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Review

Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)

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ABSTRACT — Perfluoroalkyl substances (PFASs) are persistent environmental contaminants. Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) are representatives of PFASs. Recently, the U.S. Environmental Protection Agency (US EPA) set the health advisory level as 70 parts per trillion for lifetime exposure to PFOS and PFOA from drinking water, based on the EPA's 2016 Health Effects Support Documents. Then, a monograph on PFOA was made available online by the International Agency for Research on Cancer, where the agency classified PFOA as "possibly carcinogenic to humans" (Group 2B). The distinction between PFOS and PFOA, however, may not be easily understood from the above documents. This paper discussed differential toxicity between PFOS and PFOA focusing on neurotoxicity, developmental toxicity and carcinogenicity, mainly based on these documents. The conclusions are as follows: Further mechanistic studies may be necessary for ultrasonic-induced PFOS-specific neurotoxicity. To support the hypothesis for PFOS-specific neonatal death that PFOS interacts directly with components of natural lung surfactant, *in vivo* studies to relate the physicochemical effects to lung collapse may be required. PFOA-induced DNA damage secondary to oxidative stress may develop to mutagenicity under the condition where PFOA-induced apoptosis is not sufficient to remove the damaged cells. A study to find whether PFOA induces apoptosis in normal human cells may contribute to assessment of human carcinogenicity. Studies for new targets such as hepatocyte nuclear factor 4α (HNF4α) may help clarify the underlying mechanism for PFOA-induced carcinogenicity.

Key words: PFOS, PFPA, Toxicity

INTRODUCTION

Perfluoroalkyl substances (PFASs) are persistent, bioaccumulative and detected in humans. Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) are representatives of PFASs.

In May 2016, the US EPA set the health advisory level as 70 parts per trillion for lifetime exposure to PFOS and PFOA from drinking water, based on their developmental toxicities for rats and mice, respectively (USEPA, 2016a, 2016b). The detailed descriptions are shown in the EPA's 2016 Health Effects Support Documents (HESD) for PFOS and PFOA, respectively (USEPA 2016c, 2016d).

The US EPA (2016a) summarizes PFOS as follows: The developing fetus and newborn are particularly sensitive to PFOS-induced toxicity. The level for PFOS of 0.07 micrograms per liter (µg/L) is based on a reference dose (RfD) derived from a developmental toxicity study in rats, where the critical effect was decreased pup body weight following exposure during gestation and lactation. For carcinogenicity, the US EPA (2016a) has concluded as follows: Although some human studies suggest an association with bladder, colon, and prostate cancer, the literature is inconsistent and some studies are confounded by failure to control for risk factors such as smoking. The evidence for cancer in animals was judged to be too limited to support a quantitative cancer assessment (i.e., no dose-response).

The US EPA (2016b) summarizes PFOA as follows: The level for PFOA of 0.07µg/L is based on an RfD derived from a developmental toxicity study in mice, where the critical effects included reduced ossification in proximal phalanges and accelerated puberty in male pups following exposure during gestation and lactation. For

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carcinogenicity, the EPA (2016b) summarized as follows; there is suggestive evidence of carcinogenic potential for PFOA. Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. Two chronic bioassays of PFOA support a positive finding for the ability of PFOA to be tumorigenic in one or more organs of rats, including the liver, testes, and pancreas. Without fully understanding the mode of carcinogenic action of PFOA, the EPA (2016b) estimated a cancer slope factor of 0.07 $(mg/kg/day)^{-1}$ based on testicular tumors, and confirmed that the level of 0.07μg/L based on noncancer effects is protective of the cancer endpoint.

On July 19, 2016, the monograph on PFOA was made available online by the International Agency for Research on Cancer (IARC), where the agency has classified PFOA as "possibly carcinogenic to humans" (Group 2B), based on limited evidence in humans that it can cause testicular and kidney cancer, and limited evidence in lab animals (IARC, 2016)

PFASs are a family of perfluorinated chemicals that consist of a carbon backbone typically 4 to 14 in length and a charged functional moiety, such as sulfonate and carboxylate. The effects of PFAS carbon chain length on the toxicity and toxicokinetics are described elsewhere (Lau *et al.*, 2007; Takahashi *et al.*, 2014). PFOS and PFOA have the same eight -carbon backbone and different charged functional moieties of sulfonate for PFOS and carboxylate for PFOA, respectively.

The above three documents reviewed current information for PFOS and PFOA. The distinction between PFOS and PFOA, however, may not be easily understood from the above documents. For the better understanding of toxicity of PFASs, delineation of different aspects of both chemicals may be of some help. Therefore, this paper discusses differential toxicity between PFOS and PFOA, focusing on neurotoxicity, developmental toxicity and carcinogenicity; mainly based on the above three documents. For an effective discussion, a summary of the above mentioned documents is first introduced. Then, further consideration is made by adding new published information and our unpublished data.

NEUROTOXICITY

HESD description

Epidemiology

Hoffman *et al.* (2010) examined the associations between perfluorochemicals and diagnosis of attention deficit hyperactivity disorder (ADHD) using the NHANES data from 1999-2000 and 2003-2004. Serum PFOS was positively associated with parental reports of ADHD. PFOA was also positively associated with parentally reported ADHD. Data interpretation was limited by the cross-sectional study design, other potential confounders (e.g., alcohol consumption) that were not included in the available data, and measurement error resulting from using current PFOS or PFOA levels as proxy measures of etiologically relevant exposures.

