

**ORAL TWO WEEK (3X/WEEK) DOSE RANGE
FINDING STUDY OF DECITABINE AND
TETRAHYDROURIDINE IN CD-1 MICE**

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ABSTRACT

The purpose of this study was to determine the target organ toxicity and reversibility of decitabine (DAC) and tetrahydrouridine (THU; administered 1 hour prior to DAC) in mice treated orally 3 times/week for up to 7 doses followed by a 2-week recovery period. Twelve animals/sex received both THU vehicle and DAC vehicle, or 167 mg/kg THU followed by DAC at 0, 0.4, 2, or 10 mg/kg. Treatment with four or five doses of 167 mg/kg THU/10 mg/kg DAC resulted in moribundity or mortality in all mice. Clinical findings in these animals included hunched and languid appearance, rough haircoat and decreased body weight; decreased leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, hematocrit, platelet and reticulocyte counts. Treatment with 167 mg/kg THU/2 mg/kg DAC resulted in similar clinical observations and moribundity/mortality in 1 male and 3 females. Gross pathology was generally unaffected in early death animals; histopathology was not performed. All surviving animals dosed with 167 mg/kg THU alone or 167 mg/kg THU/0.4 to 2 mg/kg DAC showed no adverse clinical signs, body weight effects, gross necropsy findings or changes in serum chemistry. At terminal sacrifice (SD 16), decreases in leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin and hematocrit values were noted in all THU/DAC treated animals. Following the recovery period, all parameters were normal, suggesting reversibility. C_{max} for DAC was attained at 30 minutes postdose and both C_{max} and AUC values were generally proportional to dose. In conclusion, seven doses (3x/week) of 167 mg/kg THU administered alone or 167 mg/kg THU/0.4 mg/kg DAC were generally well-tolerated in male and female CD-1 mice, with the major target of DAC being the hematologic system.

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INTRODUCTION

The DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine (decitabine; DAC), is a promising therapy for the hemoglobinopathies, which include sickle cell disease and β -thalassemia. At low doses, decitabine appears to hypomethylate DNA without causing cytotoxicity.

It has previously been demonstrated that decitabine reactivates fetal hemoglobin (HbF) expression in baboons following intravenous, subcutaneous and oral administration (DeSimone J., 1985; Lavelle D., 2006; Lavelle D., 2007) and in patients with sickle cell disease following intravenous and subcutaneous administration (Koshy M., 2000; DeSimone J., 2002; Sauntharajah Y., 2003).

Development of an oral formulation of decitabine, in combination with the cytidine deaminase inhibitor tetrahydrouridine (THU), is being explored for the treatment of sickle cell disease and β -thalassemia.

The purpose of this study was to determine the target organ toxicity of decitabine (DAC) and tetrahydrouridine (THU), its reversibility and to establish doses for a long term toxicity study in male and female CD-1 mice. Mice were treated orally 3 times per week for up to 2 weeks (up to 7 total doses) followed by a 2-week recovery period.

Mice were administered either THU vehicle (sodium phosphate buffer) or 167 mg/kg THU via oral gavage, followed 1 hour later by oral gavage of either DAC vehicle (potassium phosphate buffer) or 0.4, 2.0, or 10.0 mg/kg DAC. Toxicity endpoints included mortality, clinical observations, body weights, serum chemistry, hematology, and gross pathology.

MATERIALS AND METHODS

Test Animals and Husbandry

The Institutional Animal Care and Use Committee (IACUC) of AVANZA approved this protocol and found it to be in accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.

Table 1. Test Animal Details

	Males	Females
Species and Strain	CD-1 mice	
Supplier	Charles River Laboratories; Raleigh, NC	
Method of Identification	Ear tag	
Number of Animals Received	152	152
Number Used on Study	138	138
Age at First Dose	9 weeks	
Weight Range at First Dose	30.62 – 36.92 g	30.62 – 36.92 g
Disposition of Extra Animals	Transferred to the AVANZA stock colony	

