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REVIEW ARTICLE

Emerging endocrine disrupters: perfluoroalkylated substances

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Summary

In recent years, polyfluorinated chemicals (PFCs) have increasingly been used as surfactants in various industry- and consumer products, because of their unique properties as repellents of dirt, water and oils. The most well-known PFCs are perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and their derivatives belonging to the group of perfluoroalkylated substances. The PFCs are very persistent in the environment, and some of them have been discovered as global pollutants of air, water, soil and wildlife and even found in remote polar areas. Bioaccumulation occurs also in humans, and everybody in our society has traces of these PFCs in their blood and internal organs such as the liver, kidneys, spleen, gall bladder and testes. In the blood, PFOS and PFOA are bound to serum proteins. The acute toxicity of the polyfluorinated substances is moderate but some substances can induce peroxisome proliferation in rat livers and may change the fluidity of cell membranes. Some of these PFCs, such as PFOS and PFOA, are potential developmental toxicants and are suspected endocrine disruptors with effects on sex hormone levels resulting in lower testosterone levels and higher oestradiol level. Other PFCs have oestrogenic effects in cell cultures. The industrial production of PFOS and its derivatives stopped in 2000, and the European Union has banned most uses from the summer of 2008. However, hundreds of related chemicals: homologues with shorter or longer alkyl chain, PFOA and telomers, which potentially may degrade to perfluoroalkanoic (carboxylic) acids, are not regulated.

Introduction

Polyfluorinated chemicals (PFCs) are synthetic chemical substances that have very unique properties, such as high stabilities and extremely low surface tensions. Most PFCs are insoluble in both water and organic solvents, and they repel dirt, water and oils. Although these chemicals are rather expensive several hundreds different of them are increasingly used as surfactants in various industry- and common consumer products, including 'nanoproducts'. Important are uses in paints and for impregnation of textiles, clothes, footwear, furniture and carpets. Other uses are in lubricants, waxes for floors and cars, and in fire-fighting foam for oil fires at airports, harbours, oil platforms and oil refineries. Common trade names for commercial products are: Baygard (Bayer AG, Leverkusen, Germany), Scotchgard (3M, St. Paul, MN, USA), Gore-Tex (W. L. Gore & Associates Inc., Newark, DE, USA), Zonyl (E. I. du Pont de Nemours and Company, Wilmington, DE, USA) and Stainmaster (INVISTA, Wichita, KS, USA) (Hekster *et al.*, 2003; Poulsen *et al.*, 2005; OECD, 2006).

The perfluoroalkylated substances (PFAS), such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are most persistent in the environment and have been discovered as global pollutants of air, water and soil, and even found in remote polar areas (Giesy & Kannan, 2002). PFOS is most abundant and have been determined in blood and liver samples from various aquatic mammals (seals, otters, sea lions, dolphins, polar bears and minks) and birds, fish and humans. The highest levels of PFOS have been found in polar bears from the Arctic (Bossi *et al.*, 2005; Smithwick *et al.*, 2005a,b, 2006; Betts, 2007).

Chemistry and properties

The most well-known perfluoroalkyl substances are PFOS, PFOA and their derivatives. The chemical structures for some compounds are shown in Fig. 1.

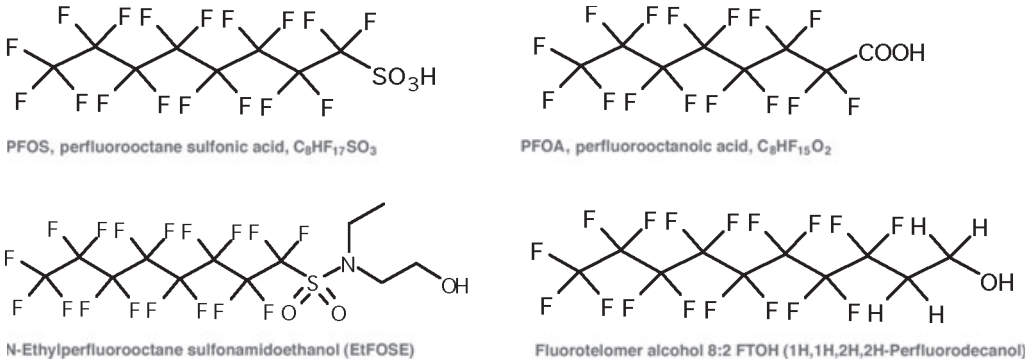

Figure 1 Chemical structures of selected polyfluorinated chemicals.

