

Clinical Research Department

NutraSweet Company

**Final Clinical Research Report**

Pharmacokinetics and Metabolism of [14-C]-Beta-Aspartame  
in Healthy Subjects

Protocol No. N20-85-02-150

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## I. ABSTRACT

Beta-aspartame (beta-APM) is formed to a minor extent during the manufacturing process of aspartame and during storage of aspartame-containing beverages. The objective of this open-label study was to determine the pharmacokinetics and metabolism of orally administered [14-C]-beta-APM in healthy male subjects.

Initially, a pilot study consisting of one subject was done to develop analytical methods and to determine if the dose was appropriate. Five more subjects were then dosed. The pilot subject received an oral dose of 40 mg of [14-C]-beta-APM (approximately 200  $\mu$ Ci) in aqueous solution. The other five subjects received a dose of 32 mg (approximately 160  $\mu$ Ci).

Plasma, red blood cell, urine, and fecal specimens were collected at baseline and at regular intervals after dosing throughout the seven-day study period and analyzed for total radioactivity and major metabolites.

The subjects underwent routine clinical evaluations prior to dosing and again after completing the seven day study period. The subjects were monitored for vital signs and adverse experiences during the study period.

The results of the 5-subject study were as follows:

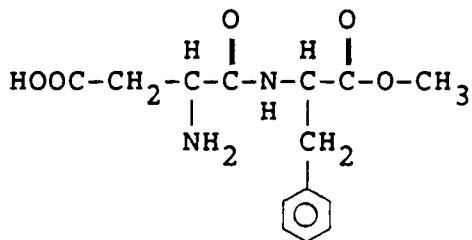
- . About 90% of the radiolabel was absorbed, as indicated by the recovery of less than 10% of the dose in the feces.
- . Total plasma radioactivity was eliminated in two phases: the shorter phase represented beta-APM metabolites, while the longer phase, as expected, represented incorporation of the label, as [14-C]-phenylalanine, into plasma protein.
- . Total red blood cell radioactivity increased gradually, indicating the incorporation of the label into red blood cell protein.
- . The methyl ester bond was extensively metabolized as seen by the absence of intact beta-APM in the plasma and less than 0.015% of the dose as beta-APM in the urine.
- . A small amount of beta-aspartylphenylalanine (beta-AP) was present in the plasma and urine (6.97% of the dose was recovered as beta-AP in the urine).
- . Metabolites identified in the plasma were beta-AP, phenylacetylglutamine (PAGln), and phenylalanine (Phe).

- . The major route of excretion of total radioactivity was the urine (42.0% of the dose over 7 days). During the first 24 hours after dosing, the major urinary metabolites were PAGln (30.8% of the dose) and beta-AP (6.97% of the dose).
- . Fecal excretion of total radioactivity was 9.56% of the dose over 7 days. The only metabolite identified in the feces was Phe.
- . The study subjects did not demonstrate any significant clinical effects which the investigator could attribute to beta-APM administration.

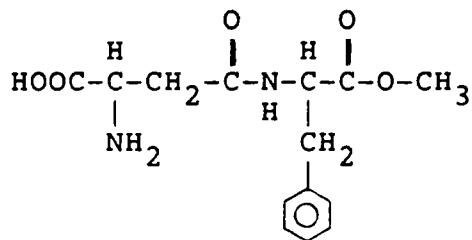
In conclusion, the metabolism of [14-C]-beta-APM in man was similar to that observed in animals. Beta-APM is metabolized to its constituent amino acids and methanol with a small amount being absorbed as beta-AP. Beta-AP is among the beta-aspartyl-dipeptides that have been identified as occurring endogenously in human urine. There were no significant clinical effects related to [14-C]-beta-APM administration.

