Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies

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Abstract

The wide use of perfluorooctane sulfonate (PFOS) has led to increasing concern about its human health risks over the past decade. In vivo and in vitro studies are important and effective means to ascertain the toxic effects of PFOS on humans and its toxic mechanisms. This article systematically reviews the human health risks of PFOS based on the currently known facts found by in vivo and in vitro studies from 2008 to 2018. Exposure to PFOS has caused hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity in laboratory animals and many in vitro human systems. These results and related epidemiological studies confirmed the human health risks of PFOS, especially for exposure via food and drinking water. Oxidative stress and physiological process disruption based on fatty acid similarity were widely studied mechanisms of PFOS toxicity. Future research for assessing the human health risks of PFOS is recommended in the chronic toxicity and molecular mechanisms, the application of various omics, and the integration of toxicological and epidemiological data.

Keywords: PFOS; human health risk; in vivo; in vitro

Contents

1. Introduction

Perfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been produced and used globally since the 1940s (Paul et al., 2009). The excellent thermal stability, chemical stability, and surfactant activity of these substances enable them to be widely used in various industrial processes and products (Buck et al., 2011). Perfluorooctane sulfonate (PFOS) is one of the most widely used PFAS. The substance contains a hydrophobic and lipophobic perfluoroalkyl chain and a sulfonic acid group that adds the polarity (the inset of Fig. 1). These structural characteristics support their applications as water and oil repellents, firefighting foams, lubricants, surfactant additives, and coating agents (Paul et al., 2009). The wide use of PFOS arouses concern on its toxic effects and human health risks, which is reflected by the increasing number of publications on the related topic in the past decade (Fig. 1). Due to the long perfluoroalkyl chain and stable carbon-fluorine (C-F) bonds, PFOS is difficult to be transformed and degraded naturally, resulting in their persistence in the environment and human body. PFOS have been found in food, drinking water, various environmental compartments, and even human tissue (Sharma et al., 2016; Domingo and Nadal, 2017; Dalahmeh et al., 2018; Jian et al., 2018). In a study about the accumulation of PFAS in human tissues, Pérez et al. (2013) confirmed the occurrence of PFOS in brain, kidney, liver, and lung, and found that PFOS was more prevalent in the liver. According to biological monitoring data of PFAS concentrations in blood, hair, milk, nail, and urine, PFOS was predominantly found in human blood (Jian et al., 2018). People are mainly exposed to PFOS through the contaminated food and

drinking water, use of consumer products containing PFOS, and occupational exposure in the production of PFOS or related products. Considering the human exposure and accumulation of PFOS, it is significant to study their human health risks.

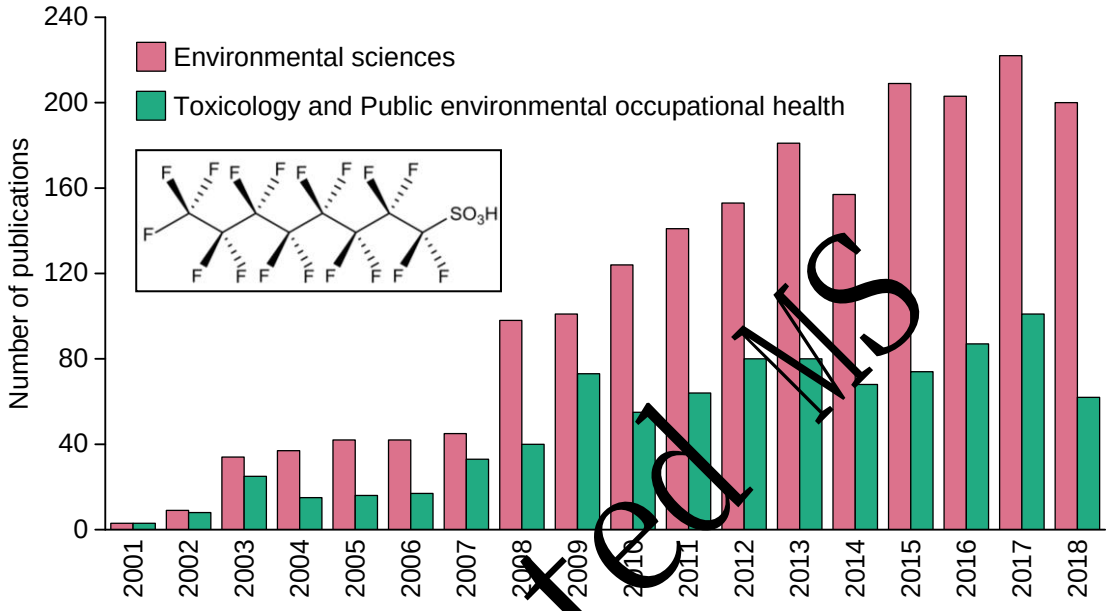

Fig. 1. Number of publications on PFOS in the field of environmental sciences, toxicology and public environmental occupational health from 2001 to the present. The data were extracted from Web of Science Core Collection in September 2018 by searching publications containing "perfluorooctane sulfonate" or "PFOS" in the topic and refined by Web of Science Categories. The inset shows the chemical structure of PFOS.

In order to investigate the toxic effects and mechanisms of PFOS, the studies were mainly conducted with animal models under simulated conditions of human exposure, and then the results were extrapolated to human based on the similarities between humans and laboratory animals in physiological processes and metabolism of PFOS. Generally, these experiments can be categorized into in vivo and in vitro studies. In vivo study is performed with the whole living animal, and can be applied

for investigating various toxic effects (e.g., acute toxicity, chronic toxicity, and cumulative toxicity). Dissociative organs, cells, or organelles are utilized for in vitro study, which mainly reveals the specific toxic mechanisms and metabolic processes. Many in vivo and in vitro studies have suggested that exposure to PFOS may lead to adverse effects on human health, such as hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity (Mao et al., 2013; Chou et al., 2017; Soloff et al., 2017; Tang et al., 2017; Chen et al., 2018a; Chen et al., 2018b; Han et al., 2018b). Among these toxic effects, the studies of hepatotoxicity, neurotoxicity, reproductive toxicity, and immunotoxicity were relatively more. However, due to the high complexity of human body and PFOS metabolism, the toxic effects and mechanisms are not fully understood (Kariuki et al., 2017; Lai et al., 2017a; Liang et al., 2017; Xu et al., 2018). It is necessary to study the human health risks of PFOS in more detail.

In this article, the human health risks of PFOS are systematically reviewed based on the currently known facts found by in vivo and in vitro studies from 2008 to 2018. Study selection is conducted based on PRISMA guidelines (Liberati et al., 2009), and the process is outlined in Fig. 2. Main toxic effects of PFOS include hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid disruption, and cardiovascular toxicity. For each toxic effect, the PFOS-induced symptoms and pathological changes are first introduced, and then the possible mechanisms proposed in the reviewed articles were analyzed and illustrated. Epidemiological evidence that supports the results from in vivo and in vitro studies of PFOS toxicity is discussed,

and some future research needs are proposed.

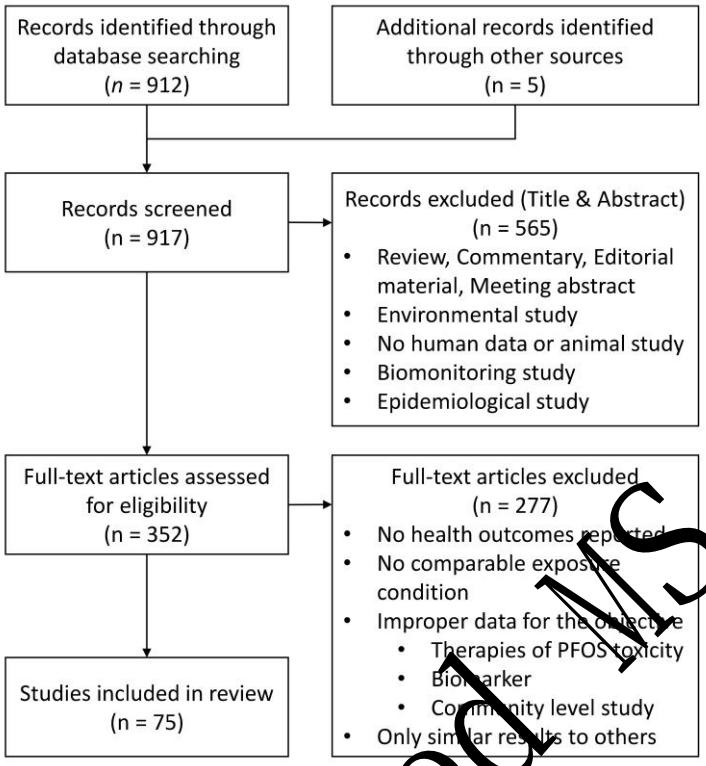

Fig. 2. Flow diagram of screening and selecting studies for this review.