Animal studies

Available *in vivo* and *in vitro* studies for PFOS neurotoxicity focused on mechanistic endpoints to a greater extent neurobehavioral indications of neurotoxicity. Effects observed included altered levels of excitatory amino acids in the brain of rats (Yang *et al.*, 2009), and changes in neurotransmitter levels and increases in miniature post-synaptic currents (mPSC) along with inward calcium currents shown in cultured Sprague-Dawley rat hippocampal neurons (Liao *et al.,* 2009). One study by using the Morris water maze found effects on learning and memory in mice at approximately 2 mg/kg/day (Long *et al.,* 2013). Developmental neurotoxicity studies in rats found increased motor activity and decreased habituation and increased escape latency in the water maze test following in utero and lactational exposure to PFOS (Butenhoff *et al.*, 2009). Ten-day old male mice exposed to PFOS showed affected habituation up to 4 months of age (Johansson *et al.*, 2008). One animal study (Johansson *et al.*, 2009) suggests a potential effect on habituation and activity patterns in mice treated on postnatal day 10 with a single dose of PFOA and evaluated at 2 and 4 months of age. The *in vivo* observations were supported by changes in the expression of a variety of neurologically active brain proteins in the treated pups. The results of an *in vitro* study of hippocampal synaptic transmission and neurite growth showed that 50 and 100 μM PFOA increased spontaneous synaptic current and had an equivocal impact on neurite growth (Liao *et al.,* 2009).

Further consideration

Sato *et al.* (2009) reported distinct difference in neurotoxicity between PFOS and PFOA. Single oral administration of PFOS or PFOA did not cause any neurotoxic symptoms up to their sub lethal doses in young adult rats and mice, when examined 14 days after the dosage by the detailed functional observational battery (FOB) including excitability such as Straub tail, tremors, twitches, convulsions, restlessness, alertness, motor activities; and by using the method of Irwin (Irwin, 1967) for startle response, touch response, pain response, righting reflex, visual placing, abdominal tone and limb tone. However,

fatal tonic convulsions appeared in the PFOS-treated rats (250 mg/kg or more) and mice (125 mg/kg or more) when ultrasonic stimulus was applied to the animals. The same ultrasonic stimulus never induced convulsions in the control animals or PFOA-treated animals. No morphological changes were detected by histopathological examination of the brain. There were no changes in concentrations of norepinephrine, dopamine, serotonin, glycine, 4-aminobutylic acid, and glutamic acid in the brain. Kawamoto *et al*. (2011) treated rats (5-week old at the start) with dietary PFOS for 13 weeks. PFOS did not show any neurotoxic symptoms when examined by detailed FOB or Irwin method; however, 5 out of 6 rats showed tonic convulsions in the 6th week when ultrasonic stimulus was applied to the 128 ppm rats (corresponding to the total PFOS dose of 338 mg/kg/6 weeks). Histopathological examinations and electron microscopic examinations could not detect any abnormality in the brain. Because the acute oral convulsive dose of PFOS was 250 mg/kg (Sato *et al.,* 2009), the convulsion induced by PFOS seemed to depend on its total dose regardless of treatment schedule. The information provided by Liao *et al.* (2009), that the potency on increases in the frequencies of spontaneous mPSC of perfluorinated carboxylates was less pronounced than that of perfluorinated sulfonates, is supportive to this PFOS-specific neurotoxicity. Kawamoto *et al.* (2008) reported as follows: PFOS (15 μM or higher) but not PFOA caused backward swimming of paramecia, which was induced by intracellular free $Ca2^{+}$ at 0.2 μM and higher. The surfactant activity of PFOS is stronger than PFOA shown by the critical micelle concentrations of PFOS and PFOA at around 8 mM and 25 mM, respectively (Harada *et al.*, 2005). Thus, effects of various surfactants including PFOS and PFOA on the swimming behavior of paramecia were compared with the hemolysis of mouse erythrocytes as an indicator of surfactant activities. The hemolysis did not correlate with their swimming behavior. A voltage-clamp study indicated that PFOS had no direct effect on the depolarization-induced Ca^{2+} influx responsible for the action potential. PFOS induced cell membrane excitability, and the action potential of the paramecium was induced at lower current intensity with PFOS. The ultrasonic-induced fatal tonic convulsion of rodents might be related to the PFOS-specific cell membrane excitability shown by Liao *et al.* (2009) and Kawamoto *et al.* (2008).

The PFOS-specific ultrasonic-induced neurotoxicity in rodents was observed at much higher levels of exposure than those expected to humans. Thus, the relation with human neurotoxicity is unclear. However, the effect is PFOS-specific, cumulative; and not detected by any of the ordinary behavioral observations, the biochemical analysis and the histopathology including the electron microscopic examination. Younger mice pups were more sensitive to PFOS exposure as shown for oxidative damage (Liu *et al.,* 2009). As described in HESD, PFOS is not readily eliminated from humans, as evidenced by the estimated average half-life values of 4.1-8.67 years. In contrast, half-life values for the rat and mouse are 48 days and 37 days, respectively. If the steady-state body burden is solely dependent on the excretion, the human body burden should be nearly 100 times as high as mice. The cause of ADHD is not well known, but the association of child-specific ADHD with PFOS exposure is suggested in epidemiological studies. Therefore, further study may be necessary for this ultrasonic-induced PFOS-specific neurotoxicity.

DEVELOPMENTAL TOXICITY

HESD description

Epidemiology

Epidemiological studies suggest a correlation between higher PFOS levels and decreases in female fecundity and fertility, in addition to decreased body weights in offspring, and other measures of postnatal growth. The epidemiology studies did not find associations between PFOA and neurodevelopmental effects, or preterm birth and other complications of pregnancy.

