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DATA EVALUATION REPORT

STUDY TYPE: 4-week rangefinding and 90-day subchronic study in mice
TOX. CHEM. NO.: 573S
ACCESSION NUMBER: 263757
MRID NO.: ?

TEST MATERIAL: INM- 6316

SYNONYMS: Harmony

STUDY NUMBER(S): 466-83

SPONSOR: Dupont de Nemours

TESTING FACILITY: Haskell laboratory

TITLE OF REPORT: 4-week rangefinding and 90-day feeding study in mice with 2-thiophenecarboxylic acid, 3[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino] carbonyl]amino] sulfonyl]-, methyl ester,(INM-6316)
AUTHOR(S): L.S. Mullin

REPORT ISSUED: June 15, 1984

CONCLUSIONS: NOEL > 7500 ppm. No effects were seen at any of the doses tested. However, it meets the MTD criteria in mice (7000ppm top dose)

Core classification: supplementary. The study is missing clinical chemistry data, ophthalmological examinations and raw data.

A. MATERIALS:

1. Test compound: Harmony, Description: tan crystalline solid Batch #7427-167, Purity,93.6%,

2. Test animals: Species: Mouse, Strain: CD-1, Age: 4-weeks, Weight: males-18.4-29.5 gms, females- 13.7-22.6 gms. Source: Charles River Breeding Laboratories, Kingston, N.Y.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 90-days male	female	Interim Sac. 4 weeks male	female
1 Cont.	0	10	10	10	10
2	50			10	10
3	500	10	10	10	10
4	2500	10	10	10	10
5	5000			10	10
6	7500	10	10	10	10

2. Diet preparation

Diet was prepared weekly and stored at refrigerator temperature. Samples of diet freshly prepared, or freshly prepared and stored at refrigerator temperature for 10 days, or stored at room temp for 24 hours or 10 days were collected and analyzed for concentration and stability. In addition, freshly prepared diet was analyzed at week 5.

Results - Concentration in the diet at the beginning of the study exceeded the nominal concentrations by 11 to 40%. But by the end of the study they were within 10% of nominal concentrations.
Stability data are on appended page 4. The compound in the diet was relatively stable under the conditions tested.

3. Animals received food and water ad libitum.

4. Statistics - Statistical treatment of the data are on appended page 1.

5. Quality assurance report was signed and attached to the study.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected weekly for signs of toxicity and mortality. In addition, each mouse was handled individually once weekly and examined for abnormal appearance or behavior.

Toxicity/Mortality (survival)
No compound-related effects were evident in the animals either at the 4-week sacrifice or by the end of the 90-day study.

Mortality: 2 females died, one in the 500 ppm and one in the 7500 ppm group. Both deaths were accidental and unrelated to compound administration.

2. Body weight

Animals were weighed weekly.

Results: There were no compound-related effects either at the 4-week interim sacrifice or at the 90-day termination of the experiment. Growth curves are on appended pages 2 and 3.

3. Food consumption and compound intake

Consumption was determined weekly, and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Food consumption/Food Efficiency/Compound Intake
No compound-related effects were seen. Mean daily intake when calculated from analytical results was 97, 528 and 1427 mg/kg for males, and 123, 690, and 2287 mg/kg for females over the 13-week period.

4. Ophthalmological examinations- were not performed.

5. Blood was collected in males at 43 and 89 days and in all surviving male mice, and in females on days 47 and 90 and all surviving females for hematology. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count (blood smears were taken but not evaluated)
	Blood Clotting Measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results:
No compound-related changes were evident at any of the blood sampling time periods.

b. Clinical Chemistry - ~~no~~ evaluations were done.
All animals that died and that were sacrificed on schedule

