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Fluorochemicals used in food packaging inhibit male sex hormone synthesis

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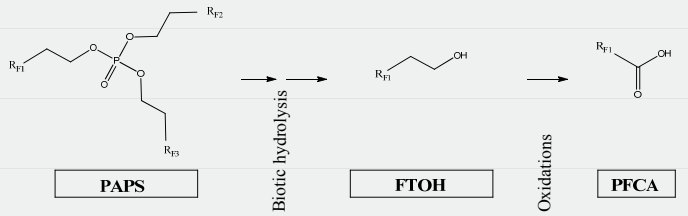
Background & Aim

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. Metabolic products of PAPS, fluorotelomer alcohol (FTOH) and perfluorocarboxylic acids (PFCAs) have shown potential to interfere with the endocrine system and thus 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.



Metabolic pathway of polyfluoroalkyl phosphate surfactants (PAPS) in rat, mice and isolated rat hepatocytes into fluorotelomer alcohol (FTOH) and perfluorinated carboxylic acids (PFCAs). R_{F1}, R_{F2} and R_{F3} represent fully fluorinated carbon chains of varying lengths.

Results

Hormone profiles, including eight steroid hormones, generally showed that 8:2 diPAPS, 8:2 monoPAPS and 8:2 FTOH led to decreases in testosterone, dehydroepiandrosterone, and androstenedione in the H295R steroidogenesis assay. Decreases were observed for progesterone and 17-OH-progesterone as well. None of the compounds showed effects in the AR reporter gene assay.

		8:2 triPAPS	10:2 diPAPS	8:2 diPAPS	8:2 monoPAPS	8:2 FTOH	PFOA
Progesterone[a]	QE	↓	↓	↓	↓	↓	n.s.
	LOEC (µM)	12.5□	50	3.1	50	3.1	n.s.
	E_{max} (%)	26 ± 10	22 ± 16	60 ± 14	42 ± 10	45 ± 18	n.s.
17-OH- Progesterone[b]	QE	↓	↓	↓	↓	↓	n.s.
	LOEC (µM)	12.5□	3.1	12.5	3.1	3.1	n.s.
	E_{max} (%)	14 ± 6	28 ± 3	50 ± 10	67 ± 3	55 ± 6	n.s.
Cortisol[b]	QE	n.s.	↓	↑	n.s.	n.s.	n.s.
	LOEC (µM)	n.s.	3.1□	50	n.s	n.s.	n.s.
	E_{max} (%)	n.s.	25 ± 15	38 ± 13	n.s.	n.s.	n.s.
DHEA[b]	QE	n.s.	↓	↓	↓	↓	n.s.
	LOEC (µM)	n.s.	3.1	50	12.5	12.5□	n.s.
	E_{max} (%)	n.s.	29 ± 3	56 ± 8	72 ± 3	39 ± 9	n.s.
Androstenedione[b]	QE	n.s.	↓	↓	↓	↓	↓
	LOEC (µM)	n.s.	12.5	12.5	12.5	3.1	12.5□
	E_{max} (%)	n.s.	17 ± 3	45 ± 6	65 ± 3	54 ± 4	19 ± 9
Testosterone[a]	QE	↓	n.s.	↓	↓	↓	n.s.
	LOEC (µM)	50	n.s.	25	25	3.1	n.s.
	E_{max} (%)	34 ± 21	n.s.	63 ± 10	56 ± 20	53 ± 16	n.s.
Estrone[b]	QE	↑	↑	↑	↑	↑	↑
	LOEC (µM)	12.5	50	50	50	12.5□	12.5
	E_{max} (%)	34 ± 8	30 ± 11	121 ± 9	137 ± 82	146 ± 24	75 ± 9
17β-estradiol[a]	QE	n.s.	n.s.	n.s.	n.s.	↑	↓/↑
	LOEC (µM)	n.s.	n.s.	n.s.	n.s.	12.5	1.6/50
	E_{max} (%)	n.s.	n.s.	n.s.	n.s.	41 ± 19	24 ± 19/17 ± 22

Overview of hormone effects in the H295R steroidogenesis assay. The qualitative effect (QE) (↓ or ↑), the lowest observable effect concentration (LOEC) and the tentative efficacy (E_{max}) given in % change are shown. All values have a significance level of $p < 0.05$. a) immunoassay data, b) HPLC-MS/MS data.

