

$\beta$ -Aspartame (SC-19129)

Biology Summary

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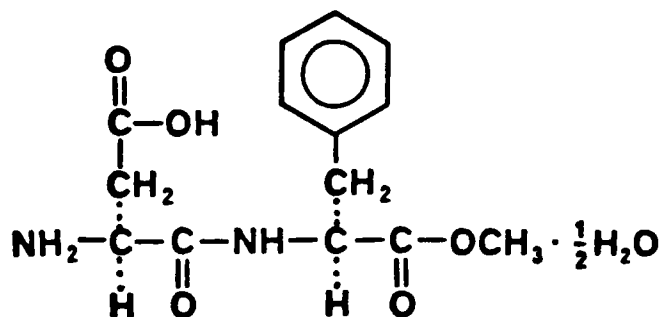
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## I. INTRODUCTION

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) is a known minor constituent of aspartame ( $\alpha$ -APM) (Figure I.1).  $\beta$ -APM is also formed from aspartame under certain storage conditions. A series of studies was undertaken to evaluate the possible effects of  $\beta$ -APM on various organ and pharmacological systems.

Studies were conducted in standardized primary screening tests to determine the potential effects of  $\beta$ -APM on the central nervous system, cardiovascular and renal systems, gastrointestinal system, and additional pharmacological parameters. Where appropriate the methods employed in these studies have been cross-referenced to their Department of Biological Research screening test protocol number.

Figure I.1

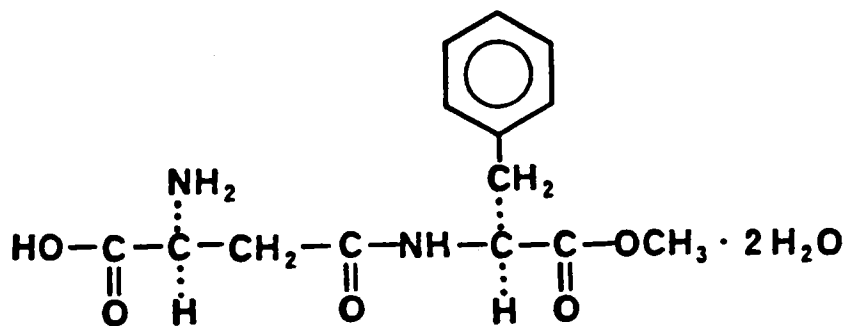


Aspartame

SC-18862·½ H<sub>2</sub>O

"α-APM", "L,L-APM"

N-L-α-aspartyl-L-phenylalanine, 1-methyl ester



beta-aspartame

SC-19129·2H<sub>2</sub>O

"β-APM"

N-L-β-aspartyl-L-phenylalanine, 1-methyl ester

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## II. Evaluation of the Effects of $\beta$ -Aspartame on the Central Nervous System

M.E. Nevins, M.S., S.M. Arnolde, L.M. Prieto

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a series of screening tests to determine potential central nervous system (CNS) effects. These tests included evaluations of general symptomological effects, motor coordination, spontaneous locomotor activity, hexobarbital interactions, analgesic effects, narcotic antagonist effects, and anti- and proconvulsant effects.

In all tests male mice weighing between 20-30 grams (HAM/ICR CD-1; Charles River Breeding Laboratories; Kingston, NY) were used. Mice were housed 10 per cage and had ad libitum access to food and water until time of testing.

For all tests  $\beta$ -APM (SC-19129, lot #CD-134-89A) was dissolved in 2% polypropylene glycol/Tween 80 (v:v; 50/50) and normal saline.  $\beta$ -APM was administered intragastrically (i.g.) at a dose of 60 mg/kg in a volume of 10 ml/kg of body weight. Unless otherwise noted there were 10 mice in each  $\beta$ -APM and vehicle control group except the hexobarbital interaction test in which there were 16 mice per group.

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## **II.A. General Symptomatology in Mice**

Methods: Mice were dosed with either  $\beta$ -APM (60 mg/kg, i.g.) or control vehicle and observed for overt behavioral symptomatology (Figure II.A.1). All mice were observed 0.5, 1, 2 and 24 hours after dosing. Additionally, at each observation interval the animals were placed on a horizontal traction wire and on an inclined plane and evaluated for motor coordination. The mice were given two opportunities to stay on the horizontal traction wire (0.12 in dia.) for 40 s. The mice were also placed in the middle of a smooth plexiglass plane inclined at 30°. Mice that were either unable to remain on the traction wire for 40 s or maintain their positions on the inclined plane for 40 s were considered to exhibit a motor coordination deficit. Details of the symptomatology screen are available in the central nervous system screening files.

Results: No symptomological differences could be distinguished between the vehicle and  $\beta$ -APM. The results of the horizontal traction wire and the inclined plane challenges are summarized in Table II.A.1. No biologically relevant effects were noted.

## **II.B. Effects on Motor Coordination in Mice**

Methods: The effects of  $\beta$ -APM on motor coordination in mice was evaluated using a rotating

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**Figure II.A.1**

Test No. _____	TEST DATA	Tech. _____
SC- _____		Animal _____
N. B. Ref. _____		Date _____ Sex _____
Chemist _____		Vehicle _____ Rte. _____

## TOXICITY AND SYMPTOMATOLOGY

[illegible]

**COMMENTS:**

**Approx: LD - 50:** \_\_\_\_\_

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Table II.A.1

Effects of  $\beta$ -APM on the Horizontal  
Traction Wire and Inclined Plane<sup>a</sup>

Time Post Injection (hr)	<u>Percent of Mice Failing to Meet Criteria</u> <sup>b</sup>			
	<u>Traction Wire</u>		<u>Inclined Plane</u>	
	Control	$\beta$ -APM	Control	$\beta$ -APM
0.5	15	15	0	0
1	25	30	0	0
2	20	20	0	0
24	0	10	0	0

a Mice were treated with  $\beta$ -APM 60 mg/kg, i.g. and evaluated for their ability to stay on a horizontal traction wire and an inclined plane for 40 sec.

b N=20 in all groups.

Searle Data Reference: Symptomatology File/K-221.

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rod test (2cl screening test protocol). Thirty minutes after  $\beta$ -APM or vehicle administration, the mice were placed on a 1 inch diameter wooden rod rotating at 4.4 rpm. The mice were given two opportunities to remain on the rod for 60 s. If 20% or more of the test animals failed to stay on the rod, the compound was considered to have caused a loss of motor coordination.

Results: In two trials, a total of three out of 20 mice (15%) tested failed to remain on the rod for 60 s. One out of 20 controls (5%) failed to remain on the rod for 60 s. This failed to meet the minimum criteria of 20% for activity. Searle Data Reference: CF/2cl/K221.

#### **II.C. Effects on Spontaneous Locomotor Activity in Mice**

Methods: The effects of  $\beta$ -APM on spontaneous locomotor activity in mice was evaluated. The mice were placed into automated activity monitors (Digiscan, Model RXYZCM(16), Omnitech Electronics, Columbus, OH) for a 30 min. baseline period, then administered either  $\beta$ -APM or control vehicle and immediately replaced into the activity monitors for an additional one hour. Activity was monitored continuously by infrared light beams and the data was

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accumulated in 10 min. intervals.