## II. INTRODUCTION

Beta-aspartame (beta-L-aspartyl-L-phenylalanine methyl ester, beta-APM), an isomer of aspartame (alpha-APM, NutraSweet®), is formed to a minor extent during the manufacturing process and in storage of aspartame-containing beverages. In its food additive petition (FAP 2A3661), G. D. Searle & Co. submitted stability studies which indicated that storage of aspartame in liquids may result in the formation of small amounts of its beta isomer. The amount of aspartame that converts to the beta isomer depends on temperature, pH, and length of storage. Structurally beta-APM differs from alpha-APM because the amino group of aspartic acid resides on the carbon atom beta to, rather than alpha to, the carboxyl group forming the amide bond.



ASPARTAME



BETA-ASPARTAME

Beta-APM has been extensively evaluated in a variety of preclinical safety studies. Acute toxicology studies have been done in rats and mice. The minimum lethal dose was greater than 5000 mg/kg in both species (1). In 4 and 26 week diet admix toxicity studies done in rats (2,3) and dogs (4,5) at dosages of 250, 500, and 1000 mg/kg/day, there were no adverse effects at any dose. In a teratology study (6), pregnant rabbits were given once daily, by gavage, doses of 250, 500, or 750 mg/kg/day. There was no embryotoxicity, fetotoxicity, or teratotoxicity at any dose; however, maternotoxicity was evident at 750 mg/kg. This maternotoxicity was less pronounced when the same amount of beta-APM was given in divided doses (7) as opposed to single doses (8) as above. A two-generation reproduction/teratology study (9) in which beta-APM was given in the diet at dosages of 250, 500, and 750 mg/kg/day, was done in rats. There were no treatment-related effects in F<sub>0</sub>, F<sub>1</sub>, or F<sub>2</sub> animals other than a slight decrease in body weight gain in F<sub>1</sub> males after weaning at the 750 mg/kg dose. This decrease was associated with a decrease in food consumption. The effects noted at 750 mg/kg/day in the rabbit and the rat above were not evident at 500 mg/kg/day which is 2000 times the estimated maximum dietary consumption of the beta isomers<sup>1</sup>. The mutagenicity of beta-APM was evaluated in Salmonella typhimurium and Escherichia coli (11,12) and in a mammalian cell assay using Chinese Hamster ovary (CHO) cells (13). The potential for beta-APM to cause DNA damage was evaluated in the Rec-assay using Bacillus subtilis (12). The potential of beta-APM to induce chromosomal aberrations was evaluated in CHO cells (14). In addition, its potential to cause chromosomal aberrations in vivo was also determined at dosages up to 1000 mg/kg/day in mice (15). The above assays were all negative, thus indicating that beta-APM is not genotoxic.

Beta-APM has also been evaluated in several preclinical pharmacological studies (16). Beta-APM has been studied in vivo after a single oral dose of 60 mg/kg and/or in vitro on various organ systems. A battery of central nervous system screening tests, including evaluation of general

<sup>1</sup>The maximum dietary consumption of the beta isomers, based on the 14-day average, 99th percentile of aspartame consumption, across all ages, for aspartame users in the U.S., assuming 20% degradation of aspartame in all carbonated beverages consumed, was estimated to be less than 0.25 mg/kg/day (10).

symptomatology, motor coordination, locomotor activity, hexobarbital interaction, analgesia, narcotic antagonism, anticonvulsant, and proconvulsant effects, have been done. Beta-APM has been evaluated for effects on the gastrointestinal system by measuring gastric secretion in pyloric-ligated rats and charcoal meal transit time in rats. Cardiovascular and renal system effects were evaluated in such tests as autonomic function in conscious rats, hemodynamic effects in the conscious normotensive dog, renal function in unanesthetized dogs, inhibition of angiotensin I converting enzyme, and in vitro inhibition of human renin. In the above studies and other pharmacology studies (16), beta-APM did not have any biologically significant effects.