PFOA, PFOS and their salts are water-soluble and may be spread globally by sea water currents (Prevedouros *et al.*, 2006).

The substances most used in practice are more complex derivatives of these basic chemical structures, e.g. sulfonamides and fluorotelomers (Fig. 1).

These complex derivatives may be degraded to either PFOS, PFOA or perfluoroalkanoic acids with shorter or longer perfluorinated alkyl chain; the perfluorinated 'tail' is not degraded in the body or in the environment (Dinglasan *et al.*, 2004; Lau *et al.*, 2007).

The complex derivatives are insoluble in water and lipids but somewhat volatile and may be transported over long distances by air from temperate to arctic areas (Wallington *et al.*, 2006). The use of fluorotelomer derivatives in fire-fighting foam has an especially high potential for environmental release and human exposure to PFCs (Moody & Field, 2000; Moody *et al.*, 2002).

Human exposure

Lipophilic persistent organic pollutants (POPs), such as DDT, polychlorinated biphenyls (PCB), dioxins and brominated diphenyl ethers, accumulate in fatty tissue, and the main route of exposure for the general population is from food intake (UNEP, 2001). However, PFCs are both lipophobic and hydrophobic and, therefore, they will not accumulate in lipids but mainly accumulate in blood, liver and kidneys, which are not so common food ingredients. A study from Canada has, however, reported PFAS in fast food composites (Tittlemier *et al.*, 2006).

The most publicly well-known occurrence of PFCs is probably as impurity in the non-stick surface layer of Teflon (E. I. du Pont de Nemours and Company) treated cookware, such as frying pans, and as greaseproof additive in pop corn microwave bags, from which the chemicals can be released gradually during cooking and leak into the food (Sinclair *et al.*, 2007).

The major human exposure is probably from PFCs used as surfactants for impregnation of consumer goods, such as textiles, foot wear, furniture and carpets, which then releases PFCs to the indoor air and contaminate indoor dust, which then is inhaled by humans. Indoor air contains in general 25–100 times more PFAS than outdoor air with maximum levels [e.g. 8 : 2 fluorotelomer alcohol (FTOH)] of 28 $\mu g/m^3$, and house dust can be very contaminated with up to 75 ppm *N*-ethyl perfluorooctane sulfonamidoethanol (EtFOSE), 16 ppm 8 : 2 FTOH, 5 ppm PFOS and 3.7 ppm PFOA (Shoeib *et al.*, 2004, 2007). Moreover, children, who may be more in contact with considerable amounts of house dusts during play on the floor, will collect such dusts on the fingers, which subsequently can be ingested. Relative to body weight children have a 5–10 times larger intake of PFCs indoor than adults (Shoeib *et al.*, 2005).

Human levels and half-lives

The PFAS are readily absorbed and bind to proteins in blood serum and accumulate mainly in organs such as liver, kidney and spleen, but also in testicles and brain (Vanden Heuvel *et al.*, 1992; Austin *et al.*, 2003; Jones *et al.*, 2003). The renal clearances of PFOA and PFOS is almost negligible in humans, contrary to a large active excretion in experimental animals (Harada *et al.*, 2005). The half-lives of different polyfluorinated compounds vary but are generally very long. The half-lives in blood serum have been estimated to be 3.8, 5.4 and 8.5 years for PFOA, PFOS and perfluorohexane sulfonamide, respectively (Olsen *et al.*, 2007) – and in the organs, the half-lives are probably even longer. Levels in serum are, in general, 2–3 times higher than in whole blood (Taniyasu *et al.*, 2003; Ehresman *et al.*, 2007).

