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Fluorochemicals used in food packaging inhibit male sex hormone synthesis

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ABSTRACT

Polyfluoroalkyl phosphate surfactants (PAPS) are widely used in food contact materials (FCMs) of paper and board and have recently been detected in 57% of investigated materials. Human exposure occurs as PAPS have been measured in blood; however knowledge is lacking on the toxicology of PAPS. The aim of this study was to elucidate the effects of six fluorochemicals on sex hormone synthesis and androgen receptor (AR) activation *in vitro*. Four PAPS and two metabolites, perfluorooctanoic acid (PFOA) and 8:2 fluorotelomer alcohol (8:2 FTOH) were tested.

Hormone profiles, including eight steroid hormones, generally showed that 8:2 diPAPS, 8:2 monoPAPS and 8:2 FTOH led to decreases in androgens (testosterone, dehydroepiandrosterone, and androstenedione) in the H295R steroidogenesis assay. Decreases were observed for progesterone and 17-OH-progesterone as well. These observations indicated that a step prior to progestagen and androgen synthesis had been affected. Gene expression analysis of StAR, Bzrp, CYP11A, CYP17, CYP21 and CYP19 mRNA showed a decrease in Bzrp mRNA levels for 8:2 monoPAPS and 8:2 FTOH indicating interference with cholesterol transport to the inner mitochondria. Cortisol, estrone and 17β-estradiol levels were in several cases increased with exposure. In accordance with these data CYP19 gene expression increased with 8:2 diPAPS, 8:2 monoPAPS and 8:2 FTOH exposures indicating that this is a contributing factor to the decreased androgen and the increased estrogen levels.

Overall, these results demonstrate that fluorochemicals present in food packaging materials and their metabolites can affect steroidogenesis through decreased Bzrp and increased CYP19 gene expression leading to lower androgen and higher estrogen levels.

Introduction

Fluorochemicals are a group of manmade compounds that possess the unique ability to repel water and oil simultaneously (Kissa, 2001). This makes them ideal for multiple purposes including in coatings for paper and board (Begley et al., 2008). In a recent study 61% of selected food contact materials (FCMs) made of paper and board were found to contain fluorochemicals and of these 57% contained polyfluoroalkyl phosphate surfactants (PAPS) (Trier, 2011) (Fig. 1). The technical mixtures of paper coatings contain dialkylated (diPAPS), monoalkylated (monoPAPS) and trialkylated (triPAPS) homologues (Trier et al., 2011). Among these diPAPS has been reported to migrate from FCMs into foods (Begley et al., 2008). Despite these findings there are no specific EU legislation for the use of PAPS in paper and board FCMs (Trier et al., 2011).

Reports of diPAPS in human sera collected in the United States suggested that not only are these chemicals used, but humans are exposed and can absorb these across the gastrointestinal tract. The reported total concentration of diPAPS homologues (4:2, 6:2, 8:2 and 10:2 diPAPS) was 4.5 μg/L (D'eon et al., 2009). Metabolism of PAPS can lead to the formation of highly persistent perfluorinated carboxylic acids (PFCAs) (Fig. 1), such as perfluorooctanoic acid (PFOA), which has a half-life in humans of 3.8 years (Olsen et al., 2007). In male rats PAPS metabolize into PFCAs possibly through the intermediate fluorotelomer alcohol (FTOH) (D'eon and Mabury, 2007, 2011). FTOH oxidize into PFCAs in isolated rat hepatocytes (Martin et al., 2005). Several body compartments have been found to contain PFCAs, including human blood/serum (Houde et al., 2006), umbilical cord blood (Kim et al., 2011; Monroy et al., 2008) and breast milk (So et al., 2006). PFOA has been measured at mean serum/plasma/whole blood concentrations ranging from 3 to 213 nM

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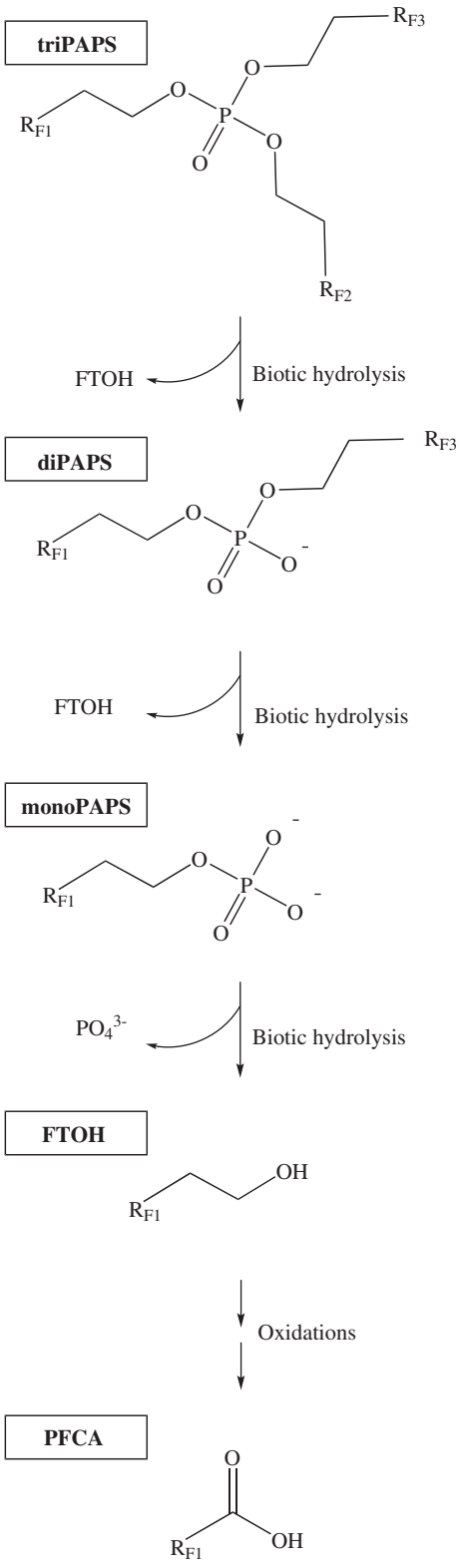