2. In vivo and in vitro studies for risk assessment of PFOS

In vivo and in vitro studies are basic and effective ways to assess the human health risks of chemicals. For assessing the toxic effects of PFOS, many studies have been conducted with various in vivo and in vitro models (Fig. 3).

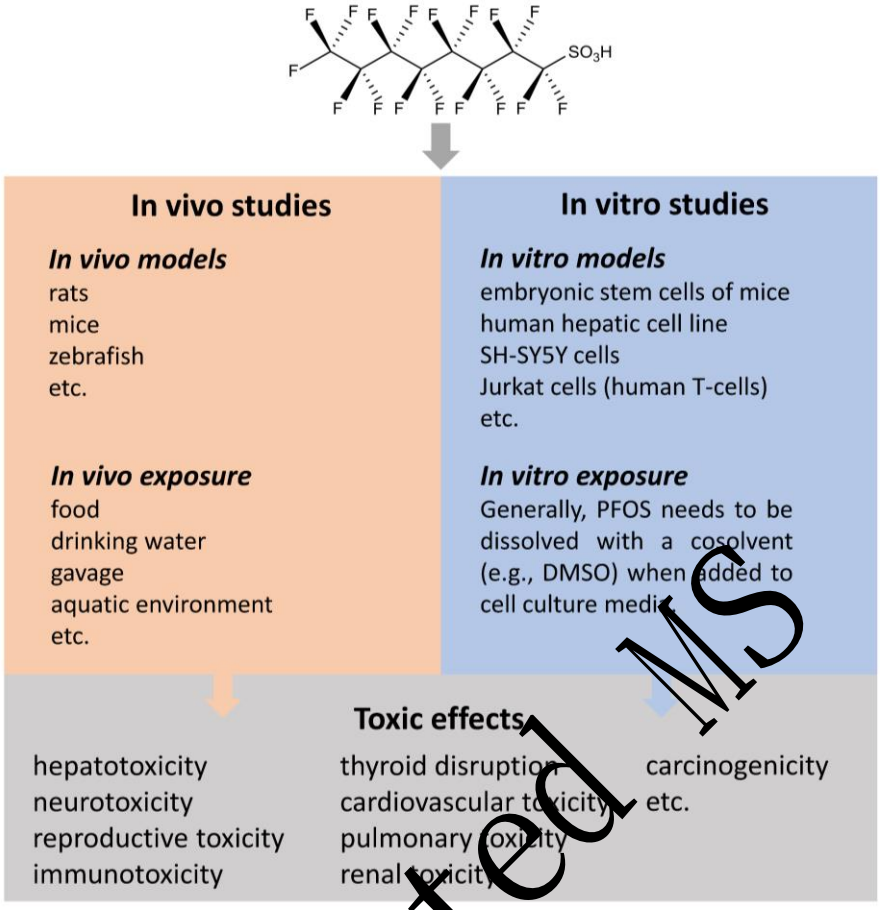

Fig. 3. Assessing the human health risks of PFOS by in vivo and in vitro studies.

2.1. In vivo studies

In vivo studies use the whole animals for toxicological experiments, and can reflect multiple types of toxic effects (e.g., acute toxicity, subacute toxicity, and chronic toxicity) with strictly controllable exposure conditions. For in vivo studies of PFOS toxicity, rats, mice, and zebrafish are the most widely used models, as these animal models show high anatomical, pathological, and genetic similarity to humans (Lieschke and Currie, 2007). Generally, rats and mice are exposed to PFOS via food, drinking water, or gavage, while zebrafish are exposed to PFOS through the aquatic environment for their living. Due to the characteristics of hydrophobicity and

lipophobicity, PFOS has to be first dissolved in water containing an organic cosolvent when being added to the food or water. Dimethylsulfoxide (DMSO) and Tween 80 are commonly used cosolvents. After exposure to PFOS, the body weight, body length, organ weight, and specific toxic symptoms of experimental animals are usually measured or recorded. Based on different objectives of the toxicity studies (e.g., hepatotoxicity, neurotoxicity, reproductive toxicity, and immunotoxicity), various toxicity indicators can be further determined with biochemical analysis of serum and histopathological examination. For example, in a study about the hepatotoxicity of PFOS, Wan et al. (2012) used mice as in vivo models. In their experiments, PFOS was dissolved in DMSO solution and then mixed with corn oil. The mice in experimental group were fed with corn oil containing PFOS, while those in control group were fed with corn oil containing only DMSO. The body weight and liver weight were measured on the designated dates to assess the fat accumulation in liver, and histological examination of liver sections was further conducted with hematoxylin staining to show the cytoplasmic vacuolations after PFOS exposure.

2.2. In vitro studies

In vitro studies are conducted with dissociative organs, cells, or organelles. Compared with in vivo studies, in vitro studies can be simpler, faster, and more economical. Additionally, another important advantage of in vitro studies is that human cells can be involved, which provides a way to solve the problem of species differences in assessing the toxicity to humans. Thus, for the in vitro studies of PFOS

toxicity, many human cells or cell lines are used. For example, SH-SY5Y, a human derived cell line, has been used as an in vitro model of neuronal function and differentiation in PFOS neurotoxicity tests (Chen et al., 2014; Chen et al., 2018b). For the in vitro exposure, PFOS is added to cell culture media. The final concentration of the cosolvent (e.g., DMSO) in culture media is usually kept below 0.1% (v/v) to minimize the cytotoxic effects of solvent (Du et al., 2013). After exposure to PFOS, the cytotoxicity, apoptosis, oxidative stress, and inflammatory cytokines are generally determined to elucidate the toxic mechanisms. In a study of PFOS-induced neurotoxicity, Chen et al. (2018c) used astrocytes as in vitro models and exposed them to PFOS dissolved with DMSO. The authors determined the cell viability and the secretion of interleukin-1 beta (IL-1β, a pro-inflammatory cytokine) to assess the physiological effects of PFOS on astrocytes. They further conducted the Western blot analysis and discussed the signaling pathway by which PFOS mediated the secretion of IL-1β in astrocytes. However, in vitro studies lack the dynamic processes in whole animals, and are difficult for assessing the chronic toxicity of PFOS. In vivo and in vitro studies each have their own advantages and disadvantages. They should complement and verify each other in the toxicity tests of PFOS.

3. Toxic effects of PFOS

3.1. Hepatotoxicity

Hepatotoxicity is chemical-driven liver injury (Mahmoud et al., 2017). Liver is a large organ of many animals and humans, and plays a vital role in metabolism and

detoxification. Many studies have shown that liver is the major target organ for PFOS bioaccumulation (Fai Tse et al., 2016; Wan et al., 2016; Han et al., 2018b). PFOS can cause hepatotoxicity and result in hepatic steatosis, hepatomegaly, hepatocellular hyperplasia, and oxidative damage of hepatocytes (Du et al., 2009; Wan et al., 2012; Huang et al., 2014; Fai Tse et al., 2016; Lai et al., 2017b; Xu et al., 2017). Hepatic steatosis (also known as fatty liver disease) is a condition in which excess fat accumulates in liver cells, and is often observed after PFOS exposure. Main functions of the liver in fat metabolism include oxidation of fatty acids for body energy supply, synthesis of cholesterol, phospholipids and lipoproteins, and transformation of proteins and carbohydrates to fat (Mourya et al., 2018). Wan et al. (2012) found that excess fatty acids and triglycerides were accumulated in the hepatocytes of mice and the liver weights were significantly increased after oral gavage of 10 mg/kg/day PFOS for over 3 days. Cheng et al. (2016) measured the content of triglyceride and cholesterol in zebrafish liver after chronic exposure to 0.5 μM (~0.25 mg/L) of PFOS for 5 months, and observed a significant increase of triglyceride in all zebrafish but a cholesterol increase only in male zebrafish. Hepatocellular hyperplasia is an increase in the amount of hepatocytes that results from abnormal cell proliferation, and is commonly a preneoplastic response (Evan and Vousden, 2001). In a study of PFOS hepatotoxicity to human hepatocytes, Cui et al. (2015) found that PFOS could stimulate the cell proliferation in vitro at the doses of 50, 100, 150, and 200 μM but inhibit the cell viability at the doses of 300, 400, 500, and 600 μM (1 μM ≈ 0.5 mg/L). Both the in vivo and in vitro studies have suggested that exposure to PFOS can cause