The pharmacokinetics and metabolism of beta-APM have been evaluated in studies with rats (17-19), rabbits (20), dogs (21), and rhesus monkeys (22) by means of [14-C]-beta-APM. The major conclusions from these studies were:

1. The ester bond of beta-APM was extensively hydrolyzed by intestinal or liver enzymes in all species studied such that little if any intact beta-APM reached the systemic circulation;
2. Absorption of beta-aspartylphenylalanine (beta-AP), the resulting metabolite of esterase action, was slow and the oral bioavailabilities of beta-AP from doses of 10 mg/kg of beta-AP or beta-APM administered as solutions was 7-9% in rats, rabbits, and monkeys;
3. Peak plasma concentrations of beta-AP following 10 mg/kg oral doses of beta-AP or beta-APM were approximately 0.4 to 0.8  $\mu$ g/ml and did not differ markedly in rats, rabbits, and monkeys. Peak concentrations were reached at 2 hours or later after dosing;
4. Absorption of total radioactivity from oral 10 mg/kg doses of beta-AP and beta-APM in the rabbit, and beta-APM in the monkey, was greater than 90%. Absorption of total radioactivity was somewhat lower in the rat (65% for beta-AP and 88% for beta-APM);
5. Absorption of total radioactivity was approximately proportional to dose when [14-C]-beta-APM was administered in a diet admixture to pregnant rats at doses of 5.8, 180, and 950 mg/kg;
6. Total radioactivity from an oral dose of [14-C]-beta-APM in rats reached the highest concentrations in the organs of digestion and elimination (stomach, intestines, liver, and kidneys). The tissue to plasma ratios ranged from approximately 3 to 30 for these tissues. Peak tissue to plasma

ratios for testes, eye lens, bone, brain, and red blood cells were less than 1;

7. The major metabolites of beta-AP were N-acetyl-beta-AP, phenylalanine, and amino acid conjugates of phenylacetic acid. The latter compounds, phenylacetylglutamine in the rat and rabbit, and phenylacetylglutamine in the monkey, were major metabolites following oral administration;
8. Beta-AP was rapidly cleared from the plasma in rats and rabbits following i.v. administration, with an elimination half-life of about 0.5-0.6 hours;
9. Total radioactivity was eliminated very slowly in rats and rabbits with terminal plasma half-lives ranging from 80 to 200 hours. This slowly cleared radioactivity was due to incorporation of [14-C]-phenylalanine into plasma and tissue proteins; and
10. Total radioactivity was excreted primarily in the urine, either as intact beta-AP and N-acetyl-beta-AP following i.v. administration in rats and rabbits or as amino acid conjugates of phenylacetic acid following oral administration in rats, rabbits, and monkeys. Elimination by oxidation to  $^{14}\text{CO}_2$  (expired in breath) was also a significant route of excretion, accounting for 4-6% of the dose in the first 7 hours after dosing in monkeys. The fecal excretion of radioactivity, aside from that resulting from unabsorbed beta-AP, accounted for the elimination of less than 10% of oral doses in rabbits and monkeys.

As a result of metabolism in the gastrointestinal tract or, to a lesser extent, upon hydrolysis in beverages, beta-APM is converted to beta-AP, among other metabolites. Beta-AP belongs to a group of compounds known as beta-dipeptides which occur naturally in both animals and plants. Humans produce beta-dipeptides as part of their normal metabolism. At least 14 beta-aspartyl-dipeptides have been identified in human urine (23-27). Possible sources of beta-dipeptides include dietary protein, products of tissue degradation, non-protein components of meats and plants (28,29), and pharmaceuticals (30). Beta-AP is among a number of beta-aspartyl-dipeptides that have been identified as occurring endogenously in human urine (31). The daily urinary excretion of beta-AP by healthy human subjects who had not consumed aspartame was found to be about 0.8 mg.

The above toxicology, pharmacology, and metabolism studies have confirmed that beta-APM and beta-AP are safe for consumption.



### III. OBJECTIVE

The objective of the study was to determine the pharmacokinetics and metabolism of orally administered [14-C]-beta-aspartame in healthy male subjects.

