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Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers *in vitro*

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HIGHLIGHTS

- Effect on oxidative stress factors of seven long-chained PFAS was investigated.
- The selected PFAS were: PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA and PFDoA.
- Four of the PFAS showed dose-dependent increase in DNA damage.
- Six PFAS increased ROS generation and the increase were dose-dependent for 2 PFAS.
- PFOA significantly decreased the total antioxidant capacity.

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ABSTRACT

Perfluoroalkylated substances (PFAS) have been widely used since 1950s and humans are exposed through food, drinking water, consumer products, dust, etc. The long-chained PFAS are persistent in the environment and accumulate in wildlife and humans. They are suspected carcinogens and a potential mode of action is through generation of oxidative stress. Seven long-chained PFAS found in human serum were investigated for the potential to generate reactive oxygen species (ROS), induce DNA damage and disturb the total antioxidant capacity (TAC). The tested PFAS were perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluoroctanoic acid (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA). Using the human hepatoma cell line (HepG2) and an exposure time of 24 h we found that all three endpoints were affected by one or more of the compounds. PFHxS, PFOA, PFOS and PFNA showed a dose dependent increase in DNA damage in the concentration range from 2×10^{-7} to 2×10^{-5} M determined by the comet assay. Except for PFDoA, all the other PFAS increased ROS generation significantly. For PFHxS and PFUnA the observed ROS increases were dose-dependent. Cells exposed to PFOA were found to have a significant lower TAC compared with the solvent control, whereas a non-significant trend in TAC decrease was observed for PFOS and PFDoA and an increase tendency for PFHxS, PFNA and PFUnA. Our results indicate a possible genotoxic and cytotoxic potential of the PFAS in human liver cells.

1. Introduction

Over the past 60 years perfluoroalkylated substances (PFAS) have been widely used in industrial and commercial applications. Long-chain PFAS are environmentally widespread, persistent, and accumulative in nature, animals and humans (Giesy and Kannan, 2001; Fromme et al., 2009). Humans are mainly exposed to the long-chain PFAS through food intake, house dust, and indoor air (Haug et al., 2011), and the average serum half-lives for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), and perfluorooctanoate (PFOA) are 8.8, 5.4 and 2.3–3.8 years, respectively (Olsen et al., 2007; Bartell et al., 2010).

PFOA and PFOS are the most predominant PFAS and have been intensively studied although mainly in rodents. Hepatotoxicity, immunotoxicity, hormonal effects and, a possible carcinogenic potential are some of the observed effects in rodents (Lau et al., 2007). PFAS are potential endocrine disruptors and affect the

Abbreviations: DMSO, dimethyl sulfoxide; EtOH, ethanol; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; PBS, phosphate buffered saline; PFAS, perfluoroalkylated substances; PFCA, perfluorinated carboxylic acids; PFDA, perfluorodecanoate; PFDoA, perfluorododecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluoroctanoic acid; PFOS, perfluorooctane sulfonic acid; PFOSA, perfluorooctanesulfonamide; PFSA, perfluorinated sulfonic acids; PFUnA, perfluoroundecanoate; ROS, reactive oxygen spices; TAC, total antioxidant capacity; tBuOOH, tert-butyl hydroperoxide.

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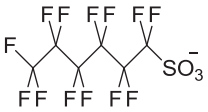
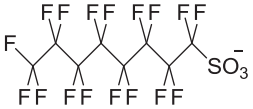
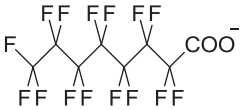
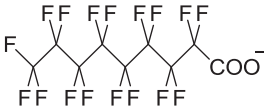
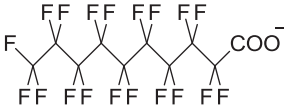
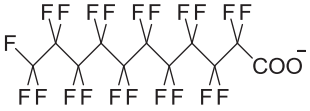
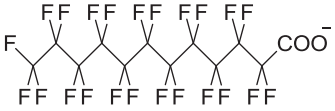
Table 1
Tested PFAS and their cytotoxicity in the HepG2 cells. As a measurement for cytotoxicity lactate dehydrogenase (LDH) leakage from the cells were measured (see Section 2.7).

