

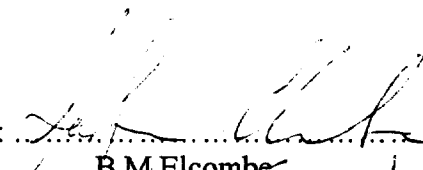
AR226-0143

CENTRE FOR XENOBIOTIC RESEARCH
University of Dundee, Biomedical Research Centre, Level 5, Ninewells Hospital and
Medical School, Dundee, DD1 9SY

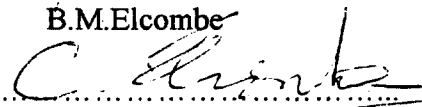
Study Report

Determination of cyanide insensitive Palmitoyl-CoA oxidation in samples from 3M
Environmental Laboratory – Covance Studies 6329-183 and 6329-212

Study Number XR0108

Author: .....
B.M. Elcombe

Date: 18/2/99.....

Approved: .....
C.R. Elcombe

1 Introduction

The purpose of this study was to investigate the levels of PCoA oxidation in the frozen liver samples provided by 3M Environmental Laboratory from Covance studies 6329-183 and 6329-212.

2 Test Substance

6329-183: PFOS, 20ppm, administered by Covance until week 4.

6329-212: EtFOSE-OH, 100 and 300ppm, administered by Covance until week 4.

3 Testing Facility

Centre for Xenobiotic Research, University of Dundee, Biomedical Research Centre, Level 5, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK.

4 Homogenate Preparation

4.1 Reagents

SET buffer: 0.25M Sucrose, 5mM EDTA and 20mM Tris-HCl, pH 7.4

4.2 Instruments

Potter-Elvehjem homogeniser

4.3 Method

Frozen liver samples were thawed on ice, blotted and weighed. A 25% homogenate was prepared in ice cold SET using a Potter-Elvehjem homogeniser (6 passes).