6. Urinalysis- No evaluations were done, however, they are not required in the guidelines for subchronic studies.

7. Sacrifice and Pathology:
All animals that died and were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
| |	Tongue	X |	.Aorta*	XX	.Brain*†
X |	.Salivary glands*	XX	.Heart*	| |	Periph. nerve*#
X |	.Esophagus*	X |	.Bone marrow*	| |	Spinal cord (3 levels)*#
X |	.Stomach*	X |	.Lymph nodes*	X |	.Pituitary*
X |	.Duodenum*	XX	.Spleen*	X |	Eyes (optic n.)*#
X |	.Jejunum*	X |	.Thymus*		Glandular
X |	.Ileum*		Urogenital	XX	.Adrenals*
X |	.Cecum*	XX	.Kidneys*†	| |	Lacrimal gland#
X |	.Colon*	X |	.Urinary bladder*	| |	Mammary gland*#
| |	.Rectum*	XX	.Testes*†	X |	.Parathyroids*††
XX	.Liver*†	XX	Epididymides	X |	.Thyroids*††
X |	Gall bladder*#	X |	Prostate		Other
X |	.Pancreas*	| |	Seminal vesicle	X |	Bone*#
	Respiratory	X |	Ovaries*†	X |	Skeletal muscle*#
X |	.Trachea*	X |	.Uterus*	| |	Skin*#
X |	.Lung*	X |	Vagina	X |	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

All control and high dose animals were examined microscopically.

All gross lesions and heart, liver and kidneys from low and intermediate dose groups were also examined microscopically.

Bone marrow smears were prepared on all mice at terminal sacrifice, but were not evaluated.

a. Organ weight - 10/group (6 groups) were sacrificed on days 33 and 34 and organs were weighed. The remaining animals except those in the 50 and 500 ppm groups continued on test until the 90-day sacrifice when those animals sacrificed had organs weighed.

Results: 4-week interim sacrifice: There was a decrease in absolute liver weight noted in female mice in the 50 and 500 ppm groups, however, this was not considered to be compound-related, and no other effects were seen.
90-day sacrifice: There was an increase in relative kidney weight in

5

Appended Page 1

Chemicals Department for residue analyses. The results of any of these analyses will appear in a separate Agricultural Chemicals Department report.

