January 20, 2006

The Honorable Stephen L. Johnson, Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: SAB Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts

Dear Administrator Johnson:

EPA's Office of Pollution Prevention and Toxics (OPPT) requested that the U.S. EPA Science Advisory Board (SAB) review the Agency's *Draft Risk Assessment of Potential Human Health Effects Associated with Perfluorooctonoic Acid (PFOA) and Its Salts*. PFOA is a synthetic (man-made) chemical and does not occur naturally in the environment. It is used in the manufacture of several commercially important products. The draft risk assessment document evaluates the potential human health effects associated with exposure to PFOA and its salts. An internal dose measure from human biomonitoring studies was used to estimate PFOA exposure levels for children and adults in the general population. Margin of Exposure (MOE) values for non-cancer effects were then estimated by comparing the human exposure levels to exposure levels from animal studies showing no observable adverse health effects. The mode of action (MOA) for tumor formation in animals was discussed and a variety of health effects were considered. The SAB PFOA Risk Assessment Review Panel was asked to comment on the scientific soundness of the Agency's draft risk assessment.

In response to the Agency's request, the SAB PFOA Review Panel met face-to-face and subsequently via telephone. The SAB panel, considering only peer-reviewed published evidence, commented on the Agency's proposals related to pharmacokinetics and cross-species extrapolation and inclusion of non-cancer health effects in its risk assessment approach. The SAB panel also provided recommendations with respect to the weight of evidence supporting the Agency's proposed MOA for liver tumors in rats, the appropriate carcinogenicity descriptor, appropriate endpoints for inclusion in the risk assessment, and the usefulness of human biomonitoring data for estimating exposure and identifying health effects. The SAB panel agreed that further research on PFOA was needed and provided specific recommendations as follows:

- The Agency has proposed that there is sufficient weight of evidence to establish that the mode of action for liver tumor induction in rats following exposure to PFOA is peroxisome proliferator-activated receptor **"** (PPAR**"**) agonism. The predominant view of the SAB was that while the current data support the Agency's proposed MOA for liver tumor induction, other mechanisms that may be relevant to humans could also play a role and should be considered. A few members, on the other hand, believed the evidence was sufficient to support the proposed MOA as a sole basis for liver tumor induction.

- The Agency has proposed that the PFOA cancer data may be best described as providing "*suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*" under the EPA Guidelines for Carcinogen Risk Assessment. The predominant SAB view was that the experimental weight of evidence regarding the human carcinogenic potential of PFOA was more consistent with the Agency's descriptor of "likely to be carcinogenic" as described in the EPA Guidelines for Carcinogen Risk Assessment since laboratory studies in rats show that PFOA is a multi-site and multi-gender carcinogen. A few members did not find the weight of evidence sufficient to support the 'likely' descriptor and agreed with the Agency's "suggestive" descriptor.
- The Agency has proposed the use of several endpoints from different life stages, species and genders for the risk assessment. For adults, endpoints were selected from the non-human primate and rat studies; the endpoints included liver toxicity and possibly mortality for the non-human primates and decreased body weight for rats. The SAB agreed with the Agency's inclusion of non-cancer endpoints. They also recommended that cancer endpoints (liver, testicular, pancreatic acinar, and mammary), as well as, other endpoints i.e., neurotoxicity, immunotoxicity and hormonal effects of PFOA be included in the risk assessment. Additionally, the SAB recommended that occupational biomonitoring data be included for identifying potential human health effects.
- In the draft assessment, the Agency uses internal dose metrics from animal toxicology studies and measures obtained from human biomonitoring studies to derive the MOE values. Since there are a variety of approaches that may be appropriate, the SAB advised the Agency to present a clearer rationale for the approach selected, and, more importantly, to describe the impact of how the selected internal dose measure affects the magnitude of the MOE. The SAB also suggested that bench mark dose (BMD) methodologies would be preferable to the reliance in the draft document on the No-Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL) for the calculation of MOE values.
- Given the use of internal doses from animal toxicology studies and human biomonitoring studies to estimate exposure, the Agency sought advice on the need to retain or modify the default uncertainty value for cross species extrapolation. While the SAB considered the use of internal dose metrics in this analysis a significant step toward reducing uncertainty related to cross species extrapolation, it nevertheless believed that significant knowledge gaps still remain that merit retention of the default uncertainty value.
- The draft document utilizes currently available non-occupational human biomonitoring data in its estimation of the MOE. The predominant SAB view recommends that further analysis of biomonitoring data from populations living near exposure sources be conducted and used to calculate MOEs for these highly exposed populations. Some, however, felt that the data were equivocal and therefore not amenable for use in calculating MOEs.

Finally, the SAB wishes to strongly encourage the Agency to continue to strengthen its risk assessment by incorporating additional data as it becomes available. EPA is to be complimented for using a novel approach to estimate internal dose. We look forward to working with the Agency to improve these efforts in the future.

Sincerely,

Dr. Granger Morgan, Chair
EPA Science Advisory Board

Dr. Deborah Cory-Slechta, Chair
PFOA Risk Assessment Review Panel
EPA Science Advisory Board

Executive Summary

EPA's Office of Pollution Prevention and Toxics (OPPT) requested that the Science Advisory Board review the "Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctonoic Acid (PFOA) and its Salts" (hereafter referred to as the "draft PFOA risk assessment document"). The PFOA Review Panel of the EPA Science Advisory Board met in February 2005 at which time nine charge questions raised by OPPT were deliberated. These questions focused on four issues including, a peroxisome proliferator-activated receptor **"** (PPAR-alpha) mode of action (MOA) for rodent liver tumors, carcinogenicity descriptors, useful models for evaluation of health effects, toxicokinetic considerations and reliance on currently available human biomonitoring exposure data for calculation of margins of exposure (MOEs). Further discussions of the entire Panel were held during a conference call in July 2005.

This Executive Summary highlights the outcome of the Panel's deliberations. It includes the context for the charge questions and issues raised for consideration by OPPT, and the conclusions reached by the SAB panel. It is important to note that all of the key findings and recommendations from the Panel deliberations were based on currently available peer-reviewed, published data with the understanding that further risk assessment will proceed as more data on PFOA health effects become available.

Issue 1. Rodent PPAR-alpha Mode of Action for Hepatocarcinogenesis:

In rats, PFOA induces liver adenomas, Leydig cell tumors (LCT) and pancreatic acinar cell tumors (PACT). The draft document concludes that these tumors constitute a triad and are the result of a PPAR-alpha agonism MOA. In this MOA, activation of PPAR-alpha leads to cell proliferation and decreased apoptosis, clonal expansion of preneoplastic foci and subsequent tumors. The draft document premises its conclusions about this MOA on studies showing that PFOA is a potent peroxisome proliferator in liver of rats and mice and, like other peroxisome proliferators, induces hepatomegaly in rats. In addition, requisite dose-response and temporal associations for some key events for this MOA have been reported.

Comment on the Weight of Evidence and Adequacy of the Data Available to Identify the Key Events for the PPAR-alpha agonist-induced Rodent Liver Toxicity and Hepatocarcinogenesis for PFOA.

The Panel's charge was to determine whether it agreed with the weight of evidence supporting a PPAR-alpha MOA for rodent liver toxicity and hepatocarcinogenesis. Panel members agreed that, considered collectively, evidence to date was consistent with an interpretation that liver tumor induction likely results from a PPAR-alpha MOA. This is based on the observations that PFOA activates the receptor, results in peroxisome proliferation, increases beta-oxidation and produces hepatomegaly, with dose and temporal responses consistent with the PPAR-alpha MOA. These events, moreover, depend upon a functional PPAR-alpha receptor, and no other known MOA, e.g., DNA reactivity or mutagenicity, has been identified.

However, with respect to uncertainties and limitations related to concluding that PPAR-alpha is the ***sole*** MOA for rodent liver tumor induction and toxicity, Panel views

diverged.

Most Panel members believed that at the current time, sufficient uncertainties and limitations of the data still exist with respect to reaching such a conclusion, given that: 1) In contrast to what would be predicted, administration of PFOA, but not the prototype PPAR-alpha agonist WY-14,643, increased liver weights in PPAR-alpha receptor knockout mice, i.e., in mice where PPAR-alpha activation was precluded, raising the possibility that PFOA-induced liver tumors could occur by PPAR-alpha independent effects. The significance of this finding currently remains uncertain in the absence of a corresponding assessment of histopathology or replication by another laboratory. 2) There is as yet no published evidence that the induction of PPAR-alpha by PFOA results in clonal expansion of pre-neoplastic foci which is considered a critical step in the proposed MOA. 3) There are no data demonstrating increased cell proliferation and/or decreased apoptosis in the liver of PFOA-treated rats, key causative events in the proposed MOA.

These Panel members also viewed two additional issues as requiring further consideration. One is the relevance of the PPAR-alpha MOA to humans. Given that human exposures to PFOA and related chemicals appear ubiquitous, uncertainties and limitations of the data for children have not been adequately characterized to be able to conclude that the PPAR-alpha MOA is not operative in this young age group. A secondary issue thought to require additional characterization in the PFOA response was the potential role of Kupffer cells, resident macrophages in the liver that do not express PPAR-alpha, but are activated by peroxisome proliferators.

A different view expressed by a few Panel members was that the observation in PPAR-alpha knockout mice of increased liver weights in response to PFOA, but not to the prototype PPAR-alpha agonist WY-14,643, was not sufficiently significant to undermine the view that PPAR-alpha agonism is the sole MOA for PFOA-induced rodent liver tumors.

In summary, Panel members agreed that collectively the weight of evidence supports the hypothesis that liver tumor induction in rodents by PFOA is mediated by a PPAR-alpha agonism MOA. Most Panel members, however, also felt, based on current evidence, that it is possible that PPAR-alpha agonism may not be the sole MOA for PFOA, that not all steps in the pathway of PPAR-alpha activation- induced liver tumors have been demonstrated, that other hepatoproliferative lesions require clarification, and that extrapolation of this MOA across the age range in humans is not supported. A few panel members did not share these reservations about a PPAR-alpha agonism MOA for PFOA-induced rodent liver tumors.

