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Effect of acute FC administration on catalase and acylCoA oxidase expression

The following report summarizes the effects of *in vivo* administration of a single acute dose of N-EtFOSE or PFOS on catalase and acylCoA oxidase gene expression and enzyme activity in liver tissue from exposed rats and guinea pigs. Tissues analyzed in this study were derived from the 3M in-house study "NTBK 116471, pg 100" (a copy of the 3M "Study/Record" is attached) and included approximately 1 g of frozen liver from each of two male and two female rats and guinea pigs that received a single injection of 40 mg/kg PFOS, 160 mg/kg N-Et-FOSE, or an equivalent volume of 2% tween 80 (vehicle control) on March 1, 1999. All animals were killed on March 4, three days following dosing. Portions of the respective livers were quick frozen in liquid nitrogen and shipped on dry ice to Duluth for analysis.

Enzyme Sample Preparation – The enzyme fraction consisted of the 6,000 g supernatant of a 10% (wt/vol) homogenate of 0.5-1.0 g frozen liver tissue in 300 mM mannitol-10 mM HEPES-1 mM EGTA (pH 7.2). Protein concentration was estimated according to the method of Bradford using commercial bovine serum albumin as standard.

L-CoA Oxidase Assay – The equivalent of ca., 5 µg/ml tissue homogenate was suspended in 60 mM KH_2PO_4-0.02 % Triton X100 (pH 7.4) containing 1 mM *p*-hydroxyphenylacetate (PHPA), 4 units/ml peroxidase, 20 µM FAD, and 60 µM lauryl-CoA (LCoA). The reactions were allowed to incubate at 37°C for 30 min in a shaking water bath and terminated by adding 3 volumes of 2 mM KCN in 100 mM sodium carbonate (pH 10.5). The concentration of H_2O_2 generated during the reaction was estimated from the fluorescence of PHAP as measured with an excitation wavelength of 317 nm and emission at 405 nm. The fluorescence was calibrated with commercial H_2O_2 and the results are expressed as nmol peroxide generated/min/mg mitochondrial protein (Table 1). Protein was quantitated by the Bradford method.

Catalase Assay - The activity of catalase was estimated by a modification of the original method published by Claiborne and Fridovich (J. Biol. Chem. **254**, 4245-52, 1979), which is based on the direct measurement of H_2O_2 disappearance as quantified spectrophotometrically at 240 nm. In this procedure, the tissue sample was diluted in 50 mM potassium phosphate (pH 7.0). The medium was warmed to 27°C and the reaction initiated by adding 10.3 mM H_2O_2. The progress of the reaction was monitored at 240 nm for 5 min. Catalase activity was estimated from the initial linear rate (E^{240}=43.6 $mM^{-1}cm^{-1}$) and expressed as units/mg protein (Table 1). One unit of activity is defined as that amount of enzyme which catalyzes the decomposition of 1 µmole of H_2O_2 per min.

Northern Blot Analyses - Quantitation of mRNA for both acylCoA oxidase (ACoAO) and catalase were performed by Northern blot analysis of quick frozen liver samples from treated rats and guinea pigs. Approximately 1 g of frozen liver was powderized in liquid nitrogen using a mortar/pestle. Total RNA was recovered using the PERFECT RNA™ isolation kit and the concentration quantified spectrophotometrically at 260nm. The RNA was electrophoresed on a 1% agarose gel, blot transferred to a cellulose membrane and hybridized to the corresponding randomly [^{32}P] labeled oligonucleotides that were PCR amplified from primers to ca., 350 base sequence of the respective rat liver gene. mRNA band density was quantified autoradiographically using phospho-imaging software.

RESULTS:

Rats – Both male and female rats express LCoAO and catalase activities, and there is no substantial difference in enzyme activities between the sexes. The same is true for the constitutive expression of mRNA for both enzymes, with the exception that unexposed male rats do not express the message for PCoAO.

Acute exposure to N-Et-FOSE causes a doubling of the specific activity of LCoAO in liver from both male and female rats, but catalase activity is unchanged. Associated with this is a proportionate 2-fold increase in the concentration of mRNA encoding for PCoAO, but no change in catalase mRNA, in livers from both sexes. Again, there is no remarkable sex difference in response to N-Et-FOSE exposure, at-least with this limited number of animals tested (n=2).