Animal studies

The US EPA derived a reference dose (RfD) for PFOS of 0.00002 mg/kg/day based on decreased neonatal rat body weight from the two-generation study by Luebker *et al.* (2005). To help characterize the mechanism of PFOS-induced neonatal mortality, Grasty *et al.* (2003) examined critical windows of exposure by treating rats with a high dose of PFOS (25 mg/kg/day) for a 4-day period during various stages of pregnancy. Neonatal mortality occurred after all treatment periods, but the incidence of neonatal death increased when exposure occurred later in gestation. Mortality was highest when treatment occurred on gestation days (GDs) 17-20, identifying late gestation as a critical exposure window for increasing the risk of neonatal survival. Neonatal death was shown to be a direct effect of PFOS on the lung surfactant. Currently, the leading hypothesis for the mode of action (MOA) of PFOS-induced neonatal mortality is that PFOS interacts directly with components of natural lung surfactants (Xie *et al*., 2010a, 2010b). PFOS interacts with the major phosphatidylcholine components of pulmonary surfactants and cell membranes and, therefore, has the potential to alter the dynamic properties of lung surfactant (Xie *et al.,*

2010a). PFOS partitions into phospholipid membranes to increase membrane fluidity in several cell types (Xie *et al.,* 2010b). This high tendency of PFOS to partition into phosphatidylcholine lipid bilayers is consistent with its resemblance to medium chain fatty acids and may be responsible for interfering with the normal physiological function of pulmonary surfactant.

For PFOA, developmental effects observed in animals include decreased survival, delayed eye opening and reduced ossification, skeletal defects, altered puberty (delayed vaginal opening in females and accelerated puberty in males), and altered mammary gland development. Wild -type and peroxisome proliferator-activated receptor (PPAR) α-null mice were used in studies to determine if PFOA-induced developmental toxicity was mediated by PPARα (Abbott *et al.,* 2007). The authors concluded that survival of PPARα-null pups and deaths of heterozygous pups born to PPARα-null dams indicates that expression of PPARα is required for PFOA-induced postnatal lethality; however, early prenatal lethality was independent of PPARα. Delayed eye opening and reduced postnatal weight gain appeared to be mediated by PPARα, but other mechanisms might also contribute.

Further consideration

PFOA-induced neonatal death is, at least in part, mediated by PPAR-α, as stated in the review of Lau *et al*. (2007) that PPARα signal is required for PFOA-induced postnatal lethality and expression of one copy of the gene is sufficient for this effect.

However, "the leading hypothesis" for the MOA of PFOS-induced neonatal mortality that PFOS interacts directly with components of natural lung surfactant may prompt a naive question. Why does PFOS, a surfactant, cause lung atelectasis by its direct effect on the lung?

The PFOS-induced neonatal death was found by Lau *et al.* (2003) as follows: Prenatal PFOS exposure significantly reduced the postnatal survival of rat pups in a dose-dependent manner. At parturition, all animals were born alive and pink in color, and all appeared to be active. However, in the 10 mg/kg group, the neonates became pale, inactive, and moribund within 30-60 min, and all died soon afterward. Similar to the rat, prenatal PFOS exposure reduced the postnatal survival of the mouse in a dose-dependent manner. Most offspring exposed to 15 or 20 mg/kg PFOS did not survive for 24 hr after birth. Lau *et al.* (2006) then reported PFOA-induced neonatal death in CD-1mice as follows: Exposure to PFOA during pregnancy slightly increased the average time to parturition, by up to half a day in the high-dose group. Most offspring were born alive, but the incidence of stillbirth and neonatal mortality was increased markedly by PFOA treatment, particularly in the high-dose groups (up to 30%). Most of the neonates exposed to 10 or 20 mg/kg PFOA did not survive the first day of life. Lau *et al.* (2007) concluded about the PFOS- and PFOA-induced neonatal deaths as follows: When neonatal survival was evaluated in this reproductive study with PFOA, a pattern of neonatal mortality mirroring that obtained with PFOS was observed.

On the other hand, the difference between PFOS- and PFOA-induced neonatal deaths was found later as follows: Yahia *et al*. (2008) gave ICR mice 1, 10 or 20 mg/kg PFOS daily by gavage from gestational day (GD) 0 to the end of the study. Almost all fetuses at 20 mg/kg were alive on GD18 and showed normal lung structure by histopathological observation; but at parturition, all neonates were inactive and weak, showed severe lung atelectasis (almost complete collapse) and severe dilatation of intracranial blood vessel when examined histopathologically, and died within a few hours. At 10 mg/kg, all neonates were born alive, 27% showed slight lung atelectasis, all of them had mild to severe dilatation of the intracranial blood vessel, and 45% of neonates died within 24 hr. The authors suggested that the mechanistic studies are necessary to examine whether the dilated intracranial blood vessel might have pressed the respiratory center of the brain, resulting in preventing the lungs to normally respire after birth. Then, Yahia *et al.* (2010) gave pregnant ICR mice 1, 5 and 10 mg/kg PFOA daily by gavage during their gestation days in order to compare with the PFOS effects. PFOA treatment reduced the fetal body weight at 5 and 10 mg/kg. Teratological evaluation of fetuses showed delayed ossification of the sternum and phalanges, and delayed eruption of incisors at 10 mg/kg, but did not show intracranial blood vessel dilatation. Postnatal evaluation revealed that PFOA reduced the neonatal survival rate at 5 and 10 mg/kg. At 5 mg/kg pups were born alive and active and 16% died within 4 days' observation, while 58% of pups were stillborn and all died within 6 hr after birth at 10 mg/kg without showing intracranial blood vessel dilatation. The histopathological examination of neonates showed no changes between the control and exposed mice either in the lung or brain.