Issue 2: Descriptor for Carcinogenic Potential

The draft document reaches the conclusion of 'suggestive' evidence of carcinogenicity but not sufficient to assess human carcinogenic potential of PFOA. This conclusion was based upon: 1) a PPAR-alpha MOA for liver tumors in rodents that was considered not relevant to humans because of their decreased sensitivity to PPAR-alpha agonism when compared to rodents, 2) the absence of hepatic cell proliferation in a 6 month study of PFOA administration in cynomologous monkeys, the species considered closest in physiology to humans; 3) the absence of a strong association between PFOA exposure and tumors in human studies as interpreted in the draft document; 4) the belief

that the LCT and PACT tumors produced by PFOA in rats were probably not relevant to humans based on the lower levels of expression of the mediators leutinizing hormone (LCT) and choleocystokinin growth factor receptors (PACT) in humans, as well as differences in quantitative toxicodynamics between rats and humans; and 5) the view that mammary fibroadenomas reported in female rats are equivocal based on their comparable rates of occurrence relative to a historical control group.

Comment on the Proposed Descriptor for the Carcinogenic Potential of PFOA

Most Panel members concluded that the experimental weight of evidence with respect to the carcinogenicity of PFOA was stronger than proposed in the draft document, and suggested that PFOA cancer data are consistent with the EPA guidelines descriptor 'likely to be carcinogenic to humans'. According to EPA's Guidelines for Carcinogen Risk Assessment[1] (also known as EPA's Cancer Guidelines), this descriptor is typically applied to agents that have tested positive in more than one species, sex, strain, site or exposure route, with or without evidence of carcinogenicity in humans. Conclusions of these Panel members were based on the following:

- While human data are ambiguous, two separate feeding studies in rats demonstrate that PFOA is a multi-site carcinogen.
- Uncertainties still exist (see Issue 1 comments) as to whether PPAR-alpha agonism constitutes the ***sole*** MOA for PFOA effects on liver. This was based on the fact that PFOA, but not the prototypical PPAR-alpha agonist, WY-14,643, increases liver weights in PPAR-alpha knockout mice, a finding of uncertain significance in the absence of liver histopathology and replication of this finding. Further, mitochondrial proliferation was suggested in the document as a basis of liver toxicity in monkeys exposed to PFOA.
- The exclusion of mammary tumors in the draft document based on comparisons to historical control levels from other laboratories was deemed inappropriate, since the most appropriate control group is a concurrent control group. Using that comparison, increases in both fibroadenomas (22%, 42% and 48% for rats treated with 0, 30 and 300 ppm APFO (ammonium perfluorooctanoate or C8, the ammonium salt of PFOA), respectively) and adenocarcinomas (15, 31% and 11%, respectively) were seen in the Sibinski *et al.* (1987) 2 yr PFOA feeding study.
- Insufficient data are currently available to determine the MOA for the observed Leydig cell tumors, pancreatic acinar cell tumors and mammary gland tumors. In the absence of a defined MOA for these tumor types, they must be presumed to be relevant to humans, as suggested by EPA's Cancer Guidelines.

Given the current data base, these Panel members were not willing to ascribe an associated probability value to the potential for PFOA-induced carcinogencity. Nevertheless, based on available evidence to date, most Panel members believed that risk assessments for each of the PFOA-induced tumors are appropriate at the current time.

[1] In March 2005, EPA published final Cancer Guidelines and Supplemental Guidance which can be found at the following URL: http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283

A different view expressed by a few Panel members was that currently available evidence does not exceed the descriptor "suggestive" of carcinogenicity, based on the belief that PPAR-alpha agonism does serve as the sole MOA for PFOA-induced rodent liver tumors (Issue 1) and that mammary tumors were not demonstrated in animals when compared to historical controls. Thus, these members did not believe the evidence exceeded the draft document descriptor of "suggestive".

Issue 3: Selection of Endpoints

The draft document proposes the use of multiple endpoints from several life stages, species and gender for risk assessment. No specific recommendations on the most appropriate parameters are stipulated at the current time.

Comment on the:

Selection of Toxicity Endpoints for the Risk Assessment
The Most Appropriate Lifestage/Gender/Species for Assessing Human Risk
The Appropriateness of the Available Animal Models

The Panel agreed with the current approach of inclusivity, given the current uncertainties noted above with respect to carcinogenicity, as well as the paucity of information on potential PFOA effects on non-cancer endpoints. Similarly, no exclusion of species should be considered at present, and differences between genders as demonstrated in rat studies again suggest multiple MOAs for PFOA. The use of multiple animal models is appropriate particularly in light of the reported differences in toxicokinetics in rats, non-human primates and humans. Resolution of most appropriate parameters must await additional research, but the process will be facilitated by the ability to measure internal dose.

Panel members did not reach full agreement as to endpoints that should be included for risk assessment and the significance of occupational biomonitoring data. Most Panel members supported the inclusion of multiple cancer endpoints and liver histopathology as well as consideration of the data from occupational and epidemiological studies. While the draft document notes that the occupational studies suffer from the fact that they involve multiplicity of exposures, other studies have shown a high correlation among fluorinated compounds in biological samples from the general population and occupational cohorts. Therefore, these human studies could be advantageous for assessing potential interactions among these compounds that may be associated with adverse human health effects. These Panel members also believed that epidemiologic and occupational studies could not be disqualified without disqualifying virtually all such studies in the risk assessment process. Moreover, it is clear that occupationally-exposed populations have experienced the highest levels of exposure and therefore reported health effects in these studies merit consideration.

A contrasting view expressed by a few Panel members was that the outcomes from studies of human health effects of PFOA were equivocal, and thus these endpoints should not be incorporated into the risk assessment process.

Panel members agreed in encouraging additional research, including PPAR-alpha mediated and independent effects of PFOA. Non-carcinogenicity endpoints merit additional attention for several reasons. It is not yet known whether carcinogenicity will represent the most sensitive endpoint for PFOAs. Immunotoxicity has been reported, and

derivations of MOEs for such effects were encouraged by many Panel members. Given the prevalence in brain of PPAR receptors, including PPAR-alpha, effects on nervous system structure and function warrant attention. Moreover, no information currently exists with respect to critical periods; therefore, it is important to evaluate effects across age groups. The observations of hormonal alterations in treated animals also deserve further study to assess their importance.

Issue 4: Risk Assessment Approach

Issue 4a: Pharmacokinetic Modeling and Use of AUC as a Measure of Internal Dose

The draft document compares internal dose metrics from animal toxicology studies and human biomonitoring studies for purposes of ultimately generating margin of exposure (MOE) information. Area under the concentration curve (AUC) was calculated from PFOA serum levels in human biomonitoring studies assuming a steady state. In some of the rat studies, serum PFOA concentrations were available, or it was considered that sufficient pharmacokinetic information was available to estimate serum levels. For this purpose, AUC was estimated from a pharmacokinetic model. Specifically, compartmental modeling of serum concentrations using single dose rat oral exposure studies were used to estimate internal dosimetry for the longer term dosing studies based upon the premise that pharmacokinetic information for rats and humans is sufficient for this purpose and that this approach does not exceed the limits of the available data.

Comment on the Use of the One Compartment Pharmacokinetic Model

The Panel concluded that the empirical model used in the draft document was adequate for predicting blood levels resulting from repeated dosing, but that this fitting procedure is specific to this limited data set and this particular application. Concern was expressed, therefore, that use of the descriptor "one compartment" to describe PFOA pharmacokinetics in the draft document is misleading, given the actual complexities in many of the available datasets, and the term should be removed or replaced unless carefully qualified.

Comment on the use of the AUC as a Measure of Internal Dose for Rats and Humans for Calculation of the MOE

The Panel observed that while calculating blood AUC may be an appropriate method to estimate internal dose, it is important to note that at the current time information on PFOA health effects is limited. As additional data become available, other measures may also be appropriate, such as the C_{max}, the integrated dose above a minimum concentration, etc. Regardless of the choice for the measure of internal dose, a clearer rationale needs to be presented for the approach taken, and, importantly, for any choice adopted, the impact of the internal dose measure on the magnitude of the MOE should be described. The Panel also believes that caution should be exercised in assuming that the form of PFOA in blood, i.e., free compound or PFOA bound to various proteins or lipids, is constant in serum across the period of observation, given the current information on metabolism.

Issue 4b: Cross Species Extrapolation

In extrapolating data from animal experiments to humans, a default value of 10 is typically applied, with a factor of 3 for differences in toxicodynamics and a value of 3 for toxicokinetic differences. In the PFOA draft risk assessment document, internal doses from animal toxicology studies and human biomonitoring studies were compared. Derivation of data from animal toxicology studies included both measured PFOA serum levels from non-human primates and derived values from pharmacokinetic modeling from rat studies. The reliance on internal dose metrics was considered by OPPT to be sufficient to reduce uncertainties and therefore raised the question of the ability to either eliminate or reduce the default values for cross species extrapolation.

Comment on the Need to Use or Modify the Default Value of 10 for Cross Species Extrapolation Given the Pharmacokinetic Analysis

The use of internal dose metrics in this analysis was considered by the Panel to be a significant step toward reducing uncertainty related to the toxicokinetic uncertainty associated with interspecies extrapolation. Nevertheless, it did not believe that the direct use of blood concentration in the assessment sufficiently reduced the overall uncertainly to eliminate or modify the current default value. Significant uncertainties still remain, including the measured internal dose that best predicts adverse effects in human and other species, the bias inherent in measurement/modeling errors, the lack of information about non-cancer endpoints, developmental vulnerability and the impact of gender, and the multiple PFOA environmental exposures that occur in humans vs. animals, among others. The assumption that PFOA serum levels are at steady state in children 2-12 years of age has not been tested and may not be valid. The Panel likewise stressed that bench mark dose methodologies would be preferable to the reliance in the draft document on LOAEL-driven MOE calculations.

Issue 4c: Human Biomonitoring Data

Currently available data on PFOA levels in humans includes occupational biomonitoring studies as well as three population studies within the U.S. The measurements from the population studies come from: 1) samples from 6 American Red Cross blood banks; 2) a study of Streptococcal A infection in children; and 3) elderly volunteers in a cognitive study in Seattle. The draft EPA document only utilizes data derived from 1 and 2 above in its calculation of the MOE. Occupational biomonitoring data were excluded from the calculation because it was stated that sample sizes were small, data on gender were not available, and that blood monitoring data obtained from 2000 would overestimate current serum levels, since PFOA exposure of this group ceased in 2002. Measured levels from the elderly population were not utilized because values were considerably lower, for unknown reasons, than those reported in the other population studies for adults and children. From the other two population studies utilized in the draft document, geometric means and 90th percentiles were calculated across genders for calculation of MOEs.