### IV. MATERIALS AND METHODS

#### A. Test Article

Twelve bottles, each containing  $200 \pm 5$   $\mu$ Ci and  $40 \pm 1$  mg of [14-C]-beta-APM (lot #MRC-332-43-1), were prepared by the Radiochemistry Group, G. D. Searle & Co., Skokie, IL (Appendix I).

The radiolabelled material was dissolved in deionized water prior to administration.

#### B. Clinical Protocol

The complete protocol and amendment are included as Appendix II. The clinical phase of the study was conducted at Quincy Research Center, Kansas City, Missouri, from April to October of 1986. Philip T. Leese, M.D. was the principal investigator.

The study population consisted of six healthy male subjects, ranging in age from 24 to 37 years and weighing from 64.5 to 79.1 kg (Table 1).

Initially, a pilot study, consisting only of Subject #01, was done. The results of the pilot study were evaluated and, based on those results, a second pilot study, consisting only of Subject #02, was done using a dose of [14-C]-beta-APM which had been decreased from 40 mg to 32 mg. Upon preliminary analysis of the results from Subject #02, four more subjects (Subjects #03-06) were enrolled in the study, also receiving a dose of 32 mg (see Amendment to Clinical Protocol, dated July 25, 1986 - Appendix II).

## 1. Clinical Evaluations

Within one week prior to test article administration, the subjects provided a medical history and underwent a physical examination. The following laboratory tests were also done: complete hematology (hemoglobin, hematocrit, RBC, WBC with differential, and platelet count), fasting state blood chemistries (calcium, inorganic phosphorus, creatinine, CPK, uric acid, cholesterol, triglycerides, total protein, albumin, total bilirubin, alkaline phosphatase, LDH, SGOT, SGPT sodium, potassium, carbon dioxide, chloride, glucose, BUN), 12-lead ECG, and urinalysis (pH, specific gravity, acetone, albumin, glucose, WBC/HPF, RBC/HPF, epithelial, and casts).

The above clinical evaluations were repeated on Day 8 following test article administration (Day 1).

## 2. Treatment Period

The subjects reported to the clinical facility on the day before test article administration and remained there during the eight-day study period. Standardized diet and activity were observed during that time. The subjects fasted for at least eight hours prior to and eight hours following test article administration.

Subject #01 ingested approximately 40 mg of [14-C]-beta-APM dissolved in 60 ml of deionized water followed by three 60 ml washes of the vessel for a total of 240 ml of water. The subject ingested another 240 ml of water at 1, 2, and 3 hours after dosing.

Subjects #02-06 ingested approximately 32 mg of [14-C]-beta-APM dissolved in 80 ml of deionized water followed by two 80 ml washes of the vessel (see page 6 of Investigator's Report - Appendix IV). They also ingested 240 ml of water at 1, 2, and 3 hours after dosing.

### 3. Vital Signs and Adverse Experiences

Supine blood pressure and heart rate were measured prior to test article administration (0 hour) and at 4, 8, 12, and 24 hours thereafter.

All adverse experiences, complaints, laboratory test values, and physical examination findings involving the study subjects were recorded.

### 4. Collection of specimens

#### a. Blood Plasma and Red Blood Cells

Subject #01: Heparinized blood samples (10 ml) containing the esterase inhibitor paraoxon (diethyl-p-nitrophenylphosphate) at a final concentration of  $10^{-4}$  M were collected prior to test article ingestion (0 hour) and at 30 minute intervals through 10 hours and at 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours thereafter. A final blood sample for Subject #01 was collected on Day 37. The plasma and red blood cells were separated by centrifugation and stored at  $-20^{\circ}\text{C}$  (plasma) or approximately  $4^{\circ}\text{C}$  (red blood cells) until analyzed.

