

Evaluating Human Health Risks from Exposure to Perfluorooctanoic Acid (PFOA):  
Recommendations to the Science Advisory Board's PFOA Review Panel

February 10, 2005

by:

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## OVERVIEW

Purpose. The purpose of this document is to provide the Science Advisory Board with information on potential human health risks from exposure to perfluorooctanoic acid (PFOA) that was omitted by the Environmental Protection Agency's (EPA's) January 2005 Draft PFOA risk assessment (1), or that we believe was dismissed in error.

We also recommend an alternate method for evaluating human health risk than that selected by the Agency. We describe an ED10 (estimated dose for 10 percent response) method for evaluating excess lifetime cancer risk for three of the tumor types found at statistically significant levels in animals dosed with PFOA. We also describe a Benchmark Dose (BMD) methodology for non-cancer risk assessment recommended by the National Academy of Sciences as the preferred approach in a situation analogous to that for PFOA, in which a dose without harmful effect has not been identified for low-dose endpoints, and for which risk levels are defined on the basis of concentrations in blood (2). This method was also selected by 3M in its recently published evaluation of human health risks from PFOA exposures (3).

Recommendations. Among the ideas that we wish the Panel to consider as you formulate recommendations to the Environmental Protection Agency on its PFOA risk assessment are the following:

- Consider the risks of mammary, pancreatic, and testicular cancers relevant to humans, and the risks of hepatic tumors relevant to fetuses and developing infants and children. This is consistent with both the scientific evidence and with recommendations from other EPA advisory boards.
- Consider including endpoints not currently in the assessment, but potentially relevant to humans, including effects to the pituitary, the immune system, non-PPAR $\alpha$  mediated liver effects, ovarian tubular hyperplasia, ataxia and skeletal malformations when determining risk.
- Use a benchmark dose (BMD) approach for assessing non-cancer risks, and an ED10 approach for assessing cancer risks, in accordance with recent applications and guidance from the Agency and the National Academy of Sciences.
- Assess cumulative risks from exposure to all perfluorochemicals (PFCs) known to occur in human serum with mechanisms of action and target organs in common with PFOA.

- **Err on the side of protection:** When facing technical choices on risk interpretation, we urge the Panel to err on the side of human health protection for at least two reasons: 1) as many as one million pages of health and exposures studies relevant to PFOA have not yet been made available to the Panel (details are provided below); and 2) PFOA is just one of 15 perfluorochemicals (PFCs) detected in human blood – many of these chemicals have been shown to cause harm through PFOA’s known mechanisms of action, and some are present in human blood at higher levels than PFOA.

Information Omitted from EPA Risk Assessment. We summarize in sections below pertinent health information we believe was inappropriately omitted from EPA’s draft risk assessment. This information is publicly available and in EPA dockets on PFOA or in the scientific literature. However, we also bring to the Panel’s attention that fact that up to one million pages of relevant health and exposure information on PFOA and related chemicals from DuPont are not yet publicly available and have not been provided to the Panel.

Court documents from a lawsuit filed by EPA over DuPont’s alleged suppression of PFOA health studies (4) indicate that the Agency has recently received at least 15 boxes of additional data from DuPont, and potentially in excess of one million pages, comprising company studies and other documents relevant to human health and exposure (5). We understand that EPA is only now processing these documents. Although the Panel presumably will not have access to this additional information during this review, we urge you to err on the side of human health protection when technical choices arise, given that there is apparently a vast amount of scientific information known only to industry, only now being divulged to EPA, that will almost certainly raise further concerns about potential health impacts from human exposures to PFOA.

## BACKGROUND ON PFOA

PFOA Uses, Properties, and Occurrence. Available scientific findings to date show that PFOA and related perfluorochemicals (PFCs) occur near universally in human blood (6-9), that they persist in the body for decades (10), that they act through a broad range of toxic mechanisms of action to present potential harm to a wide range of organs, and that they persist indefinitely in the environment with no known biological or environmental breakdown mechanism (11-14). Cancers as well as reproductive and developmental effects are of particular concern.

Perfluorooctanoic acid (PFOA) is produced synthetically and through the degradation or metabolism of other synthetically derived perfluorochemical (PFC) products used as oil, water, soil and stain repellents. It is used in the production of Teflon (polytetrafluoroethylene, or PTFE), and is a breakdown product of fluorinated telomer products like Stainmaster stain repellent and commercially available Zonyl products used to coat packaging for fast food and other prepackaged items, including McDonalds French fry boxes, microwave popcorn bags, Chinese take-out containers, and butter and pizza boxes. Telomer chemicals that break down into PFOA are also the dominant surface treatment for carpet, upholstered furniture, automobile upholstery and, increasingly, popular clothing lines like Gap for Kids, Dockers and Levis.

Numerous studies by industry and academia have detected PFOA in wildlife from Italy, the US, Japan, Russia, Belgium, and Canada, in samples from Arctic polar bears, and in places as remote as the Sand Island Wildlife Refuge in Midway Atoll (15-29). PFOA has been detected in treated tap water from Ohio, West Virginia, Georgia, and Minnesota, and more broadly in rivers, streams, and other surface water bodies (30-33). PFOA has also been detected in house dust (34), and in green beans, bread, apples, and ground beef in a limited market basket study contracted by 3M (35). Researchers from University of Toronto have detected PFOA precursors (telomers) in urban and rural air (36). And PFOA has been detected in more than 90 percent of the more than 3,000 human serum samples tested, including in 96 percent of samples from 598 children across the U.S. (7-9).