Comment on the Adequacy of the Human Exposure Data for Use in Calculating a MOE

Panel members were not in full agreement as to the adequacy of the human exposure data for inclusion in the MOE calculation. Many Panel members shared concerns about the approach adopted in the draft document. One concern related to the generality of the populations currently included in the MOE calculation. It was noted, for example, that use of the blood donor and pediatric biomonitoring data may be acceptable if the purpose is to assess whether there is a potential health effect to the "general" population, although there is some question as to the size of other non-occupational populations that might be more highly exposed and the assumption that PFOA serum levels are at steady state may not be valid for children. Many Panel members agreed that existing subpopulations of the general public are more highly exposed than those studied and results from occupational studies should be included in the MOE calculation. The concern that serum levels from occupational cohorts in 2000 would overestimate current exposure levels implies that exposure levels in the future will not reach or exceed past exposures, which cannot be assured. A differing view expressed by some Panel members was that the human biomonitoring data are equivocal and thus not useful to MOE calculation.

Three different summary statistics are presented in the draft document and used in the calculation of the MOE. Of these, the Panel questioned the use of mean values, particularly geometric means in the calculations. Additionally, no rationale was provided for the choice of the 90^{th} percentile as a summary statistic, rather than the use of a higher value. Whatever the approach adopted, justification must be provided for the chosen summary measure and an explicit objective for the MOE analysis described.

INTRODUCTION

This report was prepared by the Science Advisory Board (SAB) PFOA Risk Assessment Review Panel (the "Panel") in response to a request by EPA's Office of Pollution Prevention and Toxics (OPPT) to review their *Draft Risk Assessment of the Potential Human Health Effects Associated With Exposure to Perfluorooctanoic Acid (PFOA) and Its Salts*. According to the document, OPPT has been investigating PFOA and its salts to try to understand the health and environmental issues presented by fluorochemicals, in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS). PFOA and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses. PFOA and its salts are persistent in the environment.

OPPT identified 4 issues where they were seeking the SAB's advice and recommendations. These included the proposed mode of action, carcinogenicity descriptors, toxicological endpoints selected and the pharmacokinetic modeling methods used in the risk assessment. OPPT's assessment focused on the potential human health effects associated with exposure to PFOA and its salts. Several toxicological endpoints and hypothesized modes of action were considered. Internal dose metrics were estimated for animal toxicology studies with pharmacokinetic modeling, and were obtained from human biomonitoring studies, assuming steady state. Margin of Exposure (MOE) values were calculated from the internal dose metrics. The SAB PFOA Review Panel was asked to comment on the scientific soundness of this risk assessment.

The Panel deliberated on the charge questions during their February 22-23, 2005 face-to-face meeting and during a conference call on July 6, 2005. The responses that follow represent the views of the Panel. In all cases, there was agreement by a majority of the panel members as to a particular recommendation. In some cases, there were one or more panel members that had a differing point of view; these instances have been noted throughout the report. The specific charge questions to the Panel are as follows:

Issue 1: Rodent PPAR-alpha Mode of Action for Hepatocarcinogenesis

The postulated mode of action (MOA) of PPAR**"**-agonist induced liver toxicity and liver tumors in rodents involves four causal key events. The first key event is activation of PPAR**"** (which regulates the transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism). Activation of PPAR**"** leads to an increase in cell proliferation and a decrease in apoptosis, which in turn leads to preneoplastic cells and further clonal expansion and formation of liver tumors. Of

these key events, only PPAR" activation is highly specific for this MOA while cell proliferation/apoptosis and clonal expansion are common to other modes of action. There are also several "associative" events that are markers of PPAR" agonism but are not directly involved in the etiology of liver tumors. These include peroxisome proliferation (a highly specific indicator that this MOA is operative) and peroxisomal gene expression.

Information that provides evidence that any specific chemical is inducing liver toxicity and tumors via a PPAR" agonist MOA includes *in vitro* evidence of PPAR" agonism (i.e., evidence from an in vitro receptor assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The *in vivo* evidence should demonstrate dose-response and temporal concordance between precursor events and liver tumor formation. Other information that is desirable and may strengthen the weight of evidence for demonstrating that a PPAR" agonist MOA is operative includes data on hepatic CYP4A1 induction, palmitoyl CoA activity, hepatocyte hypertrophy, increase in liver weights, decrease in the incidence of apoptosis, increase in microsomal fatty acid oxidation, and enhanced formation of hydrogen peroxide.

OPPT has proposed that there is sufficient weight of evidence to establish that the mode of action for the liver tumors (and precursor effects) observed in rats following exposure to PFOA is PPAR" agonism.

Question 1 - Please comment on the weight of evidence and adequacy of the data available to identify the key events for the PPAR" agonist-induced rodent liver toxicity and hepatocarcinogenesis for PFOA. Discuss whether the uncertainties and limitations of these data have been adequately characterized.

Issue 2: Descriptor for Carcinogenic Potential

Carcinogenicity studies in Sprague-Dawley rats show that PFOA induces a "tumor triad" similar to a number of other PPARα agonists. This "tumor triad" includes liver tumors, Leydig cell tumors (LCT), and pancreatic acinar cell tumors (PACT). OPPT has proposed that there is sufficient evidence to conclude that the liver tumors are due to PPAR"-agonist MOA, and that this MOA is unlikely to occur in humans based on quantitative differences between rats and humans. In addition, the LCT and PACT induced in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative toxicodynamic differences between the rat and the human. Overall, based on no adequate human studies and uncertain human relevance of the tumor triad (liver, Leydig cell and pancreatic acinar cell tumors) from the rat studies, OPPT has proposed that the PFOA cancer data may be best described as providing "*suggestive evidence of carcinogenicty, but not sufficient to assess human carcinogenic potential*" under the interim 1999 EPA Guidelines for Carcinogen Risk Assessment, as well as the 2003 draft EPA Guidelines for Carcinogen Risk Assessment.

Question 2 - Please comment on the proposed descriptor for the carcinogenic potential of PFOA.

Issue 3: Selection of Endpoints

OPPT has proposed the use of several endpoints from several life stages, species and gender for the risk assessment. For this draft assessment, OPPT has not made specific recommendations on the most appropriate endpoint/lifestage/species/gender. Rather, all have been presented to provide transparency.

For adults, endpoints were selected from the non-human primate and rat studies; the endpoints included liver toxicity and possibly mortality for the non-human primates and decreased body weight for rats.

For developmental endpoints, OPPT relied upon the definition of developmental toxicity outlined in the Agency's Developmental Toxicity Risk Assessment Guidelines. These guidelines state that the period of exposure for developmental toxicity is prior to conception to either parent, through prenatal development and continuing until sexual maturation. (In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism). Based on this definition of developmental exposure, OPPT considered developmental effects in the rat two-generation reproductive toxicity study to include reductions in F1 mean pup body weight (sexes combined) on lactation days 1, 5 and 8, an increase in mortality during the first few days after weaning (both sexes), a delay in the timing of sexual maturation (both sexes), and a reduction in mean body weight postweaning (F1 males only).

Question 3 - Please comment on the selection of these toxicity endpoints for the risk assessment.

Question 4 - Given the available data to date, please comment on the most appropriate lifestage/gender/species for assessing human risk.

Question 5 - Please comment on the appropriateness of the available animal models. Please comment on whether additional animal models should be investigated, and if so, what information would better enable us to ascertain potential human risks.

Issue 4: Risk Assessment Approach

A margin of exposure (MOE) approach can be used to describe the potential for human health effects associated with exposure to a chemical. The MOE is calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL or LOAEL. In this risk assessment there is no information on the sources or pathways of human

exposure. However, serum levels of PFOA, which are indicative of cumulative exposure, were available from human biomonitoring studies. In addition, serum levels of PFOA were available for many of the animal toxicology studies or there was sufficient pharmacokinetic information to estimate serum levels. Thus, in this assessment internal doses from animal and human studies were compared; this is analogous to a MOE approach which uses external exposure estimates.

Issue 4a: Pharmacokinetic Modeling and Use of AUC as a Measure of Internal Dose

As noted above, internal dose metrics from animal toxicology studies and human biomonitoring studies were compared in this draft assessment. For humans, the area under the concentration curve (AUC) was calculated from measured PFOA serum levels in human biomonitoring studies, assuming steady state. For the rat toxicology studies, the area under the concentration curve (AUC) and C_{max} were estimated from a pharmacokinetic model. The pharmacokinetic analysis could be done using a number of approaches including non-parametric analysis, physiologically based pharmacokinetic (PBPK) modeling, and classical compartmental modeling. Each has strengths and limitations given the available data. Non-parametric analyses provide a description of the data that have been collected, but have fairly limited ability to make predictions across species or to account for variations in exposures. PBPK modeling is perhaps the ideal approach for addressing PFOA for purposes of cross-species extrapolation. Extensive pharmacokinetic studies have been undertaken in rats demonstrating complex phenomena including high tissue concentrations in liver, kidney and serum and enterohepatic recirculation of the parent compound. These could be addressed using PBPK modeling for the rats, but the more limited information in monkeys and humans would either require substantial assumptions or preclude use of this approach. Classical compartmental modeling can be used to analyze the existing data on blood concentrations in rats, monkeys, and humans. Currently, the available pharmacokinetic information for rats and humans is sufficient to support compartmental modeling. Comparisons of serum protein binding across species indicated a high degree of binding in all species but also interspecies differences in the percentage of unbound PFOA in plasma. In light of the documented differences in clearance of PFOA across sexes in rats and across species, compartmental modeling of serum concentrations provides a sound approach for estimating internal dosimetry without exceeding the limits of the available data, so this approach was selected for this risk assessment.

Question 6 - Please comment on the use of the one compartment pharmacokinetic model.

Question 7 - Please comment on the use of the AUC as a measure of internal dose for rats and humans for calculation of the MOE.

Issue 4b: Cross Species Extrapolation

Judgments about the "adequacy" of a MOE are based on many considerations including uncertainty associated with cross species extrapolation. Typically, a value of

10 is considered which consists of a value of 3 for toxicodynamics and a value of 3 for toxicokinetics. Each of these can be decreased or increased if there are data to warrant it. In this draft assessment, internal doses from animal toxicology studies and human biomonitoring studies were compared. For humans, the internal doses were based on measured PFOA serum levels in human biomonitoring studies. For the non-human primate toxicology studies, internal doses associated with the NOAEL and/or LOAEL were based on measured PFOA serum levels. For the rat toxicology studies, pharmacokinetic modeling was used to estimate an internal dose metric associated with a NOAEL or LOAEL.