Subjects #02 - 06 (see Amendment to Clinical Protocol - Appendix II): heparinized blood samples (10 ml) were collected at 0, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 504 (Day 22) hours after test article ingestion. Heparinized blood samples (20 ml) were also collected at 7.5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes after test article ingestion. The plasma and red blood cells were separated and stored as described above.

#### b. Urine

The total volume of urine excreted was collected and measured for each of the following periods: -12 to 0 hours (predose), 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 8, 8 to 12, 12 to 24, and for each of the six subsequent 24-hour periods following test article administration. The urine sample or aliquots therefrom were stored at  $-20^{\circ}\text{C}$  until analyzed.

**c. Feces**

Individual fecal collections were made for the predose interval and for each of the seven 24-hour intervals following test article administration. Each specimen was weighed and stored at -20°C until analyzed.

**C. Analytical Techniques**

All analytical procedures on the test article and plasma, red blood cell, urine, and fecal samples were done by the Department of Drug Metabolism, G. D. Searle & Co., Skokie, IL. The description of the methods used and the results of the analyses are contained in their analytical report which is attached as Appendix III.

**D. Data Analysis**

See Analytical Report (Appendix III)

**V. RESULTS**

See Analytical Report (Appendix III)

**VI. CLINICAL FINDINGS**

The Investigator's Report is included as Appendix IV.

**A. Laboratory Tests**

Laboratory values that fell outside of the normal range are listed in Table 2. The principal investigator rated the following values as clinically significant:

Subject #01 had a Day 8 SGPT value of 63 IU/L. When the subject returned for follow-up testing 30 days later, the SGPT had returned to the normal range (0-50).

Subject #02 had an elevated SGPT value (250 IU/L) and an elevated SGOT value (126 IU/L) on Day 8. The principal investigator rated the relationship of these values to test article administration as unknown. Hepatitis serology was negative. Upon follow-up testing two weeks later, both values had returned to the normal range (0-50 for SGPT and 0-44 for SGOT).

Subject #03 had an elevated CPK level (329 IU/L) on Day 8 (normal range = 44-295). However, the initial baseline value was 411 with a repeat value of 178. The subject failed to respond to requests for follow-up testing and was subsequently lost to follow-up.

Subject #04 had a significantly low platelet count on Day 8 (115,000/mm<sup>3</sup>) (normal range = 172,000-418,000). At baseline the value had also been low (155,000), but the repeat value was 220,000. Upon follow-up testing six weeks later, the count was again low (145,000), but not considered clinically significant by the investigator.

Subject #06 had an elevated eosinophil count (11%) on Day 8 which the investigator rated as clinically significant (normal range = 0-6). At baseline the value had been elevated (12%) with a repeat value of 4%. At the time of follow-up testing five weeks later, the count was normal.

None of the other changes observed in laboratory values were considered to be clinically significant.

#### **B. Vital Signs**

Systolic and diastolic blood pressure and heart rate were recorded prior to and following test article ingestion (Table 3). No clinically significant changes were observed.

#### **C. Physical Examinations and Electrocardiograms**

The investigator did not report any clinically significant changes from baseline upon final physical examination of the subjects. Also, the 12-lead electrocardiograms taken prior to and one week after test article ingestion were normal for all six subjects.

#### **D. Adverse Experiences**

Two of the subjects reported adverse experiences during the 7 day period following test article administration (Table 4). Subject #01 complained of headaches and nightmares and Subject #06 complained of lightheadedness, sweating, upset stomach, and diarrhea. The principal investigator did not consider the adverse experiences clinically significant or related to test article ingestion.



#### E. Protocol Deviations

The protocol inadvertently did not indicate that RBC counts would be done as part of the hematology. These counts were done.

The procedure for preparing the test article for administration to Subjects #02-06 as described in the Protocol Amendment dated July 25, 1986 (Appendix II) was changed slightly. The actual procedure used is described on page 6 of the Investigator's Report (Appendix IV).