Table 2. Animal Husbandry Details

Feed	Certified Global Harlan Teklad Laboratory Diet 2018 (pellets)
Water	Automatic watering system
Bedding	SaniChip® certified hardwood bedding
Housing	Individually housed in polycarbonate cages suspended on stainless steel racks. Each cage was affixed with a cage card containing pertinent animal and study information.
Temperature Range	18 to 26°C
Humidity Range	30 to 70%
Light Cycle	12-hour light/12-hour dark, interrupted as necessary for study-related events
Air Changes	Minimum of 10 air changes per hour

Neat Test and Control Articles

Table 3. Neat Test and Control Articles

Name	Purity
Tetrahydrouridine (THU, NSC-112907)	94.9%
Decitabine (deoxyazacytidine, DAC, NSC-127716)	98.6%
Sterile Water for Injection (SWFI)	NA
0.9% Sodium Chloride for Injection, USP (SCFI)	97.9%
Potassium phosphate monobasic, certified ACS	100.0%
Sodium chloride, USP	99.5%
Sodium phosphate dibasic anhydrous, USP	99.9%
Sodium phosphate monobasic monohydrate, USP	99.5-100.1% ^a

a - Both purities appear on the Certificate of Analysis, assay methods are not specified.

Formulations

All formulations were prepared prior to each dosing, were maintained on wet ice and were used within 7 hours following preparation

Tetrahydrouridine

Sodium phosphate buffer (THU vehicle) was prepared by adding the appropriate amounts of sodium phosphate dibasic and sodium phosphate monobasic to SWFI. The THU vehicle was used without further formulation for Group 1; for Groups 2 to 5, a 16.7 mg/mL solution of THU was prepared in THU vehicle.

Decitabine

Potassium phosphate buffer was prepared by adding the appropriate amounts of potassium phosphate monobasic and sodium chloride to SWFI; the pH of the solution was adjusted to 6.90 ($\pm 2.90\%$). For Groups 1 and 5 (DAC vehicle), a 1.8 mg/mL solution of sodium chloride was prepared in the potassium phosphate buffer; for Groups 2 to 4, a 2 mg/mL stock solution of DAC was prepared by adding the appropriate amount of DAC to the potassium phosphate buffer; the pH was adjusted to 6.90 ($\pm 2.90\%$). The dosing formulations of DAC (0.04, 0.2, and 1.0 mg/mL) were prepared by diluting the 2 mg/mL stock solution with SCFI.

Experimental Design

Animals were gavaged with DAC or its vehicle 1 hour \pm 5 minutes after administration of THU or its vehicle at a dose volume of 10 mL/kg as described in Table 4.

Table 4. Study Groups and Treatment

Group	Treatment	THU/DAC Nominal Dose (mg/kg)	THU/DAC Dose Concentration (mg/mL)	Number of Animals			
				Main		Toxicokinetic	
				Males	Females	Males	Females
1	THU Vehicle + DAC Vehicle	0 / 0	0 / 0	12	12	6	6
2	THU + DAC	167 / 0.4	16.7 / 0.04	12	12	18	18
3	THU + DAC	167 / 2.0	16.7 / 0.2	12	12	36	36
4	THU + DAC	167 / 10.0	16.7 / 1.0	12	12	18	18
5	THU + DAC Vehicle	167 / 0	16.7 / 0	12	12	0	0

Note: This table presents the initial study design. Due to mortality in the Group 4 animals, the first 3 surviving animals/sex were maintained for recovery following the final dose on Study Day 11, and the remaining Group 4 main study animals were transferred to the toxicokinetic group.

Animals in Groups 1, 2, 3, and 5 were dosed 3 times per week for a total of seven doses (on Study Days 1, 4, 5, 8, 11, 12, and 15). Because of toxicity in Group 4, dosing for these animals was terminated on Study Day 11 (surviving animals received a total of five doses).

Table 5: Animal Observations

Procedure	Frequency of Testing	
	Main Study Animals	Toxicokinetic Animals
Cageside Observations	\geq Twice daily	\geq Twice daily
Clinical Observations	\geq Once daily	Not required
Body Weight^a	Study Days 1, 7, 15, 22, and 29	Study Days 1, 7, and 15

a - For toxicokinetic animals, body weights were collected for the purpose of dose volume calculations only and were excluded from summary calculations and statistical analyses.

