase Standardized Mortality Ratio (SMR) of myocardial infarction.

However, a more detailed follow-up investigation indicated that the increase was clearly not related to PFOA exposure, or to any other workplace chemical (DuPont, 1981).

b. Two-Phase, On-Going Study at DuPont Washington Works Site

A comprehensive two-phase PFOA study is currently in progress at the Washington Works site. Phase I is a cross-sectional survey of 1,025 workers (781 males; 243 females) at the plant that incorporates a biomarker of PFOA exposure (serum PFOA level) and clinical and questionnaire data from physical examination by occupational physicians. The primary objective of Phase I is to describe the relationship of serum PFOA to potential health outcome variables suggested by previous animal and worker studies, taking into account potential confounders and effect modifiers.

Preliminary results from this phase of the Washington Works study have indicated no association between PFOA serum levels and nearly all of the clinical laboratory (blood and urine) parameters examined. For example, no correlation was found between PFOA exposure and liver tests, the primary target organ in laboratory studies, nor in most other laboratory tests or any cancer markers, such as PSA. One exception was a modest increase in some serum lipid parameters in the subgroup of workers having PFOA levels greater than 1000 ppb. In contrast, no associations were seen between PFOA exposure and HDL cholesterol or C-reactive protein. Two other parameters, uric acid and iron, appeared increased in the serum of employees with the highest PFOA levels. Due to the cross-sectional nature of the Phase I design, the study data did not allow conclusions as to whether PFOA was or was not the cause of the changes in laboratory tests. Studies are being designed to further investigate these observations. The complete results of the current study are anticipated by May 2005.

Phase II is a retrospective cohort mortality study of all DuPont employees ever employed at the plant site. The study's primary objective is to compare observed deaths in the historical Washington Works population to the expected numbers of deaths based on five reference populations—all U.S. DuPont employees; State of West Virginia; Wood County, West Virginia; Washington County, Ohio; and the general U.S. population. The size of

the cohort, which covers 50 years of plant operations, combined with the availability of a specific biomarker of exposure (serum PFOA levels) that can be linked to specific job tasks, which can then be used to estimate past exposures. This makes this study very relevant to the understanding of PFOA and human health. Phase II results are scheduled to be complete and available by June 2005.

c. Study Sponsored by Plastics Europe

In addition to the ongoing PFOA worker studies, a collaborative cohort mortality study for tetrafluoroethylene (TFE) workers is being sponsored by Plastics Europe, an international trade organization (Bertazzi, 2004). Data have been collected for this effort at the Washington Works facility, as well as plants in Italy, Germany, England, and the Netherlands. This study will provide a detailed examination of mortality risk associated with specific exposure to several process chemicals—including PFOA—used for the manufacture of TFE.

d. NIEHS Sponsored Study

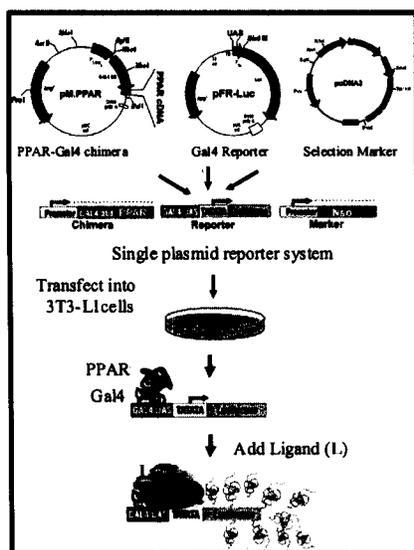
NIEHS is currently sponsoring a study in Washington County, Ohio, that is being conducted by investigators at the University of Pennsylvania. This study is designed to measure and investigate potential correlates of serum PFOA levels with several health endpoints and questionnaire responses from a random sample of the county population. These serum samples have been collected and will be analyzed by the summer of 2005.

2. Activation of Nuclear Receptors by PFOA and Naturally Occurring Fatty Acids

PFOA has been shown to be an agonist for the nuclear receptor, PPAR- α (Kennedy et al., 2004). Decreases in some lipid parameters are well-known effects of many PPAR- α agonists. Therefore, *in vitro* studies were conducted across multiple species, including humans, to (a) characterize the activity of linear PFOA on PPAR- α and other selected nuclear receptors involved in lipid metabolism; and (b) to compare the relative activity of PFOA to naturally occurring dietary fatty acids. These studies used a common testing platform of a murine-based cell line transfected with human, rat, and mouse receptors.

The analysis for the human receptors has been completed and demonstrates that:

- Naturally occurring dietary fatty acids were more potent agonists of PPAR- α than PFOA
- PFOA showed very weak or no agonism of PPAR- β , PPAR- γ , LXR- β or RXR- α



Human Nuclear Receptor	EC50 μ M				
	Positive Control	PFOA	Octanoate	Linoleate	Linolenate
PPAR- α	8.6	45.2	40.8	18.4	8.6
PPAR- β	147.5	-	-	35.0	77.7
PPAR- γ	0.1	13.3	41.4	18.4	4.4
LXR- β	0.2	-	-	-	-
RXR- α	3.1	-	-	20.3	294.1

Positive Controls: PPAR- α Ciprofibrate
 PPAR- β Tetradecylthioacetic acid
 PPAR- γ Rosiglitazone
 LXR- β T0901317
 RXR- α Methoprene

- indicates no activity

In addition to PFOA, three fatty acids were tested in these studies; these were octanoate and two essential fatty acids, linoleate and linolenate. Given the structure of the ligands studied, the nuclear receptors chosen for these studies were PPAR- α , β , and γ ; LXR- β ; and RXR- α . Details of this assay were reported previously (Bility et al., 2004). The ligand-binding domains of the various nuclear receptors were fused to the DNA-binding domain of the yeast transcription factor Gal4 under the control of the SV40 promoter. This plasmid also encoded the UAS-firefly luciferase reporter under the control of the Gal4 DNA response element. Mouse 3T3-L1 cells were subsequently transfected with the plasmid DNA (see Figure). Test ligands were dissolved in DMSO and incubated with the transfected 3T3-L1 cells for 24 hours. Fold induction of normalized luciferase activity was calculated relative to control cells treated with the DMSO vehicle. Experiments were

run as three independent samples per treatment group. Dose-response curves were developed for each ligand and EC50 values calculated from best-fit lines, these values are reported in the table.

In terms of PPAR- α , PFOA presented as a full agonist, but with less potency than linoleate and linolenate; octanoate presented as an equipotent partial agonist. In terms of PPAR- γ , PFOA presented as a very weak partial agonist, as peak activation and potency were 10- and 133-fold less than Rosiglitazone. PFOA was inactive at doses up to 200 μ M with respect to LXR- β and RXR- α .

In summary, under the conditions of this trans-activation assay, PFOA was less biologically active and more selective in its activation of nuclear receptors than naturally-occurring fatty acids. Studies are in progress to profile these same ligands against the rat and mouse receptors. Results of these studies are expected to be available in April of 2005.

3. Mammary Gland Tumors in the 2-Year PFOA Study

The incidence of mammary gland tumors seen in female rats fed PFOA in the 2-year study (Riker, 1987) is not considered to be causally related to PFOA ingestion based on the following:

- For benign tumors (adenoma and fibroadenoma):
 - There was no definitive dose-response, and there was no statistical difference in groups when data were properly analyzed
 - Tumor incidence was within the historical control values for this strain and supplier
- For adenocarcinomas, the high dose incidence was less than the control incidence and the incidence in the treated groups was not dose-related

In this study, the incidence of fibroadenomas of the mammary gland was 22, 42, and 48% at 0, 30, and 300 ppm in diet, respectively. The dose-response was such that there

was no clear incidence difference between the 30 and 300 ppm groups despite a 10-fold difference in dose. The authors concluded that this was not a treatment-related response.