oxidative damage to hepatocytes, which is mainly reflected by the production of reactive oxygen species (ROS) and alteration of oxidative stress biomarkers such as antioxidant enzymes and peroxidation products (Khansari et al., 2017; Han et al., 2018a). Additionally, in a comparative transcriptomic analysis of zebrafish fatty liver (exposed to 0.5 μg/L of PFOS for six days), 241 differential expressed genes were found to be overlapped between PFOS-exposed and mutant zebrafish (fatty liver mutant), and the zebrafish in the two groups shared genes enriched in hepatitis, fibrosis, and cirrhosis of liver cells (Fai Tse et al., 2016). PFOS and perfluorooctanoic acid (PFOA) are both saturated fluorinated chain with eight carbons. The similar chemical structure results in similar bioaccumulation potential of them in organisms. Many studies were conducted with hepatotoxicity comparison between PFOS and PFOA. Similar hepatotoxicity effects (e.g. hepatic steatosis and hepatomegaly) were also observed in PFOA exposure (Yang et al., 2016b; Wu et al., 2017; Zhang et al., 2019).

The main mechanisms of PFOS-induced hepatotoxicity involve interfering with fat metabolism, causing oxidative stress, and disturbing cell cycle progression. Hepatic steatosis usually occurs when the process of fat metabolism is disrupted and fat (or fatty acid) excessively accumulates in the liver (Reddy and Rao, 2006). Many studies have shown that PFOS can inhibit the β-oxidation of fatty acid, leading to the accumulation of excessive fatty acids and triglycerides in hepatocytes due to the structural similarity of PFOS to fatty acids (Wan et al., 2012; Cheng et al., 2016; Jacobsen et al., 2018). Fatty acid β-oxidation is an important stage of fat catabolism

(Fig. 4), and it is so named as the beta carbon of the fatty acid is oxidized to a carbonyl group in the process (Bartlett and Eaton, 2004). Through the β-oxidation process, fatty acid molecules can be broken down and generate acetyl-coenzyme A (acetyl-CoA) in the mitochondria. Then, acetyl-CoA enters the Kreb's cycle and undergoes complete oxidation (Akram, 2014). Exposure to PFOS can interfere with this vital physiological process. Wan et al. (2012) determined the rate of mitochondrial β-oxidation in mouse liver after oral PFOS exposure for 14 days and observed a significant inhibiting effect (nearly half decrease in the oxidation rate) in all treatments with various concentrations of PFOS (1, 5, and 10 mg/kg/day). Cheng et al. (2016) also reported the inhibition of mitochondrial fatty acid β-oxidation in zebrafish liver after chronic exposure to 0.5 μM (~0.25 mg/L) of PFOS for 5 months, but the expression of some key enzymes involved in the β-oxidation increased. The authors explained that the increased expression of these enzymes might result from a compensatory mechanism for the decreased β-oxidation. Oxidative stress is another cause of the hepatotoxicity of PFOS. The generation of excessive ROS in hepatocytes leads to oxidative stress and damage of hepatic cells. Mitochondrion is the main intracellular source of ROS (Turrens, 2003). The electron transport chain of mitochondrion may leak electrons to oxygen when disturbed, resulting in partial reduction of molecular oxygen to superoxide anion (a precursor of most other ROS). Khansari et al. (2017) reported that exposure to 25 μM (~12.5 mg/L) PFOS could result in the generation of ROS and lipid peroxidation in rat hepatocytes, and the oxidative stress could further lead to lysosomal membrane leakage and cellular

proteolysis. In the study by Cui et al. (2015), isobaric tags for relative and absolute quantitation were used to study the PFOS-induced cell proliferation in human hepatic cell line. The authors found that 50, 100, 150, and 200 μM (≈ 25, 50, 75, and 100 mg/L) of PFOS could increase the expression of cyclins and cyclin-dependent kinases and drive cells into G1 phase (the first phase within interphase of the cell cycle). This provides evidence for the PFOS-induced hepatotoxicity resulted from disturbing the cell cycle progression.

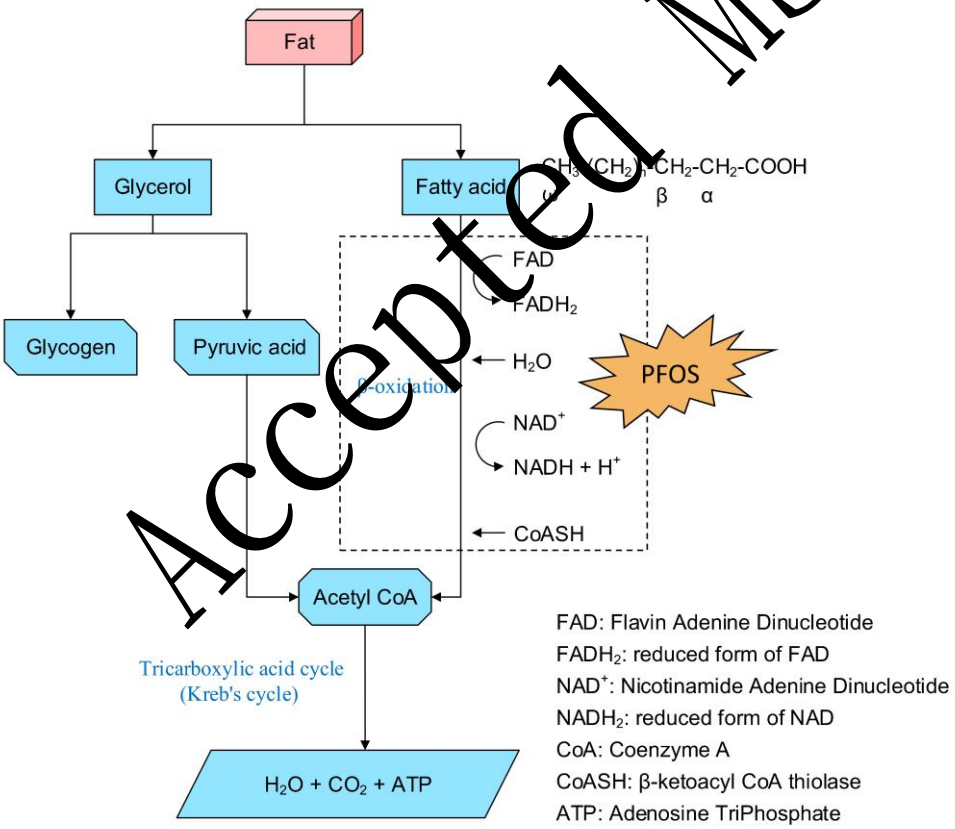

Fig. 4. PFOS targets the fatty acid β-oxidation.

3.2. Neurotoxicity

Neurotoxicity refers to that neurotoxins (natural or artificial toxic substances)

261 cause negative changes in structure and function of the nervous system (Rock and
262 Patisaul, 2018). The in vivo studies have shown that exposure to PFOS can cause
263 defects or dysfunctions in motor behavior, learning, memory, and cognition
264 (Johansson et al., 2008; Onishchenko et al., 2011; Long et al., 2013; Chen et al., 2014).
265 For example, Chen et al. (2014) studied the neurotoxicity of PFOS to *Caenorhabditis*
266 *elegans* and found that exposure to 20 μM (~10 mg/L) of PFOS for 48 h could
267 decrease the locomotor behaviors of forward movement, body bend, and head thrash.
268 However, in another study by Spulber et al. (2014), obvious spontaneous
269 hyperactivity was observed in zebrafish larvae after exposure to 1 mg/L of PFOS due
270 to a dopaminergic deficit. Long et al. (2013) used water maze tests to study the
271 neurotoxicity of PFOS to adult mice, and they found that chronic exposure to 10.75
272 mg/kg/day of PFOS for three months impaired the spatial learning ability and memory
273 as a result of hippocampus dysfunction. Similar experimental phenomena were
274 observed by Wang et al. (2015), and they explained the results in terms of the synaptic
275 plasticity. Apart from these typical neurotoxic symptoms, the in vitro studies have
276 demonstrated that PFOS can induce neuroinflammation (Chen et al., 2018b; Chen et
277 al., 2018c), as well as the damage or apoptosis of nerve cells, such as hippocampal
278 cells, neural stem cells, and SH-SY5Y cells (Long et al., 2013; Chen et al., 2014; Li et
279 al., 2015; Dong et al., 2016; Ge et al., 2016; Sun et al., 2018). PFOA can also cause
280 neurotoxicity, especially developmental neurotoxicity. However, different
281 neurotoxicity effects (both in vivo and in vitro) were observed after exposure to PFOS
282 and PFOA under the same conditions and PFOS showed greater neurotoxicity than

PFOA (Onishchenko et al., 2011; Berntsen et al., 2017; Berntsen et al., 2018).