Toxicokinetics

Sample collection tubes were prepared prior to each collection day by adding 10 μ L/tube of a 10 mg/mL THU solution. Blood samples (~0.5 mL) were collected via intra-cardiac puncture from non-fasted, anesthetized toxicokinetic animals on Study Day 1 (Group 3), Study Day 11 (Group 4), and Study Day 15

(Groups 2 and 3) at 30, 60, 90, 120, 180, and 240 minutes after administration of DAC or its vehicle.

For Groups 2 and 3, samples were collected from the first available 3 animals per time point; for Group 4, samples were collected from the first available 2 animals per time point. When there was mortality in Groups 3 and 4, animals were redistributed as necessary to allow for sample collection at all protocol-required time points whenever possible. For Group 1, samples were collected from the first 3 animals per time point on Study Day 15 immediately following and at 120 minutes following administration of the DAC vehicle.

Samples were maintained on wet ice until they were centrifuged (as soon as possible after collection), and the resultant plasma was stored frozen ($-75 \pm 15^{\circ}\text{C}$) until shipped on dry ice to The Ohio State University for analysis to determine the plasma level of decitabine via a validated LC-MS/MS method (Liu Z 2006).

C vs t data were analyzed by compartmental and non-compartment methods. WinNonlin was used to fit the data to a one-compartment oral absorption model (first-order input) with an appropriate weighting factor. C_{max} was obtained from the observed values. AUCs were calculated using the linear trapezoidal rule to the last observed time point (non-compartment) or with extrapolation to time infinity (compartment). In the case of gender comparisons, the data were truncated at the same time point.

Clinical Pathology

Blood samples were collected from fasted animals via intra-cardiac puncture as shown in **Table 6**.

Table 6: Blood Sampling Information for Clinical Pathology

Parameter	Study Day	Groups	Animals	Volume	Tubes Used
Hematology	2	1-5	1 st 3/sex/group	~0.5 mL	K ₂ EDTA
	16	1-3, 5	2 nd 3/sex/group		
	29	1-3, 5	4 th 3/sex/group		
Chemistry	16	1-3, 5	3 rd 3/sex/group	~0.6 mL	Serum separator

Additional samples were collected from non-fasted animals prior to moribund sacrifice, when possible. Hematology and chemistry samples were collected from Group 4 animals sacrificed moribund on Study Day 10; Hematology samples were also collected from moribund Group 4 animals on Study Days 12 and 16.

Termination and Necropsy

Termination

Animals were euthanized by carbon dioxide inhalation followed by exsanguination. Toxicokinetic animals that were found dead or sacrificed moribund were examined for gavage error and discarded without further necropsy. Those toxicokinetic animals surviving to their scheduled blood collection interval were subsequently euthanized and discarded without necropsy.

Scheduled necropsies for main study animals were conducted on Study Day 2 (first 3 surviving animals/sex in Groups 1 to 5), Study Day 16 (second 6 surviving animals/sex in Groups 1, 2, 3 and 5), and Study Day 29 (all surviving animals).

Necropsy

Main study animals were necropsied as soon as possible after the time of death or discovery.

Statistical Analyses

Quantitative data were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the Kruskal-Wallis ANOVA was performed on rank-transformed data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett's t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunn's test was used to delineate which groups (if any) differed from the control.

The probability value of less than 0.05 (two tailed) was used as the critical level of significance for all tests.

RESULTS

Clinical Observations

Administration of 167 mg/kg THU followed by DAC at dose levels of 2 mg/kg (Group 3) or 10 mg/kg (Group 4) resulted in mortality in both sexes. Among animals dosed with THU and 10 mg/kg DAC (Group 4), 3/3 animals for each sex survived to scheduled termination on Day 2 (no abnormal observations noted), but all remaining animals in this group did not survive to scheduled necropsy. Clinical observations and gross necropsy findings and hematology findings for all main study animals that were found dead or euthanized moribund are summarized in **Tables 7 and 9**.

Among animals dosed with THU and 2 mg/kg DAC (Group 3), 3/3 and 6/6 animals for each sex survived to scheduled termination on days 2 and 16, respectively, and 2/3 males (0/3 females) survived to the recovery sacrifice on day 29. Rough haircoat was noted in one male; there were no other abnormal findings in the surviving animals from these groups.

