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Review article

Molecular mechanisms of PFOA-induced toxicity in animals and humans: Implications for health risks

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ABSTRACT

As an emerging persistent organic pollutant (POP), perfluorooctanoate (PFOA) is one of the most abundant perfluorinated compounds (PFCs) in the environment. This review summarized the molecular mechanisms and signaling pathways of PFOA-induced toxicity in animals and humans as well as their implications for health risks in humans. Traditional PFOA-induced signal pathways such as peroxisome proliferating receptor alpha (PPARα), constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and pregnane-X receptor (PXR) may not be important for PFOA-induced health effects on humans. Instead, pathways including p53/mitochondrial pathway, nuclear lipid hyperaccumulation, phosphatidylinositol 3-kinase-serine/threonine protein kinase (PI3K-AKT), and tumor necrosis factor-α/nuclear factor κB (TNF-α/NF-κB) may play an important role for PFOA-induced health risks in humans. Both *in vivo* and *in vitro* studies are needed to better understand the PFOA-induced toxicity mechanisms as well as the associated health risk in humans.

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1. Introduction

As an emerging persistent organic pollutant (POP), perfluorooctanoate (PFOA) is one of the most abundant perfluorinated compounds (PFCs) in the environment (Goosey and Harrad, 2011). It has been widely used in industrial and consumer products such as Teflon and Gore-Tex since the 1950s (Calafat et al., 2007; Steenland et al., 2010). Due to emissions during its manufacture, usage, and disposal, PFCs are widespread in the environment. Among PFCs, PFOA has received much concern as it has been detected in cord blood and breast milk in the general population of industrial countries (Apelberg et al., 2007; Ji et al., 2012; Liu et al., 2010; Llorca et al., 2010; Mondal et al., 2012). In occupational workers, the serum concentration could reach as high as a hundred mg/L (Benford et al., 2008). In non-occupational population, the highest serum concentration of PFOA was in women from South Korea (15.0–256 μg/L, mean 88.1 μg/L) (Kannan et al., 2004).

With aromatic ring being absent in their structures, PFCs are fundamentally different from traditional POPs such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). PFCs' molecule structure consists of a hydrophilic end group and a hydrophobic alkyl chain with all carbon-hydrogen (C—H) bonds being replaced by fluorine (C—F) bonds, so PFCs have been used as surfactants. Their solubility in aqueous solution is much greater than PAHs and PBDEs, for example, PFOA has a solubility of 9.5 g/L at 25 °C (Benford et al., 2008). These physicochemical characteristics make PFCs behave differently from the traditional POPs both in the environment and living organisms.

PFOA is the most frequently used PFCs and is abundant in the environment, leading to potential human exposure through food and drinking water (Benford et al., 2008). In addition, the current water treatment methods are ineffective in removing PFOA (Takagi et al., 2011), which makes drinking water a major exposure pathway of PFOA for nearby residents (Emmett et al., 2006a). Its chemical and thermal stability are mainly due to the strong C—F bonds, making it non-reactive. Therefore, long chain PFOA is non-biodegradable and bioaccumulative in the environment (Conder et al., 2008; Steenland et al., 2010).

Once absorbed by humans, PFOA is not easily metabolized with a long half-life of 2.3–8.5 y (Benford et al., 2008; Olsen et al., 2007; Seals et al., 2011). Animal experiments show that PFOA mainly accumulates in the liver, kidney and serum (van den Heuvel et al., 1991), causing multiple toxicities in animals and humans, including hepatotoxicity, genotoxicity, immunotoxicity, and neurotoxicity (DeWitt et al., 2008). Due to its long half-life in human body (Bartell et al., 2010; Olsen et al., 2007), the environmental health risks of PFOA has received an increasing concern.

PFOA's toxicity first caught attention before the 1990's (Fig. 1, drawn by Citespace, with the 752 articles searched by key words "PFOA" and "toxicity" on apps.webofknowledge.com). During the 1970's and 1980's, several reports were made by manufactures such as 3M. However, these reports are limited to the daily observations and histological appearances without further investigation. Only after the 1990's did the impacts of PFOA on enzymes in different organs received much attention. Liver toxicity was the main focus for studies from the mid-1990s to 2010. In addition, peroxisome proliferators-activated receptors (PPARs) and their downstream functions, such as peroxisome proliferation, peroxisome β-oxidation, and microsomal cytochrome P450 activation were extensively investigated during this period. Since 2010, POFA-induced endocrine, renal and neuro toxicity has been the hot topic, including development toxicity. At the present, most studies focus on the molecular mechanisms of PFOA-induced toxicity and their corresponding treatments in animals and humans.

Several reviews focused on PFOA toxicity including the highly-cited one by Lau et al. (2007) (Fig. 1). However, still much remains unclear, especially regarding the underlying molecular mechanisms (Chang et al., 2014; Klaunig et al., 2012; Lau, 2012; Mariussen, 2012; Steenland

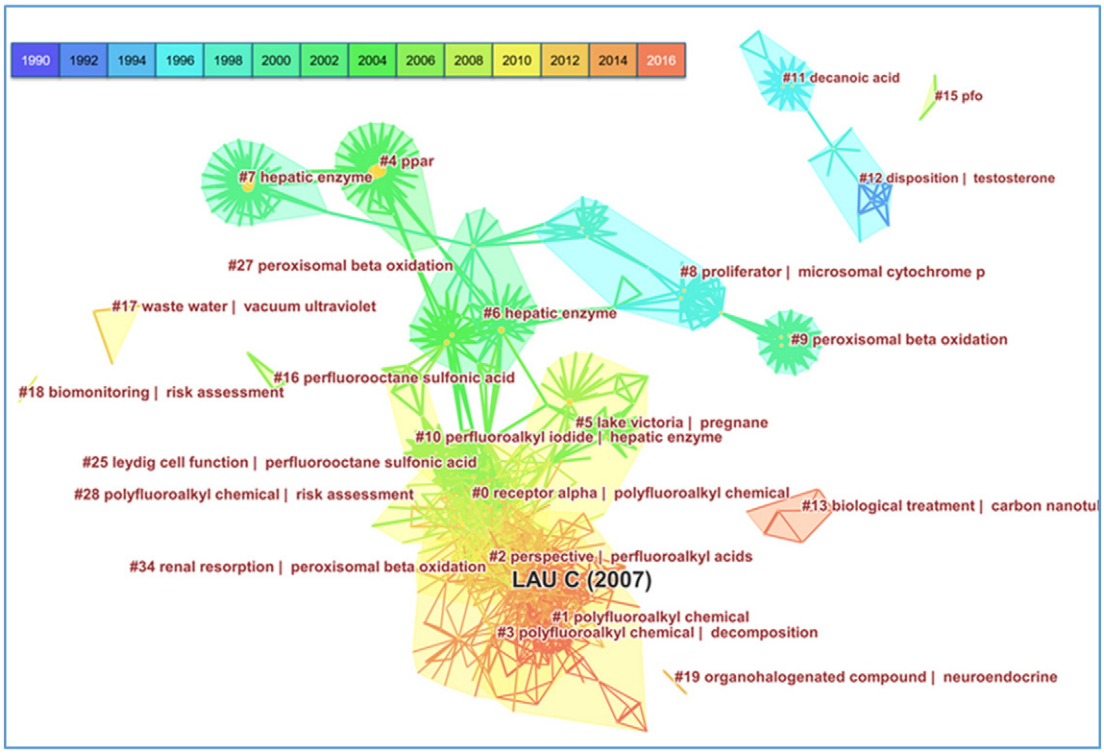

Fig. 1. Current trends on PFOA toxicity research based on a search using “PFOA” and “toxicity” (web of knowledge). Drawn by Citespace, a total of 751 publications with "PFOA" and "toxicity" in their "Title", "Abstract" or "key words" part were included (Chen, 2004). Node stands for an article, the size of a node notes citation frequency, and the color notes publication year. Lines connecting nodes show the correlation between the two articles. The shadow of the same color notes the different cluster of articles under the same topic, which is noted as “#No.” followed by cluster topic.

et al., 2010). This review mainly focuses on new information regarding the molecular mechanisms and singling pathways of PFOA-induced toxicity in animals and humans.