According to the available literature, the neurotoxic mechanisms of PFOS involve many aspects (Fig. 5). PFOS can cause oxidative damage in nerve cells by inducing the generation of ROS, such as peroxides and free radicals. These ROS may impair cell components (e.g., proteins, lipids, and DNA) and disturb normal redox signaling (Song et al., 2016a). Chen et al. (2014) determined the ROS level in SH-SY5Y cells after exposure to PFOS, and found that the treatment with 25 μM (~12.5 mg/L) of PFOS significantly enhanced the ROS generation, which could be inhibited by adding *N*-acetylcysteine (an antioxidant) before the exposure. PFOS may cause neurotoxic effects by triggering neuroinflammation. In the central nervous system, the immune cells (e.g., astrocyte) can be activated and release inflammatory cytokines to protect neurons from pathogenic factors, but sustained activation and excessive secretion of the inflammatory cytokines can cause serious nerve injury (Kim et al., 2016). In a recent in vitro study by Chen et al. (2018b), exposure to 0.02 μM (~0.01 mg/L) of PFOS brought about excessive secretion of tumor necrosis factor-α (an inflammatory cytokine that plays roles in physiological processes of nervous system, e.g., inducing apoptosis) in SH-SY5Y cells, which finally led to a rapid apoptosis. The neurotoxicity of PFOS can result from the disturbed synaptogenesis and synaptic plasticity. Synapse is the neural structure that allows a nerve cell to pass a neural signal (electrical or chemical signal) to another cell, while synaptic plasticity is the ability of synapses to strengthen or weaken in response to the changes in their activity (Bourgeron, 2015). Exposure to PFOS can disturb the

synaptogenesis and synaptic plasticity (Liao et al., 2008; Wang et al., 2015). For example, Wang et al. (2015) analyzed the genes and proteins related to synaptic plasticity in the hippocampus cells of rat offspring after prenatal exposure to PFOS via drinking water containing 15 mg/L of PFOS and concluded that the reduced spatial learning ability and memory were related to the impaired synaptic plasticity. Disturbing the calcium ion (Ca^{2+}) channel and homeostasis is an important mechanism of the PFOS-induced neurotoxicity. Calcium ion is essential to triggering the release of neurotransmitters, but PFOS can disturb the calcium homeostasis through inducing extracellular calcium influx and intracellular calcium release, resulting in calcium overload and abnormal activation of downstream signaling molecules, which eventually causes cell damage, aging, and even death (Wang and Jin, 2012). Berntsen et al. (2018) studied the excitotoxicity of PFOS in rat cerebellar granule neurons, and found that exposure to 300 μM (~150 mg/L) of PFOS for 30 min (or 60 min) could make the *N*-methyl-D-aspartate receptor (a Ca^{2+} channel) overactive and result in excess Ca^{2+} influx via the channel. In addition to the above mechanisms, PFOS may also induce neurotoxicity by altering neurotransmitter levels. Yuan et al. (2018) exposed planarians to 0.5, 1, 5, and 10 mg/L of PFOS for 1, 3, 5, 7, and 10 days, and found that the exposure could influence the expression of neuronal-related genes and acetylcholinesterase activity, leading to the changes of neurotransmitter production and cycle (specific effects depended on the PFOS dose and exposure time). This was considered as one of the mechanisms of PFOS neurotoxicity to planarians.

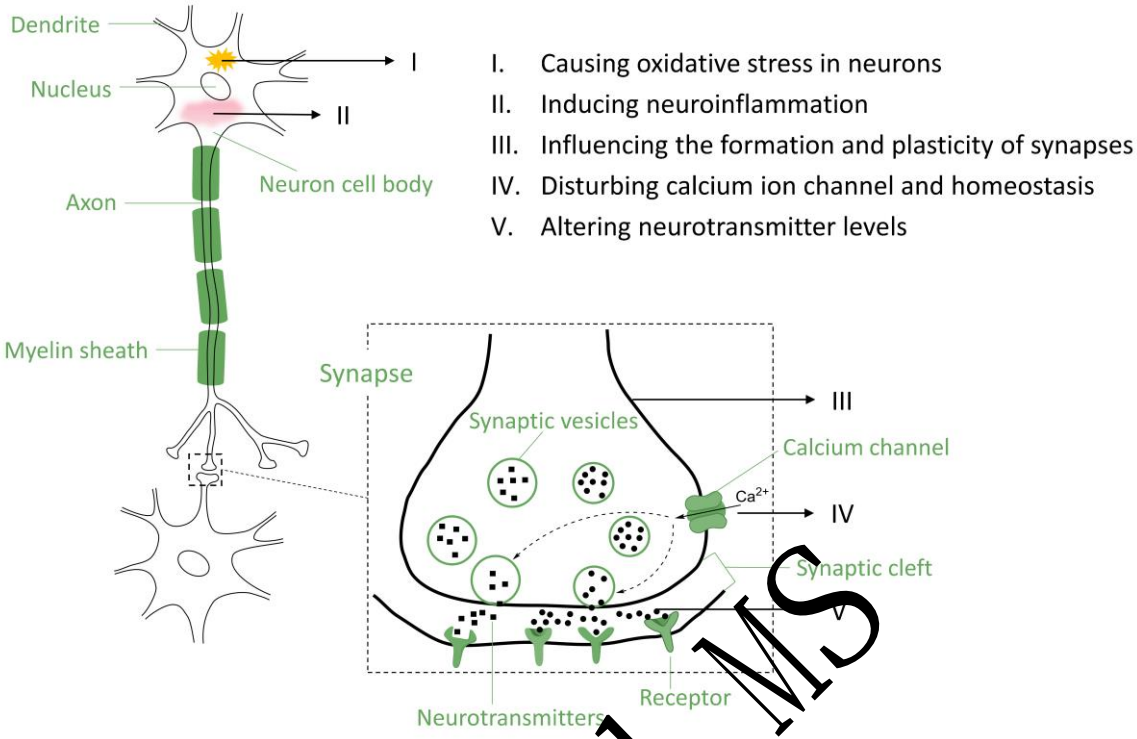

Fig. 5. The main neurotoxic mechanisms of PFOS.

3.3. Reproductive toxicity

Reproductive toxicity implies the adverse effects on the reproductive system of living organisms (Ayoka et al., 2016). Exposure to PFOS can cause damages to male and female reproductive organs, disturb related hormone secretion, and lead to poor pregnancy outcomes (Wang et al., 2011; Chen et al., 2013; Cheng et al., 2013; Lou et al., 2013; Zhang et al., 2015; Qu et al., 2016; Yang et al., 2016). Qu et al. (2016) reported that the testis weights and sperm counts of male mice were significantly reduced after oral exposure to 10 mg/kg/day of PFOS for 5 weeks. Under similar exposure condition, Wang et al. (2018) found that the dioestrus of adult female mice was prolonged but their corpus luteum was reduced. In an in vivo study of PFOS-induced reproductive toxicity, Chen et al. (2016) reported that exposure to 0.25

mg/L of PFOS for 5 months could cause structural changes in the gonads of both male and female zebrafish, and result in more mature oocytes and fewer spermatogonia in the gonads. In that study, the authors reported that the estrogen level in zebrafish (both juvenile and adult) increased and a female-biased sex ratio in zebrafish occurred after the chronic PFOS exposure. Zhang et al. (2015) reported the apoptosis of human placental syncytiotrophoblasts after exposure to 0.01, 0.1, and 1 μM (0.005, 0.05, and 0.5 mg/L) of PFOS for 24 h. Meanwhile, the treatment decreased the secretion of steroid and human chorionic gonadotropin by placental syncytiotrophoblasts. These hormones are vital to maintaining gestation and normal development of fetus. The result indicated the harmful effects of PFOS on human reproductive function. Similar toxic effects in reproduction toxicity were also observed with PFOA exposure (Yahia et al., 2010; Zhang et al., 2014; Yang et al., 2015; Lu et al., 2016).