All animals dosed with both vehicles (Group 1) or with 167 mg/kg THU followed by 0.4 mg/kg DAC (Group 2) or DAC vehicle (Group 5) survived to scheduled termination with no abnormal observations noted.

Body Weights and Body Weight Changes

Body weights and body weight gains were decreased in males and females treated with THU and 10 mg/kg DAC (Group 4); this was related to the morbidity noted in this group.

Toxicokinetics (Table 8)

At all dose levels and measured intervals, C_{\max} was attained at 30 minutes postdose. Both C_{\max} and AUC values increased with dose, but the increase was not consistently proportional. $T_{1/2}$ was approximately 30 minutes for Group 2 (0.4 mg/kg DAC, day 15) and Group 3 (2.0 mg/kg DAC, days 1 and 15). For Group 4 (10 mg/kg DAC), the $T_{1/2}$ was over 60 minutes; this longer $T_{1/2}$ for Group 4 animals may have been due to drug toxicity or longer measurable DAC levels, which could influence curve fitting. Plasma levels of DAC were higher in female mice compared to male mice in the 0.4 and 2 mg/kg dose groups. No sex-

based trend TK was evident in the 10 mg/kg group, but early mortality limited TK sampling in this group.

Table 7: Clinical Observations and Necropsy Findings on Early Death Animals

Group/ Sex	Animal	Disposition	Clinical Findings Noted Prior to Death	Necropsy Findings
3M	24709	FD (Day 20)	Minimal ataxia, hunched	None
3F	24755	FD (Day 20)	Hunched, rough haircoat, squinting, lacrimation	None
	24756	FD (Day 12)	Languid, hunched, rough haircoat, squinting	None
	24757	FD (Day 16)	Languid, cold to touch, hunched	None
4M	24797	FD (Day 17)	Hunched, rough haircoat	None
	24798	FD (Day 18)	Hunched, rough haircoat	Soft brain, enlargement and thickening of all liver lobes
	24799	MK (Day 16)	Severe head tilt, rough haircoat	None
	24802	FD (Day 10)	None	None
	24805	MK (Day 10)	Hunched, rough haircoat, squinting	None
4F	24827	MK (Day 10)	Hunched, rough haircoat, squinting	Enlargement of right mandibular lymph node
	24828	FD (Day 12)	Hunched, rough haircoat	None
	24829	MK (Day 12)	Languid, hunched, squinting	None
	24830	FD (Day 10)	None	None
	24831	MK (Day 10)	Hunched, rough haircoat, squinting	None
	24832	MK (Day 10)	Hunched, rough haircoat, squinting	None
	24833	FD (Day 12)	None	None
	24834	MK (Day 10)	Hunched, rough haircoat, squinting	None

FD = Found Dead MK = Moribund Kill
 Group 3 = 167 mg/kg THU + 2.0 mg/kg DAC
 Group 4 = 167 mg/kg THU + 10.0 mg/kg DAC

Table 8: Toxicokinetics

Group # →	2	3	3	4
DAC dose	0.4 mg/kg	2 mg/kg	2 mg/kg	10 mg/kg
Day of sample →	Day 15	Day 1	Day 15	Day 11
PK Parameter ↓				
$t_{1/2k10}$ (min)	30.43	33.12	31.68	62.74
t_{max} (min)	30	30	30	30
$AUC_{fit\ 0 \rightarrow \infty}$ (min* μ M)	58.04	590.41	412.97	2401.5
$AUC_{trap\ 0 \rightarrow t}$ (min* μ M)	44.22	510.23	316.73	2051.84
AUC (min* μ M)	M	34.83	482.94	1563.84
	F	53.64	537.38	1486.13
C_{max} (μ M)	all	0.747	7.748	18.68
	M	0.559	8.205	21.00
	F	0.935	7.290	16.36

Table 9: Summary of Day 10 Hematology Data (Early Deaths)

Group/ Sex		WBC (K/uL)	RBC (M/uL)	HGB (g/dL)	HCT (%)	PLT (K/uL)	ABRETi (10 ⁹)	ABNEUT (K/uL)	ABLYMP (K/uL)
4M	Mean	1.624	7.402	11.14	38.72	186.2	10.03	0.058	1.486
	SD	0.907	0.495	0.77	3.35	35.6	5.65	0.040	0.861
	N	5	5	5	5	5	4	5	5
4F	Mean	1.568	7.518	11.85	38.80	69.0	4.45	0.008	1.480
	SD	0.121	0.883	1.58	4.92	52.4	0.90	0.015	0.128
	N	4	4	4	4	4	4	4	4