Assessment of the mammary tumor response in this study included a comparison to historical control data from an outside laboratory. Some have questioned the use of such historical data. While the optimum historical data for a chronic study conducted at a given laboratory is derived from studies conducted at that same laboratory, valuable information can nonetheless be derived from interlaboratory data for a given species and strain. For example, the National Toxicology Program maintains a historical control database which includes animals of the same species and strain that are taken from studies conducted at different test facilities.

The value of interlaboratory data is further enhanced if the tumor type in question demonstrates similar incidences and ranges of incidences across multiple laboratories. Such is the case for mammary gland fibroadenomas in Sprague-Dawley rats. The incidence of fibroadenomas in 13 studies conducted at DuPont Haskell Laboratory ranged from 24 to 54% with a mean of 37% (Sykes, 1987). Similarly, historical control data from Charles River Laboratories, for a time period contemporary (1977-1985) to the 2-year study with PFOA, gives the average fibroadenoma incidence of 34% with a range of 15 – 58% among 11 studies conducted at different laboratories (Lang, undated). These data demonstrate that the incidence of mammary gland fibroadenoma in groups of untreated female Sprague-Dawley rats is often high and markedly variable irrespective of the laboratory from which the data are derived. Based on these considerations, the use of interlaboratory historical data for mammary tumors in Sprague-Dawley rats is entirely appropriate.

The laboratory conducting the two-year study in rats with PFOA (Riker Laboratories) did not have an adequate historical control database, as it was the only chronic study conducted at this laboratory at that time. Thus, control incidences from other laboratories, but derived from the same supplier of Sprague Dawley rats, were used to aid the assessment of mammary gland tumors in the two-year study. The incidences of

fibroadenomas in the PFOA-treated groups (42 and 48%, respectively) were within the range of historical incidences for this tumor type in both the Charles River and Haskell Laboratory historical databases. Consistent with these findings, the incidence of fibroadenomas in the PFOA-treated groups was not statistically different from the Haskell Laboratory historical control incidence ($p < 0.05$).

These data support the study authors' conclusion that the distribution of fibroadenomas in the PFOA study was a reflection of background incidence and was not related to PFOA treatment.

The SAB also discussed the findings of mammary gland adenocarcinoma in female rats in the two-year study. The incidences of adenocarcinoma were 7/46, 14/45, and 5/44 in the control, 30 ppm, and 300 ppm groups, respectively. The incidences of adenocarcinoma are not dose related and the incidence in the 300 ppm (high dose) group is actually lower than controls. Furthermore the incidence in the 30 ppm group (the group with the highest incidence of adenocarcinoma) is not statistically significant relative to controls by the Fisher's exact test ($p < 0.05$). This lack of statistical difference is true whether one uses the number of mammary glands evaluated as the denominator or the total number of animals examined ($n=50/\text{group}$). Therefore, the overwhelming weight of evidence indicates that mammary gland adenocarcinoma was not a treatment-related effect in this study.

To further clarify tumor findings in the two-year study in rats with PFOA, and in response to questions raised by the SAB, an independent pathology working group will be convened to peer-review tumor findings in mammary glands from this study and to assess causality. A report from this working group is expected by June of 2005. The relevance of mammary tumors, as well as other endpoints, is discussed in Attachment II.

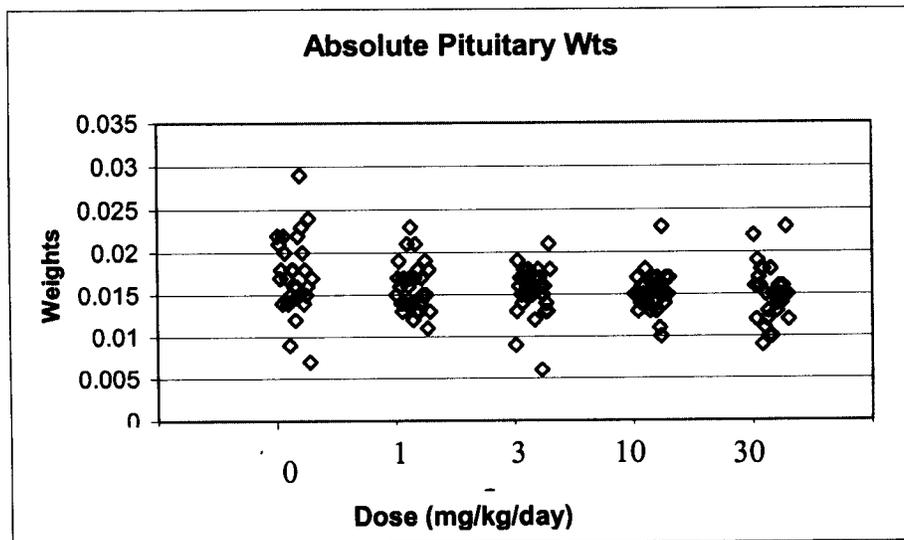
4. Pituitary Weight Changes in F₁-Females in the Two-Generation Reproduction Study

In the two-generation reproduction study in rats with PFOA (Argus Research, 2002), statistically significant but minimal decreases in pituitary gland weights were observed in F₁-generation females administered 3 mg/kg/day and above. Weight-of-evidence considerations strongly indicate that these changes, though statistically significant, were spurious and not related to the administration of PFOA. This conclusion is based on the following:

- The statistically significant weight decrements were not dose related across a large (10-fold) range of doses
- Decreases were small compared to the variation in pituitary weight parameters observed within the control group population
- At the high dose, all individual pituitary weight parameters in F₁ females were within the range of values for individual control animals
- No pituitary weight changes were seen in the P-generation females
- No treatment-related microscopic changes occurred in the pituitary of female rats of either generation at any of the doses tested
- No treatment-related organ weight or microscopic changes were seen in male rats, clearly the more sensitive gender, in either generation at any of the doses tested

Statistically significant decreases in pituitary weight parameters in PFOA treated groups administered 3, 10, or 30 mg/kg/day in the two-generation reproduction study did not occur in a dose-related manner. For example, mean pituitary absolute weights in these three groups were identical (15 mg) despite the order-of-magnitude span in dosages. Furthermore, the decreases in pituitary weights relative to controls were very small. The mean absolute pituitary weights in the three groups administered 3 mg/kg/day and above were decreased 12% compared to the control group mean. However, the coefficient of variation for absolute pituitary weight within the control population was approximately 23%. The relatively large coefficient of variation noted among individual pituitary weights is consistent with the difficulty inherent in weighing very small organs.

The minimal nature of these pituitary weight changes is further underscored by a comparison of individual pituitary weights of animals in treated groups with those of the control group. Notably, all pituitary weight parameters (absolute weight and weight relative to both body and brain weight) in the F₁ female high-dose group were within the ranges of values for the within-study control group. A scatter-plot of individual absolute pituitary weights is given in the figure below.



Consistent with the minimal and likely spurious nature of the pituitary weight changes observed in some PFOA-treated F₁ female groups, no such statistically significant changes in pituitary weights were seen in the P-generation females. Furthermore, no treatment-related microscopic changes were seen in pituitaries of either the P- or F₁-generation female rats. More importantly, no pituitary effects—including organ weight or microscopic changes—were observed in either generation of male rats at any PFOA dose tested, including doses that clearly showed other evidence of toxicity. Male rats have consistently shown greater sensitivity to PFOA-associated toxicity, irrespective of the toxicity endpoint considered. This greater sensitivity is consistent with well-established pharmacokinetic differences between male and female rats. Thus, it is highly unlikely that true compound-related effects on the pituitary gland would be seen in females at

nontoxic doses as low as 3 mg/kg/day when no such pituitary effects were observed in males at a dose (30 mg/kg/day) that was 10-fold higher and overtly toxic.

In conclusion, the weight of evidence considerations noted above strongly indicate that the pituitary weight changes noted in some PFOA-treated F₁-generation female groups were spurious and unrelated to administration of the test material.

