# Toxicological studies of PFOA (Perfluorooctanoic acid) and 8:2 FTOH (1H, 1H, 2H, 2H-Perfluorodecanol)

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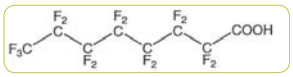
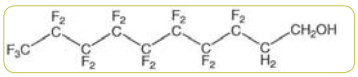
## INTRODUCTION

- Fluorinated organic compounds have been manufactured for more than 50 years. They are employed in the production of fire-fighting foams, herbicides and insecticides, lubricants, paints, adhesives and acid etching solutions (Jackson, Laikhtman et al. 1999; Kannan, Franson et al. 2001)
- These compounds have recently gained much attention because of their high stability and wide distribution in the environment.

**PFOA**




**FTOH**




## MATERIALS AND METHODS

- Male HanTac:WH rats (300g) were exposed to 25mg/kg body wt of PFOA or of 8:2 FTOH per oralt for ten successive days.
- Peroxisomal ß-oxidation (Osmundsen, Braud et al. 1998) and liver carboxylesterase was measured (Sterri, Johnsen et al. 1985)
- Catalase was assayed as disappearence of $H_2O_2$ at 240nm.
- Changes in gene-expression were monitored by using 7K Rat micro-arrays from the microarray core facility at NTNU Trondheim ( 7K-S1-62-65).

## RESULTS

### Chemical analysis in liver after PFOA and FTOH treatment

*Table 1: Each value is mean from six animals. (* p<0.05, Anova, Dunnet`s test).*

| | PFOA analyses ug/g liver-tissue | Changes in body-weight(g) | Liver-weight(g) | Liver-weight % of body-weight(g) |
|---|---|---|---|---|
| Control | 0,5 | 26,3 | 11,6 | 3,7 |
| PFOA | 50 | 8,3* | 15,8* | 5,5* |
| FTOH | 5 | 21,8 | 15,9* | 5,0* |

### β – oxidation

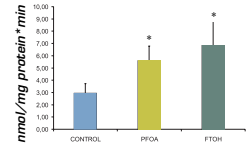



*Fig 2: Peroxisomal ß-oxidation was measured spectrophotometrically at 340 nm as rates of palmitoyl-CoA-dependent reduction of NAD+. Each value is mean + SD from six animals. The results show a significant increase in peroxisomal ß-oxidation after treatment with PFOA and FTOH (*p<0.01, Anova, Dunnet`s test).*

### Carboxylesterase

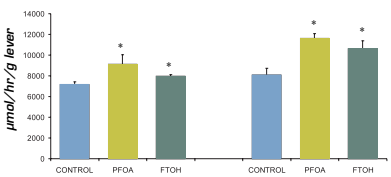



*Fig 1: Liver carboxylesterase activity was measured spectrophotometrically. The substrates, 4-nitrofenylacetat (4-NFA) (0.6M) and 4-nitrofenylbutyrat (4-NFB) (0.15M), were converted to 4-nitrofenol which was measured at 400 nm. Each value is shown as mean + SEM from six animals. The results show that the carboxylesterase-activity is significantly higher compared to control with 4-NFA and 4-NFB, for both PFOA and FTOH treated animals (*p<0.05, Anova, Dunnet`s test).*

### Catalase

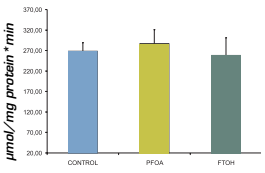



*Fig 3: Catalase activity was assayed as disappearence of $H_2O_2$ at 240nm. Each value is mean + SD from six animals. The results show that the catalase activity was not significantly changed in the PFOA or FTOH treated animals.*

### Microarray

*Fig.4: Microarray slide, shows differentially expressed genes.*

Microarray was used to monitor levels of m-RNA in rat liver. m-RNA from vehicle treated rats was labelled with Cy3 ( F 543-green color ), m-RNA from rats treated with PFOA/FTOH was labelled with Cy5 ( F633-red color). Mixture of Cy3 and Cy5 results in yellow spots. All together 7000 genes were examined.

**PFOA**

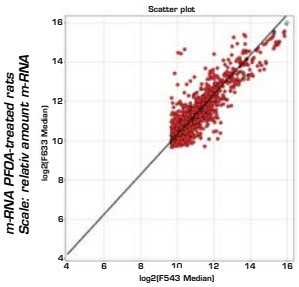



*Fig 5: Scale: relative amount m-RNA m-RNA Vehicle treated rats*

**FTOH**

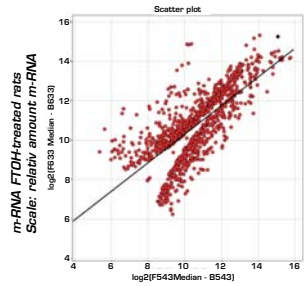



*Fig 6: Scale: relative amount m-RNA m-RNA Vehicle treated rats*

*Fig 5 and Fig 6:*

Scatterplots are shown as $\log_2$ plots of the two channel intensities, log2 633 versus log2 543. Spots that fall on the 45° diagonal represent those m-RNAs whose abundances are equal for the vehicle rats and the PFOA/FTOH treated rats. Spots away from the diagonal represent differential expressed genes.