The fact that mortality was highest when PFOS treatment occurred on gestation days (GDs) 17-20 favors the direct surfactant theory, but PFOA also showed a similar critical window as shown by Wolf *et al.* (2007) as follows: PFOA exposure during the earliest stages of gestation (GD1-6) is not required to produce the developmental toxicity observed in this study, and exposure to higher doses late in gestation (GD15-17) can be sufficient to affect postnatal weight gain.

Both PFOS and PFOA are surfactants. If PFOS directly interact with pulmonary surfactant, the lung may be inflated but not collapsed. The route of exposure appropriate for examining the direct effect of chemicals with the lung may be inhalation. Kennedy *el al.* (2004) described the effect of PFOA on acute inhalation toxicity studies by citing their own study (Kennedy *et al.*, 1986) as follows: The approximately 4-hr lethal concentration in rats was 0.81 mg/L and the LC50 was 0.98 mg/L. All death occurred within 48 hr of exposure, and dying rats has hyper-inflated lungs. No lung collapse was mentioned for PFOS inhalation in the description in HESD (2016), which cites the report of Rusch *et al.* (1979) as follows: Sprague-Dawley rats were exposed to PFOS dust for 1 hr. Rats were observed for abnormal signs prior to exposure, every 15-min during exposure, at removal from the chamber, hourly for 4 hr after exposure, and then daily for up to 14 days. All rats in the 24.09 mg/L group died by day 6. Mortality for the other groups was 0%, 10%, 20%, 80%, and 80% in the 1.89, 2.86, 4.88, 6.49, and 7.05 mg/L groups, respectively. Clinical signs observed included emaciation, red material around the nose or other nasal discharges, dry rales, breathing disturbances, and general poor condition. Necropsy results indicated discoloration of the liver and lung. Based on the findings, the acute inhalation LC50 was 5.2 mg/L (ppm). Thus, *in vivo* studies to relate the physicochemical effects to lung collapse to support the "leading hypothesis" for the MOA of PFOS-induced neonatal death that PFOS interacts directly with components of natural lung surfactant may be required.

CARCINOGENICITY

HESD & IARC descriptions

The summary of HESD description is as follows: Applying the U.S. EPA Guidelines for Carcinogen Risk Assessment, there is suggestive evidence of carcinogenic potential for PFOS and PFOA (USEPA, 2005).

In a chronic oral toxicity and carcinogenicity study of PFOS in rats, biologically significant tumors were found slightly only in high-dose rat livers. The genotoxicity data are uniformly negative. Human epidemiology studies did not find a direct correlation between PFOS exposure and the incidence of carcinogenicity in worker-based populations. Other worker and general population studies found no statistically significant trends for any cancer type. Thus, the weight of evidence for the carcinogenic potential to humans was judged to be too limited to support a quantitative cancer assessment.

Epidemiology studies demonstrated an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. Two chronic bioassays of PFOA support a positive finding for its ability to be tumorigenic in one or more organs of rats, including the liver, testes, and pancreas. Mutagenicity studies of PFOA using the S. typhimurium and E. coli system have resulted in negative results. Results of clastogenicity studies in Chinese hamster ovary cells were equivocal. Micronucleus (MN) assays were negative. PFOA is known to activate PPAR pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol and bile acid biosynthesis, and retinol metabolism genes. There are some data that provide support for the hypothesis that the PPARα agonism is the MOA for the observed liver tumors in rats. PPARα is found in human livers and, when activated, is linked through activation to a number of metabolic responses but not to the large-scale peroxisome proliferation associated with tumors in rats and other rodent species. The data support a PPARα MOA for the rat liver tumors and, thus, are indicative of lack of relevance to humans. Based on PFOA-induced transcriptional activation of many other genes in PPARα-null mice, other receptors such as the constitutive androstane receptor (CAR), farnesoid receptor (FXR), and pregnane X receptor (PXR) could be involved in PFOA-induced toxicity. CAR activation can lead to hepatocyte proliferation and hepatocarcinogenesis in animals. However, the human CAR receptor is relatively resistant to mitogenic effects and less likely to induce cancers through this mechanism. In rodents, the PXR receptor can interact with PPARα in the coordination of hepatocyte proliferation, but there are differences in the amino acid composition of the ligand binding domain of the mouse receptor and the human receptor.

A significant increase in 8-OH-dG liver levels, a biomarker for oxidative stress, was observed at ≥ 10 mg PFOA/kg in the liver but not the kidney of Fischer 344 male rats by Takagi *et al.* (1991). Work with HepG2 cells by Hu and Hu (2009) suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing reactive oxygen species (ROS), impacting mitochondria, and changing expression of apoptosis gene regulators. Zhao *et al.* (2011) used human-hamster hybrid (AL) cells (containing a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11) to determine the mutagenicity of PFOA to mammalian cells. CD59 mutation frequencies were increased in AL cells after incubation with 200 μM PFOA. There was no increase in mutations in mitochondria-deficient AL cells after incubation with 100 or 200 μM PFOA. Production of ROS, nitric oxide, and

superoxide anion was significantly increased at 100 and 200 μM PFOA. Incubation with DMSO to inhibit ROS production significantly decreased the CD59 mutation frequency caused by 200 μM PFOA. In contrast, mitochondria-deficient AL cells had no increase in ROS or superoxide production after incubation with up to 200 μM PFOA. To assess whether PFOA could induce the apoptotic pathway, caspase-3/7 and caspase-9 were examined in intact AL cells. The highest concentration significantly increased caspase 3/7 and 9 activities. Incubation with 0.5% DMSO and 0.2 mM NG-methyl-L-arginine significantly decreased the increased caspase activity induced by 200 μM PFOA. The results led the authors to suggest that mitochondrial-dependent ROS might play an important role in PFOA-induced mutagenicity and that induction of caspase activities might be mediated by reactive oxygen and nitrogen species.