5. Evaluation of PFOA for Immunotoxicity

There is no definitive evidence that PFOA produces primary adverse effects on the immune system. This conclusion is based on the following considerations:

- Numerous toxicity studies in rodents and primates have shown no primary effects on the immune system organs or peripheral blood lymphocyte counts at doses that clearly produce systemic toxicity
- Reports by a single investigator of immune system effects in mice have several deficiencies which require further study before drawing conclusions as to their biological significance

Histopathological examination of immune system tissues (e.g., spleen, thymus, lymph nodes) is considered a sensitive endpoint to identify potential immunotoxicant hazards and should serve as part of a first tier evaluation (Basketter et al, 1994; Greaves, 2000). Organ weights of selected immune system organs are also recognized as valuable endpoints when evaluated in the context of all other clinical, clinical laboratory, and histopathology data from the study. In addition, routine analysis of hematology is also important in the initial evaluation of immunotoxicity (Ennulat et al, 2005, in press). Numerous multidose toxicity studies with PFOA in rats and monkeys have included assessment of these first tier end points for immunotoxicity. Based on these studies, PFOA does not produce primary toxicity to the immune system.

a. Immune system findings in toxicity studies in rats and monkeys

In a 90-day study, rats were fed PFOA at dietary concentrations ranging from 10-1000 ppm (IRDC, 1978a). There was no effect on peripheral blood lymphocyte counts. No effect was observed on spleen or thymus weight or histopathology. Similarly, in a two-year feeding study in rats fed PFOA at dietary concentrations of 30 or 300 ppm (Riker Laboratories, 1987), no immunotoxicity was evident at either dietary concentration based on the absence of compound-related effects on peripheral blood lymphocyte counts, organ weight or histopathological changes in spleen, or histopathological changes in lymph nodes. More recently, an oral gavage 2-generation reproductive study (1, 3, 10, 30 mg/kg) was reported (Argus Research, 2002). Although clear evidence of systemic toxicity was seen in male rats based on body weight effects at the higher doses, no effects on spleen or thymus weights were observed at any dose in parental or F1 generations when organ weight was analyzed as a percentage of body weight (to account for the significant body weight decrements). In addition, no effects on spleen or thymus weight were observed in F1 or F2 pups. Thus, no effects were observed in offspring of PFOA-dosed dams at any of the doses tested.

In rhesus monkeys, PFOA administration did not affect peripheral blood lymphocyte counts or spleen or thymus weights at any dose (IRDC, 1978b) and no effects on lymphoid histopathology were observed at nonlethal levels (<30 mg/kg). In cynomolgous monkeys fed 0, 2 or 20 mg/kg PFOA for 4 weeks or 0, 3, 10 or 20/30 mg/kg PFOA for 6 months, no compound-related microscopic changes were present in spleen, thymus and mesenteric lymph nodes at any dose, including doses that produced marked systemic toxicity (Covance Laboratory, 2001). In addition, there were no treatment-related changes in peripheral lymphocyte counts.

In summary, PFOA has been evaluated in numerous toxicity studies in rodents and monkeys. Most of these studies included assessment of peripheral blood lymphocyte counts, organ weights, and histopathological findings in immune system organs at doses that clearly produced systemic toxicity. However, no evidence of primary toxicity to the immune system has been observed following PFOA exposure.

b. Studies Evaluating Immunotoxicological Endpoints in Mice

Findings suggestive of immunotoxicity following exposure to PFOA are limited to several studies in mice reported by Yang and colleagues (Yang et al., 2000; Yang et al., 2001; Yang et al., 2002a; Yang et al., 2002b). Notably, most (but not all) of these studies evaluated immunotoxicity at a very high dietary concentration (0.02%) that produced marked systemic toxicity as evidenced by, for example, body weight loss of 17% after only 5 days of dosing. Nonspecific toxicity to the immune system secondary to marked systemic toxicity is a well-established phenomenon which is not addressed in these studies (Greaves, 2000). Also, these studies typically used very low numbers of animals (usually 4 mice/group) and, for some critical parameters measured, showed marked variability, even between similarly-treated controls. For example, relative liver weights in two different control groups (but groups used under the same study protocol) differed by 17% (Yang et al., 2000, Table 1a). A number of other observations suggest that further work is needed to understand the significance of the findings in this series of studies in mice:

- In one study, extremely high titers for anti-HRBC IgM antibody were reported in mice not immunized with HRBC; this raises concern about the specificity of the IgM antibody assay.
- In these studies, IgM antibody production as assessed by splenic plaque forming cells (PFC) were evaluated on the same day as serum specific antibody. However, for a given antigen, IgM antibody production as assessed by splenic PFC generally peaks one day earlier than serum IgM, as measured by ELISA. Based on the lack of data indicating time-to-peak antibody formation for this particular antigen and this strain of mice, any effect of treatment on IgM concentration cannot be evaluated.
- No histopathology of spleen, thymus or lymph nodes was performed. (Tier 1 endpoints).

In summary, based on the absence of primary target organ toxicity to the immune system across several toxicity studies in rats and monkeys, PFOA is not an immunotoxic

compound. Further work is necessary to clarify the results of studies in mice in which high doses of PFOA were reported to produce decrements in some immune parameters. Studies are being designed to clarify the immune system findings in mice reported by Yang et al.

6. Evaluation of PFOA for Neurotoxicity

Based on the absence of clinical signs or histological findings, exposure to PFOA is not associated with neurotoxicity.

No neurotoxicity has been detected in multiple toxicity studies by multiple routes of exposure in rats and monkeys. An increase in the incidence of ataxia in female rats from a 2-year feeding study was seen primarily in moribund rats toward the end of the study (Riker, 1987). An increase was not seen in male rats from this study or a second 2-year bioassay. Further, tissue distribution studies in rats show similar PFOA concentrations in the brain of males and females following oral dosing, indicating that a sex-specific response is unlikely. The body burden at steady state in males is considerably higher than that in females due to the ability of female rats to excrete PFOA in the urine. Hence it is again less likely that the female would be more responsive than the male.

In repeated exposure studies in rats and monkeys, the individual animals are routinely handled and evaluated for changes that may be indicative of nervous system dysfunction. Such evidence includes clinical signs of tremors, convulsions, gait/coordination abnormalities, lacrimation, salivation, excessive vocalization, changes in muscle tone, breathing rate, abnormal posture, arousal (activity level), polyuria, and diarrhea. In the battery of studies conducted on PFOA, a dose-related effect was not detected for any of these signs. In addition, morphological changes were not detected in the central or peripheral nervous system (including sciatic nerve, skeletal muscle, brain or spinal cord). Therefore, nervous system dysfunction has not been associated with PFOA exposure.

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ATTACHMENT II

(U.S. EPA Public Docket AR 226-1727)

**Genotoxicity, Carcinogenicity, Developmental Effects and Reproductive
Effects of Perfluorooctanoate: A Perspective from Available Animal and
Human Studies**

**Prepared for the Association of Plastics Manufacturers of Europe and the Society of the
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Introduction

This document will describe the experimental database for genotoxicity, carcinogenicity, developmental, and reproductive effects of perfluorooctanoate (PFOA) and will provide our current understanding of the potential relationship of these toxicological endpoints to man, as supported by studies of worker populations. In addition, it provides perspective on the relationship of these toxicological endpoints to human exposure and potential human health risk. PFOA and its salts are fully fluorinated organic compounds that are used as reactive intermediates or as processing aids and surfactants. A large toxicological and epidemiological database exists for PFOA. Most of the toxicological data have been developed using the ammonium salt of perfluorooctanoic acid (APFO); however, since APFO readily dissociates and is soluble in aqueous solution, the designation PFOA will be used throughout this document. The reader is referred to the U.S.E.P.A. document, "Revised Draft Hazard Assessment of Perfluorooctanoic Acid and its Salts" (U.S.E.P.A., 2002), for a detailed presentation of the toxicological and human-health databases for PFOA. Laboratory studies designed to identify potential health hazards of PFOA demonstrate that PFOA can produce effects in animal models. By contrast, the health effects observed in laboratory studies have not been observed in worker populations either under current or past exposure conditions. Therefore, we believe that PFOA does not present an unreasonable risk to human health at the levels encountered in the workplace.