2. PFOA-induced toxicities in animals and humans

2.1. Toxicokinetics

van den Heuvel et al. (1991) studied PFOA toxicokinetics in rats using ^{14}C-labeled PFOA. After a single oral dose, PFOA peaked in blood after 1–2 h, with the liver and plasma accumulating the most PFOA in both genders. Female rats rapidly eliminated 91% PFOA in 24 h in the urine, with renal showing high distribution. However, in male rats, renal elimination was suppressed, resulting in the half-life of PFOA in female being much shorter than that in male rats (1 vs. 15 d). However, this gender difference has not been observed in primates nor in mice (Butenhoff et al., 2004; Hundley et al., 2006).

Andersen et al. (2008) reviewed the pharmacokinetics and toxicokinetics of various POPs, among them, the behavior of PFOA in humans has not been well studied. To better understand PFOA distribution in humans, Loccisano et al. (2013) developed a physiologically-based pharmacokinetic (PBPK) model based on renal resorption of PFOA in rats. The predicted plasma concentration curves of PFOA followed the trends observed in experimental data. Based on the PBPK model, it is possible that renal resorption is responsible for the long half-life of PFOA in humans. However, for a community in Ohio and West Virginia valley, USA where drinking water was contaminated by PFOA, the simple steady-state first-order pharmacokinetic model and robust regression model both showed good prediction of the relation between PFOA in the serum and drinking water, but showing no sex difference (Hoffman et al., 2011). Thus, after PFOA absorption, different animals may show different toxicity modes. Besides, for its exposure pathways in humans, PFOA in foods may not be completely absorbed as the lipid content affects the bioavailability of PFOA (Li et al., 2015).

Unlike other animals, the distribution of PFOA in humans has not been well studied. Previous studies mainly focused on determination of PFOA half-life based on blood concentrations. Olsen et al. (2007) collected blood samples from 26 fluorochemical workers 2.6 y after their retirement and followed up for 5 y. The estimated half-life of PFOA was 3.0–4.1 y, with a median of 3.5 y. However, for a non-occupational population of 200 people who were from the same community, after 1 y without the exposure, the median half-life of PFOA was 2.3 y (Bartell et al., 2010). They later proved that the half-life of PFOA was mainly determined by the years residing in the polluted district, with short-term high exposure yielding half-life of 2.9 y while chronical low exposure of 8.5 y (Seals et al., 2011). So far, only two studies reported the distribution of PFOA in humans. Maestri et al. (2006) developed the detection method of PFOA in human tissues. Lung, kidney, liver, and blood samples contained the highest level of PFOA (3.0 to 3.8 μg/kg), while neuro system was the lowest (≤0.5 μg/kg). Perez et al. (2013) examined PFOA concentrations in different human organs *via* autopsy, and found that PFOA accumulated mostly in the bone (0–234 μg/kg, median 20.9 μg/kg). While lung and liver also recorded high concentration (highest at 87.9 and 98.9 μg/kg with median at 12.1 and 4.0 μg/kg), kidneys showed low concentration (highest at 11.9 μg/kg with median at 1.5 μg/kg), with no detection in the brain samples. Further research is needed to examine PFOA toxicity in different organs using both *in vivo* and *in vitro* experiments.

2.2. Hepatotoxicity

Based on toxicokinetics studies, liver is the major organ for PFOA accumulation in animals. As early as the late 1970s, PFOA-induced liver enlargement has been observed in several studies (Christopher and Martin, 1977; Metrick and Marias, 1977). During a 90-d sub-chronic test, Crl: CDBR rats were exposed to diets containing 0, 10, 30, 100, 300 or 1000 mg/kg PFOA, equivalent to doses of 0.6, 1.7, 5.6, 18 and 64 mg/kg bw/d in males and 0.7, 2.3, 7.7, 22.4 and 76 mg/kg bw/d in females (Goldenthal, 1978). Hepatocellular hypertrophy showed in males exposed at doses ≥5.6 mg/kg bw/d, with hepatocellular necrosis occurring at ≥1.7 mg/kg bw/d. Recently, Perkins et al. (2004) used the same strain of rats and exposure period, they observed increased liver mass and necrosis of liver cells at >0.64 mg/kg bw/d. In addition, increased hepatic palmitoyl CoA oxidase activity was detected, a marker of peroxisome proliferation. Dose-dependent liver mass increase associated with mitochondrial proliferation was also observed in male cynomolgus monkeys after a 26 weeks oral exposure at 0, 3, 10 or 30 mg/kg bw/d (Butenhoff et al., 2002).

Besides short-term effect, long term studies have also been conducted. Sibinski et al. (1987) fed Crl: CDBR rats with 1.5 or 15 mg/kg/d PFOA in the diet for two years, corresponding to 1.30 or 14.2 mg/kg bw/d for males and 1.60 or 16.1 mg/kg bw/d for females. At 15 mg/kg/d, elevated serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatinine phosphokinase in males were observed, with increasing liver mass and nodules being obvious. In another chronic study, PFOA induced hepatocellular adenomas in Sprague-Dawley rats, with increasing liver mass and hepatic β-oxidation activity at 15 mg/kg/d (Biegel et al., 2001).

However, *in vitro* studies on PFOA toxicity in liver cell lines suggest that instead of tumorigenesis, PFOA induces apoptosis in liver tumor cells. Hu and Hu (2009) showed that, at 50–200 μmol/L, PFOA induced production of reactive oxygen species (ROS), dissipation of mitochondrial membrane potential and apoptosis in human hepatoblastoma HepG2 cells. Obviously, these effects are more correlated to epidemic studies conducted in humans. In the National Health and Nutrition Examination Survey, PFOA was associated with higher alanine aminotransferase and γ-glutamyltransferase levels, two markers of liver damage (Gleason et al., 2015; Lin et al., 2010). This was also proved by an investigation of mid-Ohio Valley community (Darrow et al., 2016), confirming the positive correlation between PFOA exposure and elevated serum alanine aminotransferase level. However, neither liver diseases nor cancers have been observed in these studies. Thus, the hepatic toxicity of PFOA in humans may not be as strong as it is shown in animal tests.