Question 8 - Please comment on the need to use or modify the default value of 10 for cross species extrapolation given the pharmacokinetic analysis.

Issue 4c: Human Biomonitoring Data

For this draft assessment, human biomonitoring data of PFOA serum levels were available for adults and children. Similar analytical methods were used to measure the PFOA levels in both sets of blood samples. The adult data included 645 U.S. adult blood donors (332 males, 313 females) from 2000-2001, ages 20-69, obtained from six American Red Cross blood banks located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD. Each blood bank provided approximately 10 samples per 10-year age interval (20-29, 30-39, etc.) for each sex.

The children's data included a sample of 598 children, ages 2-12 years old, who had participated in a study of group A streptococcal infections. The samples collected in 1994-1995 from children residing in 23 states and the District of Columbia were analyzed for PFOA in 2002.

Question 9 - Please comment on the adequacy of the human exposure data for use in calculating a MOE.

RESPONSES TO THE CHARGE QUESTIONS

Issue 1: Rodent PPAR-alpha Mode of Action for Hepatocarcinogenesis and Liver Toxicity

Question 1. *Please comment on the weight of evidence and adequacy of the data available to identify the key events for the PPAR alpha agonist induced rodent liver toxicity and hepatocarcinogenesis for PFOA. Discuss whether the uncertainties and limitations of these data have been adequately characterized.*

As discussed in the EPA Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and its Salts, a sequence of four key events define the mode of action (MOA) by which PPAR-alpha agonists induce rodent liver tumors. According to this MOA, the initial causal event is (1) activation of PPAR-alpha, which regulates the expression of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism. These transcriptional events lead to (2) increased cell proliferation and/or decreased cell death. The chronic increase in cell growth occurs primarily in the preneoplastic focal lesions in the liver resulting (3) in the clonal expansion of the preneoplastic lesions, which ultimately results (4) in the development of hepatocellular neoplasms. In addition, there are "associative" events that may or may not be causally linked to the PPAR-alpha MOA for hepatocarcinogenesis which include blockage of cell to cell communication, an increase in peroxisomes, an increase in peroxisomal enzymes, and liver and hepatocyte hypertrophy.

The Panel agreed that, considered collectively, the weight of evidence to date is consistent with the assertion that PFOA is a PPAR-alpha agonist and can induce liver changes in adult rats that have been associated with PPAR-alpha activation. As discussed in the draft PFOA risk assessment, some of the key elements to establish this MOA have been demonstrated by appropriate experiments. In vitro studies demonstrate that PFOA is a PPAR-alpha agonist, and treatment of rats and/or mice results in peroxisome proliferation, increased beta-oxidation, and hepatomegaly, with dose and temporal responses consistent with this MOA for liver tumor induction. Studies comparing PPAR-alpha null and wild-type mice showed that PFOA-induced peroxisome proliferation, beta-oxidation, and immunotoxicity depend on the presence of a functional receptor. Further, no other established modes of action of liver cancer-induction have been reported for PFOA. PFOA is neither DNA reactive nor mutagenic, and thus not involved in a genotoxic mode of action; nor is the liver neoplastic effect due to the induction of repeated hepatocyte death and compensatory regeneration (a cytotoxic mode of action). No PPAR-alpha independent MOA for the rat liver tumor induction has been proposed.

With respect to the weight of evidence and the adequacy of consideration of uncertainties and limitations, however, the Panel did not reach full agreement. Many Panel members believed that data gaps still exist and not all of the causal events in the PPAR-alpha MOA have been demonstrated for PFOA. These include the induction of cell proliferation in the liver at early times following PFOA treatment and/or modulation

of apoptosis in hepatocytes. They also shared the belief that while the PFOA Draft Risk Assessment in general appropriately discusses the uncertainties and limitations of the data that support a PPAR-alpha MOA for PFOA-induced liver tumors in adult rats, it fails to consider three issues contrary to this MOA in sufficient detail.

First, in a study by Yang *et al.* (2002) cited in the report in the context of the receptor dependence of PFOA immunotoxicity, PPAR-alpha null mice exhibited >2-fold increases in liver weight but no peroxisomal acyl CoA oxidase induction in response to PFOA. No increase in liver weight was observed in PPAR-alpha null mice treated with the well-characterized prototype PPAR-alpha agonist, WY-14,643. While this finding is of uncertain significance, due to the lack of histopathology and the absence of a second study showing such an effect, it nevertheless raises the possibility that PFOA may induce some of its effects in mouse liver by a PPAR-alpha-independent pathway. This observation and the associated uncertainty were not mentioned in the context of liver tumor induction in the draft PFOA risk assessment. Secondly, uncertainties exist with respect to the relevance to exposed infants and children of the PPAR-alpha agonist MOA for induction of liver tumors in adults. Humans are refractory to some, but not all, PPAR-alpha activation effects. Data from studies using PPAR-alpha receptor knockout mice have shown that these receptors are essential for the rapid induction of liver neoplasms after exposure to WY-14,643. However, humans do have functional PPAR-alpha receptors, leaving unanswered the question as to why they respond so differently from rats and mice to PPAR-alpha agonists. Available data suggest that the difference between humans and rats or mice may be a consequence of a lower number of PPAR-alpha receptors such that the PPAR-alpha MOA is not considered likely to yield a similar hepatic cancer response in adult humans. However, exposures of neonates and children to PFOA remain a potential concern. Rat studies suggest similar PPAR-alpha receptor levels in neonates and adults, but because adult humans have so few receptors, and information in neonates and children is minimal, this same extrapolation cannot be made in humans. Given that human exposures to PFOA and related chemicals appear ubiquitous, uncertainties and limitations of the data for children have not been adequately characterized to be able to conclude that the PPAR-alpha MOA is not operative in this young age group.

Second, the current draft PFOA risk assessment states (page 76 lines 15-16) that the "[a]ctivation of PPAR-alpha leads to an increase in cell proliferation and a decrease in apoptosis, which in turn leads to preneoplastic cells …" Questions were raised as to whether there is available experimental evidence that the induction of PPAR-alpha results in an increase in the number of preneoplastic foci. The effect of the PPAR-alpha activation appears to be at the level of focal lesion clonal expansion (Klaunig *et al.*, 2003), however clonal expansion of focal lesions, which is not unique to a PPAR-alpha MOA, has not been shown in rats treated with PFOA.

Thirdly, some Panel members felt that the role of Kupffer cells (shown in Figure 1, page 78 of the draft document) should be discussed in the text of the draft PFOA risk assessment. There is an extensive literature on the essential role of Kupffer cells in signaling peroxisome proliferator-induced hepatocyte proliferation. Studies have shown

that hepatocyte proliferation and peroxisome proliferation occur by different mechanisms. Parzefall *et al.* (2001) and Hasmall *et al.* (2001) demonstrated that peroxisome proliferators had no effect on DNA synthesis but still induced peroxisomal acyl CoA oxidase activity in cultured rat and mouse hepatocytes that had been purified to remove contaminating Kupffer cells. Kupffer cells, which are resident macrophages in the liver, are a major source of growth factors (tumor necrosis factor alpha, interleukins) that induce DNA synthesis or suppress apoptosis in purified hepatocytes. A key finding relevant to the proposed MOA is that Kupffer cells do not express PPAR-alpha (Peters *et al.*, 2000), but are activated by peroxisome proliferators. Prevention of Kupffer cell activation by glycine inhibited, although not completely, the development of liver tumors by the potent peroxisome proliferator, WY-14,643 (Rose *et al.*, 1999). There are no data available on the effects of peroxisome proliferators on human Kupffer cells. Recognizing the role of Kupffer cell activation in the induction of DNA synthesis and subsequent neoplastic development by PPAR-alpha agonists, some members of the FIFRA Science Advisory Panel (2003) [SAP Minutes No. 2003-05] noted that the interplay between PPAR-alpha agonism and Kupffer cells has not been characterized. Thus, the results from the PPAR-alpha null mouse are not directly applicable to the human situation in which PPAR-alpha is present and can be activated.

A different conclusion was reached by a few of the Panel members who found that the weight of evidence was adequate to support a PPAR-alpha agonism mode of action for PFOA-induced rodent liver tumors. In this view, the observation of increased liver weights in response to PFOA but not to the prototype PPAR-alpha agonist WY-14,643 in PPAR-alpha knock-out mice as reported in Yang *et al.* (2002) did not merit significance because the study was not designed to evaluate liver toxicity, and the observation represents a single replication without corresponding histopathology at the current time. Nor was the possible role of Kupffer cells considered to be significant. Based on these considerations, these Panel members believed that PPAR-alpha agonism can be considered the sole MOA for PFOA-induced rodent liver tumors.

Issue 2: Descriptor for Carcinogenic Potential

Question 2. *Please comment on the proposed descriptor for the carcinogenic potential of PFOA.*

The draft PFOA risk assessment proposes that the PFOA cancer data may be best described as providing "*suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*" under the interim 1999 EPA Guidelines for Carcinogen Risk Assessment (US EPA, 1999), as well as the 2003 draft EPA Guidelines for Carcinogen Risk Assessment (US EPA, 2003). This opinion is based on the view that human studies on PFOA do not provide adequate support of carcinogenicity, as well as on the quantitative differences between rats and humans that OPPT believes raises uncertainties about the human relevance of the "tumor triad" response (liver tumors, Leydig cell tumors, and pancreatic acinar cell tumors) of PPAR-alpha agonist activation in rats.

The determination of an appropriate descriptor for the carcinogenic potential of PFOA was discussed by the Panel in the context of the available carcinogenicity data, an evaluation of mechanistic or MOA data, and guidance on how EPA applies various descriptors for summarizing weight of evidence data. Panel members did not achieve full agreement on the appropriate descriptor. Based on the above considerations, the predominant Panel view was that the descriptor "likely to be carcinogenic" was more consistent with currently available data, while a few Panel members reached the conclusion that the current evidence fails to exceed the descriptor "suggestive" of carcinogenicity.

Cancer studies on PFOA

Carcinogenicity studies in Sprague-Dawley rats have shown that PFOA induces neoplasms at multiple sites. In male rats exposed to 0 or 300 ppm ammonium perfluorooctanoate (APFO) in the feed for 2 years, increased incidences of testicular Leydig cell tumors (LCT) (0% vs. 11%), pancreatic acinar cell tumors (PACT) (0% vs. 11%), and liver adenomas (3% vs. 13%) were observed in treated animals compared to controls (Biegel *et al.*, 2001). In a 2-year study in which male and female Sprague-Dawley rats were fed diets containing 0, 30 or 300 ppm APFO, a dose-related increase in LCT was observed (0% in controls, 4% at 30 ppm, 14% at 300 ppm) (Sibinski *et al.*, 1987). The draft PFOA risk assessment does not address the effects in the liver observed in the Sibinski *et al.* (1987) study. In that study, the incidences of hepatocellular carcinoma in male rats were 6%, 2%, and 10%, and although no adenomas were diagnosed, the incidences of hyperplastic nodules in the liver were 0%, 0%, and 6%. Hyperplastic nodules may be part of the continuum of proliferative lesions in the liver carcinogenic process.

