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# Uptake, elimination and toxicokinetic modeling of $^{13}\text{C}_4$ -8:2 diPAPs given with food to twelve males

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## Background

Polyfluoroalkyl phosphate ester surfactants (PAPs) are derived from fluorotelomer alcohols (FTOHs), and are used to make food paper and board oil and water repellent. PAPs were in 2009-2011 present in 40-57% of Danish food paper and board, particularly for popcorn and fast-food, and migrate to foods such as popcorn and butter (Begley et al. 2005, 2008, Trier and Alsing, DFVA 2012). PAPs have been measured in human serum<sup>1</sup> and breast milk (Kuwabo et al., 2013) In rats 8:2 diPAP [(F(CF<sub>2</sub>)<sub>8</sub>(CH<sub>2</sub>CH<sub>2</sub>O)]<sub>2</sub>-P(O)OH is uptaken orally (gavage: 50 mg/kg) with linear elimination kinetics (half-life: 1.7 d) and metabolise to FTOHs and perfluoroalkyl carboxylic acids (PFCAs). This is of concern, since PFCAs are linked to adverse effects in humans (Andersen et al., 2008; Kennedy et al., 2004.; Lau et al., 2007.; Steenland et al., 2010). Some PFCAs, FTOHs, and PAPs are suspected endocrine disrupters, and PAPs affects testosterone synthesis *in-vitro* (Rosenmai et al., 2013). It is therefore relevant to investigate if such FTOH derived PFAS, with diPAP being the example, are precursors and thus sources of PFCAs in humans.

## Results and Discussion

Figure 1 shows that the  $^{13}\text{C}_4$  8:2 diPAP is uptaken by humans in 0.9-6% from food as in rats (D'eon et al., 2011), but that the serum concentrations decrease linearly with time in a log-log plot, which has not previously been observed. This rules out regular linear clearance kinetics of the type  $C=Ae^{-kt}$  and suggests an empirical power-law  $C=At^{-k}$ . Non-linear pharmacokinetics has previously been observed for drugs such as the anti-cancer paclitaxel (Tuszynski et al., 2008), where the power-law signifies that two competing and saturable transport/metabolic mechanisms exist (Fig. 2).

The expected metabolites  $^{13}\text{C}_4$  8:2 FTCA, PFOA and  $^{13}\text{C}$  PFNA were found, and their concentrations increased with time. This confirms that PFCAs can stem from diPAP, and likely also other FTOH based PFAS. 8:2 monoPAP was not found, similarly to what has been found in rats (D'eon et al., 2011). The MS Scan method allowed us to search for non-target metabolites.