2.3. Renal toxicity

Although the liver toxicity of PFOA has been examined in most toxicity studies, kidney diseases are more closely related to PFOA toxicity in humans. Due to the limitation of selected markers, PFOA is not connected with kidney malfunction in initial surveys among occupational workers, no is significant correlation observed with elevated urea nitrogen or creatinine (Costa et al., 2009; Emmett et al., 2006b). However, it is later observed that PFOA occupational workers have 2-fold higher rates of kidney cancer than general population (Leonard et al., 2008). Steenland and Woskie (2012) found evidence of positive correlation between PFOA exposure and malignant and nonmalignant renal disease. This relation was also proved by an epidemic study in the mid-Ohio Valley community where a positive association between kidney cancer and the high serum PFOA concentration among residents was observed, but not with other cancers such as liver, bladder, lung, or pancreatic cancer (Vieira et al., 2013). Thus the evidence for correlation between PFOA exposure and kidney disease and cancer is convincing. However, so far, no animal models are able to mimic the findings in human studies, with most of the toxicity studies showing no evidence of kidney damage after PFOA exposure (Cui et al., 2009; Son et al., 2008). This may be attributed to urine elimination, a major elimination route of PFOA from humans (Zhang et al., 2013). However, there are major differences in renal elimination between animal species based on different elimination model of PFOA in humans and animals (Hoffman et al., 2011; Loccisano et al., 2013). Thus, a similar *in vivo* animal model is needed to better understand the kidney disease induced by PFOA exposure.

2.4. Immune toxicity

Unlike renal or liver toxicity showing differences in animal species, PFOA-induced immunotoxicity has been observed both in humans and animals. Immunotoxicity refers to pollutant-induced adverse effects on the function of the immune system. Unlike hepatotoxicity, PFOA-induced immunotoxicity was not investigated until the early 2000s (Yang et al., 2000; Yang et al., 2001; Yang et al., 2002a). They observed that PFOA interfered with immune system in male C57BL/6 mice, induced severe thymic and splenic atrophy, and decreased thymocytes and splenocytes, especially for the CD4(+) and CD8(+) population as well as T/B cells in the spleen. DeWitt et al. (2008) later proved that, after exposing to >3.75 mg/kg bw/d for 15 d *via* drinking water, PFOA dose-dependently suppressed T-cell dependent immunoglobulin M antibody responses in C57BL/6 female mice. On the other hand, after dermal exposure to >18.7 mg/kg bw/d PFOA for 4 d, BALB/c mice exhibited increased immunoglobulin E levels, which could trigger inflammation (Fairley et al., 2007).

Son et al. (2009) reported that, after exposing male ICR mice to 0.49–47.2 mg/kg bw/d PFOA in drinking water for 21 d, several cytokines including tumor necrosis factor-α (TNF-α), interleukin 6, and interleukin 1b were all increased at higher doses. Qazi et al. (2009) also found that, after exposing male C57BL/6 mice to 20 mg/kg bw/d PFOA for 10 d, increased *in vivo* TNF-α production was observed after both *in vitro* and *in vivo* lipopolysaccharide exposure. These differences show that PFOA exposure alters the cytokines *in vitro* and *in vivo*; however, the specific effect varies with animals and exposure routes.

The interference of PFOA on immune systems has also been observed in humans. Steenland et al. (2013) found that PFOA exposure was associated with significant increasing incidence of ulcerative colitis, a self-immune disease leading to multiple symptoms. Several animal studies and a recent human study suggest a link between exposure to PFCs and asthma, although few epidemiologic studies have been conducted. For example, Dong et al. (2013) found a positive association between PFC exposure and Taiwan children with juvenile asthma, another self-immune disease, which was accompanied by serum immunoglobulin E elevation. In addition, Humblet et al. (2014) showed some evidence for associations between exposure to PFCs and asthma-related outcomes in children. However, the evidence is inconsistent, so additional studies are needed.

2.5. Neurotoxicity

Neurotoxicity refers to pollutant-induced alteration in the normal activity of the nervous system, causing damage to nervous tissue. The neurotoxicity of PFOA first caught attention in the early 1980s. Sibinski et al. (1987) exposed female rats to 0, 1.6 and 16.1 mg/kg bw/d for 2 y. A dose-dependent increase in ataxia was observed, with the loss of coordination being observed in female rats. However, even at higher doses, Butenhoff et al. (2005) failed to observe similar symptoms in rodents exposed to PFOA, so the observed ataxia may not be related to PFOA in Sibinski et al. (1987).

So far, most neurotoxicity studies show neurobehavioral effects in prenatally or neonatally animals. Johansson et al. (2008) exposed mice to a single dose of PFOA at 0.58 or 8.7 mg/kg bw at postnatal day 10. Spontaneous behavior and habituation were affected in the mice exposed to the high dose after 2–4 months, while the mice in the low dose group showed a small, but significant effect on spontaneous behavior. These effects show that PFOA exposure to neonatal mice during high neuronal growth period induces behavior effects in adult mice. Johansson et al. (2009) later studied the pathogen of PFOA-induced neuro effects using mice exposed to a single dose of 8.7 mg/kg bw PFOA. One day after exposure, the animals showed increased levels of proteins CaMKII, GAP-43, synaptophysin and Tau, especially in hippocampus tissue. These proteins are all involved in neuronal growth and synaptogenesis, confirming PFOA-induced neuro effects in mice.

Cellular processes such as development of synaptic plasticity and long-term potentiation are also postulated for PFOA exposure. *In vitro* experiments also show that, at 0.1 M, PFOA induces elevation of intracellular Ca^{2+} concentrations in hippocampal neurons (Liu et al., 2011). The increase in Ca^{2+} is of both extracellular and intracellular origin, involving voltage-gated Ca^{2+} channels, ryanodine receptors and inositol phosphate-3-receptors. The disturbance in Ca^{2+}-homeostasis is followed by an increase in oxidative stress as measured with fluorescent 2′,7′-dichlorofluorescein, and an increased expression of calcineurin, which is a Ca^{2+}-activated protein phosphatase. However, though these evidence suggests that PFOA induces neurotoxicity in rodents, especially in prenatal or neonatal animals, the low concentration of PFOA accumulated in human neuro system raises the question whether PFOA ever reached hazardous concentration.

2.6. Genotoxicity

Genotoxicity refers to pollutant-induced damages in genetic information within cells, thereby causing mutations and leading to cancer. Andersen et al. (2008) reported that PFOA showed no direct genotoxicity, but others showed contradictory results. Shabalina et al. (1999) reported that PFOA induced apoptosis and perturbed the cell cycle in human HepG2 cells dose- and time-dependently. In addition, DNA nonspecific degradation and typical nucleosomal fragmentation have been observed. Yao and Zhong (2005) reported that, after being exposed to PFOA for 1 h, HepG2 cells showed a dose-dependent increase in the level of DNA strand breaks detected by the single cell gel electrophoresis assay and of micronucleus detected by the micronucleus assay. The ammonium salt of PFOA (APFO) also induced chromosomal aberrations and polyploidy in CHO cells (Chinese hamster ovary) in the absence of metabolic activation (Murli, 1996b). However, APFO did not induce gene mutations in the CHO HGPRT forward mutation assay with or without mammalian microsomal metabolic activation (Benford et al., 2008). Neither did APFO induce chromosomal aberrations in cultured human lymphocytes with and without metabolic activation up to cytotoxic concentrations (Benford et al., 2008; Murli, 1996a).