Today PFAS, such as PFOA and PFOS, are present in the blood, liver and kidneys of everybody in our society (Kannan *et al.*, 2004). In utero exposure to PFOS and PFOA is, therefore, also ubiquitous as they are transferred

to the foetus through the placenta and later also to the babies through the milk (Apelberg *et al.*, 2007a; Henderson & Smith, 2007).

Levels in blood are considered a sufficient sensitive, precise and accurate biomarker of human exposure to PFOS (Butenhoff *et al.*, 2006). Levels can be high in blood serum of the general population, as levels of more than 1500 ng PFOS/mL have been observed in American blood donors (Olsen *et al.*, 2003a). In general, average levels are, however, much lower around 20–30 ng PFOS/mL, and the levels of PFOA and other perfluorocarboxylic acids even lower. There are also geographical variations as illustrated in Fig. 2.

Today the levels are so high that exposure to polyfluorinated substances measured on basis of whole blood may be the highest human exposure to exogenous chemicals, exceeding that of more well-known environmental contaminants such as DDE (persistent and bioaccumulative metabolite of the pesticide DDT), PCBs, brominated flame retardants and even phthalates. Results from a study in the Norwegian and Russian Arctic are shown in Fig. 3.

Levels of polyfluorinated compounds in maternal blood are in general 2–3 times the levels in cord blood (Apelberg *et al.*, 2007a; Fei *et al.*, 2007; Inoue *et al.*, 2004; Tittlemier *et al.*, 2004; Midasch *et al.*, 2007). Levels in semen were 10 times lower than in blood serum (Guruge *et al.*, 2005).

Toxicology

There is limited information available on the toxicology of most PFCs (Lau *et al.*, 2007), and it will probably last several years before there is sufficient knowledge to assess the full consequences of the human exposure to these chemicals.

The acute toxicity of PFAS is moderate but increases with the chain length. The oral rat LD_{50} for PFOS is 251 mg/kg (US EPA, 2000), while the oral LD_{50} for PFOA is between 430 and 1800 mg/kg (Kennedy *et al.*, 2004).

The primary target organ is the liver, where they cause an increased weight and hepatocytic hypertrophy (Seacat *et al.*, 2003; Kennedy *et al.*, 2004). In rats, some of these substances induce lower serum glucose and cholesterol levels and increases the β-oxidation of fatty acids (Sohlenius *et al.*, 1995; Seacat *et al.*, 2003). Moreover, PFAS binds to proteins in cell membranes and change the membrane properties (fluidity), which can lead to aberrant signalling from surface receptors (Hu *et al.*, 2003).

Perfluorooctane sulfonic acid exposure (10 mg/kg bw for 2 weeks) of female rats affects the oestrous cyclicity and increases serum corticosterone level, while it decreases serum leptin concentration and norepinephrine concentration in the hypothalamus (Austin *et al.*, 2003). In utero exposure to PFOS (≥0.8 mg/kg bw/day) leads in