Exposure to PFOS mainly causes reproductive toxicity through damaging reproductive organs/cells and disrupting reproductive endocrine (Fig. 6). Intact reproductive organs and cells is the basis for maintaining normal reproduction function. For males, significant reduction in testis weight and sperm count has been observed after PFOS exposure, which is thought to result from the increased apoptosis and decreased proliferation of germ cells (Qu et al., 2016). However, few studies reported the direct damage of PFOS to reproductive organs of females that are not pregnant. The gender differences in PFOS-induced toxicity can be ascribed to the sex-dependent organic anion-transporting peptides, which govern the transport of PFOS across the cell membrane (Foresta et al., 2018). Impairment of the

363 hypothalamic-pituitary-gonadal (HPG) axis is an important cause of PFOS-induced
364 reproductive endocrine disorder (López-Doval et al., 2015; López-Doval et al., 2016).
365 The HPG axis is the key regulator of reproduction, and it involves the hypothalamus,
366 pituitary gland, and gonads (Fig. 6). Through secreting gonadotropin-releasing
367 hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and
368 gonadal hormone (e.g., estrogen and testosterone), the HPG axis regulates the
369 reproduction and maintains normal reproduction function (Maruska and Fernald,
370 2011). For example, testosterone is essential for normal spermatogenesis (Walker,
371 2011). López-Doval et al. (2014) reported the inhibition of physiological activity of
372 hypothalamic-pituitary-testicular axis in adult male rats after exposure to 6 mg/kg/day
373 of PFOS for 28 days and observed evident morphological changes of hypothalamus,
374 degeneration of gonadotrophic cells and spermatozoids, and testicular edema. In their
375 another study, the possible roles of serotonin and neuropeptide Y in the PFOS-induced
376 disruption of reproductive axis were investigated (López-Doval et al., 2015). The
377 results showed that PFOS caused an increase of serotonin concentration in
378 hypothalamus and median eminence but a decrease of neuropeptide Y concentration
379 in the hypothalamus. Serotonin and neuropeptide Y are important substances involved
380 in regulating the secretion of GnRH and LH. This result suggested that PFOS
381 inhibited the reproductive axis via changing the concentrations of serotonin and
382 neuropeptide Y. Their further study found that PFOS could disrupt the reproductive
383 endocrine by changing the gene expression related to GnRH, LH, FSH, and androgen
384 receptors (López-Doval et al., 2016). These results are valuable for determining the

reproductive toxicity mechanisms of PFOS.

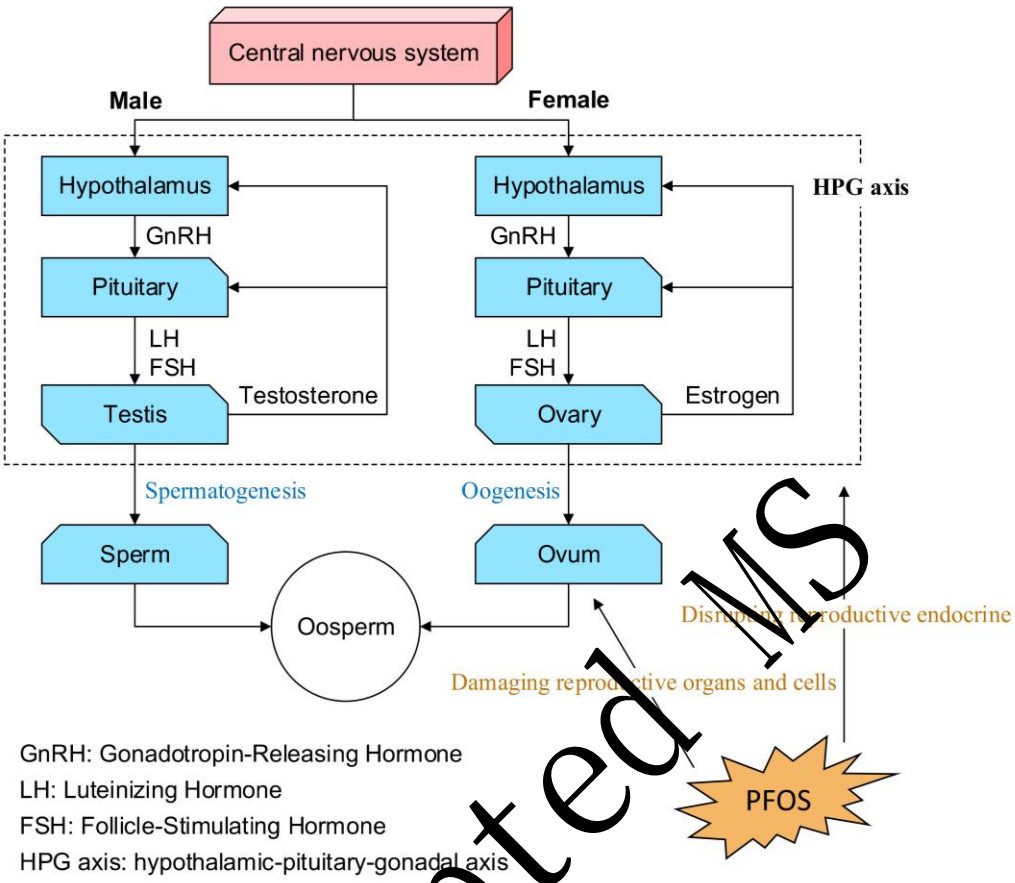

Fig. 6. PFOS causes reproductive toxicity through damaging reproductive organs and cells and disrupting reproductive endocrine (including hypothalamic-pituitary-gonadal axis regulation).

3.4. Immunotoxicity

Immunotoxicity is defined as the adverse effects on the immune system which consists of immune organs (e.g., thymus gland, bone marrow, and lymph gland), immune cells (e.g., T cells, B cells, natural killer cells, and macrophages), and immune active substances (e.g., antibodies, cytokines, and lysozymes), and usually manifests as immunosuppression, immunostimulation, hypersensitivity, or

autoimmunity (Shao et al., 2014). The immune system is a vital biological defense to avoid infection, disease, or other biological invasion. The in vivo and in vitro studies have shown that PFOS could disturb the proliferation, differentiation, and normal function of immune cells, and interfere with the release and activity of immune active substances (Dong et al., 2009; Zheng et al., 2009; Brieger et al., 2011; Fang et al., 2013; Midgett et al., 2015; Soloff et al., 2017). The effects of PFOS exposure on the proliferation of immune cells depend on the species, cell type, and exposure time and dosage. Positive, negative, and no effects of PFOS on the proliferation of immune cells were all observed in the studies (Peden-Adams et al., 2008; Wirth et al., 2014; Lv et al., 2015; Soloff et al., 2017). Exposure to PFOS has been found to be able to disturb the immune function (including innate immunity and adaptive immunity). Keil et al. (2008) reported that the activity of natural killer cells and the production of immunoglobulin M (IgM) in mice were significantly decreased at the age of 8 weeks after gestational oral exposure to 5 mg/kg/day of PFOS from gestational day 1 to 17. The natural killer cells are innate cytotoxic lymphocyte, and their activity is commonly used for evaluating the innate immunity. The IgM is a basic antibody produced by B cells, and it is widely used for evaluating the humoral immunity (adaptive immunity). The above results indicated the suppression of both innate immunity and adaptive immunity after PFOS exposure. Fang et al. (2013) found PFOS-induced immunosuppression in the larvae of marine medaka after exposure to 1 and 4 mg/L of PFOS for 25 days. In their study, bacterial lipopolysaccharide was used to trigger the host innate immunity through stimulating phagocytic cells to produce

419 pro-inflammatory cytokines (inflammatory response). With exposure to PFOS, the
420 expression of pro-inflammatory cytokines was significantly suppressed, which was
421 considered unfavorable for the immune defense. In an in vitro study of PFOS-induced
422 immunotoxicity by Midgett et al. (2015), the production of interleukin-2 (IL-2) in
423 human T cells was inhibited after exposure to 50, 75, and 100 mg/L of PFOS for 18 h.
424 The IL-2 is a type of signaling molecule (cytokine) that regulates the immune activity
425 of leukocytes, and the reduction of IL-2 is a characteristic of autoimmune diseases.
426 The result of this study suggested the adverse effect of PFOS in interfering with the
427 human immune active substances. Exposure to PFOS or PFOA could both cause
428 immunotoxicity, but the effects varied with the exposure conditions (Qazi et al., 2009;
429 Midgett et al., 2015).