Mechanisms of Action other than Peroxisome Proliferation. Studies show that PFOA exerts harm through at least five different mechanisms of action, including thyroid hormone changes, mitochondrial disruption, gap junction intracellular communication (GJIC) disruption, increased estradiol, and peroxisome proliferation. We urge to Panel to consider all mechanisms of action that may be at work in PFOA toxicity in considering EPA's charge questions. More detailed information on PFOA-related mechanisms of action is found in Appendix A.

Regulatory and Legal Issues. In April 2003 The EPA launched a major review of PFOA, still underway, that EPA's Assistant Administrator called "the most extensive scientific assessment ever undertaken on this type of chemical" (37). The Agency's January 2005 risk assessment is one outcome of the review. PFC manufacturers are also involved in ongoing litigation in a number of courts. On July 8<sup>th</sup> 2004 EPA filed a lawsuit against DuPont, alleging that the company suppressed health and pollution studies required to be reported to the Agency under the Toxics Substances Control Act. This case is pending before the Environmental Protection Agency Administrative Court. Litigation is also pending in West Virginia, Minnesota, and Alabama against DuPont and 3M in cases involving tap water pollution and alleged worker health effects (38,39).

## PFOA CANCER STUDIES

Summary. According to EPA's internal guidelines for assessing the cancer potential of a chemical (40), PFOA is a "likely" human carcinogen. The chemical meets three of five EPA cancer criteria, while a categorization of "likely carcinogen" requires that just one of these criteria is met. PFOA has been linked to multiple cancers in male and female mice, in more than one study, in tumors that are statistically significant and assumed to be relevant for humans (41-43).

The three EPA criteria that are met by PFOA and that are required for a chemical to be classified as a "likely" human carcinogen are below. A detailed discussion of the available cancer data for PFOA and its relevance to humans follows.

- An agent that has tested positive in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- A positive study that indicates a highly significant result, for example, an uncommon tumor, a high degree of malignancy, or an early age at onset;

- A robust animal tumor response in a single experiment that is assumed to be relevant to humans.

Mammary Fibroadenomas. In a 2-year bioassay sponsored by 3M, the incidence of mammary fibroadenomas in treated rats was significantly higher than that in study controls ( $p < 0.05$ , 42% in the low dose group versus 21% in the controls) (41). In a subsequent review of the study, EPA determined that these findings were significant and relevant to humans: “the increased incidences of testicular (Leydig) cell adenomas in the high-dose male rats, and of mammary fibroadenoma in both groups of female rats were statistically significant ( $P < 0.05$ ) as compared to the concurrent controls...The increases are also statistically significant as compared to the historical control indices (LCT, 0.82%; mammary fibroadenoma, 19.0%)” (44).

In the 2005 assessment that the Panel is charged with reviewing, however, the Agency dismissed the mammary adenoma findings because “[w]hen the mammary fibroadenoma indices were compared to the historical control incidence (37%) in 947 female rats in the Haskell Laboratory, however, there does not appear to be any compound related effect” (1). The downgrading of the mammary tumors goes against both solid scientific reasoning as well as the USEPA’s own guidance on historical controls in its guidelines for cancer risk assessment (40).

In this dismissal of statistically significant, elevated incidence of mammary tumors, EPA is not only reversing its own findings from an earlier review (44); the Agency is also out of accord with its own published guidance on the appropriate use of data on historical controls (40). In its guidance on the subject, the Agency dictates that “relevant historical data come from the same laboratory and same supplier, gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution” (40). The historical data used by USEPA is neither from the same lab (Riker Laboratory vs DuPont Haskell Laboratory) nor 2 or 3 years apart (Sibinski in 1983 vs 1987). (Note: The Sibinski (41) study was performed by Riker Laboratories, Inc. from April, 1981 to May 1983.)

Independent statisticians have also found scientifically indefensible the practice of dismissing significant findings based on incidence rates in historic controls (45). In practice, this inappropriate application of historic control data occurs most often in studies for which multiple endpoints are investigated over a relatively long time span within the same experiment. These many tests can lead to an increase in type 1 errors (false positives) due to the sheer number of statistical tests being performed. To compensate for this increased chance for errors, toxicologists have inappropriately compared study incidence rates to incidence rates in historical controls, declaring results within the bounds of historic control data to be false positives

The use of historical controls in this context is problematic because the amount of variability from experiment to experiment is so large that the observed difference between control and treated subjects will almost always be dwarfed so that the possibility of a type II error is extraordinarily high. Using historical control data, especially the range of incidence rates seen in controls among various studies, goes against “statistical reasoning” (45). Nevertheless, reviews of the literature have reported many of such erroneous uses of historical control data (45). EPA’s misuse of

historic control data in the case of mammary fibroadenomas and PFOA is just one case of many. In situations where there are concerns about increased risk of type I errors, researchers and reviewers can evaluate such a possible increase by analyzing the distribution of p-values, as described by (46). Perhaps this can be done with the Sibinski (41) data on mammary fibroadenomas and PFOA. Regardless, the statistically significant increase in tumor incidence should not be dismissed here.