Background on Worker Studies

Throughout this document, reference will be made to several worker studies. Studies in workers include cohorts from a PFOA production facility (Cottage Grove, MN) and two facilities that used PFOA in manufacturing processes (Decatur, AL and Antwerp, Belgium). The workers from the Cottage Grove facility are considered likely to have the highest potential for exposure since this facility manufactured PFOA since the 1940's and employees have been shown to have higher serum concentrations of PFOA than either Decatur or Antwerp plant populations. The Antwerp plant also manufactured PFOA but began in the mid 1970's. The Decatur facility routinely used PFOA but did not manufacture it until the late 1990's and Antwerp plants are facilities that manufactured other fluorochemicals and routinely used PFOA. The types of studies performed include evaluations of mortality, medical surveillance, and episodes of care. The mortality studies examined observed versus expected causes of death in the study populations. Medical surveillance included standard worker health assessments as well as evaluations of biochemical parameters that had been affected in laboratory animal studies. An episodes-of-care study examined health insurance claims data. An episode of care is defined as a series of events all related to a particular health problem that exists continuously for a period of time.

Developmental Toxicity

The developmental toxicity of PFOA has been studied in rats and rabbits by the oral exposure route and in rats by the inhalation exposure route (Gortner, 1981; Gortner, 1982; Staples *et al.*, 1984). In those studies, pregnant animals were treated with graded doses/exposures of PFOA during organogenesis. Observations of the structural integrity of the fetuses was evaluated both

externally, internally, and by skeletal examination of the fetuses obtained prior to natural delivery. For one set of oral and inhalation studies in rats, dams were allowed to litter and pups were observed through the lactation period. These studies, as discussed below, allow the conclusion that maternal exposure to PFOA during organogenesis is not uniquely hazardous to the fetus or to preweaning rat pups.

The developmental study conducted in rats by Gortner (1981) was the first to be conducted with PFOA. In this study, maternal toxicity was observed at the highest dose (150 mg/kg) and consisted of group mean body weight reductions and mortality (3 of 22 dams). Reproductive organs were unaffected by treatment. Fetal examination did not reveal any increase in embryo-fetal toxicity or structural abnormalities that were attributable to PFOA treatment. Lens abnormalities, originally attributed to PFOA treatment, were found subsequently to be an artifact of the sectioning technique.

In another oral study, rats were given 100 mg PFOA/kg of body weight by gavage from gestation day 6 through 15 (Staples *et al.*, 1984). One group of 25 pregnant rats and their litters were examined on day 21 of gestation. Another group of 12 treated dams gave birth and the resulting pups were examined on day 35 post-partum. Maternal effects including death and decreased maternal body weight gain were seen in both groups. No developmental toxicity or abnormalities were seen in the fetuses, and offspring showed normal lactational viability.

By the inhalation route, groups of pregnant rats were exposed to concentrations of either 0.14, 1.2, 9.9, or 21 mg PFOA/m³, 6 hrs/day from day 6 through 15 of gestation (Staples *et al.*, 1984). Exposure to the highest concentration resulted in the death of 3 of 12 rats with the remaining rats showing reduced weight gains and clinical signs including lethargy and chromodacyorrhea. Reduced weight gains were also seen in rats exposed to 9.9 mg/m³. No effects were seen in those exposed to either 0.14 or 1.2 mg/m³. Mean fetal body weights of surviving dams exposed to 21 mg/m³ were reduced. There were no structural abnormalities in fetuses from any of the exposure groups that could be associated with PFOA exposure.

In a rabbit developmental study (Gortner, 1982), rabbits were given oral doses of either 1.5, 5, or 50 mg PFOA/kg from gestation day 6 through 18. The number of rabbits producing litters in this study was low in all groups, a fact that affects interpretation of the study. A reduction in maternal body weight gain was observed in rabbits given 50, but not 1.5 or 5 mg/kg. No other signs of response to PFOA were observed in the pregnant rabbits. Fetuses from all treatment groups were present in the expected numbers, were structurally normal, and weighed essentially the same as their untreated counterparts. No evidence of either embryotoxicity or teratogenicity was seen. An increase in the number of fetuses with the natural and stress-related variant of thirteenth ribs was noted. This latter finding is known to be quite variable (Christian, 1987), is not a malformation *per se*, and is not likely to be relevant to humans.

Regarding reproductive development, the multigeneration reproduction study with PFOA in rats showed delays in the age at preputial separation (mean = 3.7 days) in males and the age at vaginal opening (mean = 1.7 days) in females (York, 2002). These delays are believed to be secondary to toxicity and do not represent a primary effect on organ development, as will be further discussed in the "Reproductive Effects" section that follows.

Reproductive Effects

A two-generation reproduction study in rats was conducted with PFOA (York, 2002). Rats were treated with oral gavage doses of either 1, 3, 10, or 30 mg/kg of body weight/day. In the parental rats, signs of toxicity were observed at all dose levels in the males and at 30 mg/kg in females. In males, body weight gain suppression was observed at all doses (except 1 mg/kg in the P₁ generation) along with organ weight changes (liver, kidney, and spleen). Female parental rats were relatively unaffected by treatment, with decreased kidney weights seen in P₁ females and decreased weight gains in F₁ females only at 30 mg/kg. There were no effects on any of the mating or fertility parameters in either generation. At 30 mg/kg, a number of effects in the offspring were observed including decreased pup weights, increased pup mortality (F₁ generation only), and delayed vaginal opening and preputial separation. These findings were not observed at any of the lower doses. Clearly, the effects observed in the two-generation reproduction study did not compromise the reproductive success (i.e., mating and fertility) of the rats at dosages of up to 30 mg/kg under the conditions of this study.

The two-generation reproduction study found decreased pup weights during lactation and increased pup mortality in the F₁ but not the F₂ generation. The increases in pup mortality occurring pre- and post-weaning at 30 mg/kg may be suggestive of the beginning of a dose-response curve. It is important to note that, while post-weaning mortality was not evaluated in the F₂ generation offspring (all F₂ offspring were necropsied at weaning), there were no effects on pre-weaning mortality in the F₂ offspring (pre-weaning mortality was increased in F₁ pups, but was not statistically significant). In addition, there were no effects on pup weights in F₂ generation offspring through weaning.

The increased incidence of pup mortality at 30 mg/kg is most likely a result of a general failure to thrive of the offspring, suggesting a compromised nutritional status of the offspring at pre- and/or post-weaning as reflected by reduced body weight. In support of this hypothesis, eleven of the thirteen F₁ offspring that died post-weaning died before post-weaning day eight, and these included the nine lightest pups. Although not statistically significant at all time points, pup weights were consistently decreased throughout the lactation period (90, 90, 89, 92, and 95% of control on postnatal days 1, 5, 8, 15, and 22, respectively). These effects have also been observed in reproduction studies performed with other peroxisome proliferators such as gemfibrozil, RMI 14,514, and hydrochlorofluorocarbon 123 (HCFC-123) (Fitzgerald *et al.*, 1987; Gibson *et al.*, 1981; Malinverno *et al.*, 1996). It seems likely that the compromised nutritional status of some offspring is responsible for the increased pup mortality observed in the two-generation reproduction study with PFOA.

The data from this study, discussed in more detail below, shows delayed age at preputial separation in males (mean = 3.7 days) and delayed age at vaginal opening (mean = 1.7 days) in females in the F₁ offspring. The delays in sexual maturation may have been the result of delayed growth of the F₁ offspring. As noted earlier, pup weights were consistently decreased throughout the lactation period. While the body weights of the F₁ generation offspring were similar to the controls at the time of sexual maturation, it is plausible that the delayed growth that was

observed early in lactation may have contributed to the delays that were observed in sexual maturation of the F₁ offspring.