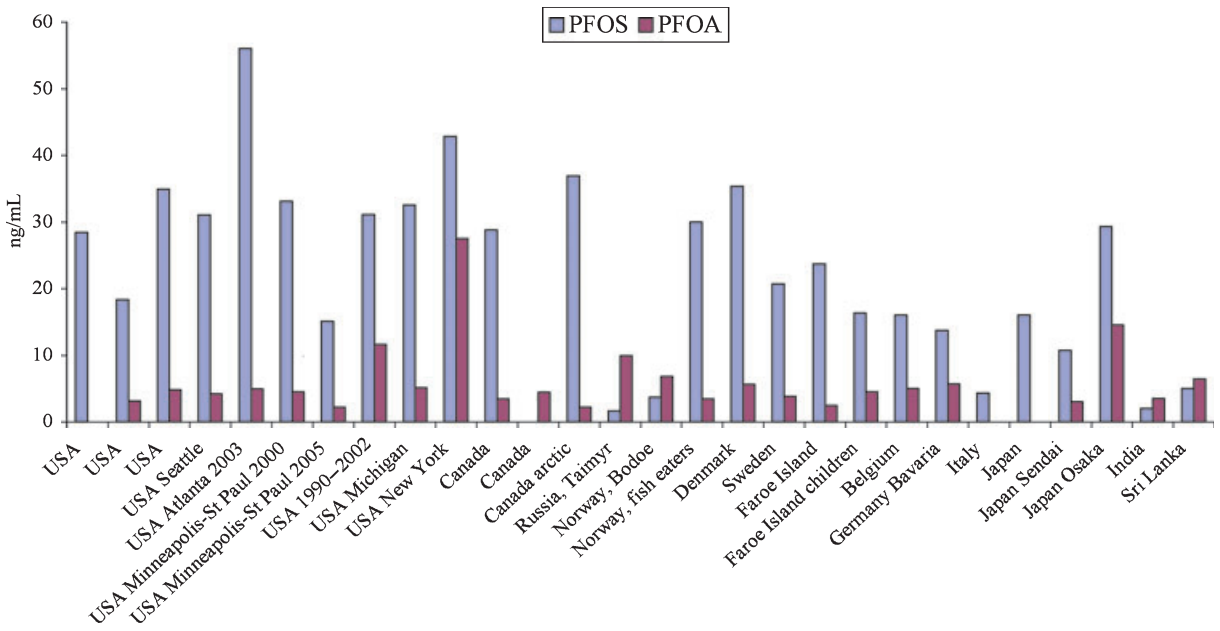

Figure 2 Typical average concentrations of perfluorooctane sulfonic acid and perfluorooctanoic acid in blood (serum/plasma) from various countries (Hansen *et al.*, 2001; Olsen *et al.*, 2003a,b, 2004, 2007; Corsolini & Kannan, 2004; Kannan *et al.*, 2004; Kubwabo *et al.*, 2004; Kuklenyik *et al.*, 2004; Tittlemier *et al.*, 2004; Guruge *et al.*, 2005; Calafat *et al.*, 2006; De Silva & Mabury, 2006; Odland *et al.*, 2006; Thomsen *et al.*, 2006; Fei *et al.*, 2007; Fromme *et al.*, 2007; Harada *et al.*, 2007; Kärrman *et al.*, 2007; Kato *et al.*, 2007).

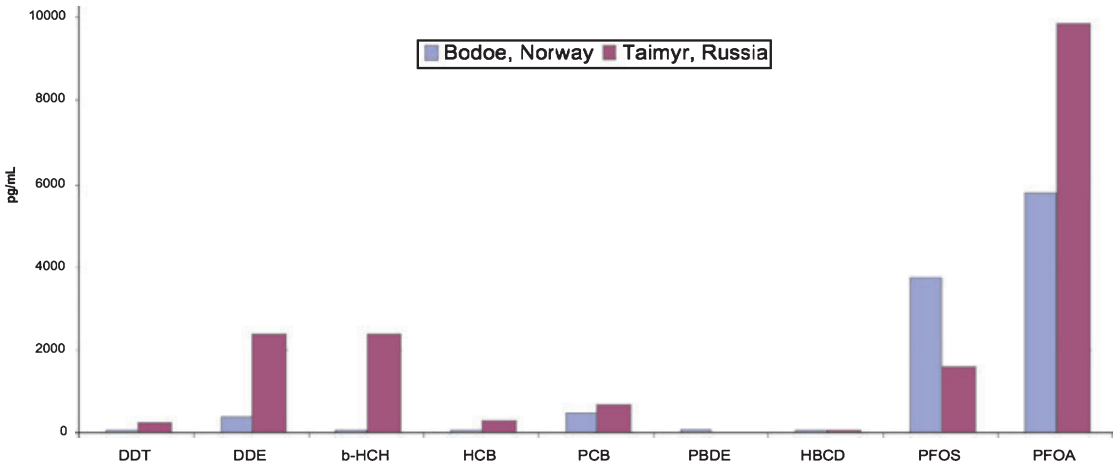

Figure 3 Persistent organic pollutants in blood plasma from pregnant women living in Norwegian and Russian Arctic (modified from Odland *et al.*, 2006).