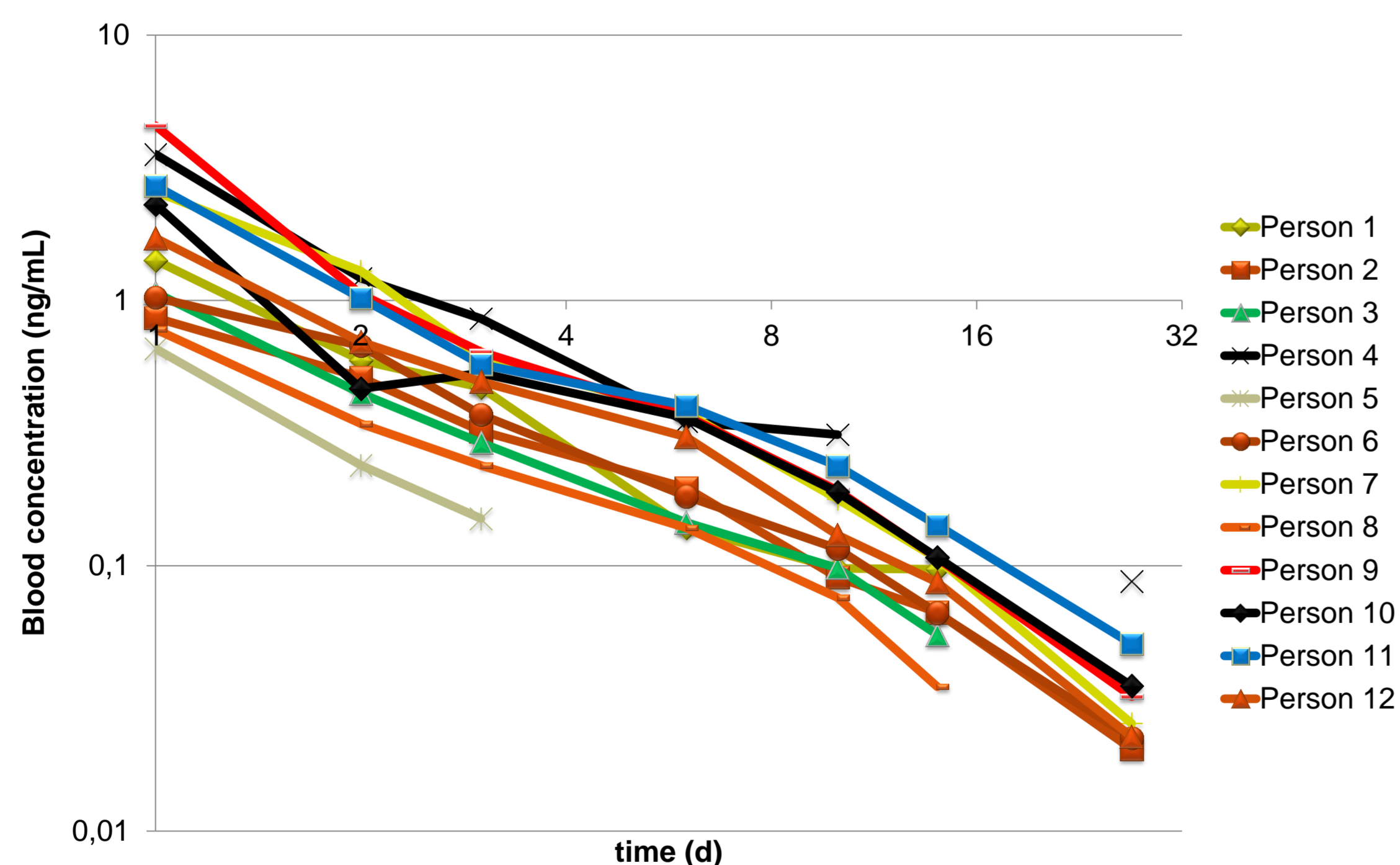


Fig. 1: Log-Log time-series of concentration C versus time t on a scale. The data suggests a power-law type of behavior  $C=At^{-k}$  with fitting constants A and k.

## Aim

The aim of the study was to assess 1) if humans uptake an FTOH-derived diPAP with food, 2) to measure uptake profiles, 3) to obtain kinetic parameters by modeling the experiment 4) if diPAPs metabolise and hence are sources of perfluorocarboxylic acids (PFCAs), in humans.

## Conclusions

The data confirms that up to 7% (preliminary result) of 8:2 diPAP is uptaken by humans with food, and metabolises to PFCAs, which confirms that FTOH based PFAS are sources of human PFCA exposure. The elimination kinetics is non-linear, and consequently the half-life is not constant and increases with a decreasing blood concentration. Half-lives of other PFAS determined in highly exposed humans might therefore be underestimated. The study suggests that diPAP might distribute to another compartment than serum.