The protocol indicated that the plasma and red blood cell fractions from the blood collections would both be stored at -20°C. The plasma was stored at -20°C while the red blood cells were stored at approximately 4°C.

For Subject #01 the plasma and red blood cell fractions were given different specimen numbers. The specimen numbers on CRF #9 refer to the plasma fraction. For Subjects #02-06 the plasma and red blood cell fractions were given the same specimen number.

## VII. DISCUSSION

Oral ingestion of [14-C]-beta-APM (labeled in the phenylalanine portion of the molecule) in man was followed by absorption of about 90% of the radiolabel, as indicated by the recovery of less than 10% of the dose in feces. The inability to detect the intact molecule in plasma and the presence of only minor amounts in urine (less than 0.015% of the dose) indicated that the methyl ester bond was extensively hydrolyzed by intestinal or liver enzymes. However, the presence of beta-AP as a metabolite in plasma and urine indicated a small amount of the compound was absorbed with the beta dipeptide bond intact (6.97% of the dose based on recovery of [14-C]-beta-AP in urine).

Total radioactivity in plasma was eliminated in two phases characterized by half-lives of 6.21 and 587 hours, respectively. The shorter half-life represented the soluble plasma 14-C fraction which included such beta-APM metabolites as free phenylalanine (Phe), beta-AP, and phenylacetylglutamine (PAGln). The longer half-life represented the incorporation of [14-C]-Phe into plasma and tissue protein. Total radioactivity of the red blood cell fraction increased gradually after ingestion of [14-C]-beta-APM. The red blood cell 14-C concentration-time curve was consistent with [14-C]-Phe incorporation into red blood cell protein (32).

The major route of excretion of total radioactivity was the urine (42.0% of the dose). The major urinary metabolite was PAGln (30.8% of the dose during the first 24 hours). PAGln was thought to be the result of bacterial metabolism of beta-AP in the lower gastrointestinal tract with the resultant formation of [14-C]-Phe and [14-C]-phenylacetic acid which is eliminated by conjugation with glutamine. Beta-AP excretion in the urine accounted for 6.97% of the dose during the first 24 hours.

Fecal excretion of total radioactivity was 9.56% of the dose. The only metabolite identified was [14-C]-Phe.

Because this study was designed primarily to determine the pharmacokinetics and metabolism of beta-APM in man and not as a placebo-controlled, crossover, safety study, it is difficult to draw conclusions regarding the out-of-range laboratory values. Most of the out-of-range laboratory values were not clinically significant. Those values that were considered significant did not appear to be related to [14-C]-beta-APM administration. In a recently reported study (33) in which healthy subjects ingested for 6 months an APM dose of 75 mg/kg/day (which contained an amount of beta-APM similar to that administered in the present study), there were no significant changes in clinical results



compared to placebo ingestion. In light of these data, it is not likely that the clinically significant out-of-range laboratory values observed in the present study were related to beta-APM administration. This is not surprising in view of the fact that extensive animal safety studies on beta-APM have not revealed any toxic effects at doses up to 2000 times the estimated maximum dietary consumption of the beta isomers. Furthermore, beta-aspartyl-dipeptides, including beta-AP, have been identified as naturally-occurring components of human urine.

In conclusion, the analytical and clinical results of this study were consistent with the results of previous studies. The metabolism of [14-C]-beta-APM in man was similar to that observed in animals and there were no significant clinical effects related to its administration.