In female rats, a dose-related increase in mammary gland fibroadenomas was reported (22% in controls, 42% at 30 ppm, and 48% at 300 ppm) by Sibinski *et al*. (1987). In addition, the incidence of mammary gland adenocarcinomas was greater in the low dose group than in controls (15% in controls, 31% at 30 ppm, and 11% at 300 ppm). The draft PFOA risk assessment did not consider the mammary gland neoplasms to represent a compound-related effect because of high background rates reported for fibroadenomas in Sprague-Dawley rats in historical control data (37%) reported for female rats in the Haskell Laboratory in 1987 (Sykes). Many Panel members did not believe that historical control comparisons are as reliable as concurrent controls. A number of parameters may contribute to inter-laboratory differences in tumor response including differences in diet, animal age at the start and termination of studies, animal supply sources and breeding practices, environmental conditions, vehicles and routes of administration, animal care procedures that may affect weight gain and survival, and the use of different substrains. Thus, the concurrent control group is the most appropriate group for evaluations of chemical-related effects. Moreover, in the historical database of Chandra *et al*. (1992), the incidence in controls of mammary gland fibroadenomas was 19.0% and the incidence of adenocarcinomas was 8.8% in female Sprague-Dawley rats. Therefore, a neoplastic effect in the mammary gland is apparent in the Sibinski study in comparison to Chandra *et al.* (1992). Many Panel members therefore believe that the

elevated tumor rates observed in female rats in the Sibinski *et al.* (1987) study raise concerns for neoplastic effects induced by PFOA in the mammary gland that should not be dismissed. In addition, many Panel members agreed that EPA should sponsor an independent histopathology review of the male rat livers and the female mammary glands from the Sibinski study and re-analyze the resulting tumor incidence data.

A few Panel members believe that the comparison of the Sibinski *et al.* (1987) mammary tumor data to the historical control data (Sykes, 1987) in the draft risk assessment document was valid.

Mode-of-action analysis, uncertainties, and human relevance

The PFOA draft risk assessment proposes that there is sufficient evidence to conclude that liver tumors induced by PFOA are due to a proposed PPAR-alpha agonist MOA (Klaunig *et al.*, 2003), and that this MOA is unlikely to occur in humans based on quantitative differences in the numbers of PPAR-alpha receptors between rats and humans. In addition, the PFOA draft risk assessment proposes that the Leydig cell tumors (LCT) and pancreatic acinar cell tumors (PACT) induced in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative toxicodynamic differences between the rat and the human. Thus, the panel examined issues related to our understanding of the MOA for the multiple tumor types induced by PFOA in rats and the impact of available information on determining the human relevance of the animal tumor responses.

Liver adenomas.

As noted under Issue 1, the Panel concurred that the collective evidence is consistent with the hypothesis of a PPAR-alpha agonist MOA for PFOA with associated peroxisomal β-oxidation activity, increases in absolute and relative liver weight, and liver tumors in Sprague-Dawley rats. Issues on which the Panel members opinions diverged related to whether a PPAR-alpha agonist MOA for liver tumor induction in rats might occur in humans and/or whether additional MOAs might be involved.

***Key events in the PPAR-alpha agonist MOA*.**

The PFOA risk assessment did not identify dose-response data showing increases in hepatocyte proliferation and suppression of apoptosis in rats exposed to PFOA. Many Panel members believed this to be a critical deficiency, because these are key events in the proposed MOA linking activation of PPAR-alpha to the liver tumor response.

Another observation that influenced most Panel members with respect to potential human relevance of the response in rats is the observation that the same early effects actually occur in monkeys exposed to PFOA. These effects include the induction of peroxisomal β-oxidation activity (2.6 fold), significant increases and positive dose-response trends for absolute and relative liver weights (1.6 fold), and the return of relative liver weight to control levels after a 13-week recovery period. Cell proliferation was evaluated in monkeys but only after 6 months of exposure. Unfortunately, neither the rat nor the monkey studies provided data on hepatocyte proliferation during the first 1-2 weeks of exposure, or direct measurements of apoptotic cells during or after exposure to

PFOA was stopped. The lack of data on cell proliferation and apoptosis in animals exposed to PFOA makes it impossible to analyze dose-response concordance between these key events and tumor induction for PFOA in relation to other PPAR-alpha agonists. Because the available data for PFOA in rats and monkeys indicate similar responses in the livers of rodents and primates (increased liver weight and induction of hepatic peroxisomal enzyme activity), many Panel members shared the view that human relevance for liver effects induced by PFOA by a PPAR-alpha agonism MOA cannot be discounted.

Some panel members, however, considered the increase in liver weight in rats exposed to PFOA and the return to control levels following an 8-week recovery period (Palazzolo, 1993) to be consistent with an increase in cell proliferation and suppression of apoptosis by PFOA during the exposure period. In addition, the lack of an increase in hepatic cell proliferation in rats after 1 month or more exposure to PFOA (Biegel *et al.*, 2001) was considered consistent with observations of a transient increase in hepatocyte proliferation with other peroxisome proliferators.

PPAR-alpha -independent liver effects.

As noted in response to Issue 1, many members of the Panel shared the view that significant uncertainties still exist with respect to the predictability of a PPAR-alpha agonist MOA for human cancer risk associated with exposure to PFOA. In a comparative study of PFOA and the prototype PPAR-alpha agonist Wy-14,643, at doses of each chemical that produced increases in liver weight and peroxisomal fatty acid acyl-CoA oxidase activity in wild-type mice, only PFOA caused a similar 2-fold increase in liver weight (but no increase in acyl-CoA oxidase activity) in PPAR-alpha null mice (Yang *et al.*, 2002). While this study was not designed to assess liver toxicity, it confirms that PFOA is a PPAR-alpha agonist for peroxisomal enzyme induction, and also indicates that liver changes induced by PFOA in rodents can occur by a mechanism that is independent of PPAR-alpha activation. The lack of liver enlargement or tumor response in PPAR-alpha null mice exposed to Wy-14,643 for 11 months has been cited frequently as evidence that liver cancer induction by peroxisome proliferators is mediated by PPAR-alpha activation (Peters *et al.*, 1997). The study of Yang *et al.* (2002) needs to be replicated, but appears to suggest that results with Wy-14,643 in PPAR-alpha null mice do not predict all of the potential liver effects of PFOA.

Further, while not diminishing the conclusion that a PPAR-alpha MOA is operative in the rodent liver carcinogenesis induced by PFOA, many Panel members expressed concern over the as yet incomplete understanding of the role of Kupffer cells in the carcinogenic process. PPAR-alpha independent stimulation of hepatocyte growth factor production in Kupffer cells appear to be essential to the mechanism of hepatocyte replicative DNA synthesis, suppression of apoptosis, and liver tumor development by peroxisome proliferators. Until the interplay between PPAR-alpha agonism and Kupffer cell activation is characterized, negative results from the PPAR-alpha null mouse may not be relevant to the human situation in which Kupffer cells and hepatocellular PPAR-alpha are present and can be activated.

A few members of the Panel believed that the finding of increased liver weights produced by PFOA in PPAR-alpha knockout mice, as noted for Issue 1, were not significant enough to undermine the PPAR-alpha agonism MOA, nor did they consider the absence of information about Kupffer cell activation to be relevant to a PPAR-alpha agonism MOA for PFOA-induced rodent liver tumors.

LCTs, PACTs, and mammary neoplasms.

Panel members did not consider the consolidation of liver tumors, LCTs, and PACTs into a triad MOA to be justified. They believed that available evidence is inadequate to support a PPAR-alpha agonist MOA for the induction of LCTs and PACTs (Klaunig *et al.*, 2003), and, at present, available data are insufficient to characterize the MOA for PFOA-induced LCTs and PACTs. As such, a specific MOA needs to be worked out for each tumor type. In addition, most members felt that the appropriate comparison for mammary neoplasms was to concurrent not to historical controls, and in that view subscribe to the interpretation that PFOA does increase mammary gland neoplasms, and no MOA data are available for the mammary tumor response. As discussed in EPA's Cancer Guidelines, in the absence of sufficient data to establish a MOA, the animal tumor responses are presumed to be relevant to humans.

A few Panel members believed, in contrast to the above view, that the comparison of PFOA-induced mammary tumor levels to historical controls was valid, and thus deemed the evidence for mammary neoplasms to be insufficient to demonstrate such tumors in response to PFOA. This served to support the view of these members that PPAR-alpha agonism represented the sole MOA for PFOA-induced rodent liver tumors.

Application of cancer descriptors

The meaning of terms such as "suggestive evidence of carcinogenic potential" or "likely to be carcinogenic to humans" may differ among some in the general public and the EPA because of differences in perception and intent. Hence, EPA recommends a weight-of-evidence narrative that explains the complexity of issues influencing an agent's carcinogenic potential in humans. Descriptors are applied to provide consistency across agents that are evaluated for their carcinogenic potential. In developing their cancer risk assessment guidelines (US EPA 1999, 2003), EPA has not provided definitive criteria for choosing a descriptor; however, examples of the types of evidence that would be covered by a descriptor are listed. EPA also cautions that terms such as "likely," when used as a weight-of-evidence descriptor, do not correspond to a quantifiable probability.

Most Panel members shared the view that while human cancer data on PFOA are inadequate to support a definitive conclusion of the presence or absence of a causal association, the animal data are much stronger than the examples summarized in the EPA's Cancer Guidelines under the descriptor "suggestive evidence of carcinogenic potential." The descriptor "suggestive" is typically applied to agents that show a marginal increase in tumors only in a single animal study or a slight increase in a tumor response at a site with a high background rate. The animal data for PFOA are consistent with the examples listed by EPA under the descriptor "likely to be carcinogenic to humans" applied to agents that tested positive in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans; or a positive

study that indicates a highly significant result and where the response is assumed to be relevant to humans. These members concluded, as described above, that data from two separate feeding studies demonstrate that PFOA is a multisite carcinogen in rats. Significant increases in tumor incidence and dose-response trends were observed in male and female rats. Some of the tumor responses were observed at sites with low background rates; the incidence of PACTs and LCTs in control rats was 0% at both sites. Because available data are insufficient to characterize the MOA for PFOA-induced LCTS, PACTs, or mammary tumors, the responses at these sites are presumed to be relevant to humans. Uncertainties also still exist for the MOA(s) for liver tumors induced by PFOA.