EWG urges the Panel to recommend that EPA consider PFOA-induced mammary tumors significant and relevant to humans.

Hepatocarcinogenicity relevance to children. To help resolve questions over the relevance to humans of liver tumors observed in laboratory studies, the EPA recently turned to its Scientific Advisory Panel, a body of outside experts mandated under federal pesticide law. After a comprehensive review in 2003, the Panel determined that, while relevance is uncertain for adults, human fetuses and neonates may, in fact, be uniquely susceptible to liver cancer risks posed by chemicals like PFOA that are known to be PPAR $\alpha$  agonists (47). EPA has not addressed this susceptible population in their risk assessment, but has instead dismissed the relevance of human liver cancer risk from exposures to PFOA. We urge the SAB to be informed by the SAP's recommendation and advise the EPA to consider liver cancer risks for fetuses and neonates exposed to PFOA.

Testicular cancer. Industry data show statistically significant, elevated risk for testicular tumors from exposures to PFOA (42,41). Although EPA determined that the tumors should be "assumed to be relevant to humans," the Agency subsequently asserted that "they probably do not represent a significant cancer hazard for humans" (1). As support for this position, EPA scientists present a hypothesis that PFOA's influence on testosterone is the mechanism of action for testicular tumors. EPA then reports that in one study, monkeys dosed with PFOA did not show statistically significant depleted testosterone levels (48). The Agency went on to conclude that, based on their hypothesis, the observation of significantly elevated testicular tumors in laboratory studies is not relevant to humans.

In drawing this conclusion, based as it is on an untested hypothesis, EPA is ignoring recent advice from their Scientific Advisory Panel (47). The SAP recently advised the Agency to consider significant findings relevant to humans except in cases for which the mechanism of action is known with certainty and is known not to be relevant to humans (47). Such is not the case for PFOA and testicular cancer. We urge the Panel to advise EPA to consider the testicular tumor findings relevant to humans.

Pancreatic cancer. Industry data shows significantly increased occurrence of pancreatic tumors for animals dosed with PFOA (42). In its 2005 draft PFOA risk assessment EPA begins the section on pancreatic acinar cell tumors in the risk assessment by noting that "[t]he mechanism by which PFOA induced pancreatic acinar cell tumors is unknown." (1). Nevertheless, the Agency proceeds to present a hypothesis for a possible increase in cholecystikinin, and then goes on to conclude that the mechanism is not active in humans. With this rationale, EPA determined that pancreatic tumors related to PFOA are not relevant to humans. Again, as the Agency's

SAP noted, it is not appropriate to dismiss the human relevance of significant effects where mechanisms of action are not known with certainty. Since EPA has noted that the pancreatic tumor mechanism is unknown, we urge the Panel to recommend that EPA consider the pancreatic tumors relevant to humans.

## CANCER RISK DETERMINATION

Methodology. We present here a preliminary cancer risk assessment for the three PFOA-related cancers discussed above. For this preliminary assessment, we derived a cancer slope factor using as a point of departure the ED10 (estimate dose for 10 percent effect rate), and assuming a linear, low-dose response. We note that the Science Advisory Board previously recommended that EPA develop cancer estimates based on both the ED10 and LED10 (lower bound on the ED10) as points of departure, and that EPA selected LED10 as the final point of departure for their recent assessment of cancer risk from arsenic in drinking water and arsenic in pressure-treated wood. We urge the Panel to make a similar recommendation for the use of an LED10 approach in the case of EPA's PFOA risk assessment.

The preliminary estimates presented here likely underestimate excess lifetime cancer risk from exposures to PFOA: while we have assumed a linear, low-dose response, in the case of all three tumor types the response appears to be supralinear. In these cases, a linear assumption yields underestimates of the cancer risk defined by a supralinear curve. We also note that a full cancer assessment that incorporates LED10 as a point of departure will also yield a higher estimated excess lifetime cancer risk than those estimated here with the less protective ED10 approach. Cancer slope factors derived via the method described here are listed in Table 1. Excess lifetime cancer risks are calculated as described below.

Estimates of excess cancer risk were performed by creating a risk curve from the PFOA serum levels provided in the three studies by 3M (7-9). Figure 2 in each of the three studies included a distribution of the number of subjects in 1 ppb serum level increments. By assuming that the distribution of PFOA serum levels was representative of the entire US population, US Census numbers were then used to estimate the total number of people at each 1ppb serum level. Serum PFOA levels from the 3M pediatric study (7) was projected to the population of children 2 to 16 years old. PFOA levels from the 3M adult study (8) was projected to the population of adults 17 to 66 years old. And PFOA levels from the 3M elderly study (9) was projected to the population of people 67 years old and older.

For example, in deriving the estimated population with PFOA serum levels of approximately 3 ppb, we first determine from 3M data that 123 of 645 study subjects had measured serum levels of 3 ppb. Then, from U.S. Census data we determined that the US population comprises 180 million adults (ages 17-66; 89.6 million males and 90.5 million females). The total number of people in the U.S. population with estimated PFOA serum level in the range of 3 ppb was then computed as  $123 \times 180 \text{ million} / 645$ , or 34.3 million adults. Using the slopes given in Table 1, we calculated the risk for the human serum levels by ppb (i.e. serum level\*slope=cancer risk for that level). The number of people over a particular cancer risk was performed by finding the

human serum level that corresponded to the cancer risk of interest and using the proportion of the population that is above that level as determined by the methods described above.