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**IX. TABLES**

Table 1  
Characteristics

<u>Subject Number</u>	<u>Age</u>	<u>Sex</u>	<u>Race</u>	<u>Height (cm)</u>	<u>Weight (kg)</u>	<u>Temp (°C)</u>	<u>Pulse (/min)</u>	<u>Blood Pressure (mmHg)</u>
01	37	M	Caucasian	176.0	69.4	36.6	80	108/80
02	37	M	Caucasian	178.0	72.6	36.0	64	120/78
03	27	M	Negro	168.0	64.5	36.1	76	120/76
04	25	M	Negro	176.0	79.1	36.5	60	130/96
05	24	M	Hispanic	165.0	64.5	36.1	76	116/72
06	24	M	Caucasian	171.0	75.0	35.2	64	110/60
N	6			6	6	6	6	6/6
Mean	29.0			172.3	70.9	36.1	70.0	117.3/77.0
S.D.	6.3			5.2	5.8	0.5	8.3	8.0/11.7
CV, %	21.7			3.0	8.2	1.4	11.9	6.8/15.2

Table 2  
Out-of-Range Laboratory Values

Subject Number	Time <sup>a</sup>	Lab Test	Value	Reference Range	Clinically Significant <sup>b</sup>
<u>Hematology</u>					
01	Baseline	Hematocrit	52.9	39.0 - 51.4 %	No
01	Final	Hematocrit	52.7	39.0 - 51.4 %	No
01	Baseline	Monocytes	5	0 - 3 %	No
03	Baseline	WBC	4.2	4.4 - 11.9 x10 <sup>3</sup> /mm <sup>3</sup>	No
03	Final	Neutrophils	34	36 - 76 %	No
03	Final	Lymphocytes	63	22 - 58 %	No
04	Final	Platelets	115	172 - 418 x10 <sup>3</sup> /mm <sup>3</sup>	Yes <sup>c</sup>
05	Baseline	Hematocrit	51.6	39.0 - 51.4 %	No
06	Final	Eosinophils	11	0 - 6 %	Yes <sup>d</sup>
<u>Clinical Chemistry</u>					
01	Final	SGPT (ALT)	63	0 - 50 IU/L	Yes <sup>e</sup>
01	Baseline	CPK	39	44 - 295 IU/L	No
01	Final	CPK	41	44 - 295 IU/L	No
01	Final	Uric Acid	3.4	3.9 - 8.7 mg/dl	No
01	Final	Sodium	149	136 - 146 mEq/L	No
02	Final	SGOT (AST)	126	0 - 44 IU/L	Yes <sup>f</sup>
02	Final	SGPT (ALT)	250	0 - 50 IU/L	Yes <sup>g</sup>
02	Final	CPK	42	44 - 295 IU/L	No
02	Baseline	Tot. Protein	6.3	6.5 - 8.9 gm/dl	No
02	Baseline	CO <sub>2</sub>	33.3	21 - 32 mEq/L	No
03	Baseline	LDH	198	0 - 185 IU/L	No
03	Final	CPK	329	44 - 295 IU/L	Yes <sup>h</sup>
03	Baseline	Cholesterol	120	124 - 276 mg/dl	No
04	Baseline	Tot. Protein	6.3	6.5 - 8.9 gm/dl	No
04	Baseline	Chloride	109	97 - 108 mEq/L	No
05	Baseline	Tot. Protein	6.0	6.5 - 8.9 gm/dl	No
05	Final	Tot. Protein	5.7	6.5 - 8.9 gm/dl	No
05	Baseline	Calcium	8.8	8.9 - 10.7 mg/dl	No
05	Final	Calcium	8.8	8.9 - 10.7 mg/dl	No
05	Final	Chloride	109	97 - 108 mEq/L	No
06	Baseline	Tot. Protein	6.2	6.5 - 8.9 gm/dl	No
06	Final	Tot. Protein	5.9	6.5 - 8.9 gm/dl	No
06	Baseline	BUN	18	0 - 17 mg/dl	No

(Continued on next page)

Table 2 (continued)