While opting for the descriptor "likely to be carcinogenic to humans" these Panel members were not willing to state an associated probability value for PFOA-induced carcinogenicity; nor do the EPA guidelines require a quantifiable probability. This group also encouraged a cancer risk assessment for each of the PFOA-induced tumors where data permit. The risk characterization narrative should address the state of knowledge and uncertainties in the MOA for each tumor site and a range of approaches should be considered in the cancer dose-response assessment.

A few Panel members did not find the weight of evidence strong enough to exceed the descriptor "suggestive". This view was based on the belief that mammary tumors were not demonstrated in animals when compared to historical controls, and the opinion that the sole MOA for the rodent-induced liver tumors was through PPAR-alpha agonism which they believe was not relevant to humans.

Issue 3: Selection of Endpoints

Question 3. *Please comment on the selection of these toxicity endpoints for the risk assessment.*

The Panel generally agreed with the Agency approach of considering multiple endpoints and developing multiple margin of exposure (MOE) values at this stage in the assessment of potential human health effects associated with PFOA. With regard to the selection of endpoints, the initial overall philosophy should be one of inclusivity. That is, endpoints should be considered unless evidence for an effect by PFOA is equivocal or the dose associated with the effect is sufficiently high that other effects will clearly be of greater concern. The reason for being inclusive is not to generate an exhaustive catalog of PFOA effects, but rather to insure that sensitive effects (i.e., effects occurring at relatively low doses) are not overlooked or prematurely excluded from the assessment.

The Panel agreed with inclusion of all of the endpoints in the current draft of the risk assessment. None were recommended for deletion. However, caveats regarding the use of organ and body weights as endpoints were offered. Organ and body weights are often among the least sensitive endpoints for chemicals that exert specific effects on physiological or developmental systems. Nevertheless, in the absence of information

with which to select more specific endpoints (e.g., biochemical or histological changes), body and organ weight changes are likely to be indicative of toxicity.

Many members of the Panel also believed that additional endpoints should be included in the risk assessment, although recognizing that this may not be possible for some endpoints because of the absence of sufficient information:

- Based on discussion in response to Question 2, PFOA has the potential to produce carcinogenic effects in humans and therefore additional cancer endpoints (liver, testicular, pancreatic acinar, and mammary) should be included in the risk assessment.
- Liver histopathology, other than cancer, should be included as an endpoint since it could not be concluded with confidence that all liver effects are mediated through PPAR-alpha agonism (see response to Question 1), and therefore liver histopathology from PFOA may be relevant to humans. The Panel recognized that interpretation of some liver changes as adverse effects may not always be apparent (e.g., liver enlargement with no other pathology), and this should be discussed in the risk assessment.
- Immunotoxicity should be considered as an endpoint addressed quantitatively in the risk assessment. The Panel recognizes that in order to be incorporated into the risk assessment, immunotoxicity data will need to be derived in rats, or approaches developed for the estimation of serum PFOA concentrations in mice.
- Consideration should be given to addition of endpoints related to lipid metabolism.

In the view of a few Panel members who believed that data for PFOA was consistent with the descriptor "suggestive" rather than "likely" to be carcinogenic in humans, the cancer endpoints noted above were not recommended for inclusion in the risk assessment as they were deemed not to be independent PFOA effects.

The Panel agreed that additional research on PFOA was needed, and encouraged studies in the areas noted below; the Panel also encouraged exploration of methods to identify critical targets for PFOA beyond a PPAR-alpha MOA:

- Other than ataxia, no data on neurotoxicity endpoints for PFOA are available. Neurotoxicity endpoints, including behavioral measures, should be added to the risk assessment. PPAR-alpha receptors, as well as other PPAR receptors, are found in both neurons and glia, and are found in multiple brain regions (frontal cortex, basal ganglia, reticular formation). It has been proposed that, in addition to their roles common to other tissues, these receptors in brain may have specific functions in the regulation of genes involved in neurotransmission (Moreno *et al.*, 2004). This would further suggest their importance in behavioral function.
- The two-generation rat study (Butenhoff *et al.*, 2004) involved both perinatal PFOA exposure and direct PFOA dosing of the F1 offspring beginning at weaning. The Panel recognized that this approach is consistent with U.S. EPA guidance regarding developmental studies. However, consideration should be given to using developmental endpoints in F1 generation animals prior to initiation of direct dosing so that potential effects associated with perinatal

exposure can be more clearly identified.

- Current data suggest that PFOA might produce hormonal effects that would be important to consider, but in most cases the significance of the observations are unclear. For example, in a 26-week study of PFOA administration to cynomolgus monkeys, serum TSH was slightly but significantly elevated in all treatment groups on the final day of the experiment, and serum thyroxin was slightly but significantly reduced (Butenhoff, 2002). It is not clear whether these observations are physiologically meaningful or whether they were strictly dependent upon treatment per se, since hormone levels appeared to change in the control animals during the course of the experiment as well. The analysis of Butenhoff data did not include a repeated measures ANOVA, so interactions were never pursued. Even that, however, would not have revealed why hormone levels changed over the course of the experiment in control animals. One study reported PFOA-induced decreases in pituitary weight in the F1 generation female rats, but the functional significance of this observation is unclear. Overall, the Panel thought that Margins of Exposure (MOEs) should not be calculated for hormonal endpoints at this time, but that additional research to clarify the hormonal effects of PFOA should be encouraged.
- Adult male rats exhibited a much slower elimination of the ammonium salt of PFOA, i.e., ammonium perfluorooctanoate (APFO or C8), than did females. This appears to be due to gonadal hormones inasmuch as castration increased APFO elimination and testosterone replacement returned the elimination rate toward normal levels. Importantly, renal elimination was blocked by probenecid, a selective antagonist of organic anion transporters (OATPs) (Shitara, 2004). Thus, gender differences in renal OATPs may account for the gender differences in renal clearance of APFO. Likewise, the slower clearance of APFO in males may account for the observation that lower doses of APFO produced adverse effects in males compared to females. For example, the NOAEL for APFO in a 13-week study of male CD rats was 0.56 mg/kg-day whereas females exhibited a NOAEL of 22.4 mg/kg-day. These results suggest that specific organs (e.g., liver, kidney, and perhaps adrenals) are targets of APFO because of the pattern of expression of the OATPs that transport it across the cells (OATP1-4 in rat). Research to identify the relationship between OATP and PFOA toxicity may offer insight into the most important targets for PFOA effects and the best endpoints for evaluation.

Question 4. *Given the available data to date, please comment on the most appropriate lifestage/gender/species for assessing human risk.*

In general, there was consensus that at this stage in the risk assessment process, no lifestage/gender/species should be excluded from consideration in predicting human risk. Moreover, absence of information identifying a "critical period" in development during which PFOA may exert adverse effects on development requires inclusion of all life stages. Biomonitoring data indicate children and adults alike exhibit measurable levels of PFOA in serum, and the half-life of PFOA appears to be around 4 years. Therefore, there is no reason to exclude any developmental period from examination.

Finally, the inclusion of data on internal dose is an important element of the dataset for PFOA which should enlighten concerns about the use of female rats, discussed below.

Two considerations arose in evaluating the current dataset for use in assessing human risk. In general, the EPA provides a margin of safety by using exposure values that produce the lowest margin of exposure based on observed effect levels, usually NOAELS or LOAELs, including those in animal models. There was general agreement that the most appropriate criterion for assessing human risk is one that produces the lowest margin of exposure (e.g., 90^{th}, 95^{th}, or 99^{th} percentile) based on a LOAEL in animal models. The second consideration related to the appropriateness of the non-human primate as a model, generally considered to be most comparable to humans.

With respect to the first, the emphasis is on having data based on the internal dose relationships (i.e., serum PFOA levels) in some of the animal studies so that interspecies differences in metabolism and clearance are taken into account. In addition, these data also allow using both males and females despite a dramatic difference in clearance rate. Also, considering empirical measures of exposures in children and adults, this view emphasizes a concern that both developmental and adult endpoints be captured, and these endpoints have not been evaluated in non-human primates. Therefore, the findings from adult male rats including the 13 week study by Goldenthal (1978) in which liver weight was significantly increased, and the F1 males in the two-generation reproductive toxicity study (Butenhoff *et al.*, 2004) in which body weight was reduced should be considered in further analysis of human health risk.

The second view emphasizes the biological similarities between non human primates and humans for risk assessment. This is particularly important in the case of PFOA because there are a number of issues with a rodent model for PFOA exposure; e.g., sexual dimorphism with respect to elimination of PFOA, and differences in sensitivity to PPAR-alpha signaling between rat and human. However, monkeys also exhibit a different half-life of PFOA than do humans, and information about the potential toxicity of PFOA on non-human primates are derived primarily from adults.

Question 5. *Please comment on the appropriateness of the available animal models. Please comment on whether additional animal models should be investigated, and if so, what information would better enable us to ascertain potential human risks.*

The available animal models are useful, but all are considered uncertain matches for humans with respect to PFOA toxicity. Thus, most Panel members supported continued use of multiple animal models and the need for additional models. As previously noted, some responses to PFOA may occur via modes of action not related to PPAR-alpha agonism. Without knowing how these PPAR-alpha independent effects are mediated, the ability to identify the specific animal models that would be most useful is limited. Some Panel members suggested the development and use of additional animal models without PPAR-alpha, such as transgenic or siRNA rats. Use of these animal models would be of assistance for more clearly identifying PPAR-alpha independent effects of PFOA.

Overall, the Panel thought that results obtained in models using female rats were informative because they currently provide the only indication of potential effects on endpoints specific to females (e.g., reproduction and developmental effects, mammary tumors). However, some concerns were noted regarding the difference in toxicokinetics of PFOA in female versus male rats and monkeys, with females exhibiting more rapid excretion.