Findings. The majority of the female population is above the 1 in 100,000 risk for mammary tumors and that the majority of those occupationally exposed are above the 1 in 10,000 risk for both Leydig cell and pancreatic acinar cell tumors. Additionally, there is estimated to be a large number of women at a high risk (1 in 1,000) of mammary tumors. These estimates imply that 1,238 of the 216,000 breast cancers diagnosed in 2004 (49) may be attributable to PFOA exposure.

Table 1. Estimated excess lifetime cancer risk and populations affected.

Cancer Type	Slope factor	Serum PFOA level corresponding to 1 in 10,000 excess lifetime cancer risk	Estimated population in excess of 1 in 100,000 excess lifetime cancer risk	Estimated population in excess of 1 in 1,000 excess lifetime cancer risk
Leydig Cell	0.000186 per ppm	0.538 ppm	374,724	-
Mammary	0.103 per ppm	0.000971 ppm	135,313,614	40,329,000
Pancreatic	0.000185 per ppm	0.541 ppm	101,374	-

Source: EWG analysis of data from [7-9] using standard EPA cancer risk assessment methods.

## SUMMARY OF SELECT NON-CANCER PFOA EFFECTS

Summary. PFOA has been linked to a wide range of non-cancer effects, most of which are discussed in USEPA (1). Below we present information on non-cancer effects that we believe was erroneously interpreted or inappropriately dismissed by EPA. The effects we discuss include immune system suppression, effects to the pituitary, non-cancer liver effects, and cholesterol changes.

Immune suppression effects. In its draft risk assessment (1) the Agency briefly discusses available laboratory studies on the impacts of PFOA to immune function, but does not address them in the determination of risk. Available data strongly suggest that PFOA suppresses the immune system, but is limited in its utility for human health risk assessment: just one of the immune studies measured functional outcomes of the immune system (50), and although the study showed strong effects – so much so that the adjective “potent” was placed in the title of the published article – just one, high dose was tested. Supporting studies include in vitro tests, and in vivo studies that assessed cellular effects but not functional endpoints. As a whole, the body of available literature supports the conclusion that PFOA likely impairs immune function in humans.

On the basis of the strength of the available data, and given that a growing number of people in the population suffer from immune disorders, we urge the Panel to recommend that USEPA discuss the possibility of immune suppression in humans in the determination of risk and sponsor or request from industry more studies on this important outcome.

Summary of immune suppression studies. PFOA causes toxicity to four organs or tissues in the immune system and at least nine cell types that regulate immune function (51-53). PFOA has long been known to damage the immune system, but recent studies have shown that exposures to PFOA early in life are more harmful than in adulthood. Studies have not identified a dose that did not functionally damage the immune system.

In the fetus and through early life, the thymus is instrumental in fostering the growth and development of the immune system. The spleen and thymus, both critical to immune function, atrophy among animals exposed in the womb and through early adulthood; splenic atrophy occurred at the lowest dose tested. Significantly, similar doses do not lead to thymus effects in animals exposed only during adulthood (51). In a consensus statement from a diverse panel of scientists implies the significance of immune system damage in early life: “Life-long capacity for immune response is determined early in development, during prenatal and early postnatal development in mammals” (54). Thus, the type of thymic damage observed with PFOA could lead to permanent decrements in immune function.

Several studies by scientists in labs at Stockholm University and the Karolinska Institute in Sweden looked at the effects of PFOA on immune cells in detail. They found that PFOA decreased the number of every immune cell subpopulation they studied — eight in all — in the thymus and spleen (53,55). Yang et al. (50) also found PFOA damaged immune cell function, a phenomenon as the cells were unable to mount a proper immune response to foreign cells.

A few of the effects of PFOA on immune system cells are due to activation of PPAR $\alpha$ . However, in studies employing PPAR $\alpha$  null mice, many of the effects of PFOA on the thymus cells remain, reinforcing the relevance of the laboratory studies for humans, and heightening concern for in utero and early life exposures to PFOA.

In workers, increased blood levels of PFOA are associated with increased white blood cells (leucocytes) (56), suggesting that workers are under stress from infection or disease (57), consistent with a picture of poor immune function.

Pituitary. A two-generational study conforming to USEPA requirements concluded that the adverse pituitary effects were the most sensitive study endpoint of the reproductive study by York (51). The current primary producer of PFOA in the U.S. (DuPont) agreed with the study authors’ interpretation; “[t]he maternal NOAEL is 1 mg/kg/day based on significant decreases in pituitary weights that were observed at 3 mg/kg/day and above” (58).