Exposure of rats to PFOS elicits a similar doubling of LCoAO activity for both sexes, and possibly a slight increase in catalase activity in liver from male rats. Hepatic catalase activity in female rats does not appear to be affected by PFOS exposure. Unlike N-Et-FOSE, which caused a doubling of mRNA band intensity for pCoAO, acute exposure to PFOS caused a 3-6 fold increase in PCoAO mRNA expression. This enhanced expression of message was more pronounced in female compared to male rats, and in both sexes resulted in no greater LCoAO enzyme activity than was observed at half the mRNA level for rats exposed to N-Et-FOSE.

Guinea Pigs - The response to guinea pigs to exposure to N-Et-FOSE and PFOS was dramatically different from that observed for rats. The constitutive activity of LCoAO in unexposed guinea pigs of either sex was very low (near LOD values) whereas catalase activity was 1.5-3 times higher. However, the activity of neither enzyme was stimulated following acute exposure to N-Et-FOSE or PFOS *in vivo*. Perhaps the most dramatic difference between species is the fact that guinea pig mRNA encoding for PCoAO was undetectable, even following exposure to N-Et-FOSE or PFOS. Part of the explanation for this observation may be that we were using hybridization probes developed against the sequence for rat liver ACoAO because the sequence of the guinea pig enzyme is not known at this time. It may be that the guinea pig sequence is sufficiently different that the PCR product lacks complementarity for adequate hybridization to the probe for the rat sequence. Although this may indeed be an artifact, the

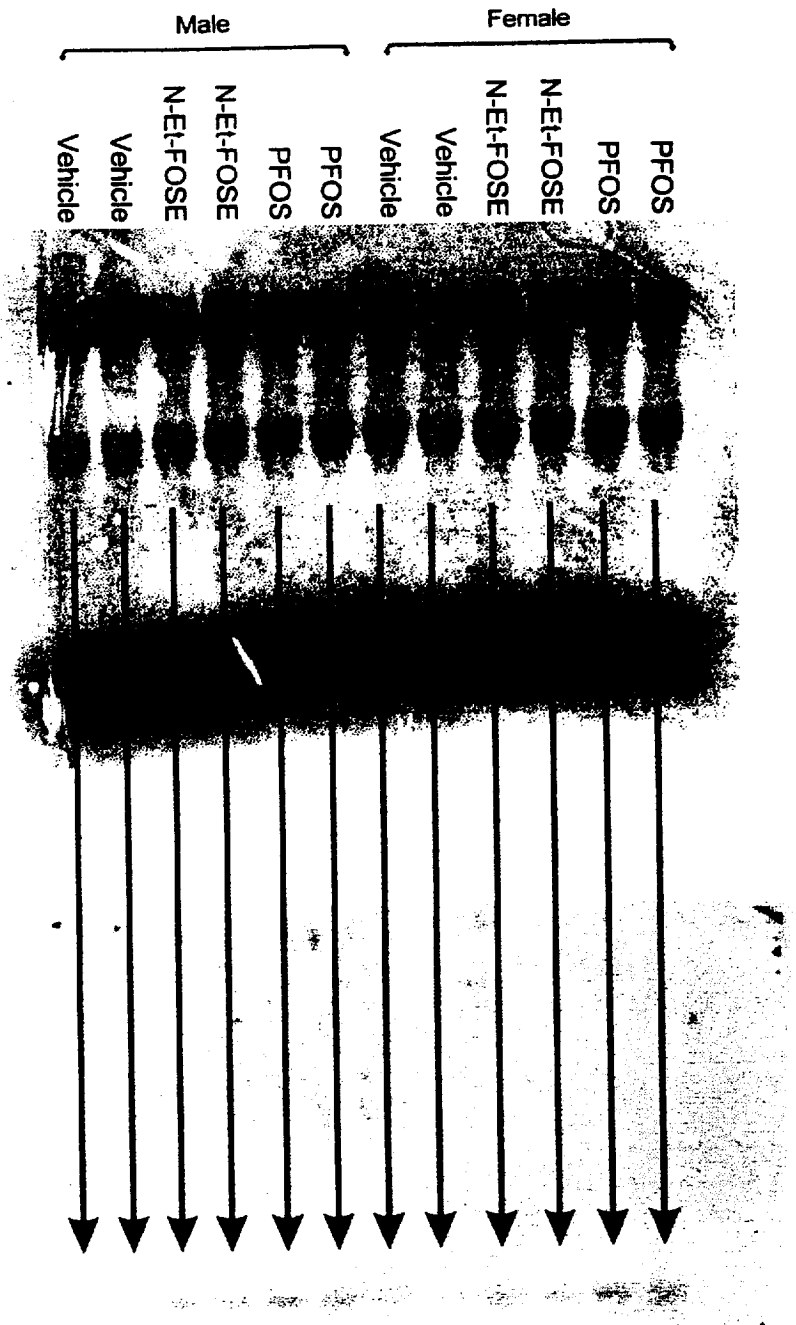
absence of constitutively expressed message and the inability to stimulate expression of PCoAO mRNA are consistent with the low and uninducible enzyme activity in guinea pigs.

