Comments on the tumor sections of the Environmental Protection Agency document entitled

**Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and Its Salts.**

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Dr. Popp has broad based experience in regulatory and experimental toxicology. Particularly germane to this statement is Dr. Popp’s 10 years of laboratory work devoted to understanding the mechanism and potential human health significance of rodent tumors related to animals exposed to PPAR alpha agonists. While providing intellectual direction to a carcinogenesis research program, Dr. Popp also served as a former Department Head and Vice President of CIIT. In more recent years, he has held leadership roles in the pharmaceutical industry and in multiple professional organizations related to toxicology. He currently and/or recently has served on multiple government advisory panels.
Summary:
As summarized in the well written Environmental Protection Agency document entitled “Draft Risk Assessment of the Potential Human Health Effects Associated with the Exposure to Perfluorooctanoic Acid and Its Salts”, there is a vast amount of data available to understand the animal carcinogenicity results of perfluorooctanoate (PFOA) and to utilize for assessment of potential risk to humans. The purpose of the current document is to stress selected points regarding animal carcinogenicity as it relates to assessing human risk.

The mode of action for the rodent liver tumors is undoubtedly related to the Peroxisome Proliferating Receptor (PPAR) alpha agonist activity of PFOA since the activation of this receptor and subsequent responses are documented through multiple data sets. Several independent review groups have come to the conclusion that liver tumors that occur as a result of activation of PPAR alpha receptor do not have relevance for humans. It is important to note that other modes of action have been appropriately excluded for the liver tumors including a likely role of hepatocellular necrosis (see below).

The mode of action of rodent Leydig cell tumors in the testis has also been extensively evaluated with resultant data demonstrating key effects of hormonal perturbation. While there is incomplete data to fully understand the mechanism of action leading to the Leydig cell tumors, there is adequate information related to the mode of action to allow an informed risk assessment for humans. The extensive data sets from humans (occupationally exposed with higher exposure than the general population) have demonstrated that perturbations of estradiol, testosterone and luteinizing hormone do not occur in exposed humans providing reassurance that Leydig cell tumors will not occur in exposed humans.

The rodent pancreatic acinar cell tumors are apparently associated with CCK induction although this is an inferential conclusion based on the effects of another PPAR agonist compound (WY14,643). It is important to note that the mode of action of the Leydig cell tumors and pancreatic acinar cell tumors are not linked to activation of the PPAR alpha receptor at this time. Therefore the risk assessment is based on an understanding of other modes of action for these 2 tumor types. In addition to the fact that the modes of action of all 3 tumor types are unlikely to occur in humans, there is a large internal exposure difference between animals with tumors and the general human population.

The final characterization of PFOA as “suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential” is the only reasonable descriptor that can be utilized from the currently available set of EPA descriptions. However, this statement appears to me to overstate the level of concern for humans when the mode of action data and internal exposure differential between humans and rodents are considered. A complete assessment of the data would suggest to me that PFOA probably does not cause a human carcinogenic response. It is interesting to note that very similar phraseology is used within the text (but not in the Executive summary) of the EPA Draft Risk Assessment: “However, the LCT (Leydig cell tumors) and the PACT (pancreatic acinar cell tumors) induced in the rat by PFOA probably do not represent a significant cancer hazard for humans…” (EPA Cancer Risk Assessment, page 84, line 7).
Comments on the human risk assessment of PFOA exposure based on individual tumor types:

Rodent Liver tumors:
PFOA has clearly resulted in a rodent liver tumor response although it is interesting to note that the response in terms of tumor incidence and in terms of the benign versus malignant tumors is rather modest compared to some other PPAR alpha agonists. The extensive review of the literature by the ILSI effort (Klaunig et al. Critical Reviews in Toxicology 33: 655-780, 2003) has resulted in the identification the key events (addressed below for PFOA) that characterize a rodent liver tumor response due to activation of the PPAR alpha receptor. The available information demonstrates that PFOA induced rodent liver tumors generally meets the criteria with several specific points to be addressed here.

The evidence that PFOA is a PPAR alpha agonist is overwhelming since the binding to the PPAR receptor has been demonstrated and the pleitropic responses are consistent with a PPAR alpha agonist. Peroxisomes are greatly induced by PFOA which is a hallmark of a PPAR alpha agonists that cause liver tumors. In addition, the livers of treated animals are enlarged to approximately 2 fold compared to control animals. While hypertrophy of hepatocytes clearly contributes to the enlarged liver, it is probable (although not demonstrated) that there is a hepatocyte proliferative response for a liver to obtain this volume.