Results: The spontaneous locomotor activity of mice treated with control vehicle or  $\beta$ -APM was evaluated and compared by separately analyzing four parameters of activity: total distance traveled, time spent in movement, number of movements made and the number of stereotypic movements made. Stereotypic movements were defined as any repetitive movement that did not involve locomotion. The dominant behaviors recorded were grooming and head weaving associated with exploratory sniffing. Data for the four activity parameters are summarized in Figures II.C.1-4. Each of the four parameters was analyzed by means of three variables: total area under the curve (Total), maximum effect (Maximum) and time to maximum effect (Tmax) using exact multivariate F-tests with  $df=3,16$ . For distance traveled, movement time and number of movements, no significant overall treatment effects were noted. For the number of stereotypic movements variable a marginal overall significant treatment effect was seen ( $p=.0508$ , exact multivariate F-test with  $df=3,16$ ). This overall effect was determined to be attributed to Tmax ( $p<.01$ , univariate F-test with  $df=1,18$ ) because Total ( $p=0.48$ ) and Maximum ( $p=0.86$ ) did not contribute to the overall effect. The analysis is summarized in Table II.C.1.

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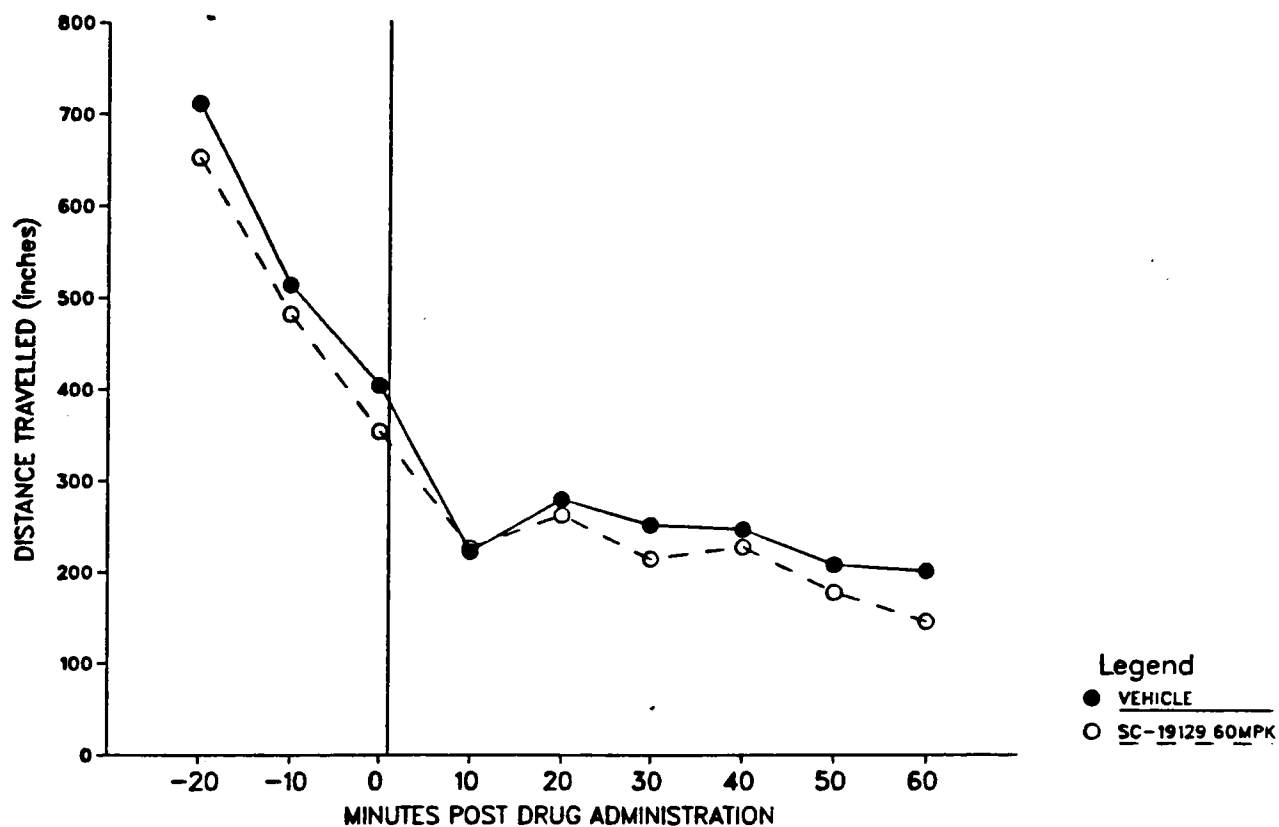
In summary, it was concluded there were no biologically relevant adverse effects in spontaneous locomotor activity in mice after  $\beta$ -APM administration.

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Figure II.C.1

Effects of  $\beta$ -APM (SC-19129) on  
Spontaneous Locomotor Activity in Mice  
(Mean Distance Traveled)

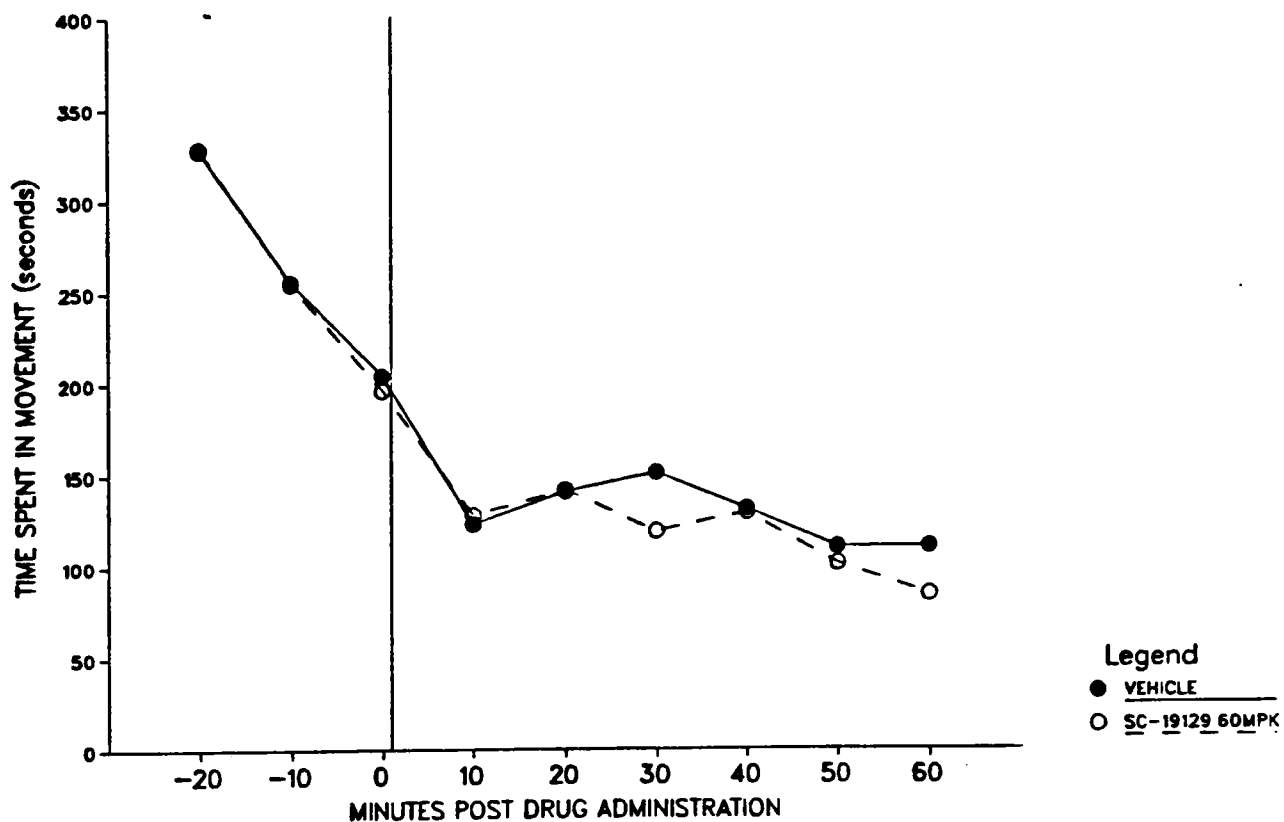


Searle Data Reference: Symptomatology File/K221

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Figure II.C.2

Effect of  $\beta$ -APM (SC-19129) on  
Spontaneous Locomotor Activity in Mice  
(Mean Time Spent in Movement)