5 Determination of Cyanide-insensitive Palmitoyl-Co Oxidation

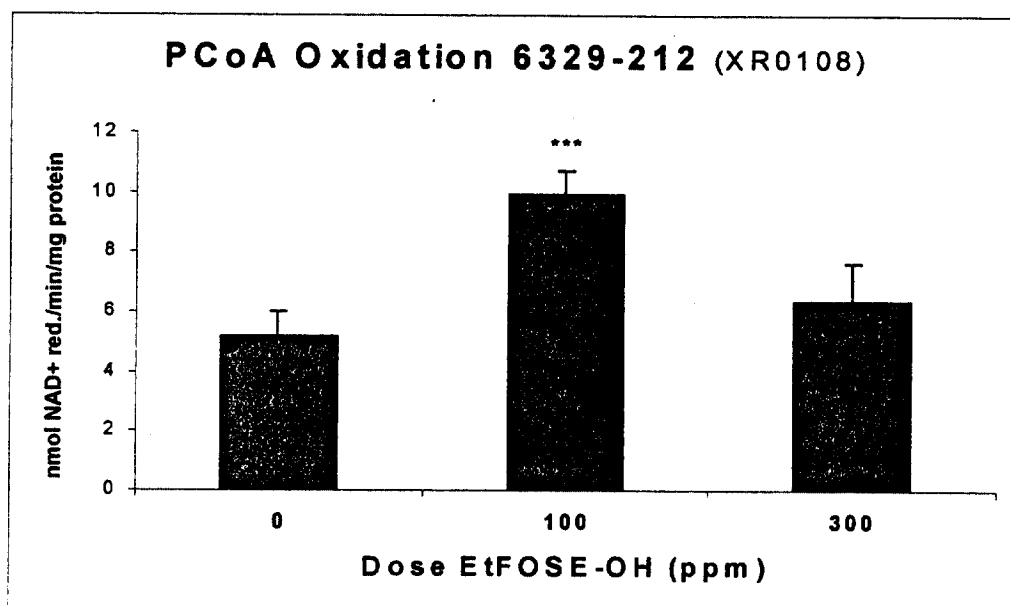
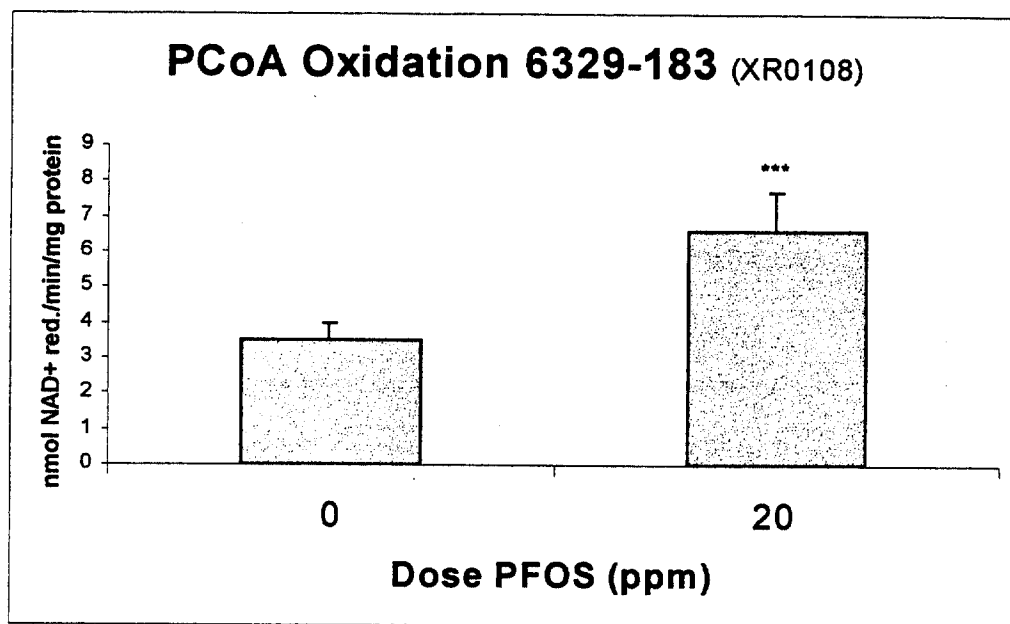
The 25% homogenate liver samples were diluted 1 part in 10 parts with ice cold SET. These diluted samples were used to determine the PCoA activity, following CXR SOP-011.

6 Statistical Analysis

A Student's T-Test (2-sided) was performed on the results; * = statistically different from control $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

7 Results

PCoA Oxidation (nmol NAD ⁺ reduced/min./mg. Protein)			
Study / Group	Dose (ppm)	Week 4 (Mean $\pm$ S.D.)	% of Control
6329-183 / 1	0	3.533 $\pm$ 0.453	100.0 $\pm$ 12.8
6329-183 / 5	20	6.634 $\pm$ 1.093***	187.8 $\pm$ 30.9***
6329-212 / 1	0	5.153 $\pm$ 0.855	100.0 $\pm$ 16.6
6329-212 / 4	100	9.901 $\pm$ 0.800***	192.1 $\pm$ 15.5***
6329-212 / 5	300	6.317 $\pm$ 1.290	122.6 $\pm$ 25.0