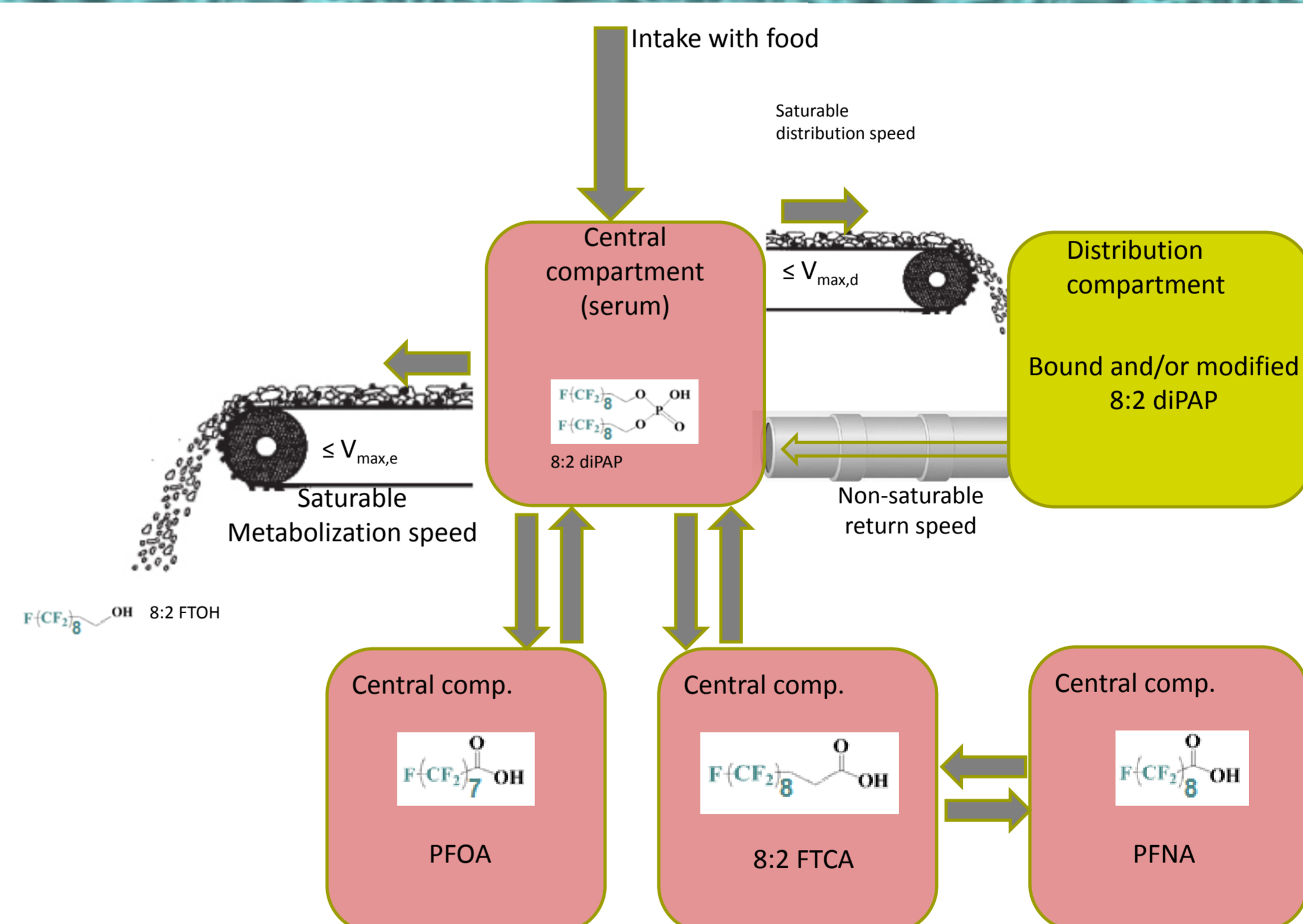


Fig. 2: Proposed kinetics and metabolic pathway for 8:2 diPAP in humans. Pipes symbolize linear kinetic pathways. Transport belts with limited speed symbolize saturable transport/metabolism. Note that two such saturable mechanisms compete for the same substrate (8:2 diPAP) in this model, as suggested by the apparent power-law behavior.

Numerical fitting of the model shown in Fig. 2 shows that the elimination, i.e. the half-life of 8:2 diPAP is not constant. Initially, at high 8:2 diPAP serum concentrations, the half-life is short (0.9-1.4 days), and similar to the half-life in rats. With decreasing serum concentrations, the elimination slows down, and half-lives become 6-11 times longer (5.7-15.4 days). If other PFAS behave similarly, the elimination of PFAS in averagely exposed humans might therefore be slower than estimated, based on half-lives determined from highly exposed humans. The identity of the second compartment remains unknown, but possibly PAPs containing a hydrophobic polyfluorinated part of diPAPs can distribute to both red blood cells and to fatty tissue as FTOH does (Bull et al., 2014).