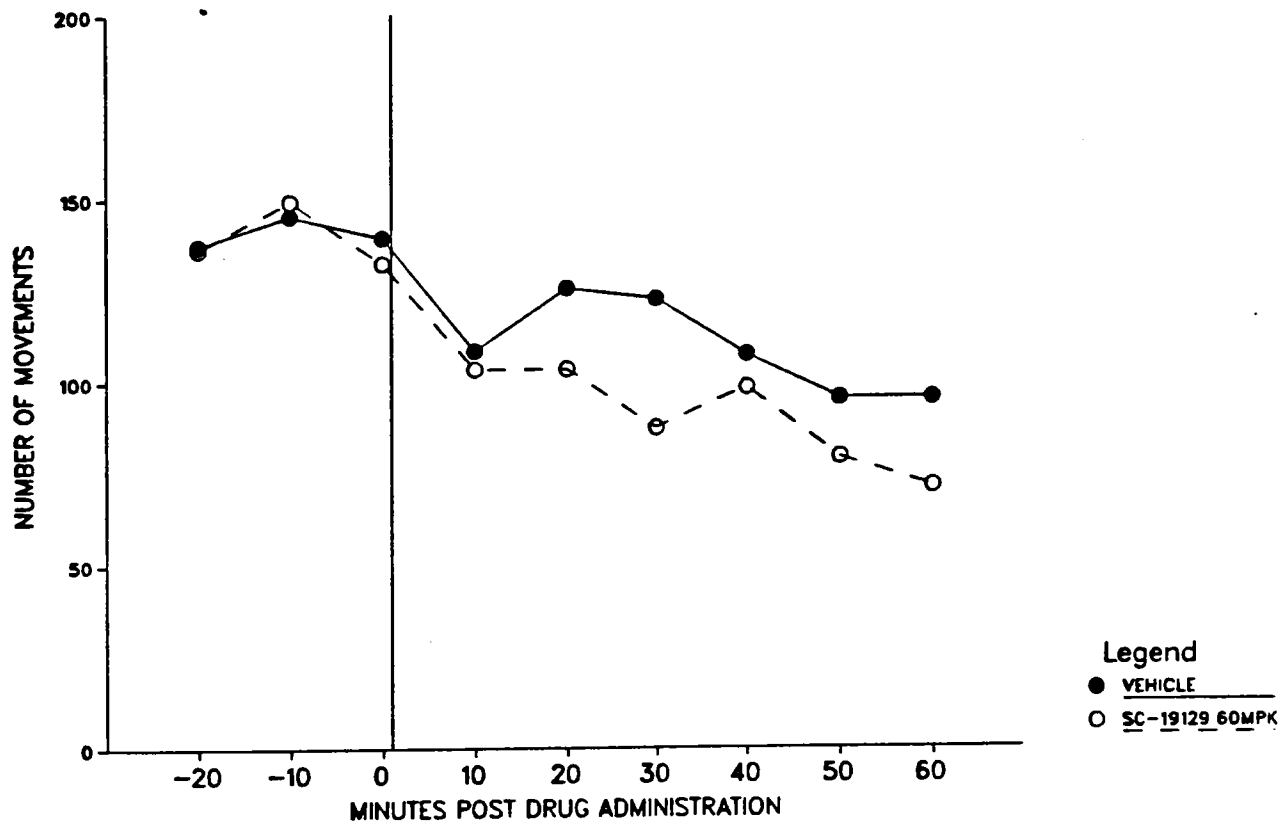


Searle Data Reference: Symptomatology File/K221

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Figure II.C.3

Effect of  $\beta$ -APM (SC-19129) on  
Spontaneous Locomotor Activity in Mice  
(Mean Number of Movements)



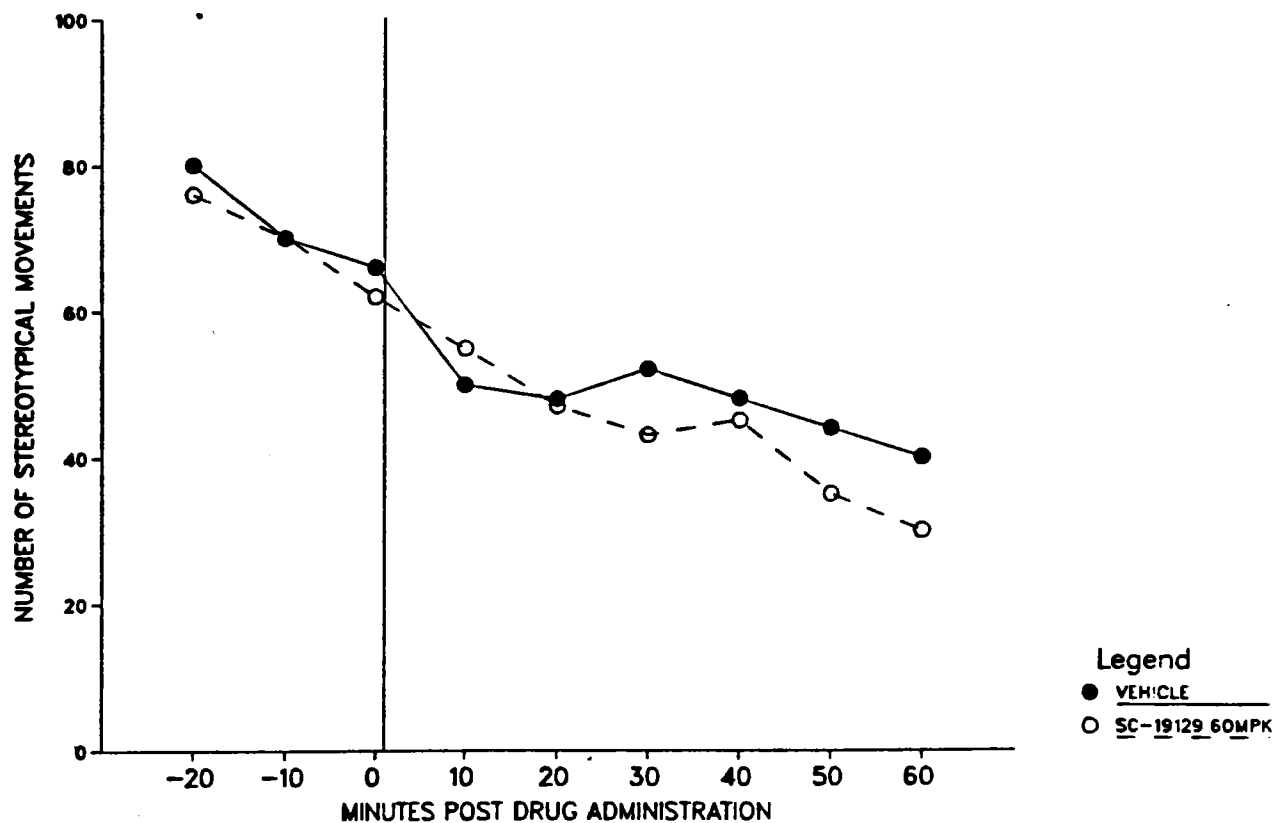
Searle Data Reference: Symptomatology File/K221