430 The immunotoxicity mechanisms of PFOS mainly cover the impacts on immune
431 cells and normal immune responses (Fig. 7). In a study of PFOS immunotoxicity with
432 bottlenose dolphins, Soloff et al. (2017) observed that in vitro exposure to 5 mg/L of
433 PFOS for 4 days stimulated the T cell proliferation and promoted proinflammatory
434 cytokine production, but the further mechanism remained unknown. Zhang et al.
435 (2013) reported PFOS-induced apoptosis in the splenocytes and thymocytes of mice
436 after orally exposed to 5 or 10 mg/kg/day of PFOS for 7 days. Apoptosis plays an
437 important role in the regulatory process of immune system. Many lymphocytes
438 undergo apoptosis at the termination of an immune response. The authors thought this
439 regulatory mechanism could be disturbed by PFOS and the PFOS-induced abnormal
440 apoptosis in the splenocytes and thymocytes was partly responsible for the

441 immunotoxicity. Dong et al. (2012) attributed the immunocyte apoptosis induced by
442 oral exposure to 0.8333 mg/kg/day of PFOS for 60 days to a p53-mediated apoptotic
443 pathway, and reported that mitochondrial dysfunction was involved in the apoptosis.
444 In an in vivo study of PFOS immunotoxicity in mice, Lv et al. (2015) found that
445 exposure to 10 mg/kg/day of PFOS for 4 weeks (including one-week recovery) could
446 reduce the proliferation of T cells by inhibiting the mitogenic reaction. In their
447 experiments, downregulation in the gene expression of cell cycle was observed with
448 PFOS exposure, which explained the possible reasons for the decreased proliferation
449 of T cells. The authors further analyzed several different pathways related to the
450 signaling transduction of immune cells, and found that PFOS inhibited
451 NRF2-mediated pathways by which the cells are protected from oxidative damage,
452 and upregulated the gene expression in T cell receptor signaling, calcium signaling,
453 and p38/MAPK signaling pathways. These signaling pathways play vital roles in
454 immunoregulation. The interference of these signaling pathways was considered the
455 underlying mechanisms of PFOS-induced immunotoxicity. Huang et al. (2015)
456 reported that exposure to 0.25 or 1 mg/L of PFOS could promote the immune
457 response in *Oryzias melastigma*. The authors analyzed the expression of genes related
458 to the immunity and observed an increased expression level of interleukin-1β at the
459 transcriptome level. Due to the complexity of the immune system and processes,
460 current knowledge on the immunotoxicity mechanism is limited and needs more
461 research.

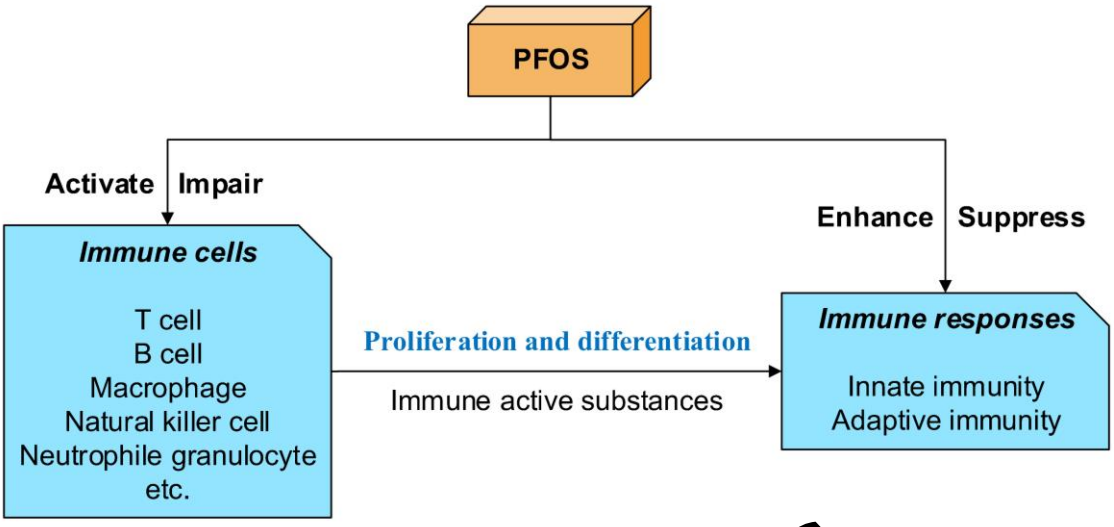

Fig. 7. Effects of PFOS exposure on the immune cells and immune responses.

3.5. Thyroid disruption

The thyroid is a large endocrine gland that regulates many physiological processes (e.g., growth, development, and metabolism) by secreting thyroid hormones (Mullur et al., 2014). Exposure to PFOS can impair the structure and function of thyroid. Coperchini et al. (2015) studied the in vitro effect of PFOS on thyroid cells and observed evident cytotoxicity (inhibited cell proliferation and increased cell death) at a PFOS concentration of 100 μM (~50 mg/L). The authors further investigated whether PFOS entered the thyroid cells and found that PFOS entered the cells via a passive diffusion mechanism. Exposure to relatively high concentration of PFOS in the culture medium was the main reason to cause the cytotoxicity. Though such a high concentration of PFOS is rare in human exposure, it is considerable to determine the thyroid disruption after PFOS exposure. Similar cytotoxicity was also observed with PFOA exposure in that study. In an in vivo study by Chen et al. (2018a), chronic

exposure to 0.25 mg/L of PFOS for 120 days changed the structure of thyroid follicular cells in zebrafish and significantly reduced the nuclear area of follicular epithelial cells. In addition, the authors found a disorder in thyroid hormone. Thyroid hormones mainly include triiodothyronine (T3) and thyroxine (T4), which are especially important for energy metabolism, inorganic ion metabolism, thermogenesis, development of central nervous system and skeleton (Ogilvy-Stuart, 2002; Mullur et al., 2014). The thyroid dysfunction generally reflects in the abnormal change of T3 and T4 level. In the above example, significant decrease in the T4 level was observed after PFOS exposure. Similar results of such a change in the T4 level were obtained in some other studies (Yu et al., 2009a; Yu et al., 2009b). Shi et al. (2009) found that the T3 level in the zebrafish larvae was significantly increased with embryo exposure to 200 and 400 μg/L of PFOS for 15 days post-fertilization, while Curran et al. (2008) reported the decrease of both T3 and T4 level in rat serum after dietary exposure to 100 mg/kg diet of PFOS for 28 days. These results suggest that the variations of thyroid hormone level depend on the species, PFOS dosage, and exposure route and time. Though the variations are not consistent, it is certain that PFOS can induce the disorder of thyroid hormones.

