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

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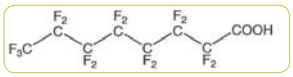
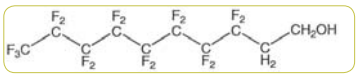
INTRODUCTION

- 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

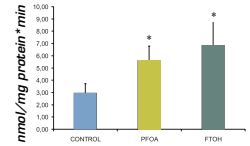


*Fig 2: Peroxisomal ß-oxidation was measured spectrophotometrically at 340 nm as rates of palmitoyl-CoA-dependent reduction of NAD+. Each value is mean + SD from six animals. The results show a significant increase in peroxisomal ß-oxidation after treatment with PFOA and FTOH (*p<0.01, Anova, Dunnet`s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

Carboxylesterase

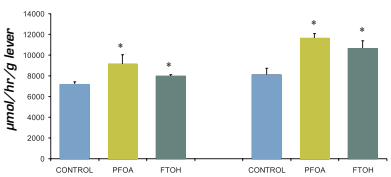


*Fig 1: Liver carboxylesterase activity was measured spectrophotometrically. The substrates, 4-nitrofenylacetat (4-NFA) (0.6M) and 4-nitrofenylbutyrat (4-NFB) (0.15M), were converted to 4-nitrofenol which was measured at 400 nm. Each value is shown as mean + SEM from six animals. The results show that 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

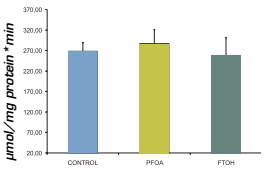


Fig 3: Catalase activity was assayed as disappearence of H_2O_2 at 240nm. Each value is mean + SD from six animals. The results show that the catalase activity was not significantly changed in the PFOA or FTOH treated animals.

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

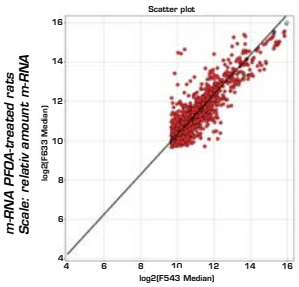


Fig 5: Scale: relative amount m-RNA m-RNA Vehicle treated rats

FTOH

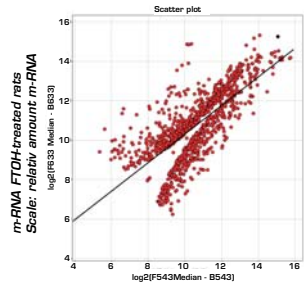


Fig 6: Scale: relative amount m-RNA m-RNA Vehicle treated rats

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.
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