Statistical Analyses

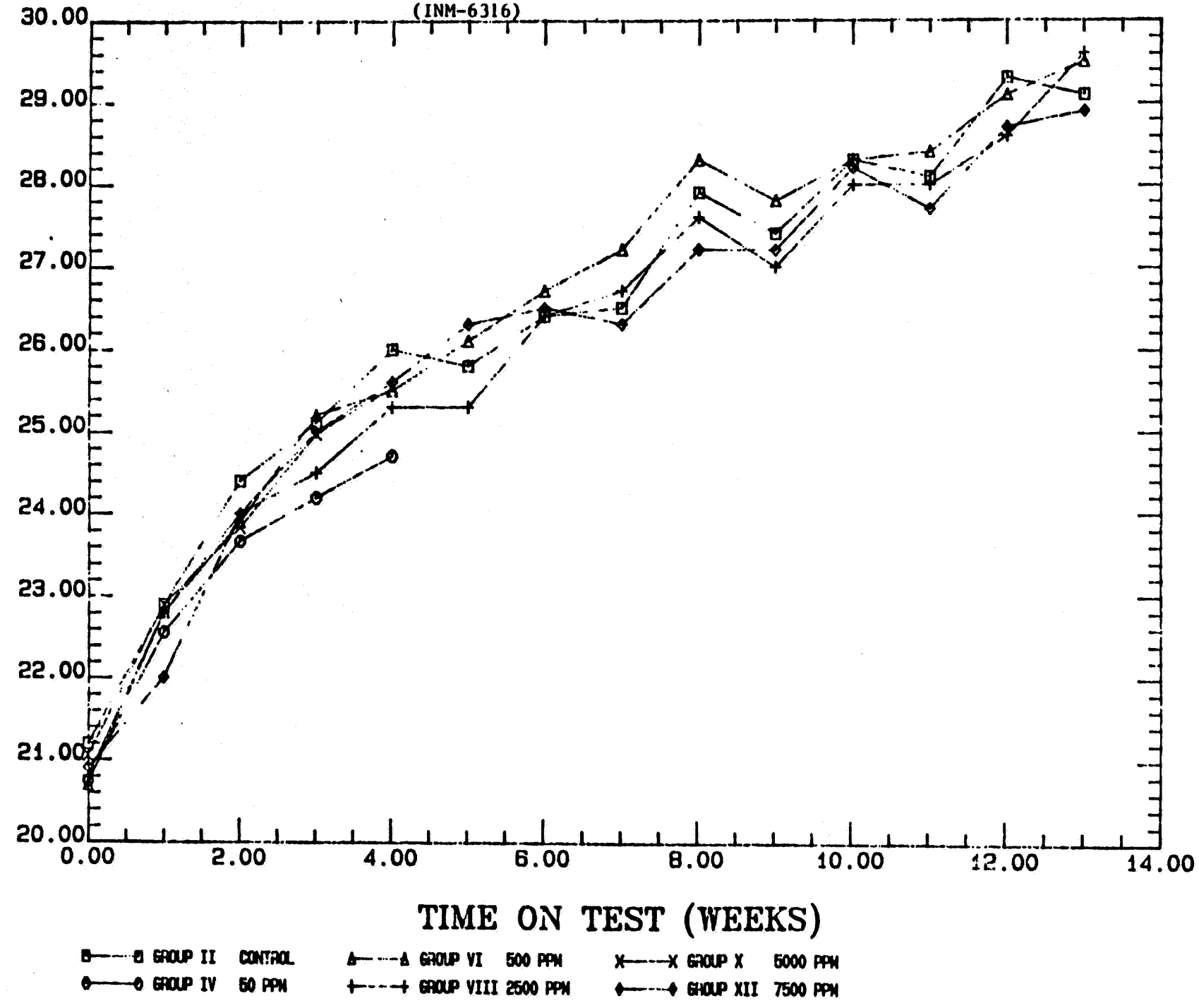
Body weights, body weight gains, absolute and relative organ weights, and clinical laboratory measurements were subjected to one-way analysis of variance. When the test for differences among group means (F-test) was significant, pairwise comparisons were made between control and test groups. For body weights and weight gains, these comparisons were made with the least significant difference (LSD) test. The clinical laboratory measurements were compared using Dunnett's test. For organ weights, the comparisons were made with both LSD and Dunnett's tests. Bartlett's test for homogeneity of variances was calculated on organ weights and clinical laboratory measurements. Significance for the comparison of means was judged at the $p \leq 0.05$ probability level.

