T-6295.28: Analysis of Liver Enzymes, Glycogen, and Perfluorooctane Sulfonate (PFOS) Concentration in Samples from T-6295.25: One-Generation Reproduction Study of PFOS – Mevalonic Acid/Cholesterol challenge and NOEL Investigation in Rats.

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Study Objective

The objective of this study was to measure the activity of glucose-6-phosphate dehydrogenase (G6PD), malic enzyme, and UDP-glucuronosyltransferase (UDPGT) and to determine the amount of glycogen and PFOS in liver specimens collected from PFOS treated rat dams and their pups.

Methods Summary

The in-life portion of this study (T-6295.25) was performed at Argus research laboratories and is detailed in the final report for that study. Briefly, female rats were dosed with 0.0, 0.4, 0.8, 1.0, 1.2, 1.6, or 2.0 mg/kg Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) for 42 days prior to mating with untreated breeder males, through confirmed mating (a maximum of 14 days), and gestation day (GD) 21 or lactation day (LD) 4. Select dams were sacrificed on GD 21 just prior to delivery and all remaining dams were allowed to litter, and sacrificed with their pups on LD 5. LD5 liver samples from dams and pups (pooled by litter) in the 0.0, 0.4, 1.6, and 2.0 mg/kg dose groups were analyzed in the current study. Details and results of the PFOS analysis are included in the T-6295.25 final report. Glycogen was analyzed in pup samples only by the method of Roe and Dailey (1966) using the anthrone reagent. UDPGT, malic enzyme, and G6PD activity were measured using $CaCl_2$ precipitation. The two cytosolic enzymes, malic enzyme and G6PD, were assayed spectrophotometrically by standard literature methods following the conversion of NADP+ to NADPH at 340 nm. The microsomal UDPGT assay followed the reduction of 4-nitrophenol at 405 nm and also used standard literature methods. All results were compared between treated and control animals and analyzed to determine statistical differences using the students T-test.

Results

G6PD

Reductions in average enzyme activity were observed in F0 samples at all dose groups analyzed (0.4, 1.6, and 2.0 mg/kg), but these reductions were only statistically significant in the 0.4 and 1.6 mg/kg dose group samples (Table 1 and Figure 1). Reductions in average enzyme activity were observed in the F1 samples at all maternal dose groups analyzed, (0.4, 1.6, and 2.0 mg/kg), but this reduction was only statically significant in the 2.0 mg/kg dose group samples (Table 1 and Figure 2).

Malic Enzyme

No statistically significant differences or trends in enzyme activity were observed in the F0 or F1 samples (Table 1 and Figures 3 and 4).

UDPGT

Statistically significant increases in activity were observed in F0 0.4 and 1.6 dose group samples but activity in the 2.0 mg/kg dose group samples did not differ from control (table 1 and Figure 5). Reductions in average enzyme activity were observed in the F1 samples at all maternal dose groups analyzed, (0.4, 1.6, and 2.0 mg/kg), but this reduction was only statically significant in the 1.6 and 2.0 mg/kg dose group samples (Table 1 and Figure 6).

Glycogen

Increases in F1 liver glycogen content were observed at the 1.6 and 2.0 mg/kg dose levels. This increase was statically significant at the 2.0 mg/kg maternal dose level only (Table 1 and Figure 7).

Discussion

Glycogen storage disease type Ia (GSD-Ia), also known as von Gierke's disease, is a condition in which G6PD levels are markedly reduced or absent, impairing or destroying the liver's ability to produce free glucose. The condition leads to abnormally large amounts of glycogen in the liver, hypoglycemia, increased dependence on fat metabolism, and increased levels of lactate and pyruvate in the blood. Without a continuous, exogenous source of glucose, severe hypoglycemia and metabolic perturbations occur that can compromise fetal outcome. Observations made in animals born with GSD-Ia include small body size and emaciation, severely enlarged pale livers, pale kidneys, and diffuse vacuolation of hepatocytes with large amounts of glycogen and small amounts of lipid.

In the current study, G6PD activity and glycogen levels were analyzed to determine if a perturbation of glycogen storage similar to that which occurs with GSD-Ia may occur upon PFOS exposure. Reductions in average enzyme activity were observed in F0 and F1 samples at all dose levels analyzed. Although not all reductions were statistically significant, these results suggest that PFOS may act to down regulate activity of this enzyme. Increased F1 liver glycogen content was also observed in this study. Although this increase was only statistically significant at the 2.0 mg/kg dose level, a wide range on concentrations was observed at the 1.6 mg/kg level and an overall increase was observed over control.