But EPA has chosen to exclude pituitary effects from their risk analysis because “there is not a clear dose-response relationship and the histologic examination did not reveal any lesions.” This rationale is faulty for two reasons. First, EPA’s conclusion directly contradicts their own guidelines. The Agency states in its Guidelines for Reproductive Toxicity Risk Assessment that “a significant increase or decrease in pituitary weight should be considered an adverse effect” (59). Second, many studies have now documented the wide variety of valid dose-response curves possible in the field of toxicology; complex curves are thought to be the result of the interplay of multiple mechanisms of action, some of which are dose-dependent, or on the differing potency of the chemical on various cell types within a target organ. The shape of the curve is not valid grounds for EPA’s dismissal of a statistically significant adverse effect.

The pituitary findings may be correlated with the small delay in vaginal patency at 30 mg/kg/day that is an indication of delayed sexual maturity. This type of delay is “usually related to delayed maturation or inhibited function of the hypothalamic-pituitary axis” (59).

Pituitary involution (weight decrease) in young adult female rats is uncommon (60). Pituitary gland weight alone is not sufficient to predict functional toxicity, but it is a sign of a toxicological effect. Given the high-dose plateau in the dose-response curve defined by study findings (Fig 1), it is possible that PFOA may be preferentially targeting a pituitary cell subpopulation (i.e. thyrotrophs, somatotrophs, corticotrophs, lactotrophs, gonadotrophs). Perhaps the plateau indicates near complete destruction and loss of function of the affected cell type. This high-dose plateau feature in the dose-response curve is observed for other PFOA-related health endpoints as well, though, and so may instead be due to some poorly understood aspect of PFOA pharmacokinetics.

To our knowledge, a careful evaluation of pituitary subpopulation histology has not been conducted for PFOA. In the rat reproduction study (51), the pituitary gland is not listed on necropsy tables, suggesting it was not histologically assessed<sup>1</sup>. Furthermore, a gross histological examination of the type typically conducted for general toxicity studies (including the chronic toxicity/cancer study) would not reveal specific pituitary pathology to individual cell populations.

The response of the pituitary is dismissed because, presumably, EPA constrained their definition of a relevant effect to those showing an increasing rate of change in the adverse effect with dose. In the case of pituitary weight changes, the absolute weight of the pituitary declines continuously with dose, but the rate of change decreases. However, supra- and sub-linear responses similar to those seen as a result of PFOA toxicity are often a combination of pharmacokinetic and pharmacodynamic parameters (61-63). A great variety of dose-response curves has been documented (64).

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<sup>1</sup> The methods state that “The following tissues were retained for *possible* histological evaluation: pituitary...” [emphasis added] York, RG (51). Oral (gavage) two-generation (one litter per generation) reproduction study of ammonium perfluorooctanoate (APFO) in rats. US EPA Administrative Record AR226-1092, Reviewed in US EPA Administrative Record AR226-1137.

The effects of PFOA on the pituitary show that the doses caused a statistically significant change yet did not follow a linear or sub-linear “dose response.” In the case of the pituitary effects observed in York (51), the null hypothesis (the results are due to chance) has been rejected through statistically analysis. The change has been determined to be due to the treatment.

From a public health perspective, we firmly believe EPA should not dismiss what is harmful but poorly understood. We urge the Panel to recommend that the USEPA include the pituitary in its evaluation of human health risks from PFOA exposures.

Non-Cancer Liver effects. The EPA has dismissed as irrelevant to humans adverse, non-cancer liver findings, a position that is incompatible with available data. EPA takes a position in the 2005 PFOA risk assessment that liver PPAR $\alpha$  activation is not relevant to humans. With this as a rationale, the Agency dismisses as irrelevant the full range adverse liver effects observed in laboratory animals.

In doing so, they also dismissed significant liver effects observed PPAR $\alpha$ -null mice, animals that could not possibly have been affected through presumed PPAR $\alpha$ - agonists, as they are missing the agonist receptors. And also in the process of dismissing all liver effects, the Agency dismissed liver effects in monkeys, animals considered similar to humans in their susceptibility to peroxisome proliferators. - increased cholesterol in humans (65), which is likely a sign of abnormal liver function.

As support for potential human relevance of adverse, non-cancer liver effects from PFOA exposures we draw on evidence from PFOA toxicity studies on PPAR null mice; other in vivo studies that specifically included considerations of peroxisome proliferation; in vitro studies of human liver cell lines; and worker studies of liver enzyme levels:

- Findings in PPAR null mice. PFOA toxicity has been investigated in PPAR null mice (55). As expected, researchers found no evidence of peroxisome proliferation in these mice. However, adverse effects presumed by EPA to stem from peroxisome proliferation were still noted in the liver (enlargement by 1.9 times the control). PFOA has also been found to have toxic effects on the liver in monkeys (48). Researchers have also found increased peroxisome enzyme activity in human liver cells following exposure to another chemical peroxisome proliferator (66). This suggests either that liver and thymic toxicity are not PPAR dependent, or that PPAR-related effects are relevant to humans, or both
- Other in vivo studies of liver damage and peroxisome proliferation. Certainly the relevance of PPAR $\alpha$ -induced toxicity to humans is a subject of current scientific debate. But we note that human PPAR relevance is supported by a number of studies, including Intrasukri (67), which found that PFOA activates both human and rat PPAR (get levels of activation); Maloney (68), which found that differential sensitivity of human versus mouse PPAR to PFOA is 2-fold or less; and (69), which found that a PFC closely related to PFOA, perfluorodecanoic acid (PFDA) causes peroxisome proliferation in human brain cells.
- In vitro studies of human liver cell lines. PFOA has been shown to disrupt the cell cycle of a human hepatic cell line (HepG2) (70) Lower doses (50 M) increased

the number of cells in G2/M and decreased S phase (decreased DNA synthesis, but increased cell division). Higher doses (100 and 150 M) increase cells in Go/G1 and decrease the number of cells in G2/M and S (decreased DNA synthesis and cell division). Changes in total cell number or apoptotic cells (sub G0/G1 content) were detected in this concentration range (50 to 150 M); apoptosis started at 200 M (70).