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Figure II.C.4

Effects of  $\beta$ -APM (SC-19129) on  
Spontaneous Locomotor Activity in Mice  
(Mean Number of Stereotypic Movements)



Searle Data Reference: Symptomatology File/K221

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Table II.C.1

Effect of  $\beta$ -APM on  
Spontaneous Locomotor Activity in Mice<sup>a</sup>

Variable	<u>Distance (inches) Traveled</u>		<u>(Mean + S.E.)<sup>b</sup></u>	
	Control		$\beta$ -APM <sup>d</sup>	
Total <sup>e</sup>	1457	+ 227	1251	+ 344
Maximum <sup>f</sup>	344	+ 45.4	305	+ 60.7
Time to Maximum <sup>g</sup>	34.0	+ 5.0	30.0	+ 4.9
-----				
Variable	<u>Number of Movements</u>		<u>(Mean + S.E.)<sup>b</sup></u>	
	Control		$\beta$ -APM <sup>d</sup>	
Total (log) <sup>e</sup>	6.5	+ 0.17	6.3	+ 0.27
Maximum (log) <sup>f</sup>	5.1	+ 0.13	5.0	+ 0.18
Time to Maximum <sup>g</sup>	30.0	+ 4.9	35.0	+ 4.3
-----				
Variable	<u>Number of Movements</u>		<u>(Mean + S.E.)<sup>b</sup></u>	
	Control		$\beta$ -APM <sup>d</sup>	
Total (sq. root) <sup>e</sup>	25.1	+ 1.5	22.7	+ 1.8
Maximum (sq. root) <sup>f</sup>	11.6	+ 0.53	11.1	+ 0.44
Time to Maximum <sup>g</sup>	34.0	+ 3.4	31.0	+ 5.7
-----				
Variable	<u>Number of Stereotypy</u>		<u>(Mean + S.E.)<sup>b,c</sup></u>	
	Control		$\beta$ -APM	
Total (sq. root) <sup>e</sup>	16.6	+ 0.84	15.4	+ 1.4
Maximum (sq. root) <sup>f</sup>	7.7	+ 0.29	7.6	+ 0.45
Time to Maximum <sup>g</sup>	38.0	+ 4.9	18.0	+ 3.6 <sup>h</sup>

a Mice were treated with  $\beta$ -APM, 60 mg/kg, i.g. and placed into an automated activity monitor. Locomotor activity was evaluated for one hour in 10 minute intervals.

b N=10 in all groups.

c Marginal treatment effect,  $p=0.0508$  (multivariate F-test,  $df=3,16$ ).

d 60 mg/kg, i.g.

e Total = total area under the curve.

f Maximum = maximum effect.

g Time to Maximum (Tmax) = time to maximum effect.

h Significant treatment effect,  $p<0.01$  (univariate F-test,  $df=1,18$ ).

Searle Data Reference: Symptomatology File/K221

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#### **II.D. Hexobarbital Interactions in Mice**

Methods:  $\beta$ -APM was tested for possible interactions with hexobarbital (2c1A screening test protocol). Thirty minutes after administration of either vehicle control or  $\beta$ -APM the mice were administered hexobarbital intraperitoneally, 100 mg/kg, and duration of sleep was measured. Sleep time was defined as the duration of time between loss of the righting reflex and the resumption of righting ability.

Results:  $\beta$ -APM did not exhibit any biologically relevant interactions on hexobarbital induced sleep time in mice (Table II.D.1).

#### **II.E. Analgesic Effects in Mice**

Methods: Potential analgesic effects were evaluated in two procedures, the hot plate test and the writhing test. In the hot plate procedure (3a2A screening test protocol) mice were placed on a hot plate (Model 35-D, IITC, Inc., Landing, NJ) on which the temperature was controlled at  $55 \pm 0.3^{\circ}\text{C}$ . The latency to lick a hind paw was measured. A maximum of 40 s on the hot plate was allowed so that tissue damage would be avoided. Reaction times on the hot plate were measured once prior to  $\beta$ -APM or vehicle control administration and at 10, 30, 60 and 90 minutes after compound administration. In the writhing

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Table II.D.1  
Hexobarbital Interactions in Mice<sup>a,b</sup>

Duration of Sleeptime ( <u>±</u> S.E.) <sup>c</sup> (minutes)	
Control	β-APM
30.9 <u>±</u> 1.5	28.4 <u>±</u> 1.5

<sup>a</sup> Mice were treated with β-APM, 60 mg/kg, i.g. 30 min. after administration of either vehicle control or hexobarbital, 100 mg/kg, i.p.

<sup>b</sup> n=16/group.

<sup>c</sup> No significant differences as measured by Student's t-test (p>0.05).

Searle Data Reference: CF/2a1A/K221

procedure (3allA screening test protocol) analgesia was evaluated by the antagonism of phenylbenzoquinone (PBQ) induced writhing. Thirty minutes after administration of  $\beta$ -APM or vehicle control, the mice were given 0.025% (w/v) PBQ intraperitoneally in a volume of 10 ml/kg body weight. Five minutes later each mouse was isolated in a large glass beaker and the number of writhes that occurred in the subsequent 10 min. was counted. A writhe consisted of dorsoflexion of the back, extension of the hind limbs, and a strong contraction of the abdominal musculature. The criterion for activity is a reduction in the number of writhes to  $\leq$  one half the median number of writhes observed in the concurrent control group. A compound is considered to have produced analgesia if 50% or more of the mice achieve this criterion.

Results: No significant analgesic activity was found for  $\beta$ -APM on the hot plate test (Table II.E.1). In the PBQ writhing test there was a significant reduction in writhing in only 20% of the mice;  $\beta$ -APM was therefore determined to have no effect on PBQ induced writhing. (Searle Data Reference: CF/3all/K219.)

## **II.F. Narcotic Antagonism Effects in Mice**

Methods: Narcotic antagonist activity was evaluated by the antagonism of morphine-induced

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Table II.E.1

Evaluation of the Analgesic Effects of  $\beta$ -APM  
on the Hot Plate Test<sup>a</sup>

Time Post Compound Administration (min)	Mean Time to Hind Paw Lick <sup>b,c</sup> (seconds)	
	Control	$\beta$ -APM
10	10.3	8.8
30	10.0	11.1
60	10.9	8.6
90	11.7	9.7

a Mice were treated with vehicle control or  $\beta$ -APM, 60 mg/kg, i.g. and placed on a 55°C hot plate. The time for the animal to exhibit a hind paw lick was measured.

b Comparisons made by a Mann-Whitney U test for each time interval. No significant differences noted ( $p>0.05$ ).

c  $n=10$ /group.

Searle Data Reference: CF/3a2A/K221

analgesia on the hot plate (3a8A screening test protocol). Fifteen minutes after  $\beta$ -APM or vehicle control administration, mice were administered morphine sulfate, 20 mg/kg, subcutaneously. The mice were then tested for analgesic response as measured by the latency to hind paw lick after being placed on a 55°C hot plate. A maximum of 40 s on the hot plate was allowed to avoid tissue damage. Latencies were measured 10,30,60 and 90 min. after morphine administration.

Results:  $\beta$ -APM was found to be devoid of any narcotic antagonist activity in mice (Table II.F.1).