Compound	Carbon atoms	Chemical structure	CAS No. [a]	Purity (%)	Cytotoxicity HepG2 (M)
Perfluorinated sulfonic acids (PFSA)					
PFHxS (perfluorohexane sulfonate)	C6		355-46-4	98	$>2 \times 10^{-4}$
PFOS (perfluorooctane sulfonate)	C8		1763-23-1	98	$>2 \times 10^{-5}$
Perfluorinated carboxylic acids (PFCA)					
PFOA (perfluorooctanoate)	C8		335-67-1	95	$>2 \times 10^{-4}$
PFNA (perfluorononanoate)	C9		375-95-1	97	$>2 \times 10^{-4}$
PFDA (perfluorodecanoate)	C10		335-76-2	98	$>2 \times 10^{-4}$
PFUnA (perfluoroundecanoate)	C11		2058-94-8	95	$>2 \times 10^{-4}$
PFDoA (perfluorododecanoate)	C12		307-55-1	96	$>2 \times 10^{-5}$

[a] The CAS No. is for the protonated acid form of the perfluoroalkylated substances.

function of thyroid hormone and functions of estrogen, androgen, and aryl hydrocarbon receptor *in vitro* (Bonefeld-Jorgensen et al., 2014). In humans, significantly higher serum levels of several PFAS including PFOS, PFOA, PFHxS, and perfluorooctanesulfonamide (PFOSA) were found in Greenlandic breast cancer patients compared with matched controls and PFOS and PFOSA were found as significant risk factors (Bonefeld-Jorgensen et al., 2011). In a prospective study of Danish women PFOSA was also found as a potential breast cancer risk factor (Bonefeld-Jorgensen et al., 2014). Another Danish prospective study did not find any association between plasma concentrations of PFOS and PFOA and the risk of prostate, bladder, pancreatic, or liver cancer, but a 30–40% increase in risk estimates for prostate cancer was observed for the three upper quartiles of PFOS compared with the lowest quartile (Eriksen et al., 2009). A significant difference in blood concentration of perfluorodecanoate (PFDA) was found between Swedish prostate cancer cases and healthy controls, with highest concentrations among the cases (Hardell et al., 2014).

The carcinogenic mechanisms of PFAS are not fully elucidated. PFAS have the ability to activate the peroxisome proliferator-activated receptor alpha and induce peroxisome proliferation in rodents, but the human relevance for this mode of action has been questioned (Klaunig et al., 2003; Andersen et al., 2008). A possible mechanism of action for PFAS in humans is generation of oxidative stress and some controversial evidence of effects in terms of oxidative stress and DNA damage exist. Several *in vitro* studies on the genotoxic and cytotoxic effects are published but the results are inconclusive (Yao and Zhong, 2005; Hu and Hu, 2009; Eriksen et al., 2010; Florentin et al., 2011; Huang et al., 2013). Some of the inconsistencies in the results may relate to differences in study and method setups.

Oxidative stress can be induced by environmental chemicals such as dioxins and heavy metals which result in increased production of reactive oxygen species (ROS) and damage of DNA (Mates et al., 2010), and similar mechanisms may be relevant for PFAS. Oxidative stress has been observed in relation to several diseases in humans, including atherosclerosis, heart attacks, chronic inflammatory diseases, central nervous system disorders, age related disorders and cancer (Aruoma, 1998; Barnham et al., 2004; Visconti and Grieco, 2009; Tsutsui et al., 2011).

The aim of this study was to assess the *in vitro* potential impact of seven long-chain PFAS on three oxidative stress endpoints: total antioxidant capacity (TAC), DNA damage, and generation of ROS. The selected endpoints were assessed using the human hepatoma cell line HepG2. The seven PFAS (PFHxS, PFOS, PFOA, perfluorononanoate (PFNA), PFDA, perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA)) were selected based on extent of human use and exposure, detection in human body, potential toxicity, and public concern (Posner et al., 2013).

2. Methods

2.1. Chemicals

PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnA were all purchased from ABCR (Germany). PFDoA was purchased from Sigma–Aldrich (Denmark). The purity of the test compounds was above 95% (specific purities and CAS No. are presented in Table 1). PFHxS, PFOS,