Gap junction intercellular communication (GJIC), a process by which cells exchange ions, messages, and other small molecules, is important for normal growth, development, and differentiation as well as for maintenance of homeostasis in muticellular organisms. Because tumor formation requires loss of homeostasis and many tumor promoters inhibit GJIC, it has been hypothesized that GJIC might play a role in carcinogenesis (Trosko *et al*., 1998). PFOA has been demonstrated to inhibit GJIC in liver cells *in vitro* and *in vivo* (Upham *et al*., 2009). However, inhibition of GJIC is a widespread phenomenon, and the effect by PFOA was neither species- nor tissue-specific. In addition it was reversible. Thus, the significance of GJIC inhibition in regard to the mode of carcinogenic action of PFOA is unknown.

IARC monograph (2016) carried out a thorough review, where genotoxicity was reviewed by citing an additional report not mentioned in HESD. The cited report showed PFOA-induced positive results in MN assay, in addition to increased levels of 8-OH-dG, ROS, and DNA strand breaks in cultured human hepatoma HepG2 cells (Yao and Zhong, 2005). The monograph concluded as follows: PFOA is not DNA-reactive, with negative results in an overwhelming number of assays for direct genotoxicity. Thus, there is strong evidence that direct genotoxicity is not a mechanism of PFOA carcinogenesis. Some studies with PFOA indicate that indirect DNA damage may result from induction of oxidative stress, however there is moderate evidence that genotoxicity overall is not a mechanism of PFOA carcinogenesis.

Further consideration

One of the differences between PFOS and PFOA is activation activity of PPARα. The activation activity of PFOA is stronger than PFOS as reported by Wolf *et al.* (2008), where concentrations predicted to produce 20% of the overall maximal responses ($C_{20\ max}$) for PFOA and PFOS are 5 and 94 μM for mouse PPARα, respectively. The activation activities of both chemicals are weaker for human than mouse, showing $C_{20\ max}$ of PFOA and PFOS are 16 and 262 μM for human PPARα, respectively. Nakamura *et al.* (2009), using humanized PPARα transgenic mouse line, showed that 2-week treatment of PFOA increased mRNA and/or protein levels of PPARα target genes cytochrome P450 Cyp4a10, peroxisomal thiolase and bifunctional protein only in the liver of wild-type mice, but not in PPARα-null or humanized PPARαmice, and suggested that human PPARα may be less responsive to PFOA than that of mice when a relatively low dose is applied.

Although the genotoxicity of PFOA was moderately denied as a mechanism of PFOA carcinogenesis in IARC monograph, the reason is not clearly stated. Thus, further consideration is made here by adding our unpublished data. Kawamoto *et al*. (2010) reported PFOA- but not PFOS-induced DNA damage using alkaline comet assay in paramecium. The DNA damage was observed at concentrations 0.01mM or more with increased ROS formation, where paramecia lost motility at 0.3mM. Nishida *et al.* (unpublished data) found PFOA-induced DNA damage in medaka as follows: PFOA induced DNA damage in both gill (300 and 1,000 ppm) and liver (100, 300 and 1,000 ppm) of medaka after 96-hr exposure measured by *in vivo* alkaline comet assay, where LC50 was 1,250 ppm for the same 96-hr exposure. The DNA damage was accompanied by an increased ROS formation only in gills at a concentration of 1,000 ppm. Kawamoto *et al.* (unpublished data) measured DNA damage in ICR male mice after exposures to PFOA using alkaline comet assay as follows: Single oral gavage administration of 500 mg/kg of PFOA did not cause DNA damage in any of the organs and tissues examined (brain, thymus, lung, liver, spleen, kidney, bone marrow; and mucosa of glandular stomach, colon, urinary bladder) 3, 6 or 24 hr after the treatment. The administrations of 120 mg/kg/day for three days increased DNA damage up to 2.5 fold only in the liver. PFOA treatment with 60 mg/kg/day for 7 days or 30 mg/kg/day for 14 days did not cause DNA damage in any of the above mentioned organs and tissues. Nakamura *et al.* (2016) reported that PFOA (at 125 mg/L and more) showed positive response (about 3 fold) in the cellular comet assay with increased ROS formation but did not show positive response in the MN test or thymidine kinase (TK) mutation assay, using $TK^{+/-}$ heterozygote of the TK6 human lymphoblastoid cell line. A

PPARα agonist, GW6471 (2 mg/L), only partly reduced PFOA-induced DNA damage while abolished the PFOA-induced ROS formation. The PFOA application also increased DNA damage (about five fold at 125 mg/L) in the nuclei measured by acellular comet assay where there is no cellular function such as PPAR. Goto *et al.* (unpublished data) also found PFOA-induced DNA damage at 125 mg/L in large nucleus of paramecium after 20min heat treatment of 80°C when measured by acellular comet assay. Whether the DNA damage observed in those acellular comet assays is a result of direct interaction of PFOA with DNA is not clear. However, negative results shown in many mutagenicity studies such as Ames tests indicate that the observed DNA damage using acellular comet assay does not induce mutation.