## **II.G. Anticonvulsant Effects in Mice**

Methods: Anticonvulsant activity was evaluated in three challenges. Thirty minutes after either  $\beta$ -APM or vehicle control administration mice were administered one of the following convulsants: pentylenetetrazol (PTZ), 35 mg/kg intravenously (2c2B screening test protocol), maximal electroconvulsive shock (ECS) applied transcorneally (2c6A screening test protocol), or minimal ECS applied transcorneally. In the maximal ECS test, 50 mA of current were applied for 0.2 s duration through electrodes applied to the cornea. The ability to block the tonic extension portion of the seizure was evaluated. In the minimal

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Table II.F.1

Narcotic Antagonism of  $\beta$ -APM in Mice<sup>a</sup>

Time Post Morphine Administration (min)	Mean Latency to Hind Paw Lick <sup>b,c</sup> (seconds)	
	Control	$\beta$ -APM
10	40.0	40.0
30	40.0	36.4
60	40.0	36.2
90	32.7	34.7

a Mice were administered either vehicle control or  $\beta$ -APM, 60 mg/kg, i.g. 15 min. prior to Morphine SO<sub>4</sub> administration, 20 mg/kg s.c. The time for the animal to exhibit a hind paw lick was measured.

b Response time comparisons were made by the means of a Mann-Whitney U test for each time interval. A 40 s cut-off time was imposed to avoid tissue damage to the mice. No significant differences noted ( $p < 0.05$ ).

c n=10/group.

Searle Data Reference: CF/3a8A/K221

ECS test, 7.5 mA were delivered for 0.2 s and blockade of the clonic portion of the seizure was evaluated. In the antagonism of PTZ-induced convulsions, as with minimal ECS convulsions, blockade of the clonic component of the seizure was evaluated.

Results:  $\beta$ -APM was found to be devoid of any anticonvulsant activity.  $\beta$ -APM treatment did not block any clonic convulsions induced by PTZ; it blocked clonic convulsions induced by minimal ECS in only 1 of 10 mice; and it did not block any tonic convulsions induced by maximal ECS. Blockade of convulsions in a minimum of 20% of the mice is required for a compound to be rated active in any of these tests. (Searle Data Reference: CF/2c1/K221, CF/2c6A/K221, CF/2c2B/K221).

#### **II.H. Proconvulsant Effects in Mice**

Methods:  $\beta$ -APM was evaluated for possible proconvulsant activity by testing for potentiation of pentylenetetrazol (PTZ)-induced clonic convulsions. Thirty min. after  $\beta$ -APM or vehicle control administration, the mice were given a subconvulsant dose (20 mg/kg, i.v.) of PTZ. If the combination of test compound and 20 mg/kg of PTZ produced clonic convulsions in 20% or more of the animals tested within 2 min., the test compound would be considered to have proconvulsant activity.

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Results:  $\beta$ -APM was tested for proconvulsant activity by testing for potentiation of PTZ-induced convulsions. One of ten  $\beta$ -APM treated mice convulsed, while none of the control vehicle treated mice convulsed. To determine if one convulsion was indicative of proconvulsant activity or was a result of random variation, the same dose (60 mg/kg) was repeated with 2 additional doses (30 and 90 mg/kg) in 10 animals each. In this follow-up test, none of the control vehicle mice convulsed. Of the  $\beta$ -APM treated mice, 1/10 convulsed at 30 mpk, 1/10 convulsed at 60 mpk, and 0/10 convulsed at 90 mpk. Because the minimum criterion for proconvulsant activity is 20% convulsions and because no dose response increase in convulsive activity was noted,  $\beta$ -APM was concluded to be devoid of proconvulsant activity. (Searle Data Reference: RB 2039)

#### II.I. Summary of Central Nervous System Effects

$\beta$ -APM was evaluated in a battery of screening tests to determine potential central nervous system effects. The tests included evaluations of general symptomatology, effects on motor coordination, locomotor activity, hexabarbital interactions, analgesic effects, narcotic antagonism effects, anticonvulsant effects and proconvulsant effects. It was concluded from these results that  $\beta$ -APM did not have any biologically relevant effects in these tests.

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### **III. Evaluation of the Effects of $\beta$ -Aspartame on the Gastrointestinal System**

R.G. Bianchi, B.S., P. Kellar and R.F. Bauer, Ph.D.

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was tested for effects on gastric secretion and the transit time of a charcoal test meal in the rat to evaluate its potential effects on the gastrointestinal system.

#### **III.A. Effects on Gastric Secretion in the Pyloric Ligated Rat Model**

Methods: Male Charles River rats [CrI: COBS, CD(SD)BR], 220-240 g body weight, were food deprived for 48 h prior to testing with drinking water available ad libitum. The rats were anesthetized with methoxyflurane and subjected to pyloric ligation. Following ligation  $\beta$ -APM, prepared in distilled water, was administered intraduodenally at a dose of 60 mg/kg in a 1.0 ml/kg volume. Control animals received distilled water. The abdominal incision was closed with wound clips and collodion applied to the area. Groups (n=6) of treated and control rats were sacrificed after 2 h and 4 h by CO<sub>2</sub> asphyxiation. The stomachs were removed intact, cut open, and the gastric contents collected and centrifuged. The volume of the contents was measured. The acid concentration of samples was determined by titrating a 0.2 ml volume of gastric juice with 0.1N NaOH to pH

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7.0. The mean  $\pm$  S.E. gastric secretion volumes, acid concentrations, and acid outputs were calculated for both treated and control groups. Statistically significant differences ( $p \leq 0.05$ ) between control and treated rats were determined by Student's t test for unpaired data.

Results: In the 2 h pyloric ligated rat test  $\beta$ -APM administered intraduodenally at 60 mg/kg did not significantly stimulate volume of gastric secretion (control 2.7 ml vs  $\beta$ -APM 2.7 ml) or affect acid concentration (control 92.1 mEq/L vs 97.2 mEq/L) (Table III.A.1).

In the 4 h pyloric ligated rat test (Table III.A.1),  $\beta$ -APM at 60 mg/kg did not significantly inhibit volume of gastric secretion (control 6.1 ml vs  $\beta$ -APM 6.8 ml) or affect acid concentration (control 120 mEq/L vs 114.7 mEq/L).

### **III.B. Effects on Rat Charcoal Meal Transit Time in Rats**

Methods: Male Charles River rats [CrI: COBS, CD, (SD)BR], 190-210 g body weight, were food deprived for 24 h prior to testing with drinking water available ad libitum.  $\beta$ -APM, prepared in distilled H<sub>2</sub>O, was administered intragastrically (60 mg/kg in a 10 ml/kg volume) one hour prior to the charcoal

Table III.A.1

Effect of  $\beta$ -Aspartame on Gastric Secretion  
in the Pyloric-ligated Rats<sup>a</sup>

Treatment	Sacrifice Time (hr)	Dose (mg/kg) (i.d.)	N	Volume <sup>b</sup> (ml/2 hr)	H <sup>+</sup> Conc. <sup>b</sup> (mEq/L)
Control	2	--	6	2.7 $\pm$ 0.6	92.1 $\pm$ 9.1
$\beta$ -APM	2	60	6	2.7 $\pm$ 0.5	97.2 $\pm$ 7.0
Control	4	--	6	6.1 $\pm$ 0.6	120.0 $\pm$ 8.5
$\beta$ -APM	4	60	6	6.8 $\pm$ 0.9	114.7 $\pm$ 6.8

a Groups of rats received vehicle or  $\beta$ -APM and sacrificed either 2 or 4 h later.

b The volume and acid concentration of the gastric juice were measured and the mean  $\pm$  SEM values calculated. The values obtained with  $\beta$ -Aspartame were not statistically different from control values (Student's t test,  $p > 0.05$ ).