In a recent study by Eriksen et al. (2010), PFOA increased the intracellular ROS production by 1.5-fold without generating DNA damage shown in the study of Yao and Zhong (2005). After intraperitoneal injection to Fisher 344 rats at 100 mg/kg/d PFOA for 8 d, significant increases of 8-OHdG in the liver were observed. The increased oxidative DNA damage was most likely to be secondary to peroxisomal proliferation (Takagi et al., 1991). These findings indicate that the genotoxic effects observed in these cells are likely to be induced indirectly by oxidative DNA damage caused by intracellular ROS.

2.7. Development and endocrine disruption toxicity

PFOA-induced adverse effects associated with the development of pancreatic and testicular in rats were first observed by Sibinski et al. (1987) based on a 2 y study. In mice, gestation exposure to PFOA led to early pregnancy loss, compromised postnatal survival, and delayed in general growth and development, and sex-specific alterations in pubertal maturation along with liver enlargement (Lau et al., 2006). Further study showed PFOA produced stronger responses of the postnatal developmental effects with early exposure in gestation, suggesting the possible sensitive period of development (Wolf et al., 2007). This observation also existed in mammary gland development (White et al., 2009). Either lactational- or intrauterine-only exposures of PFOA delayed mammary gland development as early as postnatal day 1, persisting beyond postnatal day 63. Intrauterine exposure during the final days of pregnancy caused adverse mammary gland developmental effects similar to that of extended gestational exposures and resulted in early and persistent mammary gland effects, suggesting permanent consequences.

This was later proved by a study using chronic, low-dose PFOA (5 μg/L) exposure in drinking water at concentrations approximating those found in contaminated water supplies (White et al., 2011). The lower exposure was sufficient to alter mammary morphological development in mice, and gestational PFOA exposure induced delays in mammary gland development and lactational differentiation across three generations. In a study by Yang et al. (2009) who used two strains of mice BALB/c and C57BL/6, PFOA treatment caused hepatocellular hypertrophy and delayed vaginal opening in both mouse strains. However, for mammary gland and uterine development, while BALB/c mice exhibited inhibition effect, C57BL/6 mice exhibited stimulatory effect in both organs at low dose (5 mg/kg) and inhibition effect at higher dose (10 mg/kg), suggesting these effects were strain dependent.

The extensive study on PFOA's developmental toxicity was based on the epidemic discovery of PFOA's correlation with neonatal birth weight. In a study with 1400 pregnant women and their newborns from the Danish National Birth Cohort (Fei et al., 2008), with each μg/L increase in PFOA, birth length decreased by 0.069 cm and abdominal circumference decreased by 0.059 cm. These findings were further proved by a second study in Danish National Birth Cohort, which involved 1010 children of 5 and 12 months of age. Using multiple linear regression models for analyses, maternal PFOA concentrations (μg/L) were inversely related to children's weight in the first year of life (Andersen et al., 2010). In a cohort of 1250 term singleton infants (≥37 week gestation) (Lenters et al., 2016), born to 1250 mothers from Greenland, Poland, and Ukraine (2002–2004), PFOA was associated with lower birth weight. There are also studies from others with similar conclusions. Based on the Navigation Guide Systematic Review Methodology, Johnson et al. (2014) and Koustas et al. (2014) believe that there are sufficient evidence to link fetal developmental exposure to PFOA and reduction in fetal growth in both humans and animals.

Besides birth weight, serum PFOA was also positively associated with pregnancy-induced hypertension (n = 106) in a Mid-Ohio Valley community who were exposed to high levels of PFOA through drinking contaminated-water during 2005–2010 (Darrow et al., 2013). In addition, based on 169 male offspring (19–21 y of age) of a pregnancy cohort established in Aarhus, Denmark in 1988–1989, *in utero* exposure to PFOA was also associated with lower sperm concentrations with higher levels of luteinizing hormone and follicle-stimulating hormone, suggesting *in utero* exposure to PFOA may also affect adult human male semen quality and reproductive hormone levels.

3. Molecular mechanisms of PFOA-induced toxicity

Though extensively studied, the molecular mechanisms of PFOA-induced toxicity are still uncertain. The structural difference of PFOA with other POPs makes it unique in toxicity mode. With no aromatic ring, they don't activate the aryl hydrocarbon receptor (AhR) like traditional POPs. With a similar structure to octanoic acid, PFOA is a peroxisome proliferating receptor alpha (PPARα) agonist, making PPARα the most extensively studied signal pathway for PFOA exposure.

However, studies on PFOA-induced toxicity in primates and PPARα-null rodents suggest the presence of other signal pathways. Some researchers suggest these mechanisms are probably more important than the PPARα signal pathway, which plays a limited role in POFA-induced toxicity in humans.

3.1. PPARα signal pathway

The PPARs, belonging to the superfamily of nuclear hormone receptors, are ligand-activated transcription factors that play an important role in lipid metabolism. So far, there are three known PPAR subtypes: PPARα, PPARβ/δ, and PPARγ (Rakhshandehroo et al., 2010). The PPAR-activated transcription steps include ligand binding to PPAR, binding of PPAR to the target gene, removing corepressors and recruiting coactivators, remodeling the chromatin structure, and finally facilitating gene transcription (Desvergne and Wahli, 1999). Natural ligands that can bind PPARs include various fatty acids as well as numerous fatty acid derivatives and fatty acid compounds (Rakhshandehroo et al., 2010). With similar structure to octanoic acid, PFOA is a PPAR agonist.

The peroxisome proliferating activity of PFOA was first found in the mid 1980s (Ikeda et al., 1985; Pastoor et al., 1987), which is earlier than the discovery of the first PPAR-PPARα in the early 1990s (Issemann and Green, 1990). Based on the fact that PFOA affected peroxisomal fatty acid β-oxidation and other activities in mice liver, Sohlenius et al. (1992) proposed that PFOA was a potent PPARα activator. This was supported by a 7-d diet exposure study where PFOA increased the activity of hepatic peroxisomal acyl-coA oxidase in wild-type but not in PPARα-null mice (Yang et al., 2002b). Perkins et al. (2004) also observed that PCoAO activity was increased in male Crl:CD rats after exposing to >0.64 mg/kg bw/d. These results support that PFOA acts as a PPARα-agonist in rodents.

PPARα is initially labeled as a hepatic lipid metabolism governor by Issemann and Green (1990). However, it also controls the metabolism of glucose, lipoprotein, xenobiotics and amino acid, and liver inflammation and hepatocyte proliferation in addition to other hepatocyte transformations in rodents (Chinetti et al., 2000; Desvergne and Wahli, 1999). Recently, genomic and other biomolecular technologies such as Western blot, real-time quantitative PCR (RT-qPCR), and microarray have been used to evaluate PFOA-induced toxicity. Based on *in vitro* tests, Bjork and Wallace (2009) showed that exposure to PFOA caused a dose-dependent stimulation of the expression of *Acox*, *Cte*/*Acot1*, and *Cyp4a1*/*11* transcripts, indicative of peroxisome proliferation in primary rat hepatocytes. Guruge et al. (2006) used microarray to test the transcriptome changes in rat liver after exposing to PFOA at 1, 3, 5, 10, or 15 mg/kg bw/d for 21 d. PFOA exposure significantly changed the transcription gene profile categorized for transport and metabolism of lipids, especially fatty acids and proteins, which are genes controlled by PPARα. Later, Martin et al. (2007) administered five different organic contaminants including three triazole antifungals and two perfluorinated chemicals including PFOA and perfluorooctane sulfonate (PFOS) to male rats for 1, 3, or 5 d, and tested the expression profile of various genes correlated with oxidative stress, PPAR, and xenobiotic metabolism using microarray and RT-qPCR. Among the five compounds, PFOA at 20 mg/kg bw/d caused the largest number of significantly altered transcript levels, with PFOA treatment had ~40% greater gene perturbations than PFOS treatment. Cytochrome P450 family proteins (Cyp4a14, Cyp7a1, Cyp7b1, Cyp8b1, and Cyp17a1) and fatty acid β-oxidation enzymes (Cpt-1, Cpt-2, Acaa1, Hadha, and Hadhb) were all upregulated significantly. In addition, PFOA-induced toxicity matched well with the hepatotoxicity-related genomic signatures, which was consistent with PPARα activation. The changed genes mainly involve in cholesterols and lipid hyperaccumulation, and peroxisome proliferation. These changes demonstrate that PFOA exposure affects lipid and cholesterol metabolism, which is consistent with the dramatic decreases in serum cholesterol level following PFOA exposure (>50%). Based on those evidence, they conclude that PFOA is a PPARα agonist in rodents.