CONCLUSIONS: These data provide strong evidence that: 1) N-Et-FOSE and PFOS stimulate both the transcriptional and translational expression of acylCoA oxidase in rats *in vivo*, and 2) there is a marked difference in the response of rats and guinea pigs to *in vivo* exposure to these two fluorochemicals. These results are very consistent with the suggestion that these fluorochemical compounds are "peroxisome proliferators" in rats and, much like what has been demonstrated for the classical "peroxisome proliferator" chemicals, guinea pigs are resistant to this effect of fluorochemical exposures. We were not given the data for organ weights or necropsy findings and thus cannot comment on whether exposure to either agent caused hepatomegaly or other indications that are associated with peroxisome proliferation. However, based on our data, we suspect that the evidence will reveal the classical signs of peroxisome proliferation in rats, but not guinea pigs, caused by these acute exposures.

NTBK 116471 dosed 3/1/99 40 mg/kg PFOS
sac 3/4/99 160 mg/kg N-Et-FOSE
2% tween 80

Animal	SPECIES	Sex	Tx	protein (mg/g)	Activity LCoAO (nmol/min/mg protein)	Activity Catalase (Units/mg protein)	mRNA pCoAO mRNA (% Band Intensity)	mRNA Catalase (% Band Intensity)
9R00463	rat	M	Veh	151.8	4.7	219.9	0	127
9R00464	rat	M	Veh	145.6	5.3	158.5	0	73
9R00469	rat	F	Veh	188.3	4.7	141.1	100	92
9R00470	rat	F	Veh	153.3	6.8	204.7	100	108
9R00465	rat	M	N-Et-FOSE	157.5	10.5	219.5	290	140
9R00466	rat	M	N-Et-FOSE	134.9	11.9	204.3	292	98
9R00471	rat	F	N-Et-FOSE	165.8	10.3	186.1	253	118
9R00472	rat	F	N-Et-FOSE	215.8	9.3	171.9	320	147
9R00467	rat	M	PFOS	115.4	10.9	252.0	482	155
9R00468	rat	M	PFOS	153.0	13.1	236.9	277	116
9R00473	rat	F	PFOS	143.1	9.6	204.1	630	180
9R00474	rat	F	PFOS	238.6	11.2	155.6	404	147
9G00045	Gpig	M	Veh	175.2	2.3	314.7	0	103
9G00046	Gpig	M	Veh	251.7	1.9	390.7	0	97
9G00051	Gpig	F	Veh	180.3	2.6	550.6	0	91
9G00052	Gpig	F	Veh	222.1	1.2	488.0	0	109
9G00047	Gpig	M	N-Et-FOSE	212.7	3.0	349.3	0	?
9G00048	Gpig	M	N-Et-FOSE	269.1	1.4	379.3	0	92
9G00053	Gpig	F	N-Et-FOSE	176.4	2.6	416.8	0	105
9G00054	Gpig	F	N-Et-FOSE	218.3	1.4	478.1	0	104
9G00049	Gpig	M	PFOS	153.2	ND	413.0	0	?
9G00050	Gpig	M	PFOS	222.1	1.0	460.8	0	97
9G00055	Gpig	F	PFOS	127.8	ND	672.9	0	103
9G00056	Gpig	F	PFOS	171.6	2.2	531.6	0	98

Northern Hybridization of Rat Liver Tissue



Northern Hybridization of Guinea Pig Liver Tissue