Searle Data Reference: RB 2635/4,5

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test meal. Control animals received distilled H<sub>2</sub>O. All rats received, intragastrically, a mixture of 5% charcoal in a 1% aqueous methylcellulose solution at a volume of 10 ml/kg. Groups (n=6) of treated and control rats were sacrificed by CO<sub>2</sub> asphyxiation 30 min. and 60 min. after administration of the test meal. The total length of the small intestine and the distance traversed by the charcoal meal was measured. The mean differences of the length of intestine and the traversed length between control and treated groups was statistically analyzed using Student's t test.

Results: In the 30 min. rat charcoal meal test  $\beta$ -APM administered intragastrically at 60 mg/kg did not significantly stimulate the passage of a test meal through the small intestinal tract (control 69.0% vs  $\beta$ -APM 69.9%) (Table III.B.1).

In the 1 h rat charcoal meal test (Table III.B.1)  $\beta$ -APM at 60 mg/kg did not significantly inhibit the passage of a test meal through the small intestinal tract (control 86.2% vs  $\beta$ -APM 87.1%).

### III.C. Summary of Gastrointestinal Effects

$\beta$ -APM was evaluated for effects on the gastrointestinal system by measuring gastric secretion in pyloric ligated rats and charcoal meal transit time in rats. No significant differences were noted as compared to vehicle controls.

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Table III.B.1

Effect of  $\beta$ -Aspartame on Gastrointestinal  
Transit of a Test Meal<sup>a</sup>

Treatment	Sacrifice Time (min.)	Dose (mg/kg) (i.g.)	N	Mean Percentage <sup>b</sup> Distance Traversed
Control	30	--	6	69.0
$\beta$ -APM	30	60	6	69.9
Control	60	--	6	86.2
$\beta$ -APM	60	60	6	87.1

a Groups of rats received vehicle or  $\beta$ -APM followed by administration of a charcoal test meal. The groups were sacrificed either 30 or 60 min. after the gastric instillation of the test meal.

b The distanced traveled by the test meal was measured. The values obtained with  $\beta$ -APM were not statistically different from control values (Student's t test,  $p>0.05$ ).

Searle Data Reference: RB 2539/78,79

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#### **IV. Evaluation of the Effects of $\beta$ -Aspartame on the Cardiovascular and Renal Systems**

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$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a series of tests to determine potential effects on the cardiovascular and renal systems. These tests included evaluation of autonomic function in conscious rats, hemodynamic effects in the normotensive dog, evaluation of renal function in unanesthetized dogs, effects on the inhibition of angiotensin I converting enzyme, and effects on the in vitro inhibition of human renin.

##### **IV.A. Effects on Autonomic Cardiovascular Function in Conscious Rats**

Experimental Methods:  $\beta$ -APM was evaluated to determine its potential effects on cardiovascular autonomic function in conscious rats. A series of challenge agonists were administered to conscious rats while blood pressure and heart rate were recorded. Arterial pressure and heart rate responses to challenges were compared before and after administration of  $\beta$ -APM or placebo. The direct effects of  $\beta$ -APM on baseline values of arterial pressure and heart rate were also evaluated and

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compared with those of the placebo treatment.

Male Sprague-Dawley rats (350-450 g) from Charles River were used in this study. Rats anesthetized with ether were instrumented with cannulae in both the right carotid artery and external jugular vein. These cannulae were filled with heparinized saline and externalized at the back of the neck. A rodent jacket (Alice King Chatham Medical Arts, Los Angeles, CA) with a swivel (Spaulding Medical Products, Los Angeles, CA) connected to the arterial cannula was employed to protect both cannulae and leave the rats unrestrained in individual cages. The rats were allowed at least one day to recover from the surgery.

The arterial cannula was used for measuring arterial pressure (mmHg) while the venous cannula was used for intravenous (i.v.) administration of challenge agonists. The carotid arterial catheter was connected to a pressure transducer (Statham P23Db, Gould Statham Instruments Inc., Puerto Rico) to measure pulsatile blood pressure. Patency of the arterial cannula was maintained throughout the entire period of experiment with a continuous infusion of heparinized saline (20 units/ml) at a rate of 5  $\mu$ l/min. into the arterial catheter system via a T-tube. Heart rate (beats/min.) was determined by counting the number of arterial pressure pulse waves

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in a given time period. Blood pressure was recorded on a Gould 480 recording system.

$\beta$ -APM was dissolved in distilled water. A total dose of 60 mg/kg of  $\beta$ -APM was prepared as a solution to allow administration of 5 ml per kg body weight.  $\beta$ -APM was administered intragastrically (i.g.) while placebo-treated animals received a total volume of 5 ml/kg of distilled water.

Twelve conscious rats were used. Each rat was randomly assigned to one of two treatment groups (placebo or  $\beta$ -APM) of 6 rats each. Challenge agonist solutions, prepared fresh daily in 0.9% saline, were administered as bolus injections of 0.2 ml/kg into the jugular vein, and the doses of agonists were expressed in terms of their salts. Each rat was challenged, in a fixed sequence order of agonists, before and 4 h after i.g. administration of either  $\beta$ -APM or placebo. The 4 h time interval between test agent treatment and the beginning of post-treatment challenges was chosen for pharmacokinetic reasons. A pharmacokinetic study of  $\beta$ -APM by Ajinomoto Co., Japan, indicated that in the conscious rat after oral administration of [ $^{14}$ C]- $\beta$ -APM the plasma concentration of radioactivity increased slowly, reaching a sustained peak level at 4 h.

All 12 rats received the same sequence of

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challenges. Changes in mean arterial pressure (MAP, mmHg) were measured as blood pressure responses to all challenge agonists except isoproterenol where changes in diastolic blood pressure (DBP) were measured as the response. HR response was determined only for the isoproterenol challenge. MAP response was obtained by electronic averaging of the pulsatile arterial pressure. The autonomic agonists and doses used are listed below in the sequence of administration:

Phenylephrine hydrochloride	5 µg/kg, i.v.
Methacholine chloride	5 µg/kg, i.v.
Histamine dihydrochloride	20 µg/kg, i.v.
Dimethylphenylpiperazinium iodide (DMPP)	60 µg/kg, i.v.
Isoproterenol hydrochloride	0.5 µg/kg, i.v.

Baseline MAP (or DBP in the case of the isoproterenol challenge) and HR (to isoproterenol only) were determined immediately before each autonomic challenge. MAP and HR during autonomic challenges were measured at the point of maximum response, and were allowed to stabilize at or near baseline levels before subsequent challenges were given. After the first set of challenges was completed, the rats were treated i.g. with either  $\beta$ -APM 60 mg/kg or placebo. Four h later, the challenges were repeated at the same doses and in the same order as the pre-treatment challenges. MAP

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and HR were again measured at baseline and at the point of maximum response to these post-treatment challenges. A time period of approximately 60-80 min. was required to test all challenges in each sequence indicated above.

Statistical Methods: Statistical analyses were designed to test whether rats treated with  $\beta$ -APM differed significantly from placebo-treated animals in their responses to any of the five challenges and in their baseline values of MAP and HR.