Its PPARα agonist property could explain many toxic effects observed in PFOA-exposed rodents, as well as some epidemiologic evidence in humans, such as the association between the increasing liver enzymes and higher serum PFOA level (Leonard et al., 2008; Lin et al., 2010; Lundin et al., 2009). However, many studies also show PPARα-independent mode is also involved in PFOA-induced toxicity. Kudo and Kawashima (1997) observed that PFOA-exposed mice had fatty liver, which was not shown in other PPARα- agonist chemicals. In the study of Martin et al. (2007), PFOA-induced liver steatosis was also observed. However, the lipid accumulation contradicted with the activation of PPARα. PFOA-induced development toxicity is also related to PPARα activation. Wolf et al. (2008b) observed an overlap of PPARα activation and developmental toxicity at similar concentration ranges. Abbott et

al. (2007) showed that, after exposing to PFOA for 17 d, pregnant wild-type and PPARα-null mice delayed eye opening and were low in postnatal weight gain, both depending on PPARα expression. However, early pregnancy loss was independent of PPARα expression.

In another study using wild-type, PPARα-null, or PPARα-humanized mice (Albrecht et al., 2013), PFOA exposure induced hepatic hyperexpression of *Acox1* and *Cyp4a10* in wild-type mice but not in PPARα-null mice or PPARα-humanized mice. The percentage of mice surviving postnatally was lower in wild-type litters but not in litters from PPARα-null mice or PPARα-humanized mice, demonstrating that the developmental/postnatal effects from prenatal PFOA exposure in mice were not only mediated by PPARα, but also determined by the genotype of PPARα, *e.g.*, mouse and human PPARα. Similarly, mammary gland stimulation by PFOA is observed in PPARα-knockout and wild-type C57Bl/6 mice (Zhao et al., 2010a). PFOA exposure increased serum progesterone levels in ovary-intact mice and also enhanced mouse mammary gland responses to exogenous estradiol as well as a group of steroid hormones and growth factors. The data indicate that PFOA stimulates mammary gland development in C57Bl/6 mice by promoting steroid hormone production in ovaries and increasing the levels of growth factors in mammary glands, which is independent of the expression of PPARα.

Based on microarray, Rosen et al. (2008a) studied PFOA toxicity in the liver of wild-type and PPARα-knockout mice, which were orally dosed for 7 d at 1 or 3 mg/kg PFOA with the PPARα agonist Wy14,643 at 50 mg/kg. In wild-type mice, PFOA and Wy14,643 induced changes consistent with activation of PPARα, but not Wy14,643-exposed PPARα-null mice. However, in PFOA-exposed PPARα-null mice, changes were also observed in transcripts related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle regulation. Filgo et al. (2014) analyzed PFOA-induced liver toxicity after 18 months of exposure in strains of CD-1, 129/Sv, and PPARα-knockout mice. PFOA significantly increased non-neoplastic liver lesions in PPARa-knockout mice (hepatocyte hypertrophy, bile duct hyperplasia, and hematopoietic cell proliferation). Besides the evidence from PPARα-null rodents, the second evidence also suggests that other signal pathways are responsible for the toxicity observed in primates. In Cynomolgus monkey, hepatohytrophy caused by mitochondrial proliferation is observed, but with no alteration in peroxisomal markers (Butenhoff et al., 2002). These evidence show that besides the PPARα signal pathway, PFOA could induce toxicity *via* other signal pathways.

3.2. PPARβ/δ and PPARγ signal pathways

A potential signal pathway that can substitute PPARα is the activation of other PPARs, *i.e.*, PPARβ/δ and PPARγ. All three PPARs are comprised of distinct functional domains, including an N-terminal transactivation domain, a highly conserved DNA-binding domain and a C-terminal ligand-binding domain containing a ligand-dependent transactivation function. Despite their similarities, each PPAR isoform has unique functions *in vivo*, probably because of their distinct tissue distributions. Though PPARβ/δ shares similar functions with PPARα, while PPARα is expressed predominantly in the liver, heart and brown adipose tissue, PPARβ/δ is ubiquitously expressed and has a crucial role in fatty acid oxidation in key metabolic tissues such as skeletal muscle, liver and heart (Kersten et al., 2000; Poulsen et al., 2012). PPARγ is most highly expressed in white and brown adipose tissue where it is a master regulator of adipogenesis as well as a potent modulator of whole-body lipid metabolism and insulin sensitivity (Ahmadian et al., 2013).

Vanden Heuvel et al. (2006) investigated the activation of various PPARs in human, mouse, and rat nuclear receptors. PFOA showed activation of PPARα and PPARγ in hepatocytes of all three species while PPARβ/δ only in mouse hepatocytes, with PPARγ being much weaker than PPARα. However, Takacs and Abbott (2007) reported that PFOA activated mouse and human PPARα, and mouse PPARβ/δ, but not PPARγ in both species in transfected Cos-1 cells. Rosen et al. (2008b) also examined the expression of several transcription factors that may underline the changes in the expression of lipid metabolism in PFOA-exposed mice liver. Expression of PPARγ exhibited 3–5 fold increases after PFOA exposure whereas that of PPARβ/δ was not altered in mice liver. Though the role of PFOA is still unclear, moderate activation of the two nuclear factors of PPARβ/δ and PPARγ are certain, which are the substitutes of PPARα as supported by the gene expression profile in the liver of both wild-type and PPARα-null mice exposed to PFOA (Rosen et al., 2010).

3.3. PXR/CAR signal pathways

The constitutive nuclear receptors including constitutive androstane receptor (CAR) and pregnane-X receptor (PXR) have been characterized as xenobiotic sensors that regulate the expression of genes involved in drug metabolism and elimination (Wada et al., 2009; Wang et al., 2012). Thus PXR and CAR can manipulate the expression of a wide range of xenobiotics metabolism, such as CYP2Bs, aldehyde dehydrogenases, sulfotransferases, and UDP-glucuronosyltransferases (Aleksunes and Klaassen, 2012) (Fig. 2).