As noted by the EPA Risk Assessment document (page 80), there is a numerical increase in hepatocyte proliferation at several time points beyond one month of treatment. This effect is very modest as would be expected for most PPAR alpha agonists with the exception of a very few such as WY14,643. The most dramatic increase in hepatocyte cell proliferation occurs during the first week following the onset of treatment with PPAR alpha agonists. Such data is not available for PFOA. Therefore, the minimal chronic hepatocyte proliferation in the chronic PFOA studies should not be interpreted as data that is in disagreement with the appearance of tumors related to a PPAR agonist. Indeed such data is consistent with the hepatocyte proliferation data generated for most PPAR alpha agonists.

The clonal expansion leading to the formation of tumors and the appearance of eventual tumors is noted with all hepatocarcinogens and is not unique for PPAR alpha agonist induced liver tumors.

It is important to note that other hepatocarcinogenic modes of action have generally been excluded. The question of the role of hepatic necrosis as a contributing mode of action for the liver tumors has been raised in the EPA document. However the following points must be considered. Several studies have demonstrated hepatocyte necrosis at high doses. However, in the original carcinogenesis study (Sibinski, Two year oral Toxicity/Carcinogenicity Study of Fluorochemical FC-143 in Rats, Riker Laboratory), necrosis was noted at 1 year in high dose animals but the controls and high dose animals had similar incidence of hepatic necrosis at 2 years. It is important to note that hepatic tumors were not identified in this study.
The second carcinogenicity study (Biegel et al. Toxicology and Applied Pharmacology 60: 44-54, 2001) focused on elucidating the mode of action of extrahepatic tumors associated with PFOA exposure and therefore did not report non tumor effects in the liver. The potential of necrosis as a significant factor in the hepatic tumor response noted in this study is mitigated by the fact that liver tumors were identified yet a persistent statistically significant elevation of hepatocytic proliferation was not noted. If necrosis had been playing a significant role in the mode of action of the hepatic tumors, a more dramatic sustained increase in hepatocytes proliferation would have been expected. The ILSI effort referenced above came to the conclusion that the mode of action of PPAR alpha agonist induced liver tumors “...is not likely to occur in humans based on differences in several key steps, when taking into consideration kinetic and dynamic factors”.

**Leydig Cell tumors**

Leydig cell tumors have been identified in a 2 year study of PFOA in rats (Biegel et al. Toxicology and Applied Pharmacology 60: 44-55, 2001). While a potential role of the PPAR receptor in the induction of these tumors is unknown, it is interesting that many PPAR alpha agonists have also induced the Leydig cell tumors when the compound was tested in a strain of rat where a Leydig cell tumor response could be detected. The information on the mode of action of this tumor type is again very extensive and demonstrates hormonal perturbations that strongly suggest a mode of action in animals (Biegel et al. Toxicology and Applied Pharmacology 134: 18-25, 1995; Liu et al. Fundamental and Applied Toxicology 30: 102-108, 1996). The data set is difficult to evaluate since 2 major hormones (i.e. testosterone and estradiol) are affected, the results for any one hormone are variable depending on time of treatment, and in vivo versus in vitro evaluations may at first appear to be contradictory.

A consistent and long term hormonal effect is an unequivocal elevation of estradiol that has been demonstrated in male rats in several studies. Mode of action studies have clearly demonstrated that the estradiol elevation is related to a hepatic induction of aromatase, an enzyme that converts testosterone to estradiol (Liu et al. Fundamental and Applied Toxicology 30: 220-228, 1996). Elevated estradiol levels have been demonstrated in the circulation and even in the local environment of the testis (Biegel et al. Toxicology and Applied Pharmacology 134: 18-25, 1995). There is also an increase in the local testicular level of TGF alpha associated with the elevation of estradiol. The consequence of the elevated TGF alpha is unclear. However, there is a basic literature (unrelated to PPAR alpha agonists) that has demonstrated that TGF alpha can increase cell proliferation of Leydig cells in the developing testis. It should be noted that an increase in Leydig cell proliferation was not identified in a 2 year mode of action study. However, as with the comment above related to the lack of hepatocyte proliferation, a potentially critical time for evaluation, i.e. shortly after the initiation of chemical exposure, has not been evaluated. Therefore, there is a mode of action for the developing Leydig cell tumors related to the elevated estradiol (related to aromatase induction) and an accompanying local increase in TGF alpha.

Based on this mode of action there is a relevant human biomarker of effect, i.e. circulating estradiol concentrations, that can and has been evaluated in humans with negative findings. It is important to note that this human population is composed of
occupationally exposed individuals who have much higher exposure than the general population. This same occupationally exposed group of humans has also been evaluated for effects on testosterone, another hormone central to assessing testicular function since it is produced by the Leydig cell. The lack of effects on testosterone in PFOA exposed workers is reassuring that there is no chronic effect on Leydig cell function. However, the value of this data is less compelling than the estradiol data since no chronic effects on testosterone have been described in rodents compared to consistent and long term effects on estradiol in rodents.