Since the response to a challenge correlated with pre-challenge baseline values, a response metameter that would adjust for individual rat's differences in baseline was required (see Table IV.A.1). In order to determine an appropriate metameter, the dependence of post-challenge responses on pre-challenge baselines was studied using the pre-treatment data of both the placebo and  $\beta$ -APM groups. Based on results of these analyses, an adjusted percentage change was computed for each challenge response.

For each challenge, a separate analysis of variance was performed on the adjusted percentage changes post-treatment across the two different groups in order to evaluate whether the test compound significantly altered autonomic cardiovascular function relative to the placebo effect.

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Table IV.A.1

Parameter Estimates<sup>a</sup> using Pooled Pretreatment Data  
of Mean Arterial Pressure (MAP) and Heart Rate (HR)

Challenge	Variable	Parameter Estimates $\beta_y$	Sample Size $n^b$
Phenylephrine	MAP	0.516	11
Methacholine	MAP	0.893	11
Histamine	MAP	0.253	12
DMPP	MAP	0.380	12
Isoproterenol	MAP	0.493	11
	HR	0.584	12

<sup>a</sup> Estimates for  $\beta_y$  are obtained from linear regression on the pooled data set.

<sup>b</sup> Sample sizes vary due to the detection of outliers by using Cook's distance.

In order to determine the direct effects of the test compound on baseline values of MAP and HR, algebraic differences between pre- and post-treatment baseline measurements, obtained just prior to each challenge, were computed for each animal in both the vehicle and  $\beta$ -APM groups. An analysis of variance was performed on the baseline differences including an effect due to treatment and due to sequence of challenge administration in order to test whether these differences were significant between the placebo and  $\beta$ -APM groups, whether the differences were significantly different from zero, and if there were any time trends. The 5% level of significance was used for all analyses.

Results: Table IV.A.2 summarizes the results of MAP responses (expressed as adjusted percent changes) to autonomic challenges in the conscious rat pre- and post-treatment with placebo (5 ml/kg distilled water) or  $\beta$ -APM (60 mg/kg, i.g.). No significant differences in MAP responses were found between the placebo and  $\beta$ -APM treated groups for the challenges with phenylephrine, methacholine, histamine, DMPP and isoproterenol.

Table IV.A.3 shows the results of HR responses (expressed as adjusted percent change) to isoproterenol challenge before and after i.g. administration of

Table IV.A.2

Effect of Placebo<sup>a</sup> and  $\beta$ -APM (60 mg/kg, i.g.) on Mean Arterial Blood Pressure Responses to Autonomic Challenge in Conscious Rats

Average <sup>b</sup> (S.E.) <sup>c</sup> of Mean Arterial Blood Pressure Response Values <sup>d</sup>						
Challenge	Placebo Vehicle Treatment n=6		Beta-aspartame Treatment n=6		F-ratio <sup>e</sup>	p-value
	Pre-	Post-	Pre-	Post-		
Phenylephrine 5 $\mu$ g/kg, iv	30.4 (2.0)	23.4 (3.5)	36.1 (1.1)	31.4 (1.9)	4.21	0.07
Methacholine 5 $\mu$ g/kg, iv	-29.1 (3.3)	-28.8 (2.0)	-31.4 (1.7)	-34.7 (3.3)	2.31	0.16
Histamine 20 $\mu$ g/kg, iv	-20.5 (4.2)	-24.1 (4.6)	-22.3 (1.6)	-25.8 (2.5)	0.09	0.76
DMPP 60 $\mu$ g/kg, iv	28.3 (4.6)	29.0 (2.3)	34.0 (1.6)	31.7 (6.0)	0.18	0.68
Isoproterenol 0.5 $\mu$ g/kg, iv	-34.9 (4.5)	-36.8 (2.0)	-39.2 (1.5)	-39.0 (1.2)	0.84	0.38

a Distilled water 5 ml/kg, i.g.

b mm/Hg.

c Standard error of the mean.

d Expressed as adjusted percent change from baseline value.

e F-test for difference in post-treatment values across the two treatment groups with d.f.=(1,10)

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Table IV.A.3

Effect of Placebo<sup>a</sup> and Beta-Aspartame (60 mg/kg, i.g.)  
on Heart Rate Responses to Isoproterenol  
in Conscious Rats

Average <sup>b</sup> (S.E.) <sup>c</sup> of Heart Rate Response Values <sup>d</sup>						
Challenge	Placebo Vehicle Treatment n=6		Beta-Aspartame Treatment n=6		F-ratio <sup>e</sup>	p-value
	Pre-	Post-	Pre-	Post-		
Isoproterenol 0.5 µg/kg, iv	28.8 (4.5)	31.2 (3.3)	39.2 (2.4)	38.6 (3.1)	2.64	0.14

a Distilled water 5 ml/kg, i.g.

b Beats/min.

c Standard error of the mean.

d Expressed as adjusted percent change from baseline.

e F-test for difference in post-treatment values across the two  
treatment groups with d.f.=(1,10)

Searle Data Reference: 2563/39-44

placebo or  $\beta$ -APM. No statistical significance was found for the difference in post-treatment values of HR responses between the two treatment groups.

The resting baseline values obtained prior to administration of each agonist in both the placebo and  $\beta$ -APM groups are presented in Table IV.A.4.  $\beta$ -APM treatment had no statistically significant effect on the overall mean baseline values of either MAP or HR. Placebo treatment decreased the overall mean basal MAP ( $113.9 \pm 2.3$  mmHg) by 8.3 mmHg (7.3%) and increased the basal HR ( $380.0 \pm 20.7$  beats/min.) by 35 beats/min. (9%). These changes in the overall baseline MAP and HR after placebo treatment differed significantly from those observed after  $\beta$ -APM treatment ( $p < 0.05$  and  $p < 0.01$  in the MAP and HR baselines, respectively). During the measurement of each of the five challenge responses, the pre- and post-treatment differences in baseline MAP were similar in the placebo and  $\beta$ -APM groups, and there were no significant time trends for these differences within each treatment group.

Discussion:  $\beta$ -APM was evaluated for its effects on cardio-vascular autonomic function in the conscious rat. A series of i.v. challenges with phenylephrine, methacholine, histamine, DMPP and isoproterenol was administered before and after i.g.

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Table IV.A.4

Baseline Values of Mean Arterial Pressure (MAP, mmHg)  
and Heart Rate (HR, beats/min) Obtained Prior to Autonomic  
Challenges in Conscious Rats Pre- and Post-treatment  
with Placebo or Beta-Aspartame

Challenge Variable	Baseline Values (Mean + S.E.) Placebo (5 ml/kg Distilled Water) n=6			F-Ratio <sup>a</sup> p-value Between Group Difference
	Pre-Treatment	Post-Treatment	Difference between Pre- and Post- Treatment	
Phenylephrine	MAP 117.2(4.5)	105.2(3.4)	-12.0(3.4)	0.10
Methacholine	MAP 121.0(3.5)	110.5(3.9)	-10.5(4.1)	0.07
Histamine	MAP 116.7(3.9)	108.5(3.7)	- 8.2(3.6)	0.78
DMPP	MAP 114.0(4.7)	108.8(5.1)	- 5.2(6.4)	0.41
Isoproterenol	MAP 100.5(5.3)	94.7(3.6)	- 5.8(6.4)	0.36
	HR 380.0(20.7)	415.0(26.3)	35.0(10.6)	0.007