These findings argue that PFOA exerts toxic effects on the human liver as well as the thymus, whether via PPAR activation or an alternative mechanism. In view of the diverse, documented mechanisms of action relevant to PFOA (documented below), as well as the many supporting studies discussed above, we urge the Panel to recommend that EPA consider adverse liver effects potentially relevant to humans.

Cholesterol. Six studies now point to risk for heart attack and stroke from exposures to PFOA chemical, two of which have emerged in the last 4 months (52, 65, 71-74). Just last month (January 11 2005) DuPont released the latest in this series, a study showing elevated cholesterol levels in workers exposed to the Teflon chemical, a known risk factor for heart attack and stroke. EPA's draft risk assessment does not include this information, or discuss the relevance to the general population of occupational findings of elevated cholesterol to the general population. We ask the Panel to review these studies and recommend that they be incorporated in assessments of general population risk from PFOA exposure if possible.

#### ASSESSMENT OF HUMAN HEALTH RISKS FOR NON-CANCER EFFECTS

EPA's Method for PFOA Non-Cancer Risk Assessment. In a recent EPA interpretation of "Specific Scientific Advances" in methods for risk assessment since 1980, the Agency notes that it has transitioned away from simplistic NOAEL-RfD approaches to assessing risk, to more sophisticated BMD methods that take into account the characteristics of the dose-reponse curve (75).

EPA writes that "Since 1980, EPA risk assessment practices have evolved significantly in all of the major Methodology areas... In noncancer risk assessment, we are moving toward the use of the benchmark dose (BMD) and other dose-response methodologies in place of the traditional NOAEL approach to estimate an RfD concentration or other point of departure (POD) divided by an uncertainty factor (UF)" (75). EPA adopted or endorsed this evolved approach for numerous assessments, including studies of health risks from mercury in seafood, and from arsenic in water and pressure-treated wood.

In the case of PFOA, however, EPA has reverted back to its pre-1980 NOAEL-RfD approach. The Agency provides no explanation for this deviation from its preferred method. The Agency has not only reverted to its older method of analysis, it has also neglected to complete three of the four major steps in this approach. While it has selected a point of departure (a LOAEL in the case of PFOA, not a NOAEL), the Agency has failed to derive an uncertainty factor to account for data gaps and differing susceptibilities among humans and animals; has not adjusted the point of departure by the uncertainty factor to derive a reference dose, RfD, or a reference concentration in

the case of PFOA (an RfC, a concentration of PFOA in human serum which the Agency believes to be without adverse effect); and has failed to estimate the distribution of exposures in the U.S. relative to an RfC.

We urge the Panel to request that EPA complete all four steps in the process if results from a NOAEL-RfD method (or LOAEL-RfC in this case) are to be included in the final PFOA risk assessment. We also recommend that the Panel advise EPA to instead, or additionally, assess non-cancer PFOA health risks with the Agency's typically preferred BMD approach.

Proposed Methodology for PFOA Non-Cancer Risk Assessment. We describe below a Benchmark Dose (BMD) methodology for assessment of human health risks for non-cancer effects, an approach not only endorsed previously by EPA, but also recommended by the National Academy of Sciences as the preferred approach in a situation analogous to that for PFOA, in which a dose without harmful effect had not been identified, and for which risk levels are defined on the basis of concentrations in blood (76). A Benchmark Dose approach was also selected by 3M in its recently published evaluation of human health risks from PFOA exposures (3).

The method involves four steps, according to standard EPA procedure. First, we estimate a Benchmark Dose for a 10 percent effect rate (called the BMD10) using EPA's BMD software (77). Second, we estimate the lower bound of the 95 percent confidence interval about the BMD (called the BMDL10). Third, we apply an uncertainty factor (UF) to the BMDL10 to derive an estimated reference concentration (RfC) of PFOA in human serum, essentially a "safe" dose for humans. The uncertainty factor accounts for data gaps and differing susceptibilities between humans and laboratory animals – in the examples presented below we have applied an uncertainty factor of 100, a value in the range of those typically selected by EPA for chemicals with similar levels of available data. Lastly, we estimated the fraction of the population with PFOA serum levels in excess of the RfC, based on measured human serum levels (7-9). We repeated this process for multiple, select adverse effects. We can provide further details on our methodology upon request.