## Materials & Methods

The study included only healthy males (21-50 years), who were given one dose of 700  $\mu\text{g}$   $^{13}\text{C}_4$ -8:2 diPAP (prepared as 0.7 mg/mL in 96% ethanol) spiked to a breakfast meal and left to absorb for 1 hr. Clean food was since provided for two weeks, and instructions were given on how to otherwise minimize PFAS exposure. The study received ethical approval by the Danish Ethics Committee, since the 8:2 diPAP dose corresponded to a daily intake of 67% of a provisional limit value of 90  $\mu\text{g}/\text{kg}$  food (using the 2008 EFSA TDI value of 1.5  $\mu\text{g}$  PFOA/kg bw/day) over the two weeks. 20 mL of blood (and urine) were collected from the volunteers on Day 0 (before eating the dose), and on days 1-2-3-6-10-14-27. Blood was immediately prepared as serum and frozen until analysis. Triplicate analyses were made on Day 0, Duplicate on Days 1,3,14,27 and Single on Days 2, 6,10. An in-house method was developed and validated using 350  $\mu\text{L}$  serum, ultrafiltration for 30 min/15000 G/4°C, transferred to a frozen aluminum block, and the liquid decanted from the fat pellet into 1.5 mL PP tubes. 2 ng/mL internal standard, then 1050  $\mu\text{L}$  cold AcN were added, mixed, shaken for 10 min, centrifuged 20 min/15000 G/4°C. The supernatant was transferred to a 350  $\mu\text{L}$  tip vial, and the sample was blown down with  $\text{N}_2$  to exactly 120  $\mu\text{L}$ , and 80  $\mu\text{L}$  AcN was added (40% organic strength). The sample was filtered through 0.2  $\mu\text{m}$  PP filter vial and 50  $\mu\text{L}$  was injected onto the online-SPE-ESI-QTOF-MS (Agilent 1260-1290-6550), using C18 columns for SPE (3.0 mm id \*1.7 $\mu\text{m}$ \*20 mm) and for the analytical column (Acquity CSH 2.1 mm id \*1.7 $\mu\text{m}$ \*100 mm) and 10 mm guard column. Analytical column eluents were water/AcN (A1: 95%/5%, B1: 5%/95%), buffer: formic acid/ammonia (pH 9) and 2 mM ammonia fluoride. Online SPE eluents were A: 100% water (same strength of additives as above) and B1. The PFAS were analysed using an in-house validated MS-Scan method, with acceptance criteria of 5-10 ppm accuracy. Calibration curves were made in Bovine serum treated as the samples at levels 0.125 – 0.25 – 0.5 – 1 – 1.5 – 2.5 – 5 – 10 ng/mL. LODs of 26 PFAS ranged from 0.02 ng/mL to 0.5 ng/mL (8:2 diPAP: 0.08 ng/mL), and RSDs were 2.3 – 7%.

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## References

Andersen et al., 2008. Toxicol. Sci. 102, 3–14. / Begley et al. 2005 Food Addit. Contam. 22(10), 1023-31 / Begley et al. 2008 Food Addit. Contam. 25(3), 384-90 / Bull et al., EFSA, 2014:EN-572, 345 pp. / D'eon et al., 2009. Environ. Sci. Technol. 43, 4589–4594. / D'eon et al., 2011. Environ. Health Perspect. 119:344-350. / Kennedy et al., 2004. Crit. Rev. Toxicol. 34, 351–384. / Kuwabo et al. 2013 Chemosphere 91:77, 771-77 / Lau et al., 2007. Toxicol. Sci. 99, 366–394. / Rosenmai et al., 2013. Toxicol. Appl. Pharmacol. 266:1, 132-142. / Steenland et al., 2010. Environ. Health Perspect. 118, 1100–1108. / Trier and Alsing, Danish Food Vet. Auth. 2012 (J.Nr. 2012-20-793-00245) / Tuszynski et al., 2008 J Pharm Pharm Sci, 11 (3): 77-96