Decreased body weights can result in non-specific delays in puberty (Carney *et al.*, 1998; Glass *et al.*, 1976; Glass & Swerdloff, 1980; Kennedy and Mitra, 1963; Marty *et al.*, 1999, 2001a, 2001b, 2001c; Ronnekleiv *et al.*, 1978; Stoker *et al.*, 2000a; 2000b; Widdowson & McCance, 1960). In a recent report by Lewis and co-workers (2002), variability of sexual maturation data was evaluated in control populations of Sprague-Dawley rats. They found that the typical variability among control groups was approximately two days, a finding that was also consistent with the typical variability in age at sexual maturation reported by others (Ashby & Lefevre, 2000; Clark, 1999; Marty *et al.*, 1999; Stoker *et al.*, 2000b). Since non-specific effects on body weight can cause general delays in sexual maturation, interpreting delays in sexual maturation can be problematic in studies where generalized delays in growth occur, such as those that were observed in the current study of PFOA. Clearly, PFOA do not compromise reproductive success (i.e., mating and fertility) in rats at dosages of up to 30 mg/kg.

In summary, in the two-generation reproduction study with PFOA, paternal toxicity (P₁ and F₁) was observed at all dose levels (1, 3, 10, and 30 mg/kg) and minimal maternal toxicity was observed at 30 mg/kg. While several possible reproductive/developmental effects were observed (i.e., decreased pup weights, increased pup mortality, and delayed sexual maturation in F₁ offspring), the reproductive success of the rats was not compromised. Notably, the overall results of the first and second generation appear to be similar in that there was no apparent increase in adverse outcome(s) in the second generation. The effects that were observed could be suggestive of reproductive and/or developmental effects or they could be due to general delays in growth. Unknown mechanisms may be contributing to the effects that were observed at 30 mg/kg. At dosages of ≤ 10 mg/kg, no reproductive or developmental parameters were affected, while parental males showed clear signs of toxicity. The no-observed-adverse-effect-level (NOAEL) for reproduction in the two-generation reproduction study was 10 mg/kg, while the NOAEL for general toxicity would be < 1 mg/kg for the male parental animals and 10 mg/kg for the female parental animals. The effects that were observed with PFOA in the two-generation reproduction study are consistent with those observed in studies with other peroxisome-proliferating compounds (Fitzgerald *et al.*, 1987; Gibson *et al.*, 1981; Malinverno *et al.*, 1996).

Human Experience with Respect to Development and Reproduction

An episodes-of-care study (Olsen *et al.*, 2001b) at the 3M Decatur plant site examined reproductive outcomes associated with fluorochemical exposure (which includes potential PFOA exposure). Regarding pregnancy and its potential complications, there were 40 episodes of care reported in 13 female employees in the fluorochemical plant (44.7 expected) compared to 23 episodes of care (26.3 expected) reported in eight female employees in the film plant (a non-fluorochemical plant at the same site as the Decatur fluorochemical plant) between 1993 and 1998. This resulted in an episodes of care risk ratio of 1.0 (95% CI 0.6-1.8). The total number of female employees was 122 and 101 in the chemical and film plants, respectively. The episodes-of-care risk ratios for congenital anomalies (1.0, 95% CI 0.6-1.8) as well as perinatal disorders (0.2, 95% CI 0.0 - 2.4) were also comparable between employees in the fluorochemical

and film plants. There is no evidence from this study to suggest increases in reproductive and developmental effects associated with exposure to fluorochemicals including PFOA.

Hormones

The association of PFOA serum and hormone concentrations in workers has been studied at three production facilities (Cottage Grove, Decatur and Antwerp). The episodes-of-care study conducted only at the Decatur facility also allowed observation of episodes of care that may relate to hormonal status. Two cross-sectional studies of 111 and 80 Cottage Grove male fluorochemical production workers were conducted and measured their serum PFOA concentrations in relation to the concentrations of several hormones (testosterone, estradiol, LH, FSH, DHEAS, TSH, cortisol and sex hormone-binding globulin) (Olsen *et al.*, 1998). PFOA serum concentration was not associated with changes in hormone concentrations. Although a 10% increase in mean estradiol level was observed among employees who had the highest levels of serum PFOA, this association was confounded by body mass index and was likely not due to PFOA exposure. Further, an analysis of thyroid hormone levels in 3M Antwerp and Decatur workers did not show substantial changes in TSH, T4, free T4, T3 or thyroid hormone binding globulin associated with serum PFOA concentrations (Olsen *et al.*, 2003b). The risk ratio for disorders of the thyroid in the episodes-of-care study was comparable between Decatur fluorochemical and film plant workers (1.1, 95% CI 0.6-1.8) (Olsen *et al.*, 2001b). In addition to these human observations, a six-month oral toxicity study in male cynomolgus monkeys did not produce significant changes in either sex hormones or thyroid hormones (Butenhoff *et al.*, 2002). Therefore, there is no observed association of PFOA exposure with changes in hormone levels in man or monkeys.

Genotoxicity

The weight of evidence from studies evaluating the genotoxicity of PFOA indicates that PFOA is not genotoxic. These studies include evaluation of mutagenicity, clastogenicity and cell transformation.

PFOA has not shown a potential to effect DNA point mutations or recombinations. PFOA has shown a lack of activity in bacterial reverse mutation assays including *Salmonella typhimurium* and *Escherichia coli* strains and in yeast recombination assays (*Saccharomyces cerevisiae*) in the absence and the presence of metabolic activation (Litton, 1978; Hazleton, 1995a, 1996a). Similarly, in the Chinese hamster ovary (CHO) forward mutation assay, PFOA did not induce a statistically significant increase in the number of mutant colonies in the treated cells (Toxicon, 2002).

Chromosomal aberrations were assessed in human lymphocytes and CHO cells. PFOA did not induce significant increases in the numbers of chromosomal aberrations in human lymphocytes (Hazleton, 1996b; NOTOX, 2000). When tested in CHO cells, significant cytotoxicity was observed at the highest doses tested, and these doses also increased chromosomal aberrations.

(Hazleton, 1996c, 1996d). In view of the high toxicity, the biological significance of this positive response is questionable.

PFOA did not induce a significant increase in bone marrow polychromatic erythrocytes after oral administration to mice (Hazleton, 1995b, 1996e). There was no evidence of cell transformation using the C3H 10T1/2 cell line observed at any of the dose levels tested (Stone, 1981). The genotoxicity profile for PFOA indicates a lack of activity in a range of test systems and endpoints.

Peroxisome Proliferation

PFOA is a peroxisome proliferator (PP) in numerous studies and belongs to a widening group of substances including plasticizers and hypolipidemic drugs that are known to be PPs (Ikeda *et al.*, 1985; Just *et al.*, 1989; Pastoor *et al.*, 1987; Cook *et al.*, 1992, 1994; Biegel *et al.*, 1995, 2001).

The liver is a primary target organ for both short-term and chronic effects of PFOA in rats (Griffith & Long, 1980; Olson & Anderssen, 1983; Kennedy, 1985; Pastoor *et al.*, 1987) and cynomolgus monkeys (Butenhoff *et al.*, 2002). The increased liver weight does not appear to be a result of hepatocellular hyperplasia (no increase in nuclear DNA) and has been variously attributed to increases in peroxisomes, endoplasmic reticulum and mitochondria (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Butenhoff *et al.*, 2002; Berthiaume & Wallace, 2002; Biegel *et al.*, 2001). PFOA has been shown to activate the PPAR α receptor (Maloney & Waxman, 1999). Higher doses lead to liver degeneration and necrosis and the appearance in the serum of enzymes reflecting liver damage.

Treatment of rodents with PPs initiates a characteristic sequence of morphological and biochemical events in the liver and to a lesser extent the kidney. These events include marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, large increases in peroxisomal fatty acid β -oxidation, an obvious swelling and proliferation of the mitochondria and endoplasmic reticulum, increased cytochrome P-450-mediated ω -hydroxylation of lauric acid, and various changes in lipid metabolism (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Berthiaume & Wallace, 2002). This response is initiated by the activation of the nuclear receptor, PPAR α (Green, 1995; Ashby *et al.*, 1994; Lake, 1995). PPAR α is a steroid hormone receptor able to increase the transcription rate of responsive genes and is the major mediator of PP in rodent liver. The critical role of PPAR α in PP in mice has recently been clearly established. PPAR α -null mice do not show the typical PP-mediated responses or signs of hepatic hyperplasia or neoplasia (adenomas or carcinomas) in chronic studies with PPs (Peters *et al.*, 1997; Ward *et al.*, 1998). Long-term exposure of rodents to PPs characteristically results in an increased incidence of liver tumors (Doull *et al.*, 1999; IARC, 1995).