Malic enzyme and UDPGT activity were investigated to gain insight into decreases in free and total T3 and T4 levels observed in serum samples from the T-6295.25 study. Malic enzyme, a marker of T3 action on liver, was unchanged. This indicates that, although significant decreases in levels of free and total T3 have been observed, the action of T3 on the liver appears to be unaffected. UDPGT levels were investigated to determine if increased glucuronidation and excretion of T4 and T3 may occur upon PFOS exposure and be responsible for the observed decrease in thyroid hormone levels. The results of this analysis, although unclear, do not support the hypothesis that UDPGT activity is increased upon PFOS exposure.

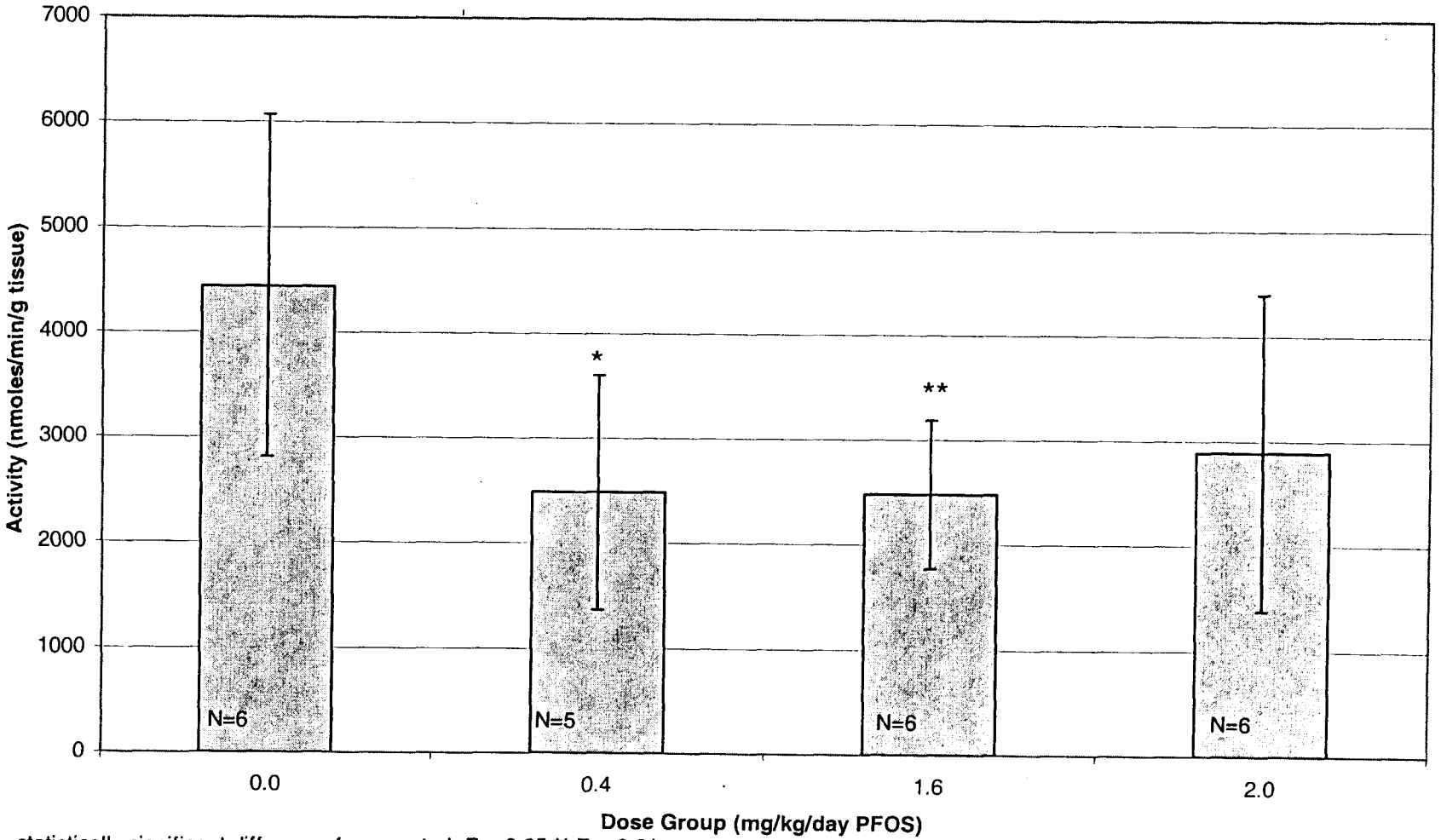
Conclusions

The results of this study indicate that perturbation of glycogen storage processes may be occurring in animals exposed to PFOS. This may related to the decreased viability observed in rat and mouse pups born to PFOS exposed dams and should be the focus of future investigations. Results also suggest T3 activity in the liver is not altered upon PFOS exposure and that increased UDPGT activity, although unclear, is most likely not the cause of decreased T3 and T4 levels observed in PFOS exposed animals.