PFOA-stimulated gene expression of xenobiotic metabolism first caught attention in transcriptome profile by Guruge et al. (2006), but PFOA activation of PXR/CAR was first proposed by Elcombe et al. (2007). They administered 23.3 mg/kg APFO in the diet to male SD rats for 1, 7 or 28 d, and tested gene expression changes using Western blot. APFO treatment increased the levels of cytochrome P450 family (CYP2B2, CYP3A1 and CYP4A1), which were controlled by PXR/CAR and PPARα. Cheng and Klaassen (2008) later used wild-type and knockout mice to determine the role of nuclear factors in PFOA-induced hepatotoxicity. PFOA is proved to be both PPARα and CAR agonist, inducing CYP2B10 and CYP4A14 expression in mice. Elcombe et al. (2010) then demonstrated that CAR and PXR expression was increased in male rats after exposing to APFO in diet for 28 d. The role of CAR/PXR in PFOA-induced toxicity was further evidenced by Rosen et al. (2008b). In the transcriptome profile of the PPARα-null mice, a large cluster of genes in CAR/PXR signal pathway show expression change upon APFO exposure. These evidence show that CAR/PXR signal pathway is an important alternative of PPARα signal pathway in PFOA-induced toxicity. CAR/PXR pathways answered the question raised in PFOA-treated PPARα-null mice, which remained hepatomegaly as opposed to PPARα-null mice treated with WY 14,643 (Wolf et al., 2008a; Yang et al., 2002a).

3.4. FXR and LXR signal pathway

Besides PXR/CAR signal pathways, PFOA may also activate other nuclear receptors such as farnesoid X receptor (FXR) and liver X receptor (LXR) due to their similar structure with fatty acids. FXR is an important regulator of bile acid and carbohydrate metabolism (Cheng and Klaassen, 2008), while LXR is a particularly important master regulator of lipid and lipoprotein metabolism similar to PPARα (Vanden Heuvel et al. 2006). PFOA treatment could affect some of these nuclear receptors to act as the substitution of PPARα possibly through the cooperation and crosstalk of these NRs (Bjork et al., 2011). However, in an *in vitro* cell study, LXR receptor from different organisms including human, mouse, and rat showed no sign of activation by PFOA, which may exclude PFOA from LXR agonist list.

In addition to direct enzyme activation test, mice knockout of a specific nuclear receptor has been often used to limit the multiplicity and redundancy of transcription regulation, which could complicate the interpretation of toxicities following PFOA treatment. The specific nuclear receptor-null mice Cheng and Klaassen (2008) used also included FXR-null mice. However, for perfluorodecanoic acid (PFDA), a homolog with even longer chain than PFOA, Cyp2B10 and 4A14 activation showed no significant difference with wild-type mice. Thus FXR had no relation

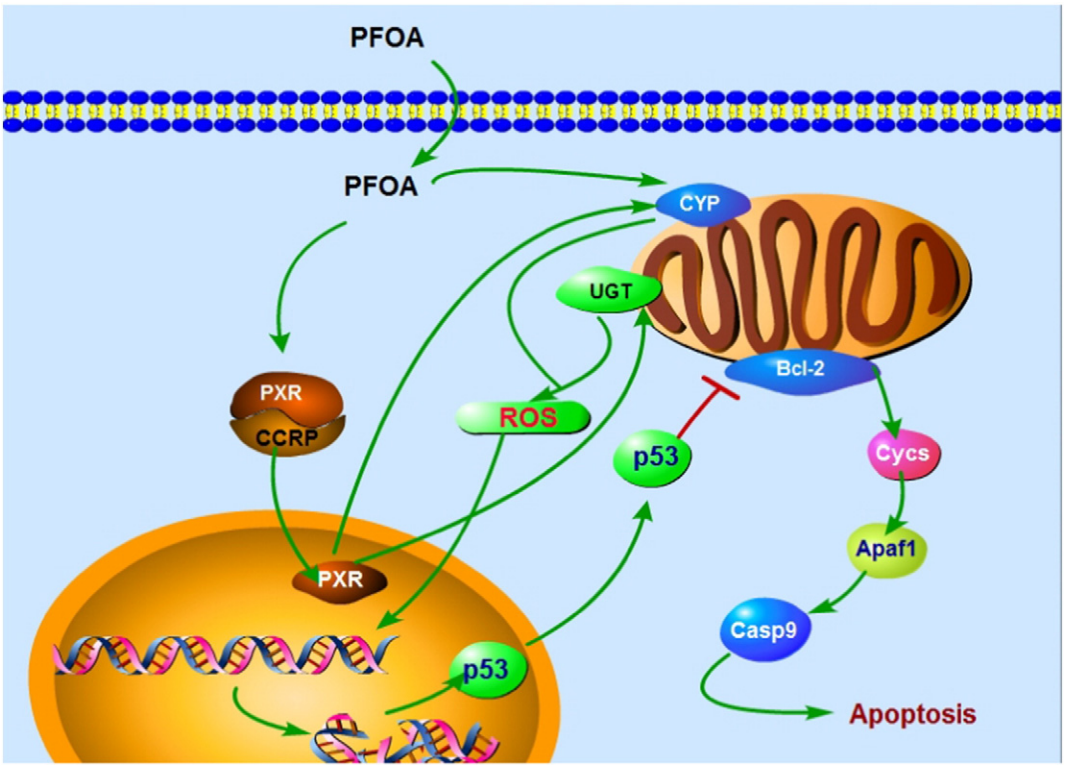

Fig. 2. PFOA induces PXR signal pathway, leading to p53 activation, cell apoptosis and cell death. Drawn by Pathway Builder 2.0.

with PFOA-induced CYP activation. This was further enhanced by primary rat and human hepatocytes culture, after PFOA treatment, both markers of *FXR*, *Abcb11* and *Nr0b2* were not altered in either species (Bjork et al., 2011). However, for the LXR tested simultaneously, after the exposure, PFOA caused a 1.7-fold increase in *Srebf1* expression in human cells, and 1.9-fold in rats, as well as a 1.9-fold change in *Abcg5*. *Srebf1* is a LXR-inducible transcription factor that controls the expression of genes involved in fatty acid synthesis and sterol metabolism while *Abcg5* is the LXR-regulated transporters involved in the biliary elimination of cholesterol and bile acids. The data implied PFOA stimulates LXR-controlled genes, hence the role of LXR in PFOA-induced toxicity warrants further investigation.

3.5. Mitochondrial pathway

The mitochondrial pathway of cell death is an intrinsic apoptosis signal pathway, representing the major pathway of physiological apoptosis in vertebrates (Oberst et al., 2008). Several steps are the key events during mitochondria-induced cell death, such as mitochondrial outer membrane permeabilization, release of cytochrome, and APAF-1-mediated caspase cascade (Elmore, 2007). Mitochondrial permeability transition, Ca^{2+} fluxes, and ROS are major stimuli for mitochondria-mediated cell death. Shabalina et al. (1999) showed that PFOA induced apoptosis in human HepG2 cells dose- and time-dependently. They later proved that the apoptosis was caused by ROS hypergeneration, with simultaneous mitochondrial transmembrane potential ($mt\Delta\Psi$) loss, leading to caspase-9 activation (Panaretakis et al., 2001). Both N-acetylcysteine (a precursor of glutathione) and Cyclosporin A (an inhibitor of MPT pore) reduce $mt\Delta\Psi$ dissipation, caspase-9 activation, and apoptosis, suggesting ROS-provoked mitochondrial pathway of apoptosis may be involved in PFOA-induced cell death.