PCoA Oxidation

Study No.: XR0108

6329-183

Date: 10/02/90

Treatment	Sample Number	Protein (mg/ml)	Sample Vol.(ul)	Protein/ml in cuvette	ABS CHANGE/MIN	nmolNAD+ Red. /min	nmol NAD+ red/min/mg	MEAN	SD	SE	% C	SD	SE	T-VALUE (VS CONTROL)
0 Control	C92506M	6.092	50	0.152	0.003021	0.486	3.189	3.533	± 0.453	0.203	100.00	± 12.820	5.733	
	C92524M	7.877	50	0.197	0.003939	0.633	3.216							
	C92526M	7.242	50	0.181	0.004776	0.768	4.241							
	C92529M	7.635	50	0.191	0.003903	0.627	3.288							
	C92546M	6.833	50	0.171	0.003966	0.638	3.732							
PFOS 20ppm	C92752M	6.001	50	0.150	0.00622	1.000	6.665	6.634	± 1.093	0.489	187.76	± 30.936	13.836	-6.860
	C92759M	6.243	50	0.156	0.004652	0.748	4.792							
	C92779M	6.773	50	0.169	0.007521	1.209	7.141							
	C92793M	6.652	50	0.166	0.007921	1.273	7.658							
	C92798M	6.425	50	0.161	0.006907	1.110	6.913							

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wry 25% homogenates 6329-183, diluted 1/10

1/51diln.

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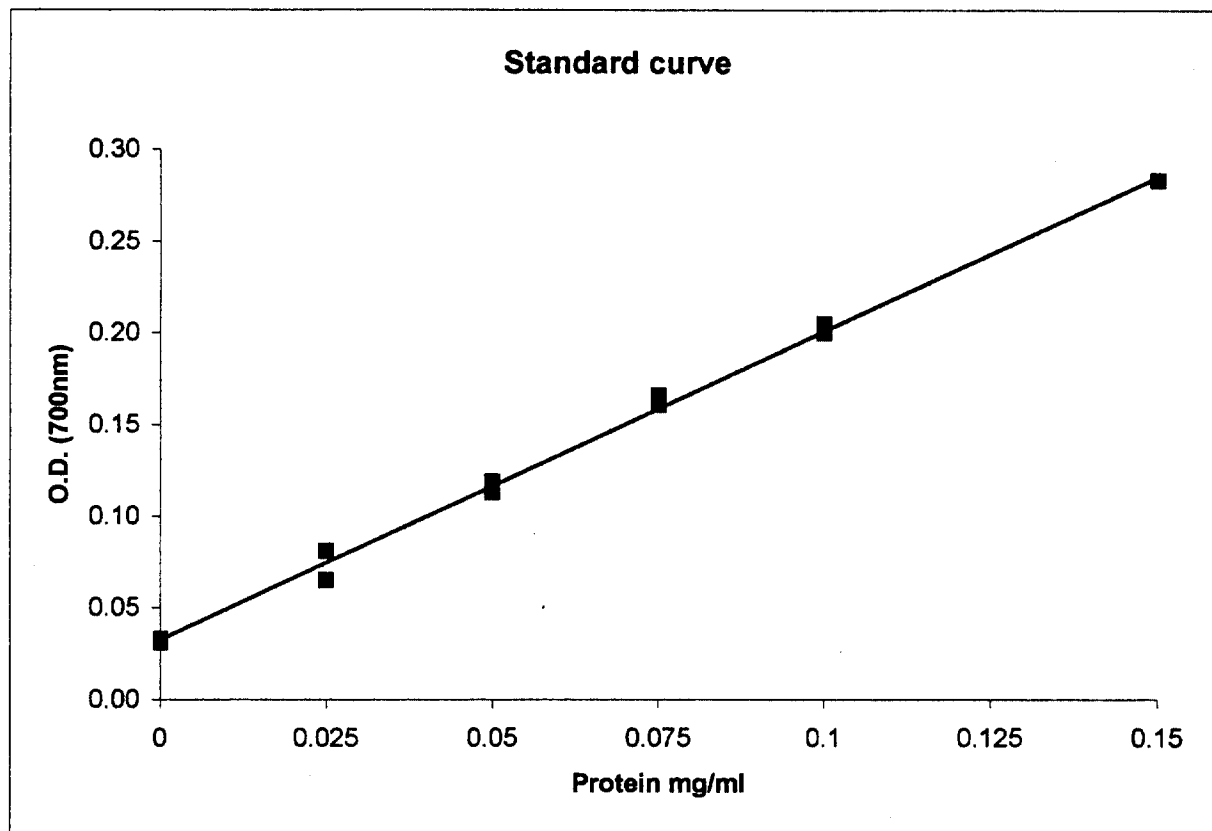
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CORRELATION COEFF 0.99857408