Selection of Adverse Effects. EWG selected multiple endpoints (adverse effects) to serve as points of departure for risk analysis. These endpoints were selected from adverse effects that occur at low doses for males and females, and from effects for which a NOAEL has not been found. The endpoints that met these criteria include hepatomegaly, decreased kidney weight, decreased spleen weight, ovarian tubular hyperplasia, ataxia, decreased pituitary weight, and skeletal malformations (increased supernumerary ribs and missing sternbrae). We also included delayed sexual maturation because it is EPA's chosen endpoint of concern in the draft risk assessment. Considerations with respect to the relevance of three of our of selected effects to humans are discussed below:

- Skeletal malformations. While there are no reports of increased supernumerary ribs or missing sternbrae in humans born to the seven highly exposed mothers who have been studied (78), the induction of skeletal malformations by PFOA may be relevant to increasing, more commonly found human skeletal malformations such as club foot, reduction deficit of limbs, or orofacial clefts of

the hard palate. Rats are susceptible to supernumerary ribs or missing sternbrae; it is possible that common human skeletal malformations, while different from those observed commonly in rats, may also be increased due to PFOA exposure.

- Ataxia. Likewise, there are no reports of neurological impairments such as ataxia in occupationally exposed persons; however, no neurological tests of any kind have been performed on this population. With that lack of information, neurological effects such as ataxia must be considered relevant to the human population.
- Ovarian tubular hyperplasia. Two reviews of ovarian slides from Sibinski (41) have been performed with different results, the original finding significant increases in ovarian tubular hyperplasia, and one failing to find such a relationship (79). These slides have not been made public to allow for independent assessment. Ovarian tubular hyperplasia (OTH) is included in this analysis on the basis of the original study that found a statistically significant, dose-dependent relationship between PFOA and OTH.

Calculation of Benchmark Dose. A graphical display of dose response curves for adverse effects (Figures 1 and 2) shows monotonic curves that plateau at high doses. This shape may be due to pharmacodynamics, but given the wide range of effects that share this shape, pharmacokinetic parameters (changes in the distribution of the chemical with time throughout the body) are also likely to play a role in defining the shape of the dose-response curve.

A BMD analysis was conducted on Leydig cell adenoma by Butenhoff et al. (3). However, Butenhoff et al. selected a model for LCA that our calculations show provides a  $\chi^2$  value of 1.53, outside the accepted bound of -1 to 1 and indicative of an inadequate model fit; this model also falls below the 90<sup>th</sup> percentile confidence interval at the lowest dose and severely overestimates the BMDL. A visual inspection of many of the PFOA dose response curves suggests that the power-restricted models fail to simultaneously fit the steep dose-response curve in the low-dose region and the flatter curve in the high dose zone. However, an unrestricted model likely overestimates risk as well with a BMDL that approaches zero. A high confidence BMDL would require a greater number of low doses that provide better curve definition in the range of the BMD.

In the absence of such additional low-dose data, however, EPA provides guidance that allows for a bounding analysis on the BMDL from the available data. An upper bound is derived by restricting analysis to the low-dose portion of the curve, which yields a near-linear model fit to the low doses. A lower bound on the BMDL is derived from a model without power restrictions, a method yielding a curve that fits all the data simultaneously.

The upper bounding analysis yields BMDL values that correlate with serum values as low as 0.442 ppm (skeletal malformations). Due to the low number of low doses and the variability of the data, the 95% confidence interval of the lower bound BMD was below 1 ppb and likely to overestimate risk. Therefore, in this case, the BMDL derived

from a linear model was used for risk calculations. Delayed sexual maturation fit a power-restricted, sub-linear, model with good fit statistics and, therefore, was not analyzed in a linear manner.

To determine a reference concentration (RfC) in a manner similar to that recommended by National Academy of Sciences for mercury (76), we applied an uncertainty factor of 100 to the BMDL<sub>10</sub>. We then compared the distribution of PFOA serum levels in the general population (79) with the derived RfC to determine what, if any, fraction of the population is exposed to PFOA at levels above the RfC.

Results. The results of the BMDL analysis are displayed in Table 2 and show that RfCs from the effects analyzed are overlapping or close to human serum levels. Of particular concern are ovarian tubular hyperplasia and potential skeletal and neurological impacts in humans that may be linked to effects seen in laboratory studies. We estimate that up to 143 million people are exposed to PFOA in excess of the RfC derived here (Table 2).

Table 2. Derived parameters in BMD analysis and population exposed to PFOA in excess of the reference concentration.

Endpoint	Sex	Study	LOAEL	BMD (ppb)	BMDL <sub>10</sub> (ppb)	RfC	% population Over RfC
Delayed Sexual maturation	F	York, 2002	30 mg/kg/day	1,008,000	147,000	1470	-
Ovarian tubular hyperplasia	F	Sibinski, 1987	1.6 mg/kg/day	2,022	980	9.8	6.78%
Decreased pituitary weight	F	York, 2002	3 mg/kg/day	21,179	12,264	123	-
Skeletal Malformation	M/F	Gortner, 1981	5 mg/kg/day	1,113	442	4.42	40.1%
Ataxia	F	Sibinski, 1987	1.6 mg/kg/day	1,117	636	6.36	19.3%

Source: EWG analysis of data from (41, 51, 80) according to standard EPA BMD analysis techniques and NAS (2000).

## CONCLUSIONS

Estimates for cancer risk presented here are at levels that warrant mitigating measures to lower human exposures and reduce risk, especially for breast cancer. Human breast cancer rates have increased for the past three decades (81); lifetime risk now stands at one in seven, heightening the importance of reducing human exposures to any suspected mammary carcinogen. For non-cancer effects, impacts to the ovary, skeletal development, and neurological effects from PFOA exposures are all of concern, based on the fact that PFOA serum levels in the general population overlap with RfCs derived by the method presented here.