There are differences in the effects exerted by different PPs. Pronounced species differences have been reported following treatment of animals with PPs *in vivo* and have been observed in hepatocyte cultures *in vitro* (Ashby *et al.*, 1994; IARC, 1995; Bentley *et al.*, 1993; Elcombe *et al.*, 1997; Lake, 1995; Maloney & Waxman, 1999). Rats and mice are highly, perhaps uniquely, responsive to the effects of PPs; whereas, Syrian hamsters exhibit an intermediate response and guinea pigs seem to be practically nonresponsive, as are primates - including both Old World and

New World (e.g., marmoset) species, and humans (Bentley *et al.*, 1993; Pugh *et al.*, 2000; Butenhoff *et al.*, 2002; Tucker & Orton, 1993; Graham *et al.*, 1994).

A large number of humans have been treated for relatively long periods of time with hypolipidemic drugs that are potent PPs in rodents. No significant changes in the peroxisome number or volume occur in humans taking substantial doses of these drugs for extended periods of time (up to 3 years) (Ashby *et al.*, 1994). Two human epidemiology studies showed no indication of an increase in cancer associated with long-term human exposure (ranging up to eight years) to hypolipidemic drugs (Ashby *et al.*, 1994).

Rodents are poor models for human risk assessment with respect to liver effects observed with PPs. The reason for the non-responsiveness of humans to PPs is not yet fully understood; although, research shows differences in amount and expression of PPAR α between humans and rodents (Cattley *et al.*, 1998; Palmer *et al.*, 1998).

Induction of liver, testicular Leydig cell and pancreatic acinar cell tumors is a common finding for PPs (Cook *et al.*, 1999). In chronic bioassays in rats, Cook *et al.* (1999) reported that 7 out of 11 PPs induced all three tumor types (Cook *et al.*, 1999), and 10 of the 11 PPs produced liver and Leydig cell tumors (Cook *et al.*, 1999).

Cancer

The oncogenicity of PFOA has been investigated in two separate two-year feeding studies in rats. PFOA was found to increase the incidence of three tumor types (liver, Leydig cell, and pancreatic acinar cell tumors-Riker, 1983, Biegel *et al.*, 2001). In the following discussion, each tumor type will be discussed in turn.

Hepatocellular Adenoma

In a chronic dietary study conducted with 156 male Sprague Dawley rats fed diets containing 300 ppm PFOA for two years (Biegel *et al.*, 2001), histopathological evaluation revealed PFOA-related increases in hepatocellular adenoma. Hepatocellular adenoma occurred at an incidence of 13 % (10/76) as compared to 3 % (2/80) and 1% (1/79) in *ad libitum* and pair-fed controls, respectively.

These liver tumors are believed to have resulted from peroxisome proliferation. Evidence for this comes from the measurement of hepatocellular peroxisome proliferation at three-month intervals during the study. Increased liver weights and hepatic β -oxidation activity were observed in the PFOA-treated rats at all time points; however, PFOA did not significantly increase hepatic cell proliferation. It is generally agreed that liver tumors in rats produced by PPs are unlikely to be relevant to humans.

Human Experience with Regard to PFOA and Liver Toxicity

Several worker studies investigated the possible association between either liver cancer or liver-related disease with PFOA exposure and have shown no association. Exposures to PFOA in

these workers, as measured by serum PFOA concentration starting in 1993, ranged from less than 1 to 114 ppm (Olsen *et al.*, 2000, 2001a, 2001c, 2003a, 2003b). PFOA was not measured routinely prior to 1993 because a total organofluorine method was used. Past serum PFOA concentrations in workers may have been higher.

Epidemiological assessments of liver cancer deaths among 3M workers with potential exposure to PFOA have not shown significantly increased Standardized Mortality Ratios (SMRs) for liver cancer; although, very few deaths from liver cancer were expected. Among 182 workers identified with definite PFOA exposure at 3M's Cottage Grove plant, there were no deaths related to liver cancer or cirrhosis of the liver during a 50-year time period (0.3 and 1.2 expected, respectively) (Alexander, 2001a). Among 1,491 workers with probable PFOA exposure, there was one liver cancer death compared to 2.0 expected (SMR = 0.5, 95% CI 0.0 - 2.0) and 6 deaths attributable to cirrhosis of the liver (6.4 expected, SMR = 1.0, 95% CI 0.4-2.1).

At 3M's Decatur plant, PFOA has been used as an emulsifier in fluoropolymer production and has also been a residual by-product of perfluorooctanesulfonyl fluoride production. PFOA production did not occur until the late 1990's. Employee serum PFOA concentrations have ranged up to 13 ppm in sampling conducted in 1998 and 2000. In this population, there were two liver cancer deaths observed compared to 0.7 expected (SMR = 3.1, 95% CI 0.4-11.1) during a 38-year study period (1961-1998) of 1,065 workers (Alexander, 2001b). It is unlikely that these observations represent a response to PFOA.

Analysis of episodes of care (health claims data) over a six-year interval (1993-1998) of a subset of these Decatur workers (n = 652) did not show differences in reported disorders of the liver (cirrhosis and hepatitis) between this Decatur fluorochemical workforce and a comparison non-exposed workforce (Decatur film plant employees) (Olsen *et al.*, 2001b). There was a nonsignificantly increased risk ratio (1.6, 95% CI 0.8-2.9) of episodes of care of disorders of the biliary tract reported in 13 individuals in the fluorochemical plant (N = 652). This episodes of care risk ratio increased to 2.6 (95% CI 1.2-5.5) when restricted to the 211 fluorochemical workers with ≥ 10 years work experience (based on eight individuals' health claims data). An episodes of care study has not been done for Cottage Grove or Antwerp fluorochemical production workers.

Hepatic clinical chemistry test results have been reported in a series of cross-sectional assessments of medical surveillance examinations for both the Cottage Grove and Decatur employee populations as well as the fluorochemical production workforce located in Antwerp (Gilliland & Mandel, 1996; Olsen *et al.*, 2000; 2003b). None of these study populations have had changes in hepatic enzyme assays or bilirubin analyses that could be associated with measured serum PFOA concentrations after adjusting for potential confounding factors including body-mass index and alcohol consumption. Serum PFOA concentrations in 3M Antwerp workers were approximately half of those measured in the Decatur workforce (Olsen *et al.*, 2001a, 2001c, 2003b).

Liver Tumor Summary

In summary, the lack of indications of increased risk of liver disease in 3M workers with exposure to PFOA suggests that the exposures encountered by non-occupationally exposed individuals should present a low risk of liver disease and, by extension, liver cancer. The lack of genotoxicity observed in genotoxicity assays and the increase in peroxisome proliferation observed in the lifetime dietary study in rats suggests a potential mechanism for the increase in hepatocellular adenoma in rats. If peroxisome proliferation is involved in the etiology of the hepatocellular adenoma observed in rats, the risk of hepatocellular adenoma developing in exposed humans is expected to be quite low due to the much lower-degree of response to PPAR α agonists in human liver.

Leydig Cell Tumors

Two chronic studies in Sprague Dawley rats have shown increases in hyperplasia and benign tumors (adenoma) of testicular Leydig cells. In the first study (Riker, 1983), the incidence of Leydig cell adenomas was 0/50, 3/50, and 7/50 at dosages of 0, 30, and 300 ppm PFOA, respectively. A second study by DuPont included numerous mechanistic endpoints (i.e., cell proliferation, hepatic enzyme measurements, hormone measurements) and was specifically designed to evaluate the mechanism of Leydig cell tumor induction (Biegel *et al.*, 2001). In this study, PFOA was administered at 0, 0-pair-fed, or 300 ppm PFOA to male rats. There was an increase in the incidence of Leydig cell hyperplasia and adenomas, with adenoma incidences of 0/80, 2/78, and 8/76 in the 0, 0-pair-fed, or 300 ppm PFOA group, respectively (Biegel *et al.*, 2001).