Kleszczynski et al. (2009) also proved that, after exposing to PFOA, human colon carcinoma HCT116 cells showed apoptosis through activation of the mitochondrial signal pathway, however, Cyclosporin A reduced the $mt\Delta\Psi$ dissipation, thus inhibiting apoptosis. Yao and Zhong (2005) showed that, after exposing to PFOA, DNA oxidative damage, DNA strand breaks, and micronucleus in HepG2 cells were dose-dependently upregulated. They also attributed these PFOA-induced genotoxic effects to intracellular ROS. Hu and Hu (2009) not only observed the activation of mitochondrial pathway, they further studied the gene expression of *p53*, *Bcl-2*, and *caspase-9* in human HepG2 cells following PFOA exposure using RT-qPCR. Differential expression of these genes proved that PFOA induced mitochondrial pathway activation through activation of the p53 signal pathway. However, these results were recently challenged by Florentin et al. (2011) as they observed a decrease of ROS generation following PFOA treatment in HepG2 cells. They argued that PFOA-induced ROS generation was probably due to improper cell culture in those studies.

This argument was supported by Walters et al. (2009) who found PFOA trans-activated PPARα and PPARγ, which in turn activated the PPARγ coactivator-1α protein. The overexpression of PPARγ coactivator-1α with a subsequent increase of nuclear respiratory factor 1 and mitochondrial transcription factor A caused a significant decrease in the intracellular accumulation of ROS (Fujimoto and Yamasoba, 2014). However, the suppression of Complex II and IV was observed while mitochondrial transcription factor A remained unchanged (Walters et al., 2009), so the results were inconclusive. *In vivo* tests also showed the evidence of PFOA-induced apoptosis in rodents. For example, Guruge et al. (2006) observed caspase-4 upregulation in the transcriptome profile in male rat livers after being exposed to PFOA daily *via* gavage of 1, 3, 5, 10 or 15 mg/kg bw/d for 21 d. Since most studies support PFOA-induced ROS generation, PFOA-induced apoptosis through mitochondrial pathway is probably reliable.

However, the source of ROS remains an unsolved issue. It is argued that PFOA as a PPARα agonist induces fatty acid β-oxidation, as well as ω-hydroxylation through CYP4A family. One consequence of up-regulation of fatty acid β-oxidation or ω-hydroxylation is hypergeneration of H_2O_2, a kind of ROS (Panaretakis et al., 2001; Zhao et al., 2010b). PPARα-induced ROS hyperaccumulation is associated with apoptosis in PFOA-exposed rodent hepatic cells (Panaretakis et al., 2001). However, as Hu and Hu (2009) and Panaretakis et al. (2001) both detected the elevation of superoxide anion O_2^-, PPARα could not be the direct source

of the ROS, as neither fatty acid β-oxidation nor CYP4A had the ability to generate this highly-active ROS species (Aubert et al., 2011; Baker et al., 2006). An indirect pathway to generate O_2^- by PPAR is through the uncoupling of mitochondrial electron transport chain (Panaretakis et al., 2001). On the other hand, PXR-controlled P450 family exhibits much stronger ROS production ability, which could also be a potent resource of O_2^- (Gu and Manautou, 2012). The important question of which source serves PFOA-induced ROS generation remains unanswered. However, the generation mode may underline potential hazards of PFOA to humans.

3.6. Lipid accumulation and PI3K-Akt signal pathway

Besides the mitochondrial signal pathway, another apoptosis-related pathogen has been suggested, *i.e.*, specific accumulation of lipid droplets in hepatocyte nuclei (Wang et al. (2013). After feeding male BALB/c mice with PFOA at 5, 10, or 20 mg/kg/d for 14 d, they observed cell apoptosis, accumulation of lipid droplets in the nucleus, and PPARγ activation, but PPARα showed no alteration. As an important location for DNA storage, replication and transcription, nuclei invaded by lipid droplet posed stress for cell survival, leading to cell apoptosis. In addition, Wolf et al. (2008a) also showed similar change in subcellular structure of PFOA-exposed mice liver dose-dependently in both wild-type and PPARα-null mice, so this could be an explanation of PFOA-induced apoptosis in mice.

Although PPARα is on the upstream of many metabolism signal pathways and in the metabolism of glucose, other signal pathway may also be involved. To a large extent, how PFOA influences glucose homeostasis is still unknown. In the last few years, the phosphatidylinositol 3-kinase-serine/threonine kinase Akt (PI3K-Akt) signal transduction pathway has emerged as one of the main signal routes that coordinate complex events leading to changes in cell metabolism, cell growth, cell movement, and cell survival. The modulation of PI3K-Atk signaling pathway in the livers of mice after 28 d of exposure to PFOA was investigated by Yan et al. (2015). Compared with normal mice, PFOA exposure significantly decreased the expression of the phosphatase and tensin homolog protein and affected the PI3K-Akt signaling pathway in the liver. Tolerance tests further indicated that PFOA exposure induced higher insulin sensitivity and glucose tolerance in mice. Biochemical analysis revealed that PFOA exposure reduced hepatic glycogen synthesis, which might be attributed to gluconeogenesis inhibition. The levels of several circulating proteins were also altered after PFOA exposure, including proteins potentially related to diabetes and liver disease. The results suggest that PFOA affects glucose metabolism and induces insulin hypersensitivity in mice.

3.7. TNF-α/NF-κB

Nuclear factor κB (NF-κB) is a transcription factor playing a pivotal role in the onset and propagation of inflammation and cancerogenesis (Vallabhapurapu and Karin, 2009). The activation of NF-κB can be triggered by a wide range of stimuli, including cytokines, oxidative stress, lipopolysaccharide, B-lymphocyte and T-lymphocyte (Hayden and Ghosh, 2008). These pathways all activate the IκB (the inhibitor of NF-κB) kinase complex (Beck et al., 2010) (Fig. 3).

Tumor necrosis factor-α (TNF) and interleukins (ILs) are widely accepted pro-inflammatory cytokine for NF-κB activation (Chu, 2013). After administering 0.5–47 mg/kg bw/d of PFOA in drinking water to male ICR mice for 21 d, Son et al. (2009) observed altered T lymphocyte populations and elevated levels of IL-6, TNFα, and IL-1b at higher doses of PFOA. Qazi et al. (2009) reported that, after exposing male C57BL/6 mice to 0.02% (w/w) PFOA in diet for 10 d, *ex vivo* TNF-α and IL-6 production was increased. Brieger et al. (2011) collected whole blood from human volunteers and measured TNF-α and IL-6 levels after incubating blood with lipopolysaccharide and 0.1–100 mg/L of PFOA. They observed that PFOA exposure increased the release of IL-6 and TNF-α. These results suggest that PFOA induces inflammation, however, there is also opposite evidence. Corsini et al. (2011) incubated human leukocytes and THP-1 cells (a human promyelocytic cell line) at 0.1–100 mg/L PFOA with lipopolysaccharide. TNFα production was suppressed in both cell types while IL-8 was suppressed in THP-1 cells. They used siRNA to interfere PPARα, and proved that the ability of PFOA to alter cytokine release was PPARα-dependent.