INTERCEPT 0.0326

SLOPE 1.686

RANGE	Y RANGE	Y CALC	No	GIVEN Y1	GIVEN Y2	protein mg/ml
0	0.031	0.033	C92506M	0.231	0.237	6.09
0	0.033	0.033	C92524M	0.336	0.250	7.88
0.025	0.081	0.075	C92526M	0.283	0.261	7.24
0.025	0.065	0.075	C92529M	0.319	0.251	7.63
0.05	0.113	0.117	C92546M	0.261	0.256	6.83
0.05	0.119	0.117	C92752M	0.231	0.231	6.00
0.075	0.166	0.159	C92759M	0.245	0.233	6.24
0.075	0.161	0.159	C92779M	0.243	0.270	6.77
0.1	0.205	0.201	C92793M	0.256	0.249	6.65
0.1	0.200	0.201	C92798M	0.244	0.246	6.42
0.15	0.283	0.286				
0.15	0.283	0.286				



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PCoA Oxidation

Study No.: XR0108

6329-212

Date: 10/02/99

Treatment	Sample Number	Protein (mg/ml)	Sample Vol.(ul)	Protein/ml in cuvette	ABS CHANGE/MIN	nmolNAD+ Red. /min	nmol NAD+ red/min/mg	MEAN	SD	SE	% C	SD	SE	T-VALUE (VS CONTROL)
0 Control	C90719M	6.032	50	0.151	0.005402	0.868	5.760	5.153	± 0.855	0.382	100.00	± 16.597	7.422	
	C90725M	6.637	50	0.166	0.005967	0.959	5.782							
	C90732M	6.682	50	0.167	0.00463	0.744	4.456							
	C90753M	6.954	50	0.174	0.006228	1.001	5.759							
	C90775M	6.985	50	0.175	0.004354	0.700	4.009							
EtFOSE-OH 100ppm	C90939M	6.122	50	0.153	0.008415	1.353	8.839	9.901	± 0.800	0.400	192.14	± 16.516	7.768	-8.581
	C90942M	6.712	50	0.168	0.01027	1.651	9.839							
	C90961M	6.712	50	0.168	0.01063	1.709	10.184							
	C90966M	6.939	50	0.173	0.01159	1.863	10.741							
EtFOSE-OH 300ppm	C90984M	6.213	50	0.155	0.006927	1.114	7.170	6.317	± 1.290	0.577	122.69	± 25.030	11.194	-1.682
	C91005M	6.773	50	0.169	0.007351	1.182	6.980							
	C91024M	7.287	50	0.182	0.005309	0.854	4.685							
	C91033M	6.758	50	0.169	0.007947	1.278	7.563							
	C91037M	6.682	50	0.167	0.005391	0.867	5.188							

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Lowry 25% homogenates 6329-212, diluted 1/10

1/51 diln.