These findings indicate that of the two tested endpoints interference with hormone synthesis is the main mechanism of effect. The characteristic hormone profiles with exposure 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.

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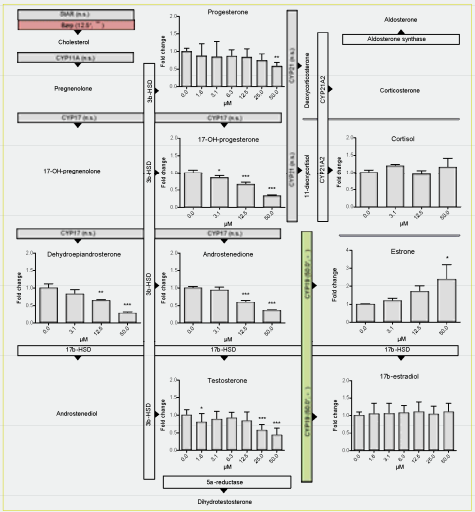
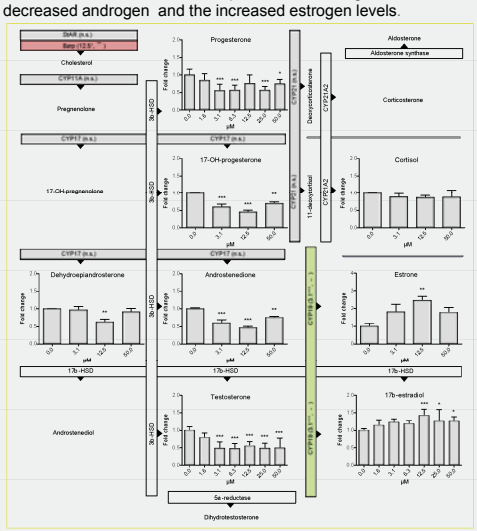
Conclusion

Overall, the results demonstrate that of the tested endpoints interference with steroidogenesis is the main target of the test compounds. Specifically, fluorochemicals used in food packaging and their metabolites can affect steroidogenesis through decreased Bzrp and increased CYP19 gene expression causing lower androgen and higher estrogen levels.



8:2 monoPAPS exposure and the hormone profile. Concentrations are given in µM and response in fold change. Concentration-effect relationship for progesterone, testosterone and 17β-estradiol are based on immunoassay data. The concentration-effect relationships for the remaining hormones are based on HPLC-MS/MS data. Bar windows (grey, green and red) represent expression of genes examined. Arrow up (↑) indicates an increase in the relative level of mRNA, arrow down (↓) represents a decrease in the relative level of mRNA and n.s. indicates that no statistically significant change was observed.

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.



8:2 FTOH exposure and the hormone profile. For further description see legend for 8:2 monoPAPS hormone profile.

Materials and Methods

Materials and methods are described in detail in the paper *Fluorochemicals used in food packaging inhibit male sex hormone synthesis* (Rosenmai et al., 2012). The tested compounds include 8:2 triPAPS, 10:2 PAPS, 8:2 diPAPS and 8:2 monoPAPS and the metabolites, 8:2 FTOH and PFOA. **H295R steroidogenesis assay.** The H295R cell line was cultured for 24 h with successive test compound exposure for 48 h. Exposure concentrations ranged from 1.6 - 50.0 µM. After 48 h exposure the supernatant was sampled for hormone analysis and cell viability was tested. Hormones were extracted from cell supernatants by SPE and were measured both by time-resolved fluoroimmunoassays and HPLC-MS/MS. Effects on gene expression were assessed by qRT-PCR. mRNA isolation and conversion to cDNA were performed with TaqMan® Fast Cells-to-CT™ Kit. cDNA was analyzed on a PCR system using TaqMan® Gene Expression Assays. **Androgen receptor reporter gene assay.** The assay was performed as previously described by Vinggaard *et al.* (2002). All plasmids were generous gifts from Dr. Albert Brinkmann (Erasmus University, Rotterdam). Test chemicals were added leading to final exposure concentrations ranging from 0.2 – 50.0 µM on agonism, antagonism and toxicity plates.

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