Actually, the negative crosstalk between PPARα and NF-κB has long been discussed. PPARα negatively regulates the vascular inflammatory gene response by suppressing NF-κB (Delerive et al., 1999). Cytokines such as IL-1b and TNFα can also suppress PPARα through activation of NF-κB (Lim et al., 2013; Stienstra et al., 2010). This is supported by

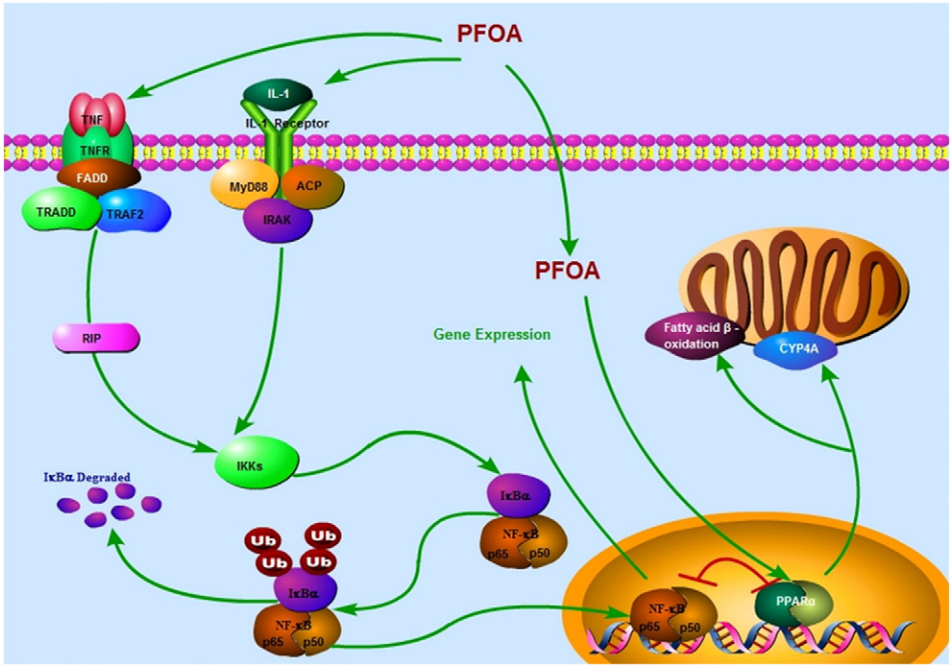

Fig. 3. PFOA induces negative crosstalk between PPARα and NF-κB signal pathways. Drawn by Pathway Builder 2.0.

immune suppression in wild-type mice absent or attenuated in PPARα-null mice after PFOA exposure (Yang et al., 2002b). The competition between PPARα and NF-κB could also be the reason for the contradictive results in PFOA-induced immune toxicity studies. If inflammation induced NF-κB activation, PPARα would be suppressed, leading to lipid accumulation and steatosis and verse versa.

However, there are exceptions. Yang et al. (2002a) demonstrated that suppression of immunoglobulin G and immunoglobulin M by PFOA didn't depend on PPARα activation, as immunoglobulin M suppression is similar in wild-type and PPARα-null mice. The exception implies there may be other pathways involved in PFOA-induced immune suppression, one such possibility is through the negative crosstalk of PXR and NF-κB (Gu et al., 2006; Li et al., 2012; Zhou et al., 2006).

4. Health risks of PFOA in humans

As discussed above, PFOA-induced toxicity is activated *via* several signal pathways based on *in vivo* and *in vitro* evidence. Their toxic modes have been thoroughly discussed in several studies. Elcombe et al. (2013) conducted a comprehensive review and assessment of the potential human relevance of liver tumorigenesis that is mediated by nuclear receptors, including PPARα, CAR, PXR, and AhR. They concluded that, for PPARα agonists including PFOA, the following sequence of key events are identified in the mode of action for hepatic tumor induction in rodents: 1) PPARα activation is in the liver; 2) alteration of cell growth pathways is in the liver; 3) perturbation of hepatic cell growth and survival leads to the formation of new preneoplastic liver cells and the induction of new focal liver lesions; 4) selective clonal expansion of preneoplastic foci; and 5) transformation and outgrowth of preneoplastic liver cells into adenomas (Corton et al., 2014).

However, among these steps, the PPARα activation has been demonstrated only in humans. Numerous hypolipidemic drugs targeting PPARα have been widely used, so PPARα may not induce health hazards in humans other than expression change of hepatic enzymes. Actually, for PFOA, the role of PPARα agonist has recently been challenged. In the study of Wang et al. (2013), PPARα expression change was not observed in the liver of mice after exposing to 20 mg/kg bw/d PFOA for 14 d.

For CAR agonist, a similar procedure has been suggested, with steps 2 and 3 being changed to 2) altered hepatic gene expression is specific to CAR activation; and 3) hepatocellular proliferation is increased (Elcombe et al., 2013). However, the uncertainty of CAR-induced carcinogen in humans remains, since CAR agonist - phenobarbital, also a drug used for decades, shows no cell proliferation in cultured human hepatocytes, and has not been reported to develop altered hepatic foci in human liver. For PXR agonists, data are limited, so a definite mode of carcinogenic action in rodents could not be established, but steps 1, 3, and 4 seem critical (Elcombe et al., 2013). Considering the hepatic toxicity of PFOA in primates only stays at hepatomegaly, PXR could not be an alternative pathway to lead to PFOA-induced hepatic carcinogen.

On the other hand, though PFOA-induced immunotoxicity through NF-κB is still questionable, the relationship between PFOA exposure levels and NF-κB-induced tumor metastasis has caught attention recently. Zhang et al. (2014) studied the molecular mechanisms underlying the stimulating effects of PFOA on cancer cell invasion and matrix metalloproteinases (MMP) expression. PFOA exposure at >5 nM largely enhanced the invasion ability of the breast cancer cell MDA-MB-231. Both protein and mRNA levels of MMP-2/-9 were increased in the cells after PFOA exposure, and activation of NF-κB by accelerating NF-κB translocation into the nucleus was proved by Western blot. The addition of NF-κB inhibitor in culture medium suppressed this invasiveness enhancement and MMP-2/-9 overexpression, proving that PFOA-induced cancer invasion was mediated by NF-κB activation. These results also raise a further question about the potential risk of PFOA in human carcinogenesis.

Another signal pathway could potentially be relevant to humans is the mitochondrial signal pathway. Though it only causes apoptosis, the ROS triggering this pathway could damage the balance of many cellular processes, eventually causing cancer. Recently, Zhao et al. (2010b) reported that PFOA caused exponential growth of human-hamster hybrid A_L cells, which is hybrid between CHO cells and human lymphocytes. Exposure of A_L cells to PFOA for 16 d induced significant mutagenic effects, along with the increase of ROS level. Concurrent treatment of A_L cells with ROS inhibitor significantly decreased the mutagenic potential of PFOA. These results suggest that exposure to PFOA leads to ROS generation, which induces mutagenicity in A_L cells. Since mutagenicity is often a prerequisite to the development of malignancy, this provides a direct base for PFOA-mediated cancer induction, which has implication in PFOA-induced toxicity and health risks in humans.

5. Conclusions

Though PFOA has long been considered and studied as PPARα agonist, PFOA-induced toxicity may stimulate other signal pathways. Substantial evidence shows the low potential of PPARα signal pathway on the health risk of PFOA in humans, so we should downplay the role of PPARα in future research. Actually, studies focusing on signal pathways of PFOA-induced toxicity such as PPARs and PXR/CAR may not be relevant to human health risk, while pathways like the NF-κB and mitochondrial signal pathway should receive more attention as they are more closely related to human health.

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