As described here, many studies showed PFOA-induced DNA damage or 8-OH-dG formation. However, only two studies reported by Yao and Zhong (2005) and Zhao *et al.* (2011) showed PFOA-induced mutation. In general, activation of PPAR α suppresses apoptosis as follows: Roberts *et al.* (1998) indicated that activation of rat liver PPARα provides a survival signal for hepatocytes, preventing their death in response to apoptotic stimuli. Peroxisome proliferators (PPs) such as the hypolipidaemic drug, nafenopin and the phthalate plasticiser 2-diethylhexylphthalate induce rodent hepatocyte cell proliferation and suppress apoptosis leading to tumors (Roberts *et al.,* 2002). PPAR α activations causes perturbation of cell proliferation and apoptosis (Klaunig *et al*., 2003).

PFOA is shown to induce apoptosis in many studies as follows: Shabalina *et al.* (1999) showed that PFOA exhibited a dose-and time-dependent increase in the frequency of apoptosis, starting at 200 μM in HepG2 cells; and the working group of IARC noted that these data were indicative of an antiproliferative response. Panaretakis *et al.* (2001) delineated a ROS and mitochondria-mediated pathway for induction of apoptosis by PFOA. Fernández Freire *et al.* (2008) reported that high doses of PFOA caused oxidative stress in Vero cells, which was closely linked to cell cycle arrest at the G1 phase and induction of apoptosis. Minata *et al*. (2010) reported that PFOA produced marked fat accumulation, severe cholangiopathy, hepatocellular damage and apoptotic cells especially in bile ducts in PPAR α-Null mice. Hu and Hu (2009) suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing ROS, impacting mitochondria, and changing expression of apoptosis gene regulators in HepG2 cells. However, Elcombe *et al.* (2010) failed to find PFOA-induced apoptosis that hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to PFOA occurred without affecting apoptosis through increased activation of PPARα and CAR/PXR, while a PPAR α agonist (Wy 14,643) decreased apoptosis.

The difference in PFOA-induced apoptosis among studies may be explained by the observation of O'Shea *et al.* (2011) that PFOA elicited great degree of inter-individual variability in cytotoxicity and induction of apoptosis in a study of immortalized human lymphoblast cell lines.

Thus, the positive mutations observed by two reports may have reflected the particular condition where the PFOA-induced apoptosis did not effectively manifest. Whether PFOA induces apoptosis in normal human cells or not may contribute to assessment of human carcinogenicity.

PFOA involvement of the hepatocyte nuclear factor 4α (HNF4α) inhibition as a cause of its carcinogenicity was first shown by Scharmach *et al.* (2012) in HepG2 cells by using proteomic approach as follows: Network analysis revealed that proteins affected by PFOA (25 μM) are primarily involved in lipid metabolism and cancer. HNF4α, but not PPARα, was the key regulator of the network. Subsequent western blot analysis revealed that the amount of HNF4α and its target HNF1α was downregulated by the PFOA treatment. PFOA inhibited HNF4α-dependent gene transcription as well. Buhrke *et al.* (2015) conducted a microarray analysis to screen PFOA-induced alterations in the transcriptome of human primary hepatocyte, and concluded that PFOA inhibition of HNF4α is an essential factor for liver development and embryogenesis. Beggs *et al.* (2016) indicated that PFOA- and PFOS-induced hepatic effects are mediated by HNF4α, showing that treatment of human hepatocyte with PFOA and PFOS at a concentration relevant to occupational exposure caused a decrease in HNF4α protein without affecting HNF4α mRNA or causing cell death. The studies for new targets such as HNF4α may help to clarify the underlying mechanism for PFOA-induced carcinogenicity.

CONCLUSION

1. The cause of ADHD is not well known, but association of child-specific ADHD with PFOS exposure is suggested in epidemiological studies. Therefore, further study may be necessary for this ultrasonic-induced PFOS-specific neurotoxicity.
2. To support the "leading hypothesis" for the MOA of PFOS-induced neonatal death that PFOS interacts directly with components of natural lung surfactant, *in vivo* studies to relate the observed physicochemical effects to lung collapse may be required.

3. PFOA-induced DNA damage secondary to oxidative stress may develop to mutagenicity under the condition where PFOA-induced apoptosis is not sufficient to remove the damaged cells. Whether PFOA induces apoptosis in normal human cells may contribute to the assessment of human carcinogenicity. The studies for new targets such as HNF4α may help clarifying the underlying mechanism for PFOA-induced carcinogenicity.

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REFERENCES

Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J. and Lau, C. (2007): Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicol. Sci., **98**, 571-581.

Beggs, K.M., McGreal, S.R., McCarthy, A., Gunewardena, S., Lampe, J.N., Lau, C. and Apte, U. (2016): The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol. Appl. Pharmacol., **304**, 18-29.

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

Buhrke, T., Krüger, E., Pevny, S., Rößler, M., Bitter, K. and Lampen, A. (2015): Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology, **333**, 53-62.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Farrar, D.G., Jung, R., Chang, S.C., Kennedy, G.L. and Butenhoff, J.L. (2010): Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. Arch. Toxicol., **84**, 787-798.

Fernández, Freire, P., Pérez Martin, J.M., Herrero, O., Peropadre, A., de la Peña, E. and Hazen, M.J. (2008): *In vitro* assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid. Toxicol. In Vitro, **22**, 1228-1233.

Grasty, R.C., Wolf, D.C., Grey, B.E., Lau, C.S. and Rogers, J.M. (2003): Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat. Birth Defects Res. B Dev. Reprod. Toxicol., **68**, 465-471.

Harada, K., Xu, F., Ono, K., Iijima, T. and Koizumi, A. (2005): Effects of PFOS and PFOA on L-type Ca2+ currents in guinea-pig ventricular myocytes. Biochem. Biophys. Res. Commun., **329**, 487-494.