As part of a discussion of additional sources of information and models to help ascertain potential human risks, the Panel considered observations from studies in humans. The following specific observations in regard to inclusion of the epidemiologic data as informative regarding endpoints were shared by most Panel members:

- The PFOA Draft Risk Assessment did not use the occupational biomonitoring data because "data are not available for specific occupational exposures." The Panel points out that neither are data available for "specific environmental exposures." The further claim that information on "critical factors" like gender, sampling methods and occupation are not available for the worker populations does not seem relevant. Gender differences are not considered in the PFOA Draft Risk Assessment document's MOE calculations (combined male and female values are used) because, unlike in rats, there are no apparent gender differences in PFOA elimination in humans, at least in the sparse published data available at the time of this review.
- In the PFOA Draft Risk Assessment document, limitations of epidemiological studies are emphasized, while some associations (cerebrovascular disease, triglycerides and cholesterol) are deemed less convincing, based on small numbers or inconsistencies in the results. It is undeniable that the epidemiology studies, like the toxicological ones, have some limitations, not the least of which are uncertainties regarding exposure. However, there is little doubt that these workers are more highly exposed than the general population. A special strength of epidemiological studies is that no cross-species extrapolation is needed; humans are the model. It is also true that there may be multiple exposures in the occupational studies, but this fact alone cannot disqualify them without simultaneously disqualifying virtually all epidemiological studies, which doesn't seem appropriate. If the question addressed by an MOE analysis is "how far" are existing human exposures from exposures that can cause a health effect, any health effect in the epidemiological studies imply the answer is "zero distance," *regardless of the actual serum values*.

While conceding the small numbers and short follow-up in the available epidemiological studies make the positive results less than compelling, they are not, conversely, reassuring. The evidence showing increases in cholesterol and triglyceride values in worker cohorts suggest increased risk of cerebrovascular disease mortality.

In responding to charge question #3, therefore, many Panel members shared the view that human cancer and alterations in lipid metabolism data be included in the relevant endpoints for consideration. This implies that the rich data base of occupational exposures be added to the occupational biomonitoring data to be considered. They are not

now included in the PFOA Draft Risk Assessment document because the worker epidemiological studies were not considered suitable for quantitative risk assessment.

A contrasting conclusion reached by some Panel members was that the peer-reviewed human data on health effects of PFOA were equivocal, thus there was not consensus that endpoints suggested by some epidemiologic studies should be used as endpoints in the risk assessment.

Issue 4: Risk Assessment Approach

4a: Pharmacokinetic Modeling and Use of AUC as a Measure of Internal Dose

Question 6. *Please comment on use of the one-compartment pharmacokinetic model.*

The purpose of developing a mathematical model to fit the serum PFOA time course data from the single dose rat oral dosing studies in the PFOA Draft Risk Assessment document was to estimate the AUC and C_{max} values during the longer term toxicology studies with daily dosing. The internal dose metrics calculated with this model were then compared with human serum concentrations to establish an MOE. The equations used to describe these data sets are the same as those usually employed in one-compartment models for uptake and elimination and were referred to throughout the draft document as a one-compartment model.

However, the Panel was concerned that using the "one-compartment" nomenclature without caveats and qualifications will give readers of the Draft Risk Assessment Document the impression that PFOA pharmacokinetics follow a one-compartment description when in fact they are much more complex. In a one-compartment model, the chemical distributes evenly throughout a volume of distribution that is itself in rapid equilibrium with blood. Elimination kinetics are first-order and do not change with dose level or with time. However, the data indicate that it is clearly inappropriate to describe the observed kinetics of PFOA in rats or monkeys as following a simple one-compartment model. The relatively complex pharmacokinetic behavior of PFOA is reflected in several of the pharmacokinetic data sets. For example, elimination from blood after iv dosing and tissue distribution kinetics after oral dosing are poorly characterized by the one-compartment model. In both rats and monkeys, blood levels are related in a complex manner to dosage and the duration of treatment.

Although the one-compartment model is not appropriate, the empirical model used in the document and referred to as a 'one compartment model' is adequate for predicting blood levels resulting from repeated dosing. However, the document needs to make it clear that the fitting procedure is specific to this limited data set and useful for this one application. It is strongly recommended that the terminology 'one-compartment' model should be stricken from the document unless carefully qualified.

Question 7. *Please comment on the use of the AUC as a measure of internal dose for rats and humans for calculation of the MOE.*

Calculating the 'blood' AUC (as a measure of average daily concentration of PFOA) is an appropriate method of estimating the internal dose, although it is not the only possible measure. In the absence of clear understanding of modes of action (MOA), it is also possible that the C_{max}, the integrated dose above a minimum concentration, or some other quantity may be a more plausible measure of internal dose. For example, if the MOA was receptor-based, as might be expected for interactions of PFOA with PPAR or other receptor proteins, one of these other measures of dose might also be appropriate. These alternatives include receptor occupancy or the concentration above some minimum concentration (C_{min}) where C_{min} is the concentration required to initiate activation of the receptor-mediated signaling pathway. In this latter case, the MOE would be based on the integral of (C_t-C_{min}) rather than just the integral of concentration (C_t).

In light of these other possible internal dose measures, the EPA document would be strengthened if a clear rationale for the choice of the AUC were included. Since the inclusion of this explanation may involve a detailed discussion of toxicokinetic and toxicodynamic issues, such a discussion would best be included as an appendix. While the report does provide an example of how the MOE differs when based on the C_{max} as compared to the AUC, it would be helpful if the impact on the magnitude of the MOE of using each of these other internal dose measures was explored in more detail. Calculations of MOEs based on these other measures would provide a better idea of the extent of possible variability introduced by different internal dose measures that may reflect a variety of possible MOAs.

When estimating an AUC, it is important to note the sample that is being analyzed in the various studies. AUCs can be calculated for serum, plasma or whole blood. These are very different biological matrices. The document should clearly specify the biological media measured in each study in which AUCs are reported.

Another issue to be considered is that the analyses of serum time course in the document are based on the assumption that the analyte in serum is in the same form and the proportion of free compound in blood is constant throughout the period of observation. This assumption does not always hold true. For example, with some siloxanes, the blood concentrations during and after inhalation exposure are primarily free siloxanes that are available for exhalation and metabolism. After a period of time in the body, the siloxanes in blood appear to reside in the lipid pool within the blood and although they are easily analyzed are no longer available for these other clearance processes (see Andersen *et al.*, 2001; Reddy *et al.*, 2003). A situation where the PFOA in blood at much longer times after exposure is in a distinctly different biological pool would lead to difficulties in comparing rat AUC and human AUC values to obtain a MOE. Interspecies differences in PFOA free fraction in plasma may also complicate the comparison of AUC values to obtain a MOE.

The direct use of internal measures of dose by US EPA in this document represents a promising and relatively innovative approach for risk assessments of environmental compounds compared to the more usual practice based on comparing daily dose rates by various routes of administration. This new approach reduces the need to include uncertainties introduced by the use of administered or ambient doses as measures of exposure. This type of risk assessment methodology is likely to become much more widespread due to advances in analytical chemistry and the rapid expansion of human biomonitoring activities throughout the world. Because this risk assessment is likely to serve as a prototype for future tissue-dose based risk assessments, some important issues raised by this tissue-dose based approach need to be more fully considered and adequately contrasted with the more common assessments based on comparisons of administered doses.

To address these issues, the EPA should be encouraged to develop documentation explaining their rationale guiding the use of these tissue-dose based risk assessment approaches. Such documentation should compare current methods based on daily intakes with these alternative, 'tissue-based' approaches to more explicitly address the risk characterization issues that arise in moving to this new approach. Such a document might include discussion of (1) the choices of tissue dose measures based on serum concentrations and the risk implications of each choice; (2) the impacts of utilizing direct measures of tissue dose on the magnitudes of interspecies and interindividual uncertainty factors; (3) the implications of different metrics for characterizing distributions of human tissue dose measures on estimates of MOEs; and (4) the importance of routine analysis of appropriate blood concentrations; e.g., serum, plasma, etc. in providing the information for most appropriately applying the tissue dose approach.

Issue 4b: Cross Species Extrapolation

Question 8. *Please comment on the need to use or modify the default value of 10 for cross species extrapolation given the pharmacokinetic analysis.*

The internal dose analysis used in this document is considered by the Panel to be a significant step toward reducing uncertainty related to cross species extrapolation. Although reduced, however, cross species toxicokinetic uncertainty is not eliminated. Sources of uncertainty remain, including the lack of information about the measured internal dose that best predicts adverse effect in human and other species, and the bias inherent in measurement/modeling error. While it is difficult to assign a quantitative value to the magnitude of this uncertainty reduction, it can be stated that the toxicokinetic uncertainty value for PFOA would fall within the range of one to three, based on the customary scale of a value of 3 for each aspect of cross species extrapolation, pharmacokinetics and pharmacodynamics. Pharmacodynamics aspects of PFOA cross species scaling are not addressed in a sufficient manner to alleviate the application of some type of uncertainty factor/s (addressing toxicodynamic equivalence across species). The assumption that PFOA serum levels are at steady state in children 2-12 years of age has not been tested and may not be valid. The additional complexity of multiple C-8

environmental exposures in humans versus animal experiments involving exposures to PFOA specifically, further clouds the overall uncertainty analysis.

While the pharmacokinetic modeling that is presented in the PFOA risk assessment is useful, a more comprehensive way to account for biological processes that determine internal dose is with the development of a physiologically based toxicokinetic model. The Panel encourages EPA to continue to develop toxicokinetic models as they can improve dose-response assessment by revealing and describing nonlinear relationships between applied and internal dose.

A discussion of confidence should always accompany the presentation of model results and include consideration of model validation and sensitivity analysis, stressing the predictive performance of the model. Toxicokinetic modeling results may be presented as the preferred method of estimating equivalent human doses or in parallel with default procedures (see Section 3.1.3), depending on the confidence in the modeling.

Standard cross-species scaling procedures are available when the data are not sufficient to support a toxicokinetic model or when the purpose of the assessment does not warrant developing one. The aim is to define dose levels for humans and animals that are expected to produce the same degree of effect (U.S. EPA, 1992b), taking into account differences in scale between test animals and humans in size and in lifespan. It is useful to recognize two components of this equivalence: toxicokinetic equivalence, which determines administered doses in animals, and humans that yield equal tissue doses, and toxicodynamic equivalence, which determines tissue doses in animals and humans that yield equal lifetime risks (U.S. EPA, 1992b).

It is equally important to note that pharmacodynamics aspects of PFOA cross species scaling are not addressed in a sufficient manner to alleviate the application of some type of uncertainty factor/s (addressing toxicodynamic equivalence across species). These factors may be different for each species extrapolated. By the language used in the U.S. EPA Cancer Guidelines, it seems evident that standard default values were never intended to act as complex scaling factors when internal doses in human serum are compared to animal internal doses across multiple pathways, genders, steady-state serum levels with long human half-lives and/or different life stages.