Group 4 = 167 mg/kg THU + 10.0 mg/kg DAC

Clinical and Anatomical Pathology

Clinical Chemistry

On SD 16, mean total protein and albumin values were significantly decreased in males treated with THU followed by 0.4 mg/kg DAC (Group 2), 2 mg/kg DAC (Group 3), or DAC vehicle (Group 5). These changes resulted in significantly decreased A/G ratios in males treated with THU followed by 2 mg/kg DAC (Group 3). These findings were considered non-adverse due to a lack of

correlating findings in females and because group mean values remained within the historical control ranges.

Hematology (Tables 10 and 11)

On day 2, there were no treatment-related findings.

On day 10, slight to moderate decreases were noted in leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, and hematocrit data in male and female moribund animals receiving THU and 10 mg/kg DAC (Group 4). Decreases in platelet and reticulocyte counts were considered severe and were also observed in these animals.

On days 12 and 16, decreases in these same hematology parameters were also noted in moribund animals receiving THU and 10 mg/kg DAC (Group 4); the severity tended to be greater than that observed at day 10.

On day 16, mean leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, and hematocrit values were decreased below the historical control ranges in males and females treated with THU and 2 mg/kg DAC (Group 3); these decreases were usually significantly different from the control means. Decreases were also noted for these same parameters in males and females treated with THU and 0.4 mg/kg DAC (Group 2); the differences were often significant but not always lower than the historical control ranges. Minimal to moderate decreases in reticulocyte counts were noted in all surviving females and 2/3 surviving males treated with THU and 2 mg/kg DAC (Group 3); the differences were not statistically significant.

On day 29, all hematology parameters were generally within the historical reference ranges suggesting reversibility, although mean total leukocyte counts in treated males were still significantly lower than the control mean.

Macroscopic Findings

There were no vehicle- or test-article-related effects on gross pathology in animals surviving to scheduled termination

CONCLUSION

Treatment 3 times per week (for a total of four or five doses) with 167 mg/kg THU followed by 10 mg/kg DAC resulted in mortality in all male and female CD-1 mice. Clinical findings in these animals included hunched and languid appearance, rough haircoat, squinting, and decreased body weight and decreases were noted in leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, hematocrit and platelets.

Treatment with six or seven doses (3x/week) of 167 mg/kg THU followed by 2 mg/kg DAC resulted in mortality in one male and three females; clinical findings for these animals were similar to those observed in animals treated with 10 mg/kg DAC (no clinical pathology performed).

At terminal sacrifice (day 16), surviving animals dosed 3 times per week (for a total of seven doses) with 167 mg/kg THU alone or followed by up to 2 mg/kg DAC presented with decreases in leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, and hematocrit values, most of these parameters were within normal limits on day 29, suggesting reversibility.

In conclusion, seven doses (3x/week) of 167 mg/kg THU administered alone or followed by 0.4 mg/kg DAC were generally well-tolerated in male and female CD-1 mice, with the most sensitive target of DAC being the hematologic system.