mice and rats dose-dependently to a reduced litter size caused both by reduced implantation and foetal and neonatal death (Luebker *et al.*, 2005a; Lau *et al.*, 2006). Pups of dams of mice and rats exposed by oral gavage to PFOS (≥0.4 mg/kg bw/day) showed a reduced body weight and a delay in development and neuromotor maturation (Luebker *et al.*, 2005b; Fuentes *et al.*, 2007), but there are no reports of increase in malformations (Lau *et al.*, 2007). Gestational PFOA exposure of mice is associated with altered mammary gland development in Dams and female offspring (White *et al.*, 2007).

Apelberg *et al.* (2007b) found that reductions of birth weight, ponderal index and head circumference were associated with PFOS and PFOA concentrations in blood among vaginal deliveries. A similar inverse association concerning maternal PFOA concentration and birth weight was found by Fei *et al.* (2007).

Perfluorooctane sulfonic acid, PFOA and other tested PFCs were not active in various mutagenicity test systems. However, PFOA exposed rodents developed an excess of Leydig cell adenomas (Cook *et al.*, 1992; Biegel *et al.*, 1995; Liu *et al.*, 1996). Moreover, PFOS and EtFOSE caused liver hepatocellular adenomas in female rats and thyroid follicular cell adenomas in male rodents (Thomford *et al.*, 2002). Because of these studies, the US EPA classifies PFOA as an animal carcinogen (US EPA, 2000). In accordance, a study from the work environment reports an increased incidence of urinary bladder cancer in workers with high exposure to perfluorooctane sulfonyl fluoride (Alexander *et al.*, 2003).

Thus, although PFAS are only moderately toxic, they do induce a number of adverse effects in experimental animals, which together point to potential problems with the currently very high human exposure levels.

Possible mixture effects

As exposure to PFCs is ubiquitous to humans this exposure is added to all other exposures humans may experience. This raises the question of possible mixture effects, which could increase effects from other exposures. Mixture effects have already been shown in vitro and in vivo for oestrogenic compounds (Silva *et al.*, 2002; Tinwell & Ashby, 2004) and for anti-androgens (Birkhoj *et al.*, 2004; Metzdorff *et al.*, 2007), but there are very few studies of mixture effects of compounds with different modes of action.

For PFAS, there are several reports of such effects. Co-administration of PFOS and dioxin [2378-tetrachlorodibenzo-p-dioxin (TCDD)] resulted in an increased p450 A1A expression as compared to TCDD alone (Hu *et al.*, 2003). PFAS may also increase the carcinogenicity of other chemicals as the genotoxicity of cyclophosphamide in the micronucleus assay with hamster lung V79 cells increased many fold by simultaneous exposure to PFOS (Jernbro *et al.*, 2007).

Thus, the sensitivity to other chemicals can be increased by simultaneous exposure to PFCs.

Endocrine disruption

Some PFCs have oestrogenic effects in cell cultures ('E-screen assay'; Soto *et al.*, 1995). For example, the fluorotelomer alcohols 6 : 2 FTOH and 8 : 2 FTOH induce MCF-7 breast cancer cell proliferation and up-regulates the oestrogen receptor, but PFOS, PFOA and perfluorononanoic acid had no oestrogenic effect in that test (Maras *et al.*, 2006; Vanparys *et al.*, 2006). Nevertheless, PFCs act as endocrine disruptors, because exposure of adult rats affects their endocrine system by decreasing the

testosterone level and increasing the oestradiol level (Biegel *et al.*, 1995; Shi *et al.*, 2007).

The effects on hormone levels in rodents are reflected in changes in the testis where exposure to perfluorooctanoate results in Leydig cell hyperplasia and eventually the development of Leydig cell adenomas (Biegel *et al.*, 1995). A study of testis effects in adult rats exposed to ≥5 mg perfluorododecanoic acid/kg bw daily for 2 weeks also showed a reduced gene expression of many genes involved in cholesterol transport and steroidogenesis and a reduced serum testosterone level (Shi *et al.*, 2007). Thus, it seems as if exposure to PFAS substances can severely affect proliferation and function of Leydig cells in the adult rat.