Results

A. Diet Analyses

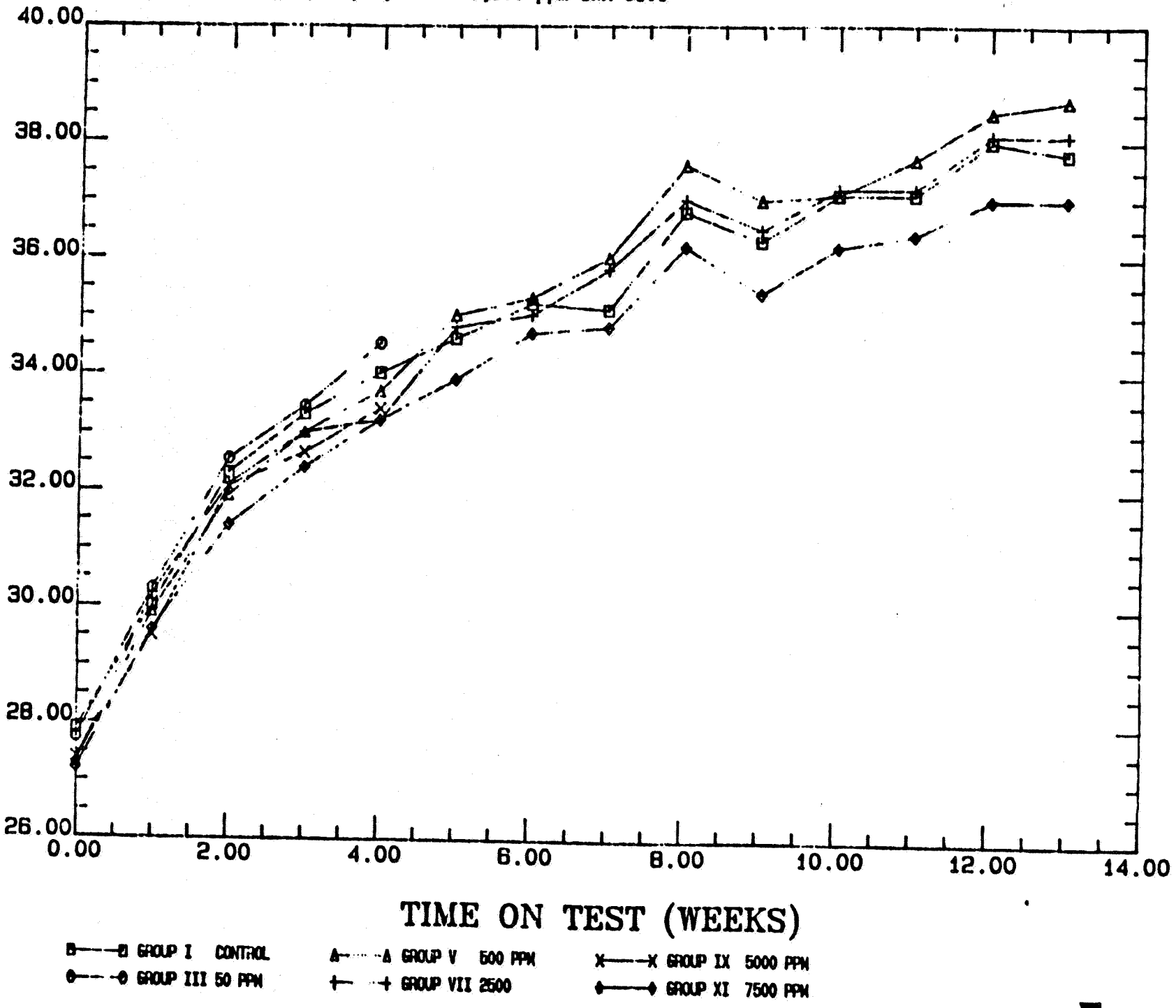
Results of diet analyses are presented in Appendix B and summarized in Table 1. The concentrations of INM-6316 in diet samples collected at the beginning of the study exceeded the nominal concentrations by 11% to 40%. The concentrations of INM-6316 in diet samples collected at study week 5

Figure 2: Growth Curves of Female Mice Fed with Diets that Contained 0, 50, 500, 2,500, 5,000 or 7,500 ppm (INM-6316)



Appendix B "1

Figure 1: Growth Curves of Male Mice Fed with Diets that Contained 0, 50, 500, 2,500, 5,000 or 7,500 ppm INM-6316



257

Appendix P84

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TABLE 1

CONCENTRATIONS OF INM-6316 IN THE DIETS FED TO MICE DURING A FOUR-WEEK RANGE-FINDING AND 90-DAY SUBCHRONIC TOXICITY STUDY

STORAGE CONDITIONS	CONCENTRATION OF INM-6316 IN DIET SAMPLES (ppm)[a]				
	NOMINAL 50 ppm	NOMINAL 500 ppm	NOMINAL 2,500 ppm	NOMINAL 5,000 ppm	NOMINAL 7,500 ppm
Fresh Frozen	68[b]	601 ± 75[c]	2,982 ± 398[c]	5,621[b]	8,773 ± 949[c]
24-Hour Room Temperature	65[b]	538 ± 25[d]	2,966 ± 659[d]	5,976[b]	7,856 ± 1,023[d]
10-Day Room Temperature	65[b]	520 ± 101[d]	2,877 ± 533[d]	5,266[b]	8,528 ± 1,412[d]
10-Day Refrig.	59[b]	564 ± 105[d]	2,863 ± 586[d]	5,739[b]	8,303 ± 2,233[d]

[a] Values are corrected for recovery.

[b] Results of samples collected at initiation of study only. These dose levels were only used for the four-week range-finding study.

[c] Mean ± 1 standard deviation for samples collected during weeks 0, 5, and 12 of study.

[d] Mean ± 1 standard deviation for samples collected during weeks 0 and 12 of study. Stability samples were not collected at week 5.

9