Given the extraordinary environmental stability and the long human half-life of PFOA, a protective approach to regulatory measures is warranted. PFOA appears to

affect the young and developing animal more than the adult. The risks include developmental and reproductive toxicity, immunotoxicity, and cancer. PFOA-related effects to functional immunotoxicity are not well characterized and need more study to determine NOAELs and to characterize mechanism of action.

It is our view that the hazards, confirmed human serum levels of PFOA, and the special considerations listed above merit a proactive approach to in reducing PFC use. Accordingly, we believe it is in the public health interest that PFOA and compounds that breakdown into PFOA be removed from the consumer market and that industrial use be limited to uses where recoveries of PFOA from wastes are complete. Additionally, given the presence of many similar PFCs found in the serum of the population, we believe that the PFC family of chemicals should be thoroughly assessed for their pharmacokinetic, toxicological, and physical chemical properties before industrial or commercial use.

We request that the Science Advisory Board's PFOA Panel urge EPA to adopt the standard risk assessment methodology we have presented here; to consider as relevant to humans mammary, testicular and pancreatic cancer findings; to consider liver cancer as relevant to the fetus; infant and child; to include ovarian tubular hyperplasia and other non-cancer low dose impacts in the risk assessment; to include in the risk assessment the many other PFCs commonly found in human serum with mechanisms of action and target organs in common with PFOA; and to err on the side of human health protection when technical choices arise.

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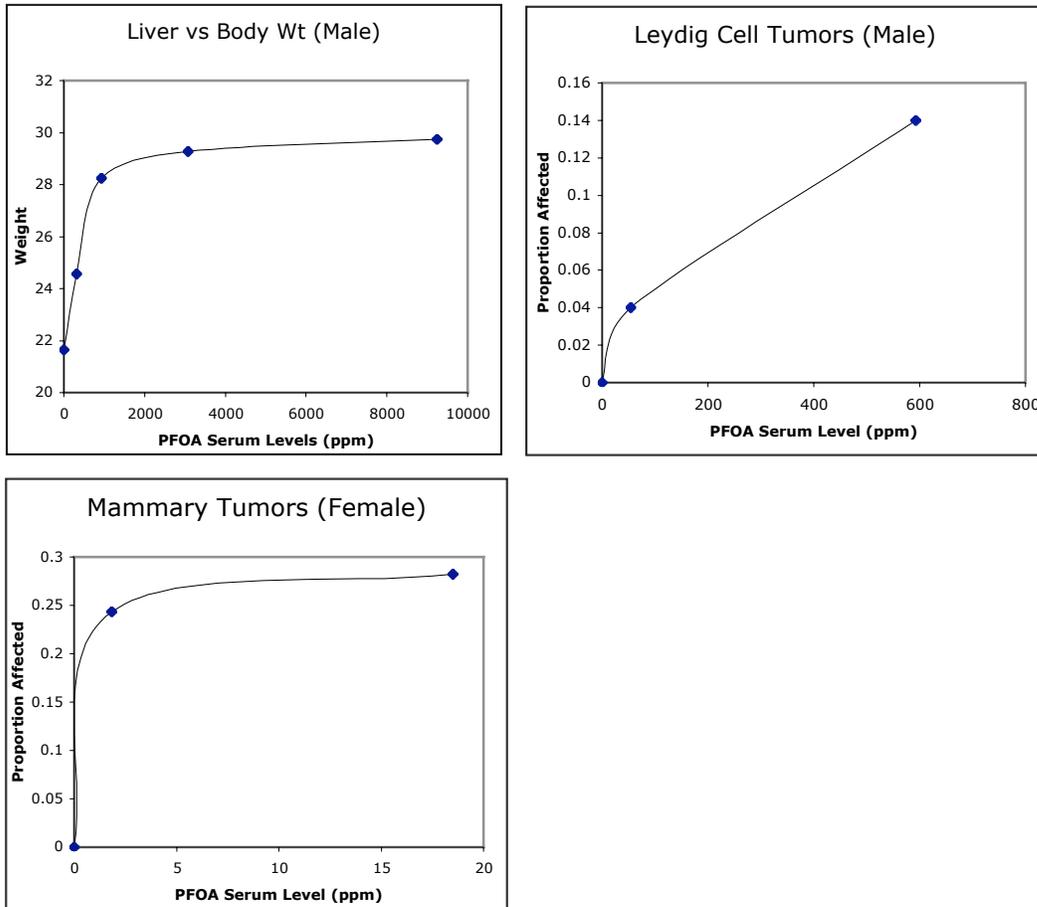
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**Figure 1.** Examples of supra-linear dose-response shapes in PFOA toxicology studies. Tumor responses are from Sibinski (1987) data; Liver vs Body weight is from York (2002) data. A steep response is seen at low doses followed by a plateau.



**Figure 2.** Examples of supra-linear dose-response shapes in PFOA toxicology studies. All graphs are derived from data in York (2002). A steep response is seen at low doses followed by a plateau. The female plateau is more dramatic than the male plateau.