List of Figures:

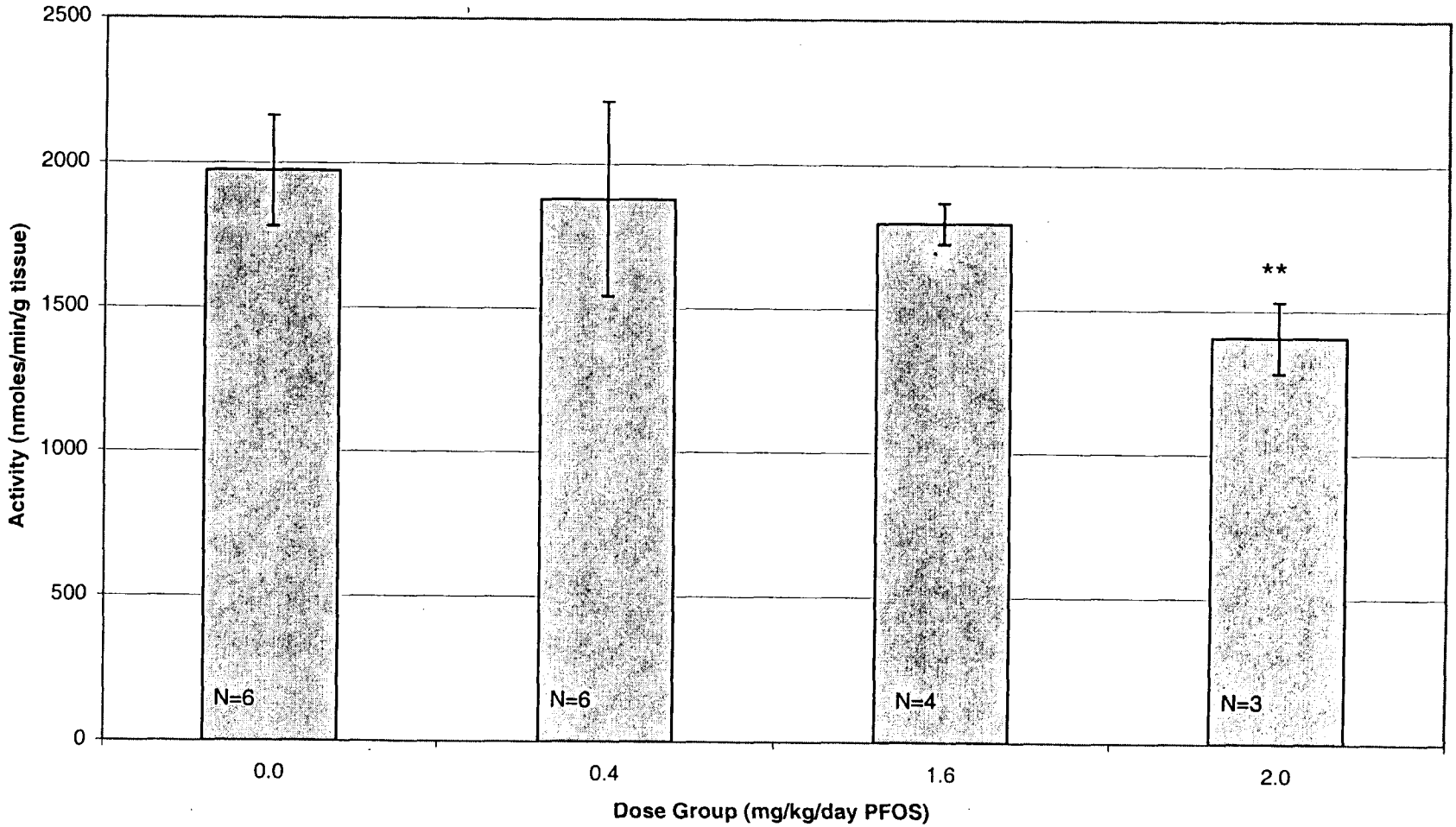
Table 1: Individual Data							
	G-6PD Enzyme Activity (nmoles/min/g tissue)		Malic Enzyme Activity (nmoles/min/g tissue)		UDPGT Enzyme Activity (nmoles/min/g tissue)		Glycogen (mg/g liver)
Maternal Dose group	F0	F1	F0	F1	F0	F1	F1
0.0	4910	2035	1459	414	254	369	0.40
	7289	2107	1373	288	286	309	0.58
	4458	2221	1251	290	179	338	2.61
	3814	1719	807	276	284	419	1.63
	2409	1783	336	145	276	433	2.89
	3760	1972	1193	199	302	217	0.66
0.4	3876	2358	1351	328	362	404	4.60
	2177	1928	627	272	303	255	0.49
	3408	2055	1035	309	309	273	0.27
	1673	1341	837	169	304	361	0.51
	1287	1783	628	155	316	266	0.33
		1811		200		182	
1.6	1442	1745	980	236	413	240	15.18
	3558	1728	1667	185	297	150	0.83
	2861	1863	1398	190	356	255	1.99
	2154	1857	1170	214	335	306	1.17
	2441		1021		279		0.15
	2422		1055		279		
2.0	2506	1398	1503	269	241	243	3.42
	1558	1288	647	177	256	217	3.84
	2723	1535	1081	289	230	189	1.85
	2633		1073		255		
	2087		830		312		
	5841		1602		218		