*Tables 2-5: Indicating different groups of genes expressed as ratio PFOA/Control, FTOH/Control*

*Table 2*

| Mitochondrial and peroxisomal genes | PFOA | FTOH |
|---|---|---|
| 2,4-dienoyl CoA reductase 1 mitochondria | 7,9 | 7,6 |
| Acetyl-Co A acetyltransferase 1 mitochondrial | 4,4 | 6,2 |
| Carnitine palmitoyltransferase 1 | 2,4 | 2,9 |
| Dodecenoyl-coenzyme A delta isomerase | 11,5 | 9,3 |
| Enoyl-coenzyme A hydratase 1 | 2,6 | 2,9 |
| Acetyl-Co A acetyltransferase 1 mitochondrial | 4,4 | 6,2 |
| Acyl Coenzyme A dehydrogenase long chain | 3,6 | 3,0 |
| Stearoyl-Coenzyme A desaturase 1 | 6,8 | 5,6 |
| Serine-pyruvate aminotransferase | 2,5 | 2,6 |
| Acyl-CoA oxidase | 10,8 | 13,0 |
| Enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A Dehydrogenase | 15,3 | 14,8 |
| Dodecenoyl-coenzyme A delta isomerase | 11,5 | 9,3 |
| Cytosolic acyl-CoA thioesterase 1 | 21,6 | 29,4 |
| Stearoyl-Coenzyme A desaturase 1 | 6,8 | 5,6 |
| Catalase | 1,3 | 1,2 |

*Table 3*

| Xenometabolism genes | PFOA | FTOH |
|---|---|---|
| Cytochrome P450 subfamily IVB polypeptide 1 | 22,0 | 25,0 |
| Cytochrome P450 subfamily IIIA polypeptide 3 | 1,5 | 5,2 |
| Cytochrome P450 subfamily 2e1 (ethanol-inducible) | 3,7 | 2,1 |
| Rat Cyp4a locus encoding cytochrome P450 (IVA3) mRNA complete cds | 6,1 | 6,1 |
| UDP glycosyltransferase 1 family polypeptide A6 | 2,8 | 3,7 |
| UDP glycosyltransferase 1 family polypeptide A7 | 3,2 | 2,7 |
| UDP-glucuronosyltransferase | 0,5 | 0,5 |

*Table 4*

| Acute phase genes | PFOA | FTOH |
|---|---|---|
| Sulfotransferase hyroxysteroid gene 2 | 0,2 | 0,0 |
| Heat shock 70 kD protein 5 | 1,6 | 0,3 |
| Serum amyloid P-component | 0,6 | 0,3 |
| Transthyretin | 0,7 | 0,4 |

*Table 5*

| Various genes | PFOA | FTOH |
|---|---|---|
| Cd36 antigen | 10,5 | |
| Metallothionein | | 0,1 |
| Aldehyd Dehydrogenase 1 subfamily A1 | 9,2 | 10,5 |
| Alcohol dehydrogenase (class 1) | 2,5 | 1,5 |

## CONCLUSION

The results show that exposure to 25 mg/kg PFOA or FTOH per oralt for ten successive days leads to increased liver weight and changes in gene-expression in rats. Gene-expression of m-RNA in liver was 2 to 30-fold increased for more than 50 genes (some of the genes with increased or decreased expression are shown in table 2-5). As an example ß-oxidation was increased 2-3 fold (and m-RNA also increased 2-30 fold), whereas no change was observed for catalase, (m-RNA increased 1,2-1,3 fold)

### References

Jackson, P. E., M. Laikhtman, et al. (1999). "Determination of trace level perchlorate in drinking water and ground water by ion chromatography." J Chromatogr A 850(1-2): 131-5.
Kannan, K., J. C. Franson, et al. (2001). "Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses." Environ Sci Technol 35(15): 3065-70.
Osmundsen, H., H. Braud, et al. (1998). "Effects of dietary treatment of rats with eicosapentaenoic acid or docosahexaenoic acid on hepatic lipid metabolism." Biochem J 331(Pt 1): 153-60.
Sterri, S. H., B. A. Johnsen, et al. (1985). "A radiochemical assay method for carboxylesterase, and comparison of enzyme activity towards the substrates methyl [1-14C] butyrate and 4-nitrophenyl butyrate." Biochem Pharmacol 34(15): 2779-85.