As shown in Fig. 8, the PFOS-induced disruption of thyroid hormone homeostasis can be mainly attributed to the damage of thyroid cells and the interference of the synthesis and transport, metabolism, and action of thyroid hormones. PFOS can enter thyroid cells via a passive diffusion mechanism and cause evident cytotoxicity (Coperchini et al., 2015). The impairment of thyroid structure

disrupts the production of thyroid hormones. In a research about the effects of PFOS on thyroid hormone status in rats, Chang et al. (2008) reported the transient increase of serum T4 level within 6 h after a single oral exposure to 15 mg/kg PFOS due to the competition for binding proteins between PFOS and T4. However, the content of serum T4 decreased to the control level within 24 h and continued to decrease in the following 8 days with oral PFOS exposure. The increased serum T4 level might enhance the utilization, metabolic conversation and excretion of T4 by peripheral tissues, which led to the resulting reduction of serum T4 level. Yu et al. (2009a) observed an significant decrease in serum T4 level after the rats were exposed to 1.7, 5, and 15 mg/L of PFOS in drinking water for 21 days. They determined some messenger RNA endpoints that relates to the biosynthesis and metabolism of thyroid hormones, and ascribed the decreased T4 level to the increased hepatic T4 glucuronidation and thyroidal conversion of T4 to T3 after PFOS exposure. The consumption of T4 can partly account for the PFOS-induced hypothyroxinemia. The competitive binding for transthyretin (TTR) between PFOS and T4 might also cause the decrease of T4 level (Weiss et al., 2009). In a study about the effects of PFOS on endocrine disruption, Du et al. (2013) conducted reporter gene assays with kidney cells of African green monkey and found that PFOS could act as a thyroid hormone receptor antagonist. In their study, PFOS was reported to cause thyroid system disruption through interacting with the T3 receptor and interfering with the T3-induced transcriptional activation of thyroid hormone receptor. PFOS can directly bind with T3 receptor through hydrophobic interaction and hydrogen bonding (Ren et

al., 2015). The structure and behavior of PFOS in organism body are similar to free fatty acids, therefore it can competitively bind to fatty acid binding proteins (Luebker et al., 2002). Additionally, the polar hydrophobic nature of C-F bond can increase the affinity of PFOS for proteins (Biffinger et al., 2004).

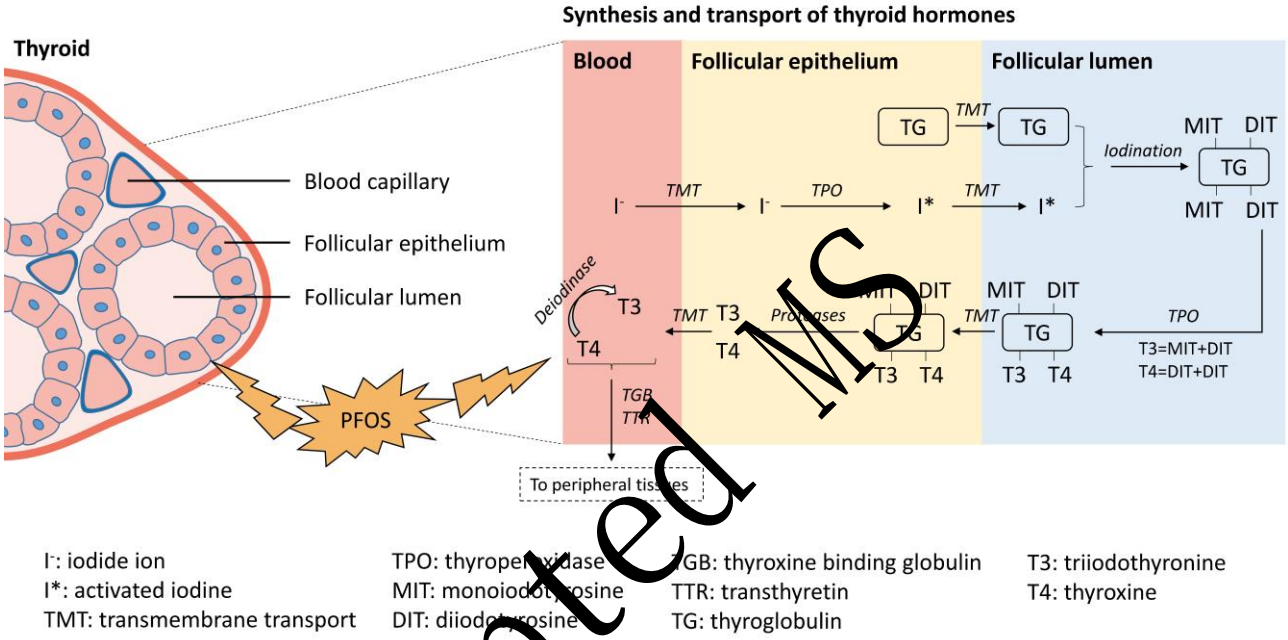

Fig. 8. Exposure to PFOS causes thyroid disruption. The left part is a diagram of thyroid including blood capillary, follicular epithelium, and follicular lumen. The right part diagrammatizes the synthesis of thyroid hormones and their transport through follicular lumen, follicular epithelium, and blood capillary.

3.6. Cardiovascular toxicity

Cardiovascular toxicity is the adverse effects on the reproductive system (including heart and blood vessels). Exposure to PFOS can cause cardiac malformation, change heart rate, and induce apoptosis of cardiomyocytes (Huang et al., 2011; Xia et al., 2011; Zeng et al., 2015; Liang et al., 2017; Tang et al., 2017). Cardiovascular system is more sensitive to chemicals during its development, thus

most studies determined the cardiovascular toxicity of PFOS in embryos (or embryonic tissue) or by adopting prenatal exposure. In the study by Huang et al. (2011), exposure to 16 mg/L of PFOS for 2, 4, 6, or 8 days increased the distance between sinus venosus and bulbus arteriosus in embryos of *Oryzias melastigma*, which reflected the PFOS-induced cardiac malformation in the positions of atrium and ventricle during heart development. Additionally, the authors observed accelerated heart rate after 8 days post-fertilization but decreased heart rate after 10 days post-fertilization with 4 and 16 mg/L of PFOS. Liang et al. (2017) found that PFOS could stimulate the heartbeat of *Daphnia magna* after exposure to PFOS for 48 h. In their experiments, the accelerated heartbeat was observed in all the experimental groups with different PFOS concentrations (30, 44, 66, and 100 mg/L). Though the heartbeat began to slow with 100 mg/L of PFOS, the heartbeat value was still higher than that of the control group. In a study of prenatal PFOS exposure, Xia et al. (2011) studied the apoptosis in heart tissue and the expressions of related genes after prenatal exposure to 2 mg/kg/days of PFOS for 19 days during the gestation, and found obvious mitochondrial vacuolization and inner membrane injury of heart tissue in rat offspring. The apoptosis of heart tissue might mainly occur via a mitochondria-mediated apoptotic pathway and the generation of ROS (Cheng et al., 2013; Zeng et al., 2015). However, the disruption of cardiogenesis is attributed to the PFOS-induced disturbance of gene expression during cardiogenesis, rather than the PFOS-induced generation of ROS (Cheng et al., 2013). Cardiovascular toxicity of PFOS was also observed in human cells. It was reported that exposure to 50 or 100

560 μM (25 or 50 mg/L) PFOS for one hour induced the generation of ROS, remodeling
561 of actin filament, and changes of endothelial permeability in microvascular
562 endothelial cells (Qian et al., 2010). The PFOS-induced generation of ROS regulated
563 the actin filament remodeling which contributed to the increase of endothelial
564 permeability, but the regulatory mechanism is unclear. Nonetheless, this demonstrated
565 direct cardiovascular toxicity risk of PFOS to humans.
566
567 *3.7. Others*
568 Apart from the above-mentioned toxic effects, several in vivo and in vitro studies
569 reported the pulmonary toxicity, renal toxicity, and the carcinogenicity of PFOS. In an
570 in vitro study about the toxic effects of PFOS on human lung cancer A549 cells, Mao
571 et al. (2013) reported the apoptosis of lung cells via a mitochondrial dysfunction
572 pathway after exposure to 50, 100, or 200 μM (25, 50, or 100 mg/L) of PFOS. Ye et al.
573 (2012) studied the pulmonary toxicity of PFOS in fetal rats with in utero exposure. In
574 their experiments, though no distinct microscopic changes of the lung tissue was
575 observed, prenatal exposure to 20 mg/kg/day of PFOS for six days altered the gene
576 expressions related to secretory proteins, cytoskeletal structure, extracellular matrix,
577 ion channel and transporting proteins, and lipid metabolism in the lung of fetal rats.
578 Wen et al. (2016) conducted an in vitro study on the renal toxicity of PFOS, and found
579 that exposure to 0.5 μM (~0.25 mg/L) of PFOS for 24 or 40 h could cause significant
580 apoptosis of renal tubular cells. Through further research, they reported new findings
581 on the PFOS-induced renal fibrosis (Chou et al., 2017). Both the two studies proposed