**Figure 1: F0 Glucose 6-Phosphate Dehydrogenase Activity
Lactation Day 5**



* = statistically significant difference from control, $P \leq 0.05$;** $P \leq 0.01$

**Figure 2: F1 Glucose 6-Phosphate Dehydrogenase Activity
Lactation Day 5**



* = statistically significant difference from control, P ≤ 0.05; ** P ≤ 0.01

Figure 3: F0 Malic Enzyme Activity Lactation Day 5

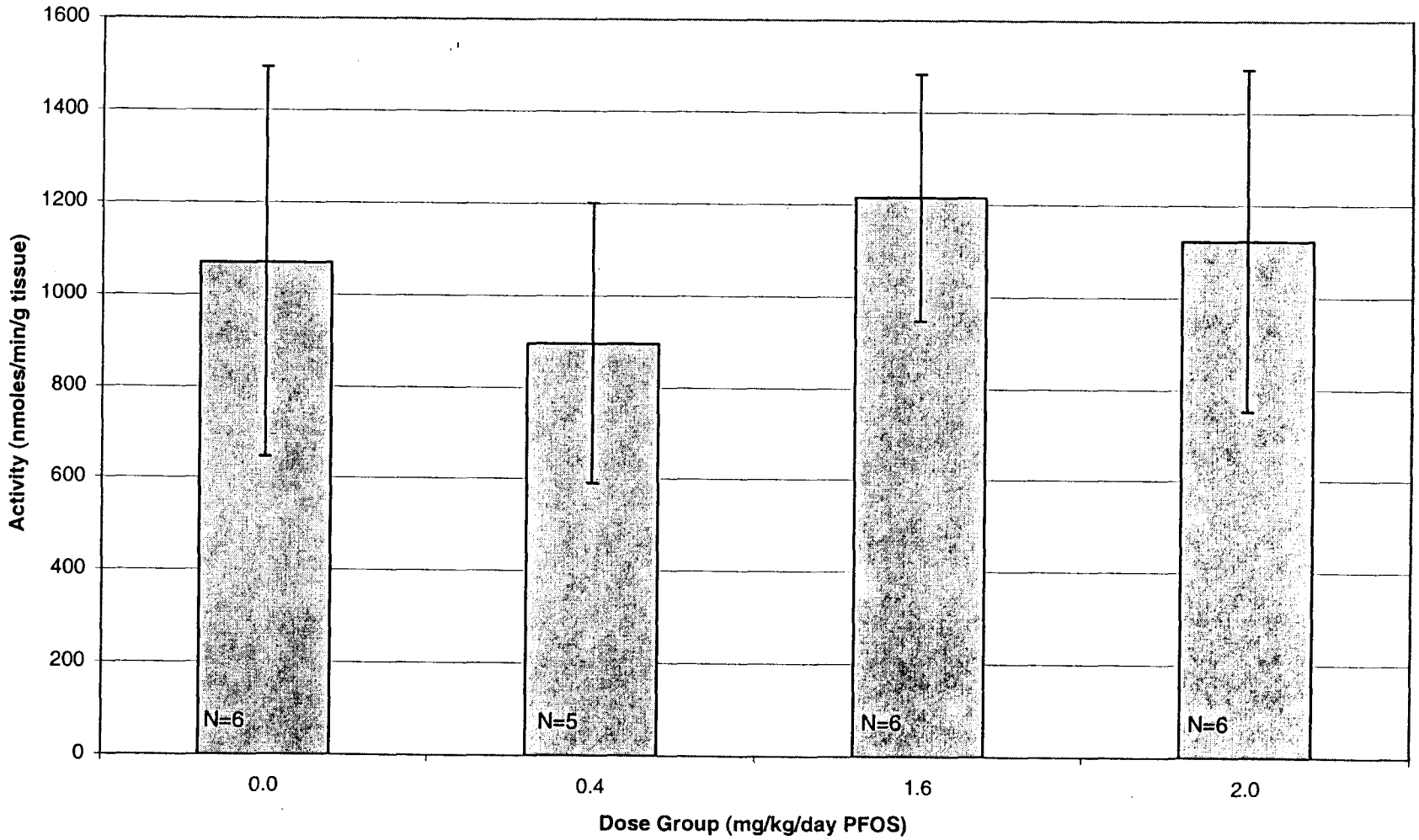


Figure 4: F1 Malic Enzyme