Table 10: Summary of Day 16 Hematology Data

Group/ Sex		WBC (K/uL)	RBC (M/uL)	HGB (g/dL)	HCT (%)	PLT (K/uL)	ABRETi (10 ⁹)	ABNEUT (K/uL)	ABLYMP (K/uL)
1M	Mean	7.343	10.260	15.77	54.23	1258.3	370.63	1.090	5.837
	SD	2.067	0.384	0.58	0.90	233.3	45.43	0.397	1.930
	N	3	3	3	3	3	3	3	3
2M	Mean	2.450	9.010*	13.63*	47.93	1838.7*	467.47	0.327	2.007
	SD	0.000	0.550	0.76	3.32	74.8	68.11	0.211	0.197
	N	3	3	3	3	3	3	3	3
3M	Mean	1.543*	7.300*	11.37*	38.97*	1182.3	130.17*	0.053*	1.437*
	SD	0.244	0.819	1.66	5.70	240.7	132.54	0.040	0.200
	N	3	3	3	3	3	3	3	3
4M	Mean	1.040	5.260	7.60	25.30	74.0	9.90	0.000	0.960
	SD
	N	1	1	1	1	1	1	1	1
5M	Mean	4.590	9.580	14.27	49.93	1078.0	403.10	0.877	3.060
	SD	0.367	0.332	0.40	1.25	294.2	44.92	0.624	0.842
	N	3	3	3	3	2	3	3	3
1F	Mean	8.013	9.760	15.60	53.07	1176.0	302.57	0.897	6.850
	SD	2.358	0.183	0.79	1.25	185.3	56.40	0.301	2.125
	N	3	3	3	3	2	3	3	3
2F	Mean	3.110*	8.300*	13.20*	44.70*	1267.5	384.20	0.265	2.615*
	SD	1.400	0.566	0.71	3.25	55.9	75.66	0.163	1.167
	N	2	2	2	2	2	2	2	2
3F	Mean	1.283*	5.943*	9.20*	29.83*	512.0	9.27	0.000*	1.240*
	SD	0.440	0.332	0.52	1.25	305.6	5.59	0.000	0.426
	N	3	3	3	3	3	3	3	3
5F	Mean	5.747	9.723	15.40	52.67	821.7	373.63	1.107	3.933*
	SD	1.298	.0451	0.46	2.14	253.5	61.99	0.532	0.656
	N	3	3	3	3	3	3	3	3

* - Significantly Different From Control Value, $p < 0.05$

Group 1 = 0 mg/kg THU + 0 mg/kg DAC (control)

Group 2 = 167 mg/kg THU + 0.4 mg/kg DAC

Group 3 = 167 mg/kg THU + 2.0 mg/kg DAC

Group 4 = 167 mg/kg THU + 10.0 mg/kg DAC

Group 5 = 167 mg/kg THU + 0 mg/kg DAC

Table 11: Summary of Day 29 Hematology Data

Group/ Sex		WBC (K/uL)	RBC (M/uL)	HGB (g/dL)	HCT (%)	PLT (K/uL)	ABRETi (10 ⁹)	ABNEUT (K/uL)	ABLYMP (K/uL)
1M	Mean	8.163	9.477	14.93	51.63	.	322.63	1.563	5.927
	SD	0.982	0.688	0.98	2.10	.	100.66	0.734	0.706
	N	3	3	3	3	0	3	3	3
2M	Mean	5.263*	9.203	14.53	52.03	1079.0	368.00	0.867	4.050
	SD	1.073	0.414	0.35	0.74	.	76.60	0.155	1.079
	N	3	3	3	3	1	3	3	3
3M	Mean	4.270*	9.375	14.00	51.45	1102.0	242.05	0.725	3.405
	SD	0.509	0.559	0.00	1.06	256.0	26.94	0.304	0.88
	N	2	2	2	2	2	2	2	2
5M	Mean	6.250*	9.100	15.03	50.70	1323.5	234.50	1.077	4.893
	SD	0.727	0.852	0.40	4.45	545.2	108.83	0.133	0.739
	N	3	3	3	3	2	3	3	3
1F	Mean	4.183	9.407	15.07	50.17	655.0	330.90	0.473	3.510
	SD	1.355	0.903	0.06	3.33	141.4	74.14	0.25	0.924
	N	3	3	3	3	2	3	3	3
2F	Mean	4.947	8.763	15.60	49.87	872.7	421.83	1.150	3.543
	SD	2.711	0.244	1.15	2.80	355.4	66.00	0.381	2.449
	N	3	3	3	3	3	3	3	3
5F	Mean	7.727	8.690	15.50	49.47	1020.0	308.13	1.000	6.507
	SD	2.548	0.885	0.20	3.43	465.3	77.96	0.785	2.058
	N	3	3	3	3	2	3	3	3

* - Significantly Different From Control Value, $p < 0.05$

Group 1 = 0 mg/kg THU + 0 mg/kg DAC (control)

Group 2 = 167 mg/kg THU + 0.4 mg/kg DAC

Group 3 = 167 mg/kg THU + 2.0 mg/kg DAC

Group 5 = 167 mg/kg THU + 0 mg/kg DAC

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