<u>Subject Number</u>	<u>Time</u> <sup>a</sup>	<u>Lab Test</u>	<u>Value</u>	<u>Reference Range</u>	<u>Clinically Significant</u> <sup>b</sup>
<u>Urinalysis</u>					
01	Baseline	Epithelial	Few	Negative	No
01	Final	Epithelial	Occ	Negative	No
02	Baseline	Epithelial	Occ	Negative	No
02	Final	Epithelial	Occ	Negative	No
03	Baseline	Epithelial	Occ	Negative	No
03	Final	Epithelial	Rare	Negative	No
04	Baseline	Epithelial	Occ	Negative	No
04	Final	Epithelial	Occ	Negative	No
05	Baseline	WBC/HPF	20	0 - 15 /HPF	No
05	Final	WBC/HPF	30	0 - 15 /HPF	No
05	Baseline	Epithelial	Occ	Negative	No
05	Final	Epithelial	Rare	Negative	No
06	Baseline	Epithelial	Occ	Negative	No
06	Final	Epithelial	Occ	Negative	No

<sup>a</sup> Baseline = predose; Final = Day 8 (Subjects dosed on Day 1).

<sup>b</sup> Principal Investigator's Opinion.

<sup>c</sup> Follow-up 6 weeks after Final; platelets = 145,000/mm<sup>3</sup>.

<sup>d</sup> Follow-up 5 weeks after Final; eosinophils = 5%.

<sup>e</sup> Follow-up 30 days after Final; SGPT = 10 IU/L.

<sup>f</sup> Follow-up 14 days after Final; SGOT = 18 IU/L.

<sup>g</sup> Follow-up 14 days after Final; SGPT = 21 IU/L.

<sup>h</sup> Subject #03 lost to follow-up.



Table 3  
Vital Signs

Subject Number	HOUR 0		HOUR 4		HOUR 8		HOUR 12		HOUR 24	
	Blood Pressure (mmHg)	Pulse (/min)	Blood Pressure (mmHg)	Pulse (/min)	Blood Pressure (mmHg)	Pulse (/min)	Blood Pressure (mmHg)	Pulse (/min)	Blood Pressure (mmHg)	Pulse (/min)
01	120/70	80	110/70	76	110/74	72	118/70	80	130/76	76
02	124/62	68	120/62	70	120/76	72	122/88	72	120/76	70
03	120/86	80	122/80	72	130/90	72	118/78	76	118/86	68
04	124/78	72	122/76	68	136/80	64	138/76	68	120/86	64
05	110/70	64	116/68	68	130/80	68	118/80	68	120/80	64
06	110/64	60	108/60	56	120/76	60	110/52	64	118/66	76
N	6	6	6	6	6	6	6	6	6	6
Mean	118.0/71.7	70.7	116.3/69.3	68.3	124.3/79.3	68.0	120.7/74.0	71.3	121.0/78.3	69.7
S.D.	6.4/9.0	8.3	6.1/7.8	6.7	9.4/5.8	5.1	9.4/12.3	5.9	4.5/7.5	5.4
CV, %	5.4/12.6	11.7	5.2/11.3	9.8	7.6/7.3	7.5	7.8/16.6	8.3	3.7/9.6	7.7

**Table 4**  
**Adverse Experiences**

Subject Number	Complaint	Dosing Date	Start		Stop		Related to Test Article (a)	Severity
			Date	Time	Date	Time		
01	frontal headache	4/9/86	4/9/86	1510	4/9/86	1700	no	mild
01	headache	4/9/86	4/12/86	1400	4/12/86	1420	no	mild
01	nightmares	4/9/86	4/12/86	0100	4/14/86	0150	no	moderate
01	headache, right side	4/9/86	4/16/86	0220	not available		no	moderate
06	diarrhea	9/23/86	9/30/86	0630	9/30/86	0900	no	moderate
06	lightheaded	9/23/86	9/30/86	0630	9/30/86	0650	no	moderate
06	sweats	9/23/86	9/30/86	0630	9/30/86	0635	no	moderate
06	upset stomach	9/23/86	9/30/86	0630	9/30/86	0650	no	severe
06	upset stomach	9/23/86	9/30/86	0650	9/30/86	0900	no	mild

<sup>a</sup> principal investigator's opinion