Activity Lactation Day 5

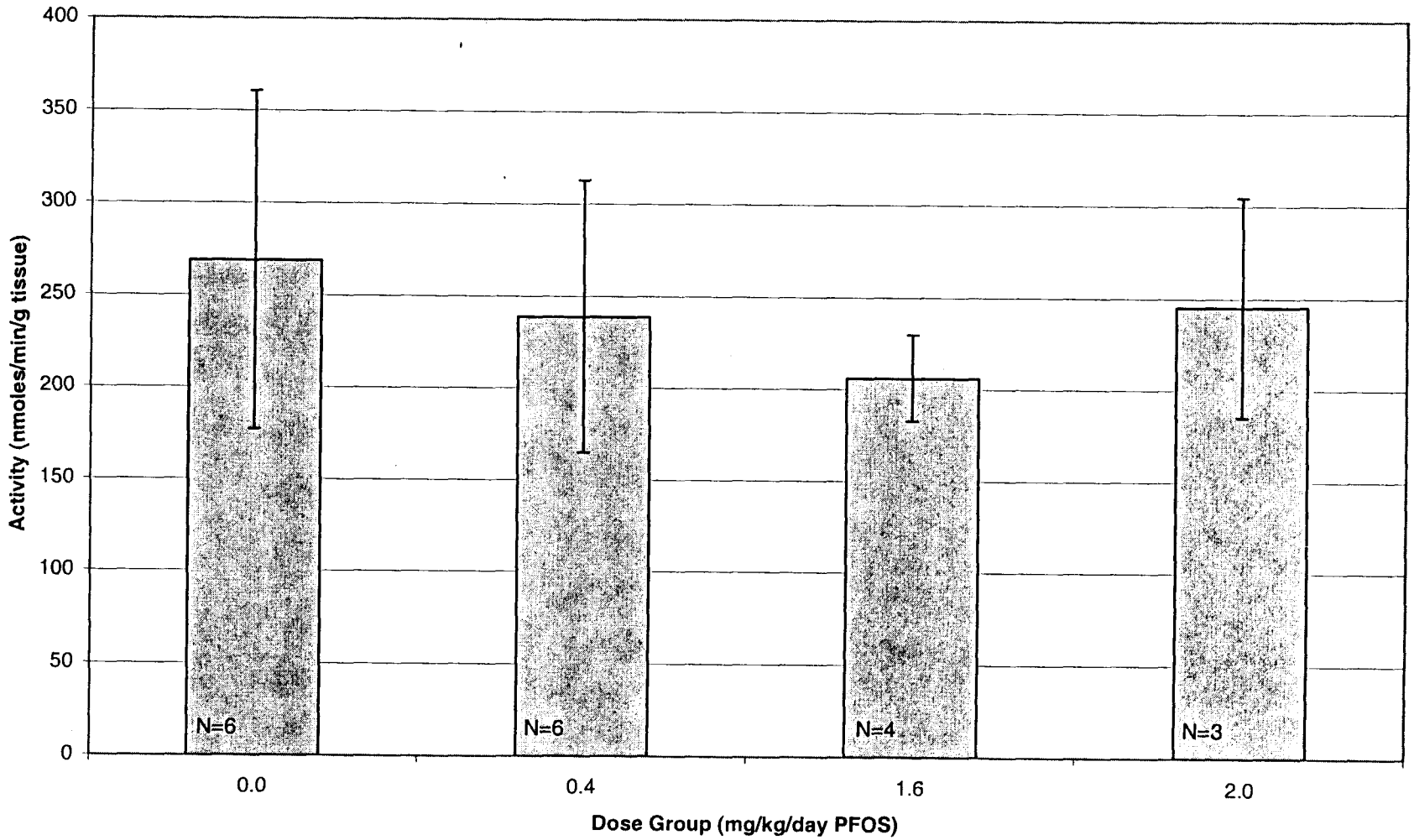
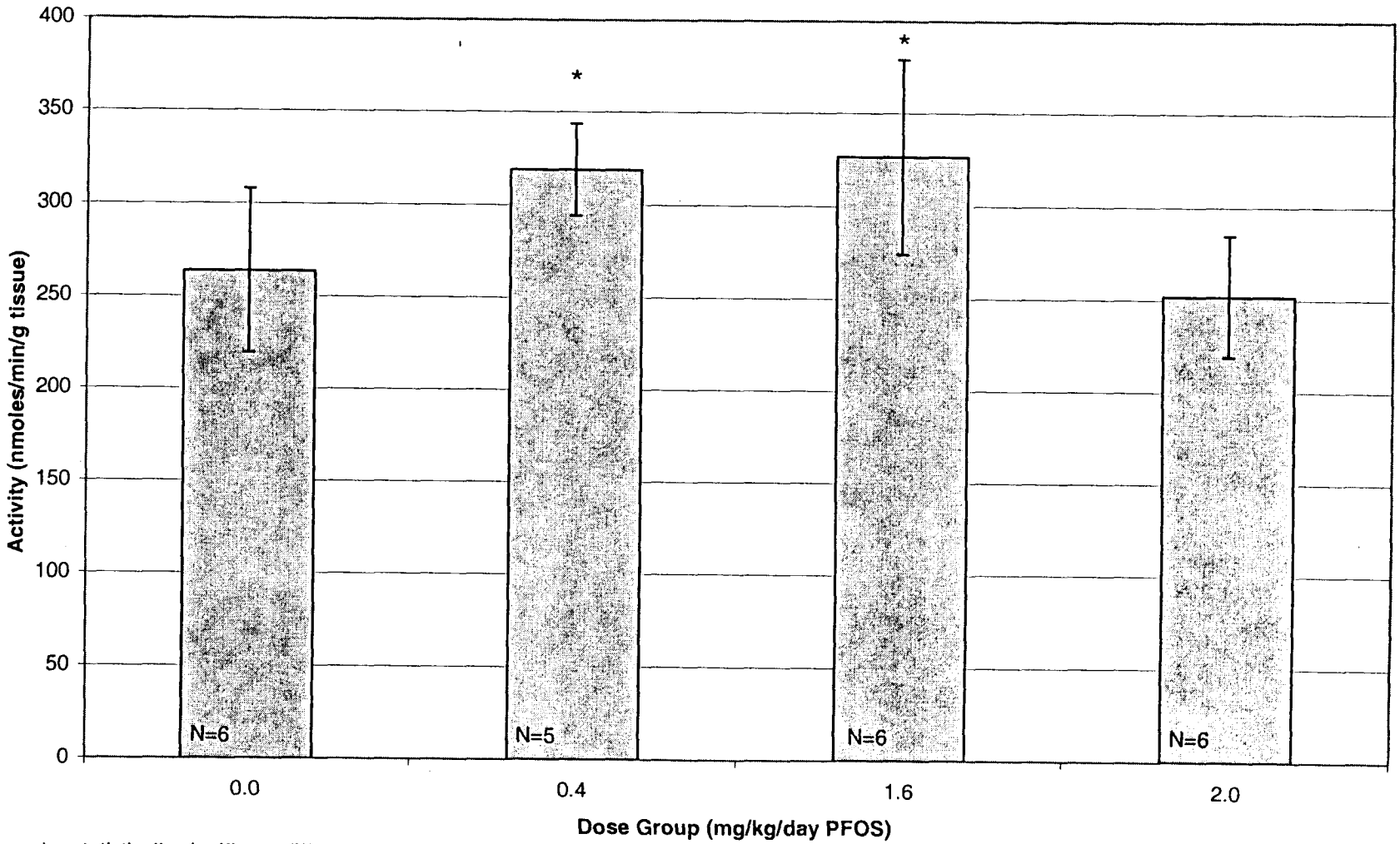
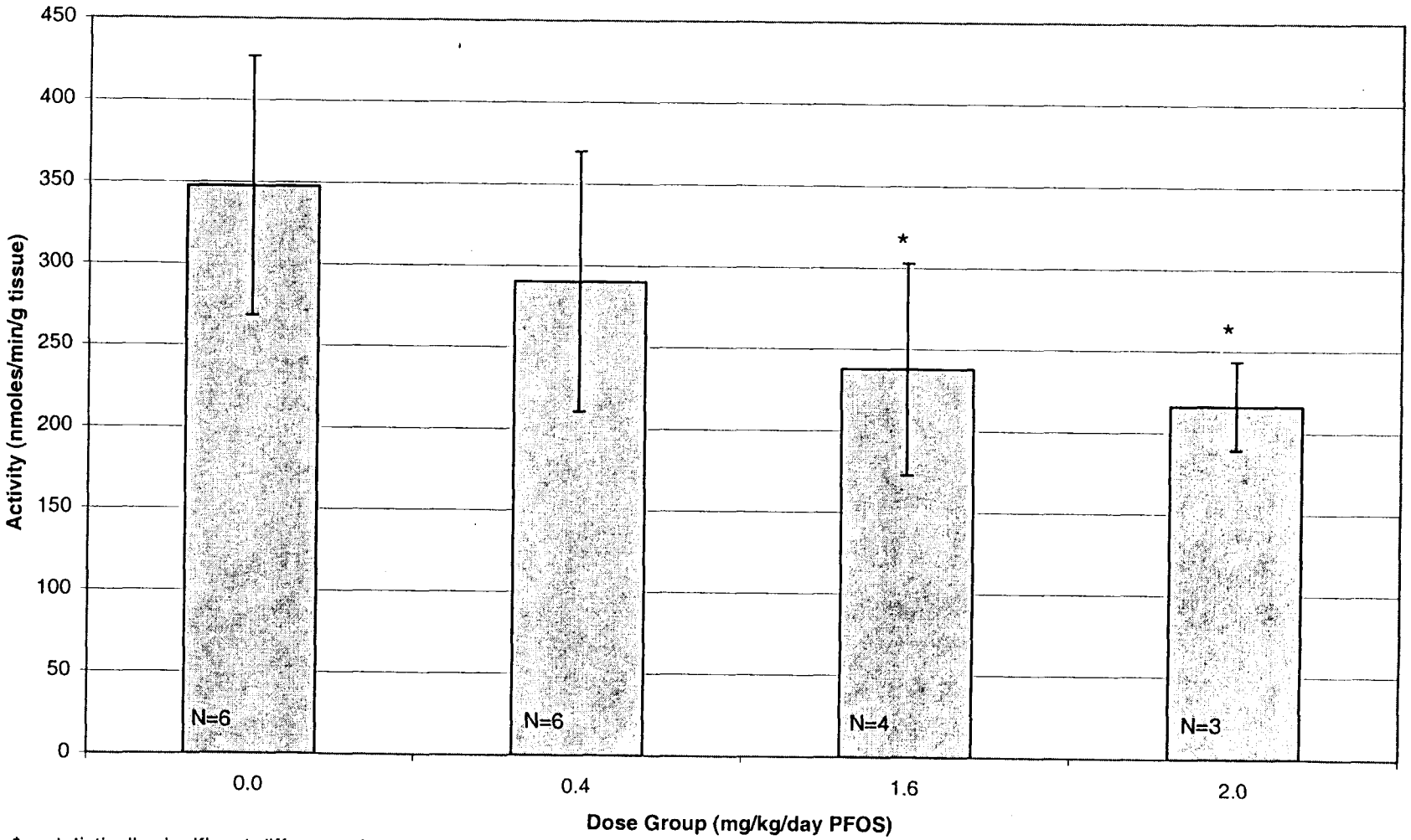