Experimental evidence for the mechanism of PFOA-induced Leydig cell tumor formation, while not conclusive, tends to support the hypothesis that a sustained increase in estradiol within the testes may be responsible for the increased incidence of Leydig cell tumors in male Sprague Dawley rats (Cook *et al.*, 1992; Biegel *et al.*, 1995; Liu *et al.*, 1996a, 1996b). The extent to which this effect may be linked to PPAR α activation is not clear. Other PPs (DEHP and clofibrate) have been shown to increase serum estradiol concentrations in male rats (Eagon *et al.*, 1994; Rao *et al.*, 1984), and several PPs (e.g., clofibrate, DEHP, gemfibrozil, dibutyl phthalate, and Wyeth 14,643) have been shown to reduce estradiol metabolism, resulting in an increase in circulating levels of estradiol (Corton *et al.*, 1997; Eagon *et al.*, 1994; Fan *et al.*, 1998; Rao *et al.*, 1984). This pattern of hormonal alteration has also been observed *in vitro*, where 10 of 11 peroxisome proliferators evaluated increased estradiol levels, and 11 of these PPs decreased testosterone levels (Liu *et al.*, 1996a, 1996b). While most PPs may increase estradiol in rats, the direct association of elevated estradiol with the production of Leydig cell tumors remains to be demonstrated. There are seven proposed mechanisms for Leydig cell tumorigenesis in rodents, all of which disrupt the hormonal milieu within the testes (Clegg *et al.*, 1997; Cook *et al.*, 1999). The attribution of sustained estradiol increase as part of the response to PPAR α activation and as the operative mechanism for PFOA-induced Leydig cell tumors as well as the relevance of these tumors to humans will require additional research.

Human Experience with Testicular Tumors

Testicular cancer is most commonly diagnosed under the age of 40 in humans (Schottenfeld, 1996). Ninety-five percent of neoplasms of the testes arise from germinal cells and are divided

clinically into the seminoma and a variety of pure and mixed types of nonseminomatous tumors. Non-germinal neoplasms constitute 5% of testicular tumors with approximately half of these being histologically classified as Leydig cell tumors. Mortality data do not adequately explain occupational risk for testicular cancer because of the high five-year survivability rates for testicular cancer (> 95% survival). Thus, it is not unexpected that there has been only one death attributable to testicular cancer among the 3M Cottage Grove fluorochemical production workers (0.4 expected) during a 50-year study period (Alexander *et al.*, 2001a) and no deaths due to testicular cancer observed among the Decatur occupational population (0.2 expected) in a 38-year study period (Alexander *et al.*, 2001b). Analysis of episodes of care among the Decatur population from 1993-1998 did find two individuals with health claims data coded to testicular cancer (0.6 expected) (Olsen *et al.*, 2001b). One of these two workers had ≥ 10 years of work experience in the fluorochemical plant.

As noted previously, there are no direct associations of PFOA exposure with changes in sex hormones. A 10% increase in mean estradiol level observed among employees who had the highest levels of serum PFOA was confounded by body mass index and likely was not due to PFOA exposure (Olsen *et al.*, 1998).

Testicular Tumor Summary

Although Leydig cell tumors have been observed in two cancer studies in rats, the occurrence of this tumor type in humans is rare. There is currently no evidence that a relationship between PFOA exposure and increased testicular cancer risk exists in humans. In addition, no hormonal changes that may be mechanistically related to testicular cancer have been observed in monkeys or humans with PFOA exposure.

Pancreatic Acinar Cell Tumors

Male Sprague Dawley rats fed diets containing 300 ppm PFOA for two years (Biegel *et al.*, 2001), exhibited an increase in pancreatic acinar cell adenoma and combined pancreatic acinar cell adenoma/carcinoma. Acinar cell adenoma incidence was 9 %, 0%, 1% in PFOA-treated rats, *ad libitum* fed controls, and pair-fed controls, respectively. A prior two-year dietary bioassay in male and female Sprague Dawley rats at 30 and 300 ppm PFOA did not result in an increase in pancreatic tumors (Riker, 1983); although, a subsequent pathology peer review has noted the presence of hyperplastic foci.

Pancreatic acinar cell tumors (Reddy & Rao, 1977) are often observed following chronic exposure of rodents to other PPs. The mechanism by which PFOA and some other PPs induce these tumors is not well understood. The development of these tumors is known to be modified and/or mediated by several factors such as steroid hormone levels, growth factors such as cholecystokinin (CCK) and dietary fat (Obourn *et al.*, 1997). Biegel *et al.*, (2001) have proposed that PFOA and other PPs could increase the fat content in the gut and stimulate CCK release that, in turn, could lead eventually to hyperplasia in the pancreatic acinar cells. It must be concluded that, at the present time, this is a speculative mechanism that is not supported by experimental evidence for PFOA (Biegel *et al.*, 2001; Butenhoff *et al.*, 2002) and its applicability to humans is uncertain (Gavin *et al.*, 1996, 1997; Cattley *et al.*, 1998; Pandol, 1998). Pancreatic

acinar cell adenomas are rare in humans (Anderson *et al.*, 1996) and when considering the relevance of this rat tumor data with regard to human health risk, the non-genotoxic mechanism (with a likely threshold), and the relatively low exposure in humans should be taken into account.

Human Experience with Pancreatic Disease

The pancreatic acinar cell tumors observed in PFOA-treated rats (Biegel *et al.*, 2001) are not commonly diagnosed in humans. Among the Cottage Grove workforce with definite PFOA exposure (n = 182), there was one death reported for pancreatic cancer compared to 0.8 expected (SMR = 1.3, 95% CI 0.0-7.4) (Alexander *et al.*, 2001a). Employees (n = 1,491) defined with probable PFOA exposure had six deaths attributable to pancreatic cancer compared to 4.8 expected (SMR = 1.4, 95% CI 0.5 - 2.7). These pancreatic cancers were likely to have been of ductular origin rather than acinar. At the 3M Decatur manufacturing site there were no deaths attributable to pancreatic cancer among the 1,065 employees with one expected (Alexander *et al.*, 2001b). One episode of care for pancreatic cancer has been reported (Olsen *et al.*, 2001b). Although the episodes of care risk ratio for acute pancreatitis was increased (2.6, 95% CI 0.6-15.8) among the fluorochemical production workforce, this effect is difficult to interpret because it is based on six health claims from just one employee.

Because a sustained elevation of CCK has been suggested as a potential mechanism for pancreatic cancer, plasma CCK levels were assayed in 74 Cottage Grove PFOA production workers participating in medical surveillance examinations in 1997 (Olsen *et al.*, 2000). CCK values (mean 28.5 pg/ml, SD 17.1, median 22.7 pg/ml, range 8.8-86.7 pg/ml) approximated the assay's reference range (up to 80 pg/ml) and were negatively, not positively, associated with employees' serum PFOA concentrations.

Pancreatic Tumor Summary

PFOA was associated with an increase in acinar cell tumors of the pancreas in rats in one of two separate two-year bioassays. This tumor type is rare in humans, and there is no epidemiological evidence for a relationship between PFOA exposure and pancreatic cancer. The relevance of acinar cell tumors of the pancreas in rats to human pancreatic cancer risk is uncertain.

Mammary Gland Tumors

In the 3M-cancer study with PFOA in Sprague Dawley rats (Riker, 1983), the incidence of fibroadenomas of the mammary gland apparently was increased in female rats (22%, 42%, and 48% at 0, 30, and 300 ppm in diet, respectively). There was no apparent difference in incidence over a ten-fold dose range. The authors of this study concluded that the mammary tumor data did not reflect an effect of PFOA.