582 a mechanism that PFOS caused renal injury via inducing the deacetylation and
583 inactivation of peroxisome proliferator activated receptor γ, which plays important
584 roles in many cell signaling processes and can protect renal cells from PFOS-induced
585 injury when over-expressed. In vivo and in vitro experiments have shown inadequate
586 evidence for the carcinogenicity of PFOS. In a carcinogenicity study of PFOS with
587 Sprague Dawley rats, an increase in the incidence of hepatocellular adenoma was
588 observed with the dietary treatment of 20 ppm PFOS, but the authors considered it an
589 incidental observation in the rats surviving to terminal sacrifice (Butenhoff et al.,
590 2012). Several other studies reported no direct or no obvious carcinogenesis of PFOS
591 (Florentin et al., 2011; Ngo et al., 2014; Arrieta-Cortes et al., 2017). Nonetheless, the
592 carcinogenic potential of PFOS should not be ignored and needs more research
593 (Jacquet et al., 2012).
594
595 **4. Human health risks of PFOS**
596 Currently available data of PFOS toxicity from in vivo and in vitro studies have
597 demonstrated the toxic effects of PFOS on experimental animals. However, these
598 results are predictive for the human health risks and have limitations when being
599 extrapolated to humans. The limitations mainly result from the differences in
600 physiological sensitivity and PFOS metabolism between experimental animals and
601 humans (Hartung, 2008). For overcoming the limitations, epidemiological
602 investigation is conducted to verify the results from animal experiments. By
603 epidemiological study, some toxic effects of PFOS on human health can be directly

observed under actual exposure conditions. Table 1 summarizes some representative epidemiological evidence that supports the results from in vivo and in vitro studies. These epidemiological results show direct associations of PFOS exposure and human health risks. For example, Gallo et al. (2012) found that the serum PFOS concentration was positively associated with the level of serum alanine transaminase (ALT) in adults. In the human body, ALT is mainly stored in hepatocytes, and the serum ALT level would significantly increase even if a few hepatocytes are damaged. Therefore, the above result associated the PFOS exposure with hepatotoxicity in humans. Vuong et al. (2016) studied the relationship between prenatal PFOS exposure and executive function in school-age children, and found that the exposure was associated with metacognition impairment and behavior regulation. Executive functions are high neurocognitive processes. Prenatal exposure to PFOS may disrupt normal neurodevelopment and cause impairment in executive functions. Their results provided epidemiological evidence for the neurotoxicity of PFOS to humans. In an epidemiological study conducted by Lin et al. (2016), it was found that the PFOS concentration was positively associated with CD31+/CD42a− (circulating endothelial microparticles) and CD31+/CD42a+ (platelet microparticles) in serum of adolescents and young adults. The CD31+/CD42a− and CD31+/CD42a+ are biomarkers of endothelial apoptosis and platelet apoptosis, respectively. This result indicated the cardiovascular disease risk of PFOS to humans. Kataria et al. (2015) investigated the association between serum PFOS and kidney function of adolescents, and found that the level of PFOS was significantly associated with the decreased glomerular

626 filtration rate and the increased serum uric acid. This result was consistent with that
627 exposure to PFOS can cause oxidative stress and damage glomerular endothelial cells
628 in laboratory studies.

629 The combination of toxicological and epidemiological studies is necessary to
630 fully understand the toxicity of PFOS to humans. For this purpose, Negri et al. (2017)
631 integrated the evidence that showed the effects of PFOS on fetal growth from
632 toxicology and epidemiology by a five-step "Epid-Tox" process. According to their
633 conclusions, both epidemiological and toxicological evidence has suggested that
634 PFOS can cause a decrease in birth weight of humans and rodents, but no quantitative
635 toxicological evidence was found to support the epidemiological results as effective
636 extrapolated concentrations of PFOS from animal experiments were generally higher
637 than those in humans. However, exposure to high doses of PFOS is required and
638 reliable method for the animal experiments to predict the risks in the general
639 population (Adami et al., 2011). More research is needed to strengthen the causal
640 inference between PFOS exposure and human health risks.

641 **Table 1** Representative epidemiological evidence that supports the human health risks of PFOS.

Toxic effect	Study area	Time span	Sample size	Main result	Reference
Hepatotoxicity	West Virginia, USA	2005–2006	47,092	Serum PFOS concentration is positively associated with the level of serum alanine transaminase (a marker of hepatocellular damage) in adults.	Gallo et al. (2012)
Neurotoxicity	Cincinnati, USA	2003–2006	242	Prenatal exposure to PFOS may be associated with both behavior regulation and metacognition impairment.	Vuong et al. (2016)
Reproductive toxicity	Avon county, UK	1991–1992	447	Higher prenatal exposure to PFOS is associated with increased weight of girls at 20 months.	Maisonet et al. (2012)
Immunotoxicity	Faroe Islands, Denmark	2007–2009	349	Prenatal and infant exposure to PFOS is associated with children's antibody concentrations against tetanus and diphtheria vaccines at the age of five.	Grandjean et al. (2017)
Thyroid disruption	New York State, USA	2005 and 2010	87	Serum PFOS concentration is positively associated with the level of free and total thyroxine in older adults.	Shrestha et al. (2015)
Cardiovascular toxicity	Taiwan, China	2006–2008	848	The higher serum PFOS level is closely associated with the increased carotid intima-media thickness.	Lin et al. (2016)
Pulmonary toxicity	Taiwan, China	2009–2010	200	Serum PFOS concentration is positively associated with impaired lung function in children.	Qin et al. (2017)
Renal toxicity	USA	2003–2010	1,960	Serum PFOS concentration is associated with the decreased kidney function within the normal range in adolescents.	Kataria et al. (2015)
Carcinogenicity	Greenland, Denmark	2000–2003	146	PFOS may be a risk factor of developing breast cancer in Inuit.	Bonefeld-Jorgensen et al. (2011)

642

643 **5. Conclusions and future research needs**

644 Potential environmental and health risks of PFOS have aroused great concern over the
645 past decade. Animal experiments conducted in vivo and in vitro are primary means to
646 ascertain the human health risks of PFOS and its toxic mechanisms. This article
647 systematically reviews the toxic effects and human health risks of PFOS based on the
648 currently known facts found by in vivo and in vitro studies from 2008 to 2018. Exposure to
649 PFOS can cause hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid
650 disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity in laboratory
651 animals and many in vitro human systems. These results and related epidemiological studies
652 confirmed the human health risks of PFOS. The widely studied toxic mechanisms of PFOS
653 mainly involve the oxidative stress (e.g., cytotoxicity) and physiological process disruption
654 based on fatty acid similarity (e.g., competitive binding with receptor protein). However, the
655 specific molecular mechanisms (including signaling molecules and pathways) still need
656 further investigation.

657 Current in vivo and in vitro studies for assessing the human health risks of PFOS face
658 the following challenges:

659 (1) Insufficient toxicological tests and data on PFOS toxicity. Though some progress has
660 been made in assessing the toxic effects of PFOS, more toxicological tests and data are
661 still needed to improve the knowledge about the long-term effects and mechanisms of
662 PFOS toxicity.

663 (2) Biomarkers for PFOS-induced injuries. Biomarkers are measurable indicators of a

biological state or condition, either normal or pathogenic (Ruiz-Romero and Blanco, 2015). It is significant to detect the structural and functional changes of human body in the levels of molecule, cell, or individual before serious injuries. In animal experiments, biomarkers can reflect the early biological effects with PFOS exposure and provide useful information on the toxic mechanisms. Currently available biomarkers for detecting various toxic effects are limited and need further development.

(3) Molecular mechanisms of PFOS toxicity. Though many studies have reported that a certain molecular mechanism is related to a PFOS-induced injury, but various signaling molecules and pathways may be involved. More systematic research on the molecular mechanisms should be conducted.

(4) Application of various omics. The toxic effect, especially chronic toxicity, of PFOS is usually the result of a continuous physiological response involving genome, transcriptome, proteome, and metabolome. Incorporating various omics into the in vivo and in vitro studies of PFOS toxicity can better elucidate the toxic mechanisms in future research.

(5) Integration of the toxicological and epidemiological data. The ultimate purpose of animal experiments is to assess the human health risks of PFOS. It is necessary to minimize the species differences in result extrapolation of animal experiments. Additionally, effective extrapolated concentrations of PFOS from animal experiments are generally higher than those in humans, which decreases the biological plausibility of causality. Sound improvement of the experimental techniques and analytical methods is needed to solve this problem.

(6) Co-exposure to multiple PFAS. In an actual situation, people may be simultaneously exposed to multiple PFAS, such as both PFOS and PFOA. The interactions and joint toxicity are unclear. Further studies are needed to develop the knowledge.

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