In the case of PFOA the strong reliance on LOAEL-driven MOE calculations instead of more appropriate Bench Mark Dose methodologies, and the absence of probabilistic approaches to assessing human exposure and risk, was considered by most Panel members as another source of dynamic uncertainty.

The use of an uncertainty factor/s based on data variability may be an alternative to the traditional scaling factors given the kinetics analysis strength and in light of the larger concerns of overall uncertainties related to dynamic analysis (as reflected in the MOE approach). This may prove more productive when comparing relatively robust toxicokinetic dose response models involving serum concentrations and/or their surrogates.

In conclusion, whereas toxicokinetic uncertainty is possibly reduced in this analysis, care must be exercised in the estimation of the overall cross species uncertainty, which further dynamic analyses may show falls below or above 10.

4c: Human Biomonitoring Data

Question 9. *Please comment on the adequacy of the human exposure data for use in calculating a MOE.*

Full agreement was not reached by Panel members with respect to the utility of the human biomonitoring data for the calculation of the MOE. Most Panel members expressed the view that the human exposure information should be utilized in these calculations while a few Panel members believed that these data were equivocal and thus not appropriate for the MOE calculations.

Populations used for MOE calculations

In addition to the occupational biomonitoring data, the PFOA Draft Risk Assessment document described three separate study populations from the United States with available individual serum PFOA levels. One consists of samples from six American Red Cross blood banks, another from a study of Streptococcal A infection in children, and a third from elderly volunteers from Seattle who participated in a study of cognitive function. Only the first two study populations were used in calculating the MOE for the risk assessment.

A question was raised about reliance on the female blood bank donor population for calculating prenatal MOEs, because the influence of pregnancy on serum PFOA levels is not known. Likewise, use of the samples obtained from the children for the age span of 2-12 years for the postweaning period MOE may not be appropriate because the assumption of steady state used in the MOE analyses may not be valid for children. Half-life issues in humans, especially when considering the impact of age at exposure (or the critical windows of exposure model), contribute to the questions about adequacy of using these samples (Pryor *et al.*, 2000; Selevan *et al.*, 2000; Sweeney *et al.*, 2001). Thus, there are a variety of possible problems with using these data to represent the general population, but the Panel agreed that they were likely to be reasonably representative and are better than data often available for exercises of this nature.

It was suggested that biomonitoring data in highly exposed groups (occupational and environmental) be included in the MOE analyses. It was noted that the existence, size and levels of exposures of populations which may differ from those studied has yet to be fully determined. Until this has been determined, it is not clear what percent of the general population is covered by the MOEs that have been calculated. Thus, the appropriateness of relying solely on the blood bank and pediatric samples for MOE calculations depends strongly on the purpose of the MOE exercise, i.e., whether it is to assess the likelihood that any people could be suffering health effects from PFOA or only the "general population." If the latter case, the biomonitoring data that were used may be appropriate, but the sizes of more highly exposed populations remains unknown and this should be acknowledged.

A few members of the Panel held the view that the human data were equivocal, based on the likely multiplicity of exposures of occupational groups, and thus should not be included in the draft PFOA risk assessment for MOE calculations for the general population.

Depiction of the biomonitoring data

The tables and summary statistics that were used in the draft PFOA risk assessment are somewhat uninformative and unsatisfactory. It is difficult to determine the distribution of population exposures from these given the method of data presentation. A preferable approach would be to use a non-parametric data-driven method to display the data (including the occupational data), using, for example, some density estimation procedure or smoother. Inclusion of the worker data in these displays would allow a clearer understanding of the relationships. Even side-by-side box plots would have been preferable to what was provided. This requires having access to the raw data, however. Because such a request is easy to satisfy, the Panel recommends that EPA provide more informative displays of the biomonitoring data.

Appropriate summary measures for MOE calculations

At least three summary statistics are mentioned in the Draft, the geometric mean, the arithmetic mean, and the 90^{th} percentile.

The rationale for the use of "means" should be explained, especially the use of the geometric means which seems the least satisfactory, since it is about 25% lower than the arithmetic mean in these data. Use of a geometric mean for population inference (to transform a lognormal to a normal distribution, for example) might be justified, but not for the purpose of calculating an MOE. Moreover, the distribution does not even seem to be lognormal, as judged by the Shapiro-Wilk test. The idea that a few censored data points are responsible for failing this test seems highly unlikely, and could have been accounted for in the test itself.

Means of any kind don't seem appropriate for a ubiquitous exposure. Of the three choices, the 90^{th} percentile seems the most appropriate in that case. At least one Panel member wondered why some even higher percentile, say 95^{th} or even a maximum value wouldn't be better. The maximum value in any of the samples is still an underestimate of the maximum value in the population. Even the upper 99.99^{th} percentile represents 30,000 people in the US.

In summary, the Panel finds that:

- Use of the blood donor and pediatric biomonitoring data may be acceptable if the purpose is to assess whether there is a potential health effect to the "general" population, although there is some question as to the size of other non-occupational populations that might be more highly exposed and the assumption that PFOA serum levels are at steady state may not be valid for children;
- Most Panel members believed that occupational biomonitoring data should be included in the MOE calculations, especially regarding additional endpoints such as alterations in lipid metabolism.
- A few members did not favor this inclusion based on the equivocal findings.

- The biomonitoring data should be presented in a more informative manner, for example, through side-by-side box plots or some other method that would better depict the range of values and distributions;
- Thought should be given to what appropriate summary statistic for the biomonitoring datasets used in MOE calculations should be. Some panelists believe that 90^{th} percentiles or higher, perhaps even maximum values might be most appropriate. In any event, justification for use of the chosen summary measure should be made and related to the explicit objective of the MOE analysis.

References

Andersen ME, Sarangapani, Reitz RH, Gallavan RH, Dobrev ID and Plotzke KP. 2001. Physiological Modeling Reveals Novel Pharmacokinetic behavior of Inhaled Octamethylcyclotetrasiloxane. Toxicological Sciences, 60:214-231.

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, and Cook JC. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicological Sciences, 60:44-55.

Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, Jung R, Kennedy G Jr., Lieder P, Olsen G and Thomford P. 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicological Sciences, 69: 244-257.

Butenhoff T, Kennedy GL Jr., Frame SR, O'Connor JC and York RG. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology, 196:95-116.

Chandra M, Riley MG, and Johnson DE. 1992. Spontaneous neoplasms in aged Sprague-Dawley rats. Archives of Toxicology, 66:496-502.

Goldenthal EI. Final report, ninety day subacute rat toxicity study on Flourad® Fluorochemical FC-143, International Research and Development Corporation, Study No. 137-089, 3M Reference No. T-3141, November 6, 1978. US EPA AR226-0441.

Hasmall S, James N, Hedley K, Olsen K, Roberts R. 2001. Mouse hepatocyte response to peroxisome proliferators: dependency on hepatic nonparenchymal cells and peroxisome proliferator activated receptor alpha. Archives of Toxicology, 75:357-61,

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, and Fenner-Crisp PA. 2003. PPAR-alpha agonist-induced rodent tumors: modes of action and human relevance. Critical Reviews in Toxicology, 33:655-780,

Moreno, S., Farioli-Vecchioli, and Ceru, M.P. 2004. Immunolocalization of peroxisome proliferators activated receptrs and retinoid X receptors in the adult rat CNS. Neuroscience, 123:131-145.

Palazzo MJ. 1993. 13-Week dietary toxicity study with T-5180 ammonium perfluorooctanoate (CAS No. 3825-26-1) in male rats. Laboratory Project Identification HWI 6329-100. Hazelton Wisconsin, Inc. US EPA AR226-0449.

Parzefall W, Berger W, Kainzbauer E, Teufelhofer O, Schulte-Hermann R, and Thurman

RG. 2001. Peroxisome proliferators do not increase DNA synthesis in purified rat hepatocytes. Carcinogenesis, 22:519-23,

Peters JM, Cattley RC, and Gonzalez FJ. 1997. Role of PPAR-alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. Carcinogenesis, 18:2029-2033.

Peters JM, Rusyn I, Rose ML, Gonzalez FJ, Thurman RG. 2000. Peroxisome proliferator-activated receptor alpha is restricted to hepatic parenchymal cells, not Kupffer cells: implications for the mechanism of action of peroxisome proliferators in hepatocarcinogenesis. Carcinogenesis, 21:823-826.

Pryor JL, Hughes C, Foster W, Hales BF and Robaire B. 2000. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environmental Health Perspectives, 108 (Suppl 3):491-503.

Reddy MB, Dobrev ID, Plotzke KP, Andersen ME, Reitz RH, Morrow P, and Utell M. 2003. A Physiologically Based Pharmacokinetic model for inhalation of octamethylcyclotetrasiloxane (D4) in humans during rest and exercise. Toxicological.Sciences, 72:3-18.

Rose ML, Rusyn I, Bojes HK, Germolec DR, Luster M, and Thurman RG. 1999. Role of Kupffer cells in peroxisome proliferator-induced hepatocyte proliferation. Drug Metabolism Reviews, 31:87-116.

SAP minutes 2003-05, USEPA, December 9, 2003, A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Proposed Science Policy: PPAR-alpha Agonist-Mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessment.

Selevan SG, Kimmel CA and Mendola P. 2000. Identifying critical windows of exposure for children's health. Environmental Health Perspectives, 108 (Suppl 3):451-455.

Sibinski LJ. 1987. Two year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats. Vol. 1-4, 3M Company/Riker Exp. No.0281CR0012.

Sweeney AM, Symanski E, Burau KD, Kim YJ, Humphrey HE and Smithci MA. 2001. Changes in serum PBB and PCB levels over time among women of varying ages at exposure. Environmental Research, 86:128-139.

US EPA. 1999. Draft Revised Guidelines for Carcinogen Risk Assessment (External Draft, July 1999). U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC.

US EPA. 2003. Draft final guidelines for carcinogen risk assessment. EPA/630/P-03/001A. NCEA-F-0644A. U.S. Environmental Protection Agency, Risk Assessment

Forum, Washington, DC.

US EPA. 2005. Guidelines for Carcinogen Risk Assessment . EPA/630/P-03/001F. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC,

Yang Q, Xie Y, Alexson SE, Nelson BD, and DePierre JW. 2002. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochemical Pharmacology, 63:1893-1900.