This is of considerable concern, because Leydig cell hyperplasia is common among infertile men (Holm *et al.*, 2003) who, as a group, also show lower testosterone levels than comparable normal controls (Andersson *et al.*, 2004). Reduced testis function has been linked to the testicular dysgenesis syndrome (TDS) (Skakkebaek *et al.*, 2001). The TDS hypothesis states that in utero exposure to endocrine disruptors can damage testis development and lead to reduced testis function in the adult, with symptoms ranging from a moderately reduced semen quality to testis cancer. The best animal model for human TDS consists of rats exposed to long-chain phthalates in a critical time window during development (Fisher *et al.*, 2003). The exposure results in testis dysgenesis with Leydig cell hyperplasia and clustering of the Leydig cells in the centre of the testis, resulting in reduced testosterone levels and compromised fertility in the adults (Sharpe, 2006; Hallmark *et al.*, 2007). The compromised Leydig cell function is reflected in a reduced expression of genes involved in cholesterol transport and steroidogenesis (Liu *et al.*, 2005). This has striking similarities to the reported effect of PFAS exposure; however, it seems as if PFAS compounds, in contrast to phthalates, can induce the effects in the adult.

Conclusion

Polyfluorinated chemicals have increasing importance in industry and consumer products because of their unique properties. However, these chemicals are persistent and have become wide-spread in the environment and accumulate in wildlife and humans.

One of the most worrying effects of polyfluorinated substances is their effect on Leydig cells in the rat testis, where the reported effects have a strong resemblance to observations from the clinic of infertile men seeking help with assisted reproduction. Such effects (Leydig cell hyperplasia, lower testosterone levels) can also be induced by exposure to other ubiquitous chemicals such as phthalates. As PFCs can induce these effects in the adult, it raises the question of what happens when a person with a moderately reduced testis function, maybe caused by TDS induced in utero, as an adult is exposed to chemicals that can elicit the same effect.

Butenhoff *et al.* (2004) have made a risk characterization of the general population exposure to PFOA concluding that the margin of safety was ranging between 1600 for liver weight increase and 8900 for Leydig cell adenomas. This assessment was based on information obtained from animal experiments. However, the facts that the half-life of the chemical in humans is much longer than in animals, and the renal clearance in humans is negligible, contrary to the active excretion in animals, makes human risk assessment based on animal experiments questionable for these chemicals.

The international awareness and concern is increasing. In 2000 the main producer, the 3M Company, voluntarily stopped the production of one of the chemicals (PFOS), and a ban of some fluorotelomers has been introduced in Canada. In Europe, the EU countries will ban PFOS and its derivatives from the summer of 2008. However, PFOS is only a small part of the problem. The family of PFCs consists of several hundreds other unrestricted chemicals. Because the exposure to polyfluorinated substances is so considerable, and uses seem to increase, there is an urgent need to resolve, what effect such exposure has on humans.

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Panel discussion

S. Swan

You noticed a reduction in testosterone and altered Leydig cell differentiation in mammals after exposure to these chemicals. Did this have an effect on the anogenital distance (AGD)?

A.A. Jensen

The AGD was not measured.

O. Söder

The high level of PFAS in polar bears is worrying. What is the source of these chemicals, for example fish or environment? We should not expect the chemicals to be present in high concentrations in the environment of polar bears.

A.A. Jensen

We do not know the origin of PFAS in polar bears. They are at the top in the food chain, but there is much less PFAS in the sea or in fish and seals. The main chemical in the bear is PFOS (perfluoro-octane sulpfonic acid) but there are also some longer chain compounds and a few lower chain compounds. The extremely high PFOS levels may be linked to the biochemistry of the polar bears with high levels of vitamin A in the liver. The PFOS is predominantly present in the blood, kidney and liver, and some is transported to the testis and brain.