There are well characterized effects of PFOA on testosterone in rodents including decreased testosterone in the whole animal in a 2 week study and demonstrated inhibition of testosterone synthesis in in vitro studies. The basis for a lack of a chronic effect on testosterone is not clear but may result from compensatory mechanisms as occurs with chemical perturbations of other hormonal systems. In summary, while the changes in testosterone noted short term in rodent studies may contribute to the Leydig cell tumors, the most convincing evidence is that the mode of action resulting in testicular tumors is through increased estradiol again providing a human biomarker of effect. The lack of hormonal alterations in workers strongly indicates that there is little to no risk of testicular tumors in the general population.

Pancreatic acinar cell tumors
As with the Leydig cell tumors, pancreatic acinar cell tumors have been noted in conjunction with liver tumors and in some cases with Leydig cell tumors with several other PPAR alpha agonists. While the association of these 3 tumor types is intriguing and may suggest a common association with activation of the PPAR alpha receptor, there is inadequate data to support a PPAR receptor mode of action for the rodent pancreatic tumors associated with PFOA or any other PPAR alpha agonist. Therefore, the mode of action of the pancreatic acinar cell tumors must be assessed independent of any association with the PPAR alpha receptor.

In rodents, pancreatic acinar cell tumors associated with non genotoxic compounds or dietary manipulation are commonly mediated through an increase in cholecystokinin (CCK), a pancreatic cell trophic factor that causes cell proliferation in acinar cells. It is important to note that sustained pancreatic acinar cell proliferation has been demonstrated (Biegel et.al. Toxicological Sciences 60:44-55, 2001) in PFOA treated rats. Attempts have been made to assess if CCK elevation provides a basis for the chronic increase in rodent acinar cell proliferation thereby providing a basis for the mode of action for PFOA induced pancreatic acinar cell tumors. The most straight forward data would be the demonstration of elevated CCK in PFOA treated rodents similar to the elevation noted with other compounds and dietary manipulation which result in pancreatic acinar cell tumors through CCK elevation. The data to support this mechanism for PFOA induced pancreatic acinar cell tumors is inferential. The experiment with PFOA has been attempted but results were not obtainable due to technical difficulties in the measurement of CCK. Apparently, the PFOA in the circulation resulted in interference in the immunoassay making assessment of CCK impossible in these rodent studies. The inferential information is supplied by a study with WY 14,643, a PPAR alpha agonist that has been demonstrated to result in pancreatic acinar cell tumors. In these studies (Obourn et.al. Toxicology and Applied Pharmacology 145: 425-436, 1997), CCK was elevated
supporting the concept that a CCK mode of action is likely involved in pancreatic acinar cell tumors in WY14,643 exposed animals and providing a distinct suggestion that a CCK mode of action is involved in the pancreatic acinar cell tumors associated with PFOA.

It should, however, be noted that chronic pancreatic acinar cell proliferation was not demonstrated in WY14,643 treated rats in another study (Biegel et al. Toxicological Sciences 60:44-55, 2001). However, as with the hepatocytes and the Leydig cells, proliferation of pancreatic acinar cells was not evaluated in the early time period following initiation of treatment. While the increase in CCK with WY14,643 provides only circumstantial support for a CCK mechanism for PFOA, it should be stressed that this is the dominant mode of action for the formation of rodent pancreatic acinar cell tumors for non genotoxic compounds. As pointed out in the EPA Risk Assessment document and in many publications, the control of pancreatic exocrine excretion in humans is neural based rather than through release of CCK based on intestinal contents. Therefore, the CCK mechanism of tumor induction is not known nor believed to occur in humans.

It is also important to note that a very high proportion of human pancreatic tumors are not of the acinar cell type but rather is a ductal cell type.

**Human exposure compared to animal exposures associated with tumor responses**

The above analysis provides a basis for assessing human risk based on mode of action where the mode of action for the various tumors are not likely to occur in humans. However, another important aspect is the margin of exposure between the internal exposures required to induce the various rodent tumor responses compared to human internal exposures. These margins of exposures are quite large and are detailed in the EPA Risk Assessment document and will not be reiterated here. Such large margins of exposure are very important ancillary information in addition to the mode of action of the tumors for supporting a conclusion that the PFOA non hepatic tumor responses in rodents “…probably do not represent a significant cancer hazard for humans…” (EPA Draft Risk Assessment Page 84, line 7). As already noted above, the ILSI reference has concluded that the mode of action of PPAR alpha agonist induced tumors “…is not likely to occur in humans based on differences in several key steps, when taking into consideration kinetic and dynamic factors”. This position is supported by the EPA Risk Assessment.