The laboratory conducting the study, Riker Pharmaceuticals, did not have an adequate historical control database. However, untreated control rats (same strain and supplier) from 13 chronic toxicity/oncogenicity studies conducted at Haskell Laboratory from 1984-87 provided 947

control rats, which were on test for at least one year (scheduled sacrifice at two years). Charles River, the supplier, also maintains a control database.

Statistical evaluation of the incidence of fibroadenomas in the PFOA-treated groups versus the Haskell Laboratory historical controls was not significant ($p = 0.3$). The incidence of fibroadenomas in the 13 reference Haskell laboratory studies ranged from 24 to 54% with a mean of 37%. In the PFOA study, the control group incidence lies just below and the test group incidences lie near the top of the control range. The incidences in the PFOA-treated groups (42 and 48%) are similar to the average of the Haskell Laboratory historical control groups (37%).

Historical control data posted on the Charles River Laboratories Web-Site, gives the average fibroadenoma incidence of 41% with a range among 24 studies of 13 - 61%. These data further support the study authors' conclusion that the distribution of fibroadenomas in the PFOA study were a reflection of background incidence and were not related to PFOA treatment.

When all mammary tumors of epithelial origin in this study are combined, there is no statistically significant increase in total tumors. Mammary tumors in rats present as a continuum from benign to malignant. In composition. They range from tumors of primarily epithelial cells to various degrees of connective tissue involvement. From a biological perspective, both adenomas and fibroadenomas are classified as benign fibroepithelial tumors, and, when combined for the PFOA study is not statistically increased. Similarly, there is no biological difference between the terms adenocarcinoma and carcinoma. The data for total malignant tumors shows a lower incidence of malignant tumors in the high-dose compared to the control animals (17, 31, 11% in the 0, 30, 300 ppm groups).

Human Experience with Breast Cancer

The available human data do not suggest an increased breast cancer risk. There have been no breast cancer deaths observed among Cottage Grove workers identified with definite PFOA exposure (0.2 expected) and two breast cancer deaths observed among those with probable PFOA exposure (3.6 expected, SMR = 0.6, 95% CI 0.1 - 2.0) (Alexander, 2001a). There have been no breast cancer deaths in the Decatur fluorochemical production workforce (0.9 expected) (Alexander, 2001b). There were two episodes of care for breast cancer (3.5 expected) among a subset of the Decatur fluorochemical production workforce compared to zero episodes of care in the comparison film plant employee population (4.0 expected) (Olsen *et al.*, 2001b). One of these individuals had worked ≥ 10 years. As for benign neoplasms of the breast, the risk ratio was 1.1 (0.4-2.8) based on nine individual episodes of care in the Decatur fluorochemical plant and ten individual episodes of care in the film plant. Non-malignant disorders of the breast were slightly higher among Decatur fluorochemical female employees as the episodes of care risk ratio was 1.6 (95% CI 0.9-2.9) based on 28 individual episodes of care in the chemical plant and 19 individual episodes of care in the film plant. The majority of these episodes of care were identified as fibrocystic disease.

Mammary Tumor Summary

In summary, the tumors seen in the mammary glands of rats fed PFOA reflect background incidence.

Prostate Tumors

An epidemiological investigation of the Cottage Grove chemical division workforce associated prostate cancer mortality with employment duration in perfluorochemical production activities (Gilliland, 1992; Gilliland & Mandel, 1993). Specifically ≥ 10 years of employment was associated with a 3.3 fold increase (95% CI 1.0 -10.6) in prostate cancer mortality relative to workers not employed in the chemical division. A major limitation of this investigation, with regard to evaluating the potential effects of PFOA exposure, was the lack of job and department specificity in the duration of employment analyses. Only one Cottage Grove employee had worked directly in the PFOA production building (Olsen *et al.*, 1998). Alexander (2001a) addressed this limitation by computerizing all work history records of Cottage Grove employees with at least one year of cumulative employment and constructing a calendar year, job- and department- specific exposure matrix from this computerized database. Alexander (2001a) did not find prostate cancer mortality associated with duration of employment among those Cottage Grove employees with definite or possible exposure to PFOA (cases observed/expected): 0 - < 1 year (0/0.1), 1 - < 5 years (2/1.4), 5 - < 10 years (0/9.8) and ≥ 10 years (4/2.9). The SMR was 1.4 (95% CI 0.4 - 3.5) for prostate cancer in the ≥ 10 year duration category. The Alexander (2001a) investigation improved upon the methods used for exposure assessment, nevertheless, some misclassification of exposure is likely. Maintenance and other mobile workers not specifically identified as definitely PFOA exposed workers may have routinely entered the areas of high exposure (drying and packaging). The extent to which this misclassification occurred and the effects on the study results is unknown.

Among the Decatur fluorochemical production workforce, there have been no prostate cancer deaths (1.0 expected) (Alexander, 2001b). In the episodes of care investigation of this same workforce with 10 or more years of experience, however, a risk ratio of 8.2 (0.8-399) was reported for prostate malignant neoplasms based on 4 episodes of care among fluorochemical workers (1.5 expected) compared to 1 episode of care among the comparison film plant workers (3.1 expected) (Olsen *et al.*, 2001b). On the other hand, there was no evidence of prostatic hypertrophy as the episodes of care risk ratio was 1.0 (95% CI 0.6-1.5) based on 24 individual episodes of care in the Decatur fluorochemical plant and 52 episodes of care in the film plant.

Conclusions

At the exposure levels encountered in either the workplace or the environment, PFOA does not appear to present a human health risk. The chemical is not genotoxic in assays measuring various endpoints and utilizing test systems ranging from bacteria to mammals. The developing fetus is not uniquely sensitive to the effects of PFOA. Indications of a fetal response are seen only under dosing/exposure conditions in which the adult animal is also responding. No evidence of structural abnormalities produced by *in utero* exposure to PFOA exists from animal tests. Clearly, the effects observed in the two-generation reproduction study (decreased pup weights, increased pup mortality, and sexual maturation delays only at the 30 mg/kg dose) did not compromise the reproductive success (i.e., mating and fertility) of PFOA-exposed rats. With

respect to the human experience there is no evidence of increases in episodes of medical care related to either developmental or reproductive health matters. In addition, evaluation of the hormonal status of 3M workers from the Cottage Grove, MN plant did not reveal any changes in sex hormones associated with PFOA exposure.

In the long-term studies with PFOA in rats, the incidence of tumors of the liver, pancreas, and testes was increased. An apparent increase in mammary fibroadenomas, seen in the PFOA-treated females, was the result of an unusually low incidence of fibroadenomas in this particular control group. The incidence of mammary tumors in all test groups was within the range expected for this strain of rat based on historical control data.

The tumors whose incidence is increased in rats treated with PFOA (liver, testes and pancreas) are frequently observed in rats treated with PPs. It is generally recognized that rats have a heightened response to peroxisome proliferators relative to other species, including man, due in part due to their higher level of expression of the nuclear receptor PPAR α . Because of the increased sensitivity of rats to PPs, the human significance of these three tumor types is not clear. With respect to the liver, tumors observed in rats result from PPAR α activation and are unlikely to be relevant to humans. The relevance to humans of pancreatic acinar cell tumors and Leydig cell tumors is also questionable. In addition, available data for humans who have had long-term treatment with hypolipidemic drugs (which are potent peroxisome proliferators in rats) show no increase in these three cancers associated with their long-term use.

Studies of workers, believed to be the highest exposed human population, have not shown an increased cancer risk. Mortality studies show no increase in any cancer that could be associated with PFOA exposure. In addition, the episodes-of-care study and clinical studies of workers do not reveal any indications of PFOA-related response of liver, testes, and pancreas.

In summary, it can be concluded from toxicological studies that PFOA is non-genotoxic, the fetus is not uniquely sensitive, and reproductive success is not compromised. The tumor types produced by PFOA in rats are associated with peroxisome proliferation, a response that is not readily induced in man. Thus, combined with comparatively lower exposures in humans, it is unlikely that PFOA will have an adverse impact on human health with regard to these endpoints.

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