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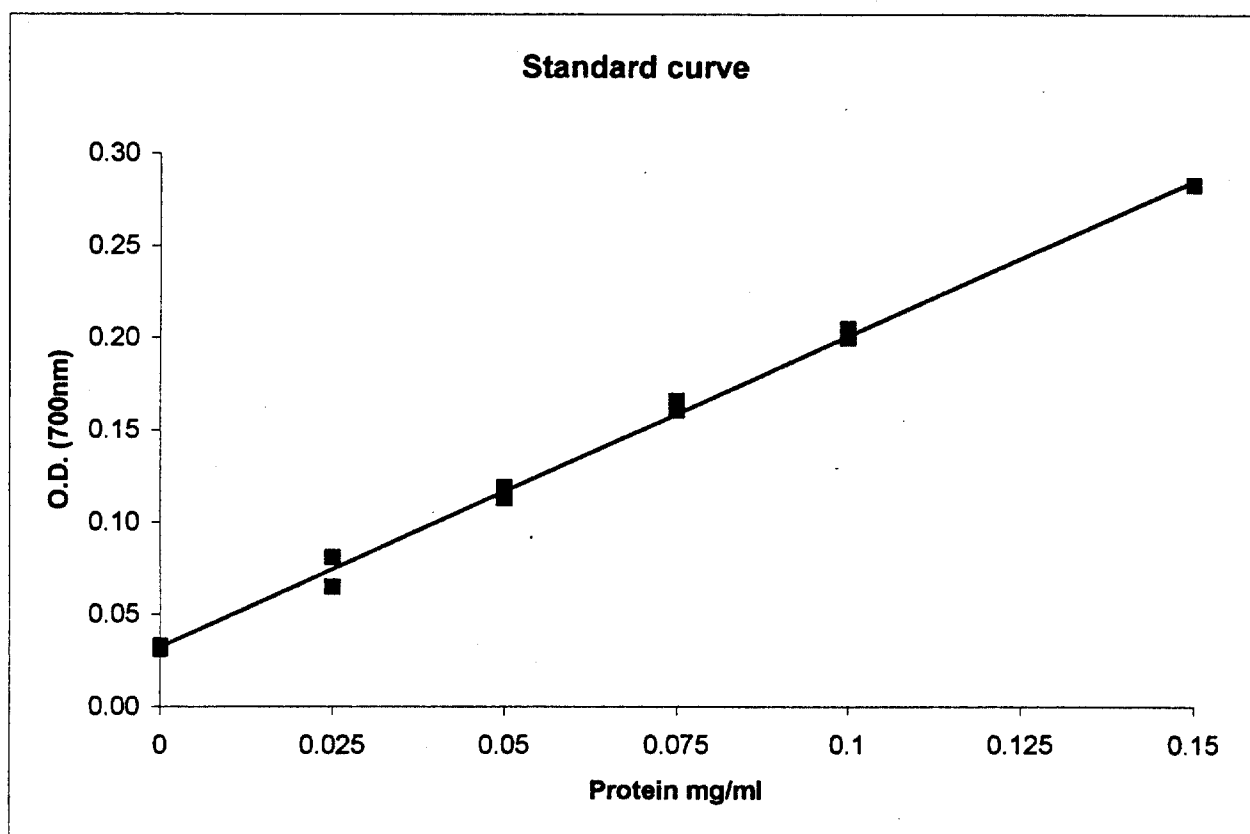
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INTERCEPT 0.0326

SLOPE 1.686

X RANGE	Y RANGE	Y CALC	No	GIVEN Y1	GIVEN Y2	protein mg/ml
0	0.031	0.033	C90719M	0.229	0.235	6.03
0	0.033	0.033	C90725M	0.255	0.249	6.64
0.025	0.081	0.075	C90732M	0.252	0.255	6.68
0.025	0.065	0.075	C90753M	0.263	0.262	6.95
0.05	0.113	0.117	C90775M	0.266	0.261	6.98
0.05	0.119	0.117	C90939M	0.235	0.235	6.12
0.075	0.166	0.159	C90942M	0.253	0.256	6.71
0.075	0.161	0.159	C90961M	0.256	0.253	6.71
0.1	0.205	0.201	C90966M	0.261	0.263	6.94
0.1	0.200	0.201	C90984M	0.244	0.232	6.21
0.15	0.283	0.286	C91005M	0.253	0.260	6.77
0.15	0.283	0.286	C91024M	0.291	0.256	7.29
			C91033M	0.261	0.251	6.76
			C91037M	0.251	0.256	6.68



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