Fig. 1. Metabolic pathway of polyfluoroalkyl phosphate surfactants (PAPS) in rat, mice and isolated rat hepatocytes into fluorotelomer alcohol (FTOH) and perfluorinated carboxylic acids (PFCAs) (D'eon and Mabury, 2007, 2011; Fasano et al., 2006; Kudo et al., 2005; Martin et al., 2005). R_{F1}, R_{F2} and R_{F3} represent fully fluorinated carbon chains of varying lengths.

in the general population worldwide (EFSA, 2008). This indicates that not only are the general population exposed to PFCAs, but also that fetuses and infants may be exposed through placental passing and/or breast feeding. Based on the above studies it seems likely that PAPS also in humans is a precursor of PFCAs and as PAPS is present in human blood, it is relevant to establish if PAPS in itself have the potential to cause adverse effects in humans.

To our knowledge no data exists on the toxicology of PAPS, whereas PFOA and perfluorooctane sulfonic acid (PFOS) have been widely reviewed (Andersen et al., 2008; Kennedy et al., 2004; Lau et al., 2007; Steenland et al., 2010). Studies conducted in male rats have shown that exposure to PFCAs of varying chain lengths, which are all potentially final metabolites of PAPS, could decrease testosterone levels *in vivo* (Feng et al., 2009; Shi et al., 2007, 2009). Furthermore decreased gene expression of key enzymes and transporters involved in steroidogenesis was observed as a result of perfluorododecanoic acid (PFdoDA) exposure in male rats (Shi et al., 2007, 2009). For PFOA a study showed a decrease in testosterone levels in isolated rat Leydig cells with exposure (Zhao et al., 2010). Finally 8:2 FTOH, a metabolite of PAPS, also showed the capacity to decrease testosterone levels in the human adrenal cortico-carcinoma cell line (Liu et al., 2010). The above mentioned studies suggest that PFCAs and 8:2 FTOH have testosterone-inhibiting potential, and that the mechanism of action is interference with sex hormone synthesis. In accordance with these data an epidemiological study investigated associations between high PFOA and PFOS exposure, semen variables and hormone levels. A statistically significant association was found between high levels of PFOA and PFOS exposure and a decreased number of normal sperms as the only parameter (Joensen et al., 2009). As PAPS resemble PFOA and 8:2 FTOH by containing fluorinated chains and being surfactant molecules, it is therefore relevant to investigate if PAPS also have endocrine disrupting potential.

The aim of this study was to examine the potential of four PAPS (8:2 monoPAPS, 8:2 diPAPS, 10:2 diPAPS and 8:2 triPAPS) and metabolic products of these, PFOA and 8:2 FTOH, to interfere with steroidogenesis and androgen receptor activation using two *in vitro* assays, the H295R steroidogenesis assay and the androgen receptor (AR) reporter gene assay, respectively. The levels of eight steroid hormones and gene expression levels of six proteins involved in the steroidogenic pathway were end-points in the H295R steroidogenesis assay.

Materials and methods

Chemicals

Test chemicals 8:2 triPAPS (65.1%), 10:2 diPAPS (94.6%), 8:2 diPAPS (97.4%), and 8:2 monoPAPS (88.7%) were synthesized by Chiran (Trondheim, Norway). 8:2 FTOH (97%) was purchased from Fluorochem Ltd. (Derbyshire, UK) and PFOA (95%) was purchased from VWR (Herlev, Denmark). The notations 8:2 and 10:2 refer to the chain length of the fluorinated chains and the CH_2 group within the molecule e.g. the notation 10:2 diPAPS reflects that the two chains contain ten carbon atoms that are fully fluorinated and two carbon atoms to which hydrogen atoms are attached (Fig. 1). Stock solutions of 20 mM were prepared in DMSO (Sigma-Aldrich, Copenhagen, Denmark).

The calibration standard solution for high pressure liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) analysis contained dihydrotestosterone, dehydroepiandrosterone (DHEA), progesterone, estrone, testosterone and 17β-estradiol, all purchased from Sigma-Aldrich, (Copenhagen, Denmark), as well as 17-OH-progesterone, cortisol and androstenedione purchased from Steraloids (Rhode Island, USA), Riedel-de Haën (Seelze, Germany) and Cerilliant (Round Rock, USA), respectively. Internal standard solution containing testosterone-d_2, 17β-estradiol-d_3 and methyltestosterone-d_3 were purchased from RIKILT (Wageningen, Nederlands).