Hoffman, K., Webster, T.F., Weisskopf, M.G., Weinberg, J. and Vieira, V.M. (2010): Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ. Health Perspect., **118**, 1732-1767.

Hu, X.Z. and Hu, D.C. (2009): Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. Arch. Toxicol., **83**, 851-861.

IARC (International Agency for Research on Cancer) (2016): Perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 110, Available at https://monographs.iarc.fr/ENG/Monographs/vol110/mono110-07.pdf

Irwin, S (1967): Drug screening and evaluation of new compounds in animals. In Animal and Clinical Pharmacologic Techniques in Drug Evaluation (Nodine, J.H. and Siegler, P.E. Ed.) pp.36-54, Year Book Medical Publishers Inc. Chicago.

Johansson, N., Fredriksson, A. and Eriksson, P. (2008): Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neuro Toxicol., **29**, 160-169.

Johansson, N., Eriksson, P. and Viberg, H. (2009): Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol. Sci., **108**, 412-418.

Kawamoto, K., Nishikawa, Y., Oami, K., Jin, Y., Sato, I., Saito, N. and Tsuda, S. (2008): Effects of perfluorooctane sulfonate (PFOS) on swimming behavior and membrane potential of paramecium caudatum. J. Toxicol. Sci., **33**, 155-161.

Kawamoto, K., Oashi, T., Oami, K., Liu, W., Jin, Y., Saito, N., Sato, I. and Tsuda, S. (2010): Perfluorooctanoic acid (PFOA) but not perfluorooctane sulfonate (PFOS) showed DNA damage in comet assay on paramecium caudatum. J. Toxicol. Sci., **35**, 835-841.

Kawamoto, K., Sato, I., Tsuda, S., Yoshida, M., Yaegashi, K., Saito, N., Liu, W. and Jin, Y. (2011): Ultrasonic-induced tonic convulsion in rats after subchronic exposure to perfluorooctane sulfonate (PFOS). J. Toxicol. Sci., **36**, 55-62.

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

Kennedy, G.L.Jr., Hall, G.T., Brittelli, M.R., Barnes, J.R and Chen, H.C. (1986): Inhalation toxicity of ammonium perfluorooctanoate. Food Chem. Toxicol., **24**, 1325-1329.

Kennedy, G.L. Jr., Butenhoff, J.L., Olsen, G.W., O'Connor, J.C., Seacat, A.M., Perkins, R.G., Biegel, L.B., Murphy, S.R. and Farrar, D.G. (2004): The toxicology of perfluorooctanoate. Crit. Rev. Toxicol., **34**, 351-384.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L. and Stevenson, L.A. (2003): Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation. Toxicol. Sci., **74**, 382-392.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B. and Strynar, M.J. (2006): Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci., **90**, 510-518.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A. and Jennifer Seed, J. (2007): Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci., **99**, 266-394.

Liao, C., Wang, T. Cui, L., Zhou, Q. Duan, S. and Jiang, G. (2009): Changes in synaptic transmission, calcium current, and neurite growth by perfluorinated compounds are dependent on the chain length and functional group. Environ. Sci. Technol., **43**, 2099-