Figure 5: F0 UDPGT Activity Lactation Day 5



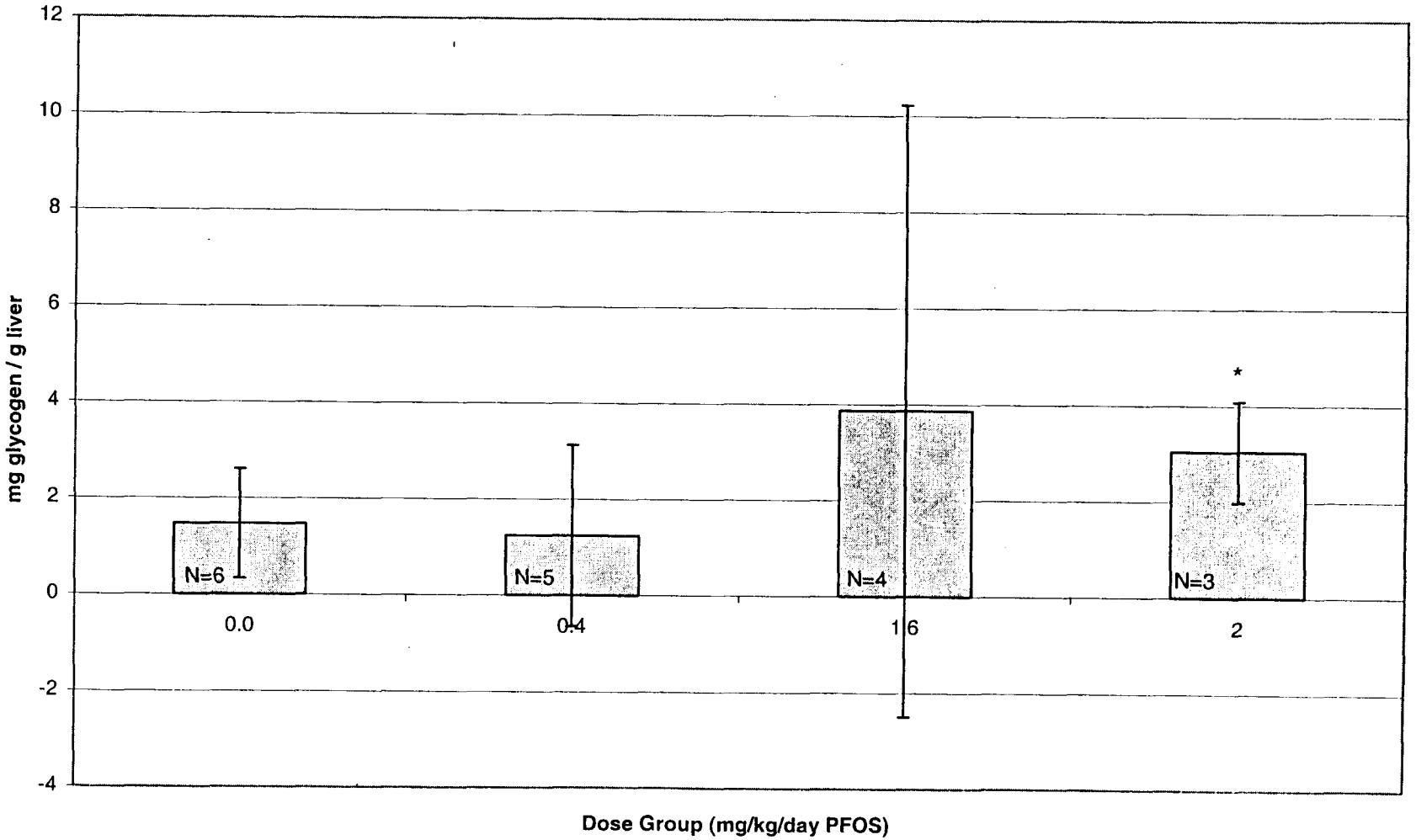
* = statistically significant difference from control, P ≤ 0.05; ** P ≤ 0.01

Figure 6: F1 UDPGT Activity Lactation Day 5



* = statistically significant difference from control, $P \leq 0.05$; ** $P \leq 0.01$

Figure 7: F1 Liver Glycogen Lactation Day 5



* = statistically significant difference from control, P ≤ 0.05; ** P ≤ 0.01

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