2104.
Liu, L., Liu, W., Song, J., Yu, H., Jin, Y., Oami, K., Sato, I., Saito, N. and Tsuda, S. (2009): A comparative study on oxidative damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages. J. Toxicol. Sci., **34**, 245-254.
Long, Y., Wang, Y., Ji, G., Yan, L. Hu, F. and Gu, A. (2013): Neurotoxicity of perfluorooctane sulfonate to hippocampal cells in adult mice. PLos One, **8**, e54176.
Luebker, D.J., York, R.G., Hansen, K.J., Moore, J.A. and Butenhoff, J.L. (2005): Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: Dose-response and biochemical and pharmacokinetic parameters. Toxicology, **215**, 149-169.
Nakamura, T., Ito, Y., Yanagiba, Y., Ramdhan, D.H., Kono, Y., Naito, H., Hayashi, Y., Li, Y., Aoyama, T., Gonzalez, F.J. and Nakajima T. (2009): Microgram-order ammonium perfluorooctanoate may activate mouse peroxisome proliferator-activated receptor alpha, but not human PPARalpha. Toxicology, **265**, 27-33.
Nakamura, M., Takahashi, T., Izumi, T., Miura, M., Kawaguchi, S., Yamamoto, A., Tsuda, S., Nakamura, T., Tanaka, S., Shimizu, N. and Sasaki, Y. F. (2016): Peroxisome proliferator activated receptor-mediated genotoxicity of perfluoroalkyl acids using human lymphoblastoid cells. Fundam. Toxicol. Sci., **3**, 143-150.
Minata, M., Harada, K.H., Kärrman, A., Hitomi, T., Hirosawa, M., Murata, M., Gonzalez, F.J. and Koizumi, A. (2010): Role of peroxisome proliferator-activated receptor-alpha in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. Ind. Health., **48**, 96-107.
OECD (Organisation for Economic Co-operation and Development) (2002): Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and its Salts. 21-Nov-02. Organization for Economic Co-operation and Development, Environ Direct, Joint Mtg Chem Comm, Working Party Chem, Pest, Biotechnol. ENV/JM/RD (2002)17/FINAL. Available at: https://www.oecd.org/env/ehs/risk-assessment/2382880.pdf
O'Shea, S.H., Schwarz, J., Kosyk. O., Ross, P.K., Ha, M.J., Wright, F.A. and Rusyn, I. (2010): *In vitro* screening for population variability in chemical toxicity. Toxicol. Sci., **119**, 398-407.
Panaretakis, T., Shabalina, I.G., Grandér, D., Shoshan, M.C.and DePierre, J.W. (2001): Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma HepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid. Toxicol. Appl. Pharmacol., **173**, 56-64.
Roberts, R.A., James, N.H., Woodyatt, N.J. Macdonald, N., and Tugwood, J.D. (1998): Evidence for the suppression of apoptosis by the peroxisome proliferator activated receptor alpha (PPAR alpha). Carcinogenesis, **19**, 43-48.
Roberts, R.A., Chevalier, S., Hasmall, S.C., James, N.H., Cosulich, S.C. and Macdonald, N. (2002): PPAR alpha and the regulation of cell division and apoptosis. Toxicology, **181-182**, 167-170.
Rusch, G.M., Rinehart, W.E. and Bozak, C.A. (1979): An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. Project No. 78-7185, Bio/dynamics Inc.
Sato, I., Kawamoto, K., Nishikawa, Y., Tsuda, S., Yoshida, M., Yaegashi, K., Saito, N., and Jin, Y. (2009): Neurotoxicity of Perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. J. Toxicol. Sci., **34**, 569-574.
Scharmach, E., Buhrke, T., Lichtenstein, D. and Lampen, A. (2012): Perfluorooctanoic acid affects the activity of the hepatocyte nuclear factor 4 alpha (HNF4α). Toxicol. Lett., **212**, 106-112.
Shabalina, I.G., Panaretakis, T., Bergstrand, A. and DePierre, J.W. (1999): Effects of the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid, on apoptosis in human hepatoma HepG2 cells. Carcinogenesis, **20**, 2237-2246.
Takagi, A., Sai, K., Umemura, T., Hasegawa, R. and Kurokawa, Y. (1991): Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. Cancer Lett., **57**, 55-60.
Takahashi, M., Ishida, S., Hirata-Koizumi, M., Ono, A. and Hirose, A. (2014): Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. J. Toxicol. Sci., **39**, 97-108.
Trosko, J.E., Chang, C.C., Upham, B.L. and Wilson, M. (1998): Epigenetic toxicology as toxicant-induced changes in intracellular signalling leading to altered gap junctional intercellular communication. Toxicol. Lett., **102-103**, 71-78.
Upham, B.L., Park, J.S., Babica, P., Sovadinova, I., Rummel, A.M., Trosko, J.E., Hirose, A., Hasegawa, R., Kanno, J. and Sai, K. (2009): Structure-activity-dependent regulation of cell communication by perfluorinated fatty acids using *in vivo* and *in vitro* model systems. Environ. Health Perspect., **117**, 545-551.
USEPA (U.S. Environmental Protection Agency). (2005): Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001B. Risk Assessment Forum, Washington, DC. Available at: https://www3.epa.gov/airtoxics/cancer_guidelines_final_3-25-05.pdf
USEPA (U.S. Environmental Protection Agency). (2016a): Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). EPA 822R16004. U.S. Environmental Protection Agency, Washington, DC. Accessed May 2016. Available at https://www.epa.gov/safewater.
USEPA (U.S. Environmental Protection Agency). (2016b): Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). EPA 822R16005. U.S. Environmental Protection Agency, Washington, DC. Accessed May 2016. Available at https://www.epa.gov/safewater.
USEPA (U.S. Environmental Protection Agency). (2016c): Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). EPA 822R16002. U.S. Environmental Protection Agency, Washington, DC. Accessed May 2016. Available at https://www.epa.gov/safewater.
USEPA (U.S. Environmental Protection Agency). (2016d): Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822R16003. U.S. Environmental Protection Agency, Washington, DC. Accessed May 2016. Available at https://www.epa.gov/safewater.
Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklenyik, Z., Bryant, X.A., Thibodeaux, J., Das, K.P., White, S.S., Lau, C.S. and Abbott B.D. (2007): Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicol. Sci., **95**, 462-473.
Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C. and Abbott, B.D. (2008): Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol. Sci., **106**, 162-171.
Xie, W., Bothun, G.D. and Lehmler, H.-J. (2010a): Partitioning of perfluorooctanoate into phosphatidylcholine bilayers is chain length-independent. Chem. Phys. Lipids, **163**, 300-308.
Xie, W., Ludewig, G., Wang, K. and Lehmler, H.-J. (2010b): Model and cell membrane partitioning of perfluorooctanesulfonate is independent of the lipid chain length. Colloids Surf. B

Biointerfaces, **76**, 128-136.
Yahia, D., Tsukuba, C., Yoshida, M., Sato, I. and Tsuda, S. (2008): Neonatal death of mice treated with perfluorooctane sulfonate. J. Toxicol. Sci., **33**, 219-226.
Yahia, D., El-Nasser, M., Abedel-Latif, M., Tsukuba, C., Yoshida, M., Sato, I. and Tsuda, S. (2010): Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. J. Toxicol. Sci., **35**, 527-533.
Yang, X., Wang, L. Sun, W. and Xue, Z. (2009): Effects of perfluorooctane sulfonate on amino acid neurotransmitters and glutamine synthetase in rats. Wei Sheng Yan Jiu, **38**, 19-21.
Yao, X and Zhong, L (2005): Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. Mutat. Res., **587**, 38-44.
Zhao, G., Wang, J., Wang, X., Chen, S., Zhao, Y., Gu, F., Xu, A. and Wu, L. (2011): Mutagenicity of PFOA in mammalian cells: role of mitochondria-dependent reactive oxygen species. Environ. Sci. Technol., **45**, 1638-1644.