Beta-Aspartame (60 mg/kg, i.g.)  
n=6

	Pre- Treatment	Post- Treatment	Difference between Pre- and Post- Treatment	
Phenylephrine	MAP 117.3(3.5)	114.3(4.0)	-3.0(3.0)	
Methacholine	MAP 122.7(3.5)	122.3(3.1)	-0.3(2.0)	
Histamine	MAP 121.3(2.6)	114.7(3.5)	-6.7(1.8)	
DMPP	MAP 114.7(2.8)	114.0(3.9)	-0.7(1.8)	
Isoproterenol	MAP 99.0(3.6)	98.2(4.4)	-0.8(2.2)	
	HR 351.7(11.4)	343.3(13.8)	-8.3(7.5)	

Test for linear trend for baseline MAP: placebo group,  $p=.15$   
F-test, d.f.=1,50; beta-aspartame group,  $p=0.74$  F-test, d.f.=1,50  
Overall mean decrease in the baseline MAP: placebo group= $8.3 \pm 2.1$  mmHg,  
 $p<0.001$ ; beta-aspartame group= $2.3 \pm 1.0$  mmHg,  $p=0.19$ ; placebo vs  
beta-aspartame group,  $p=0.02$  F-test, d.f.=1,50

<sup>a</sup> F-test for difference in the pre- and post-treatment baseline  
across the two treatment groups with d.f.=1,10

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treatment with  $\beta$ -APM or placebo. The direct effects of  $\beta$ -APM on baseline values of MAP and HR were also evaluated and compared with those of placebo treatment.

No significant differences in MAP responses (expressed as adjusted percent changes) were found between the placebo- and  $\beta$ -APM-treated groups for the phenylephrine, methacholine, histamine, DMPP or isoproterenol challenges. HR responses to the isoproterenol challenge in the rats treated with  $\beta$ -APM did not differ significantly from those of placebo-treated animals. Thus, it appears that  $\beta$ -APM does not have alpha- or beta-adrenergic blocking activity, anticholinergic activity, histamine blocking activity or ganglionic blocking activity on the cardiovascular function of conscious rats.

The baseline differences in MAP and HR between the pre- and post-treatment for each challenge were similar in the placebo and  $\beta$ -APM groups. Neither treatment had significant time trends for the baseline differences in MAP.  $\beta$ -APM treatment had no statistically significant effect on the overall mean baseline values of either MAP or HR, whereas placebo treatment produced a change of less than 10% from pretreatment control values. While statistically significant, these differences between the two groups in their effects on baseline MAP and HR are not biologically meaningful. Thus,  $\beta$ -APM has no biologically significant effect on either MAP or HR.

#### **IV.B. Hemodynamic Effects in the Conscious Normotensive Dog**

Methods: This study evaluated the effects of  $\beta$ -aspartame ( $\beta$ -APM, SC-19129) on blood pressure, heart rate, and the ECG in normotensive dogs.

Each of six female beagles (#HHNFJ243, RAQFP443, AHJ93, LNX3, OS83, KY83) weighing 6.1-9.8 kg was anesthetized with Metafane\*. The femoral artery was cannulated with Tygon\* tubing (.040"x.070") for direct blood pressure monitoring. After surgery at least one week was allowed before a dog was utilized in the study. During this week the dog was trained to lie quietly in a sling in a sound-attenuated isolation room. This training included several 1 or 2 h training periods and at least 1 training session during which a baseline 4 1/2 h study was completed. On the day of study, each dog was shaved and fitted with ECG stress electrodes (Bard, NJ) and was attached to a cable for monitoring the different parameters. The animal was allowed to stabilize for 1/2 h before a 1/2 h pretreatment period. During the pretreatment period all parameters (systolic, diastolic and mean blood pressures, P-wave duration, P-R interval, QRS duration, T-wave duration, and S-T segment length) were recorded.  $\beta$ -APM (60 mg/kg) or placebo was administered orally as a capsule and blood pressure

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and ECG were monitored for a 4 h post-treatment period. Lactose was used as a placebo and as a filler for the capsule when compound was administered.

Continuous pressure measurements were made utilizing a Micron MP-15 transducer (Micron, MA) for the aortic pressure pulse. A 3-lead ECG system was used to produce a "Lead II" type ECG recording. Both signals were displayed on a Gould oscillograph recorder and digitally analyzed by the ECGR program. This program determined systolic, diastolic and mean blood pressures, P-wave duration, P-R interval, QRS duration, T-wave duration and S-T segment length. All values determined by the program were electronically stored and transferred to Statistical Analysis System® (SAS) for later statistical analysis.

Statistical Analysis: Half-hour means of the five-minute heart rate, blood pressure (mean, systolic and diastolic), P-wave duration, P-R interval, QRS duration and S-T interval readings were computed for each animal (separately for the two crossover periods) at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 h post-treatment times. The change from pretreatment to post-treatment was expressed in terms of mean difference from pretreatment baseline values (time 0.0 to half-hour).

The changes produced by  $\beta$ -APM on heart rate,

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blood pressure and ECG interval were compared to placebo values using a repeated measures analysis of variance. In case of a significant ( $p < 0.05$ ) treatment interaction with time, treatment effects at each post-treatment hour was evaluated using appropriate contrasts in the analysis of variance. The method of moments was used to obtain an approximate two-sided, size 0.05, contrast t-test.

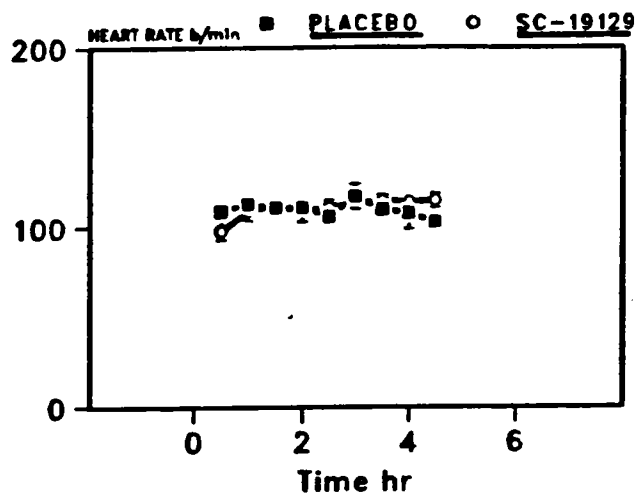
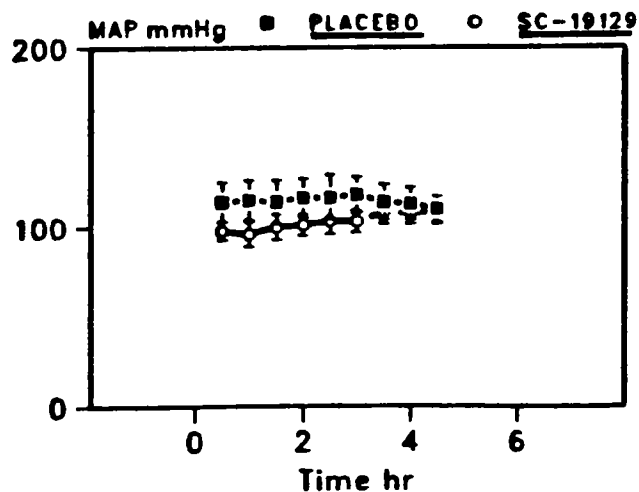
(Searle Reference: Scientific Evaluation Department Document #MP219)

Results:  $\beta$ -APM did not affect resting heart rate (Figure IV.B.1) but did cause a slight increase in MAP from the pretreatment values (Table IV.B.1). This slight rise in blood pressure from pretreatment values in the  $\beta$ -APM treatment group when compared to the vehicle group, produced a significant dose-by-hour interaction (slope) in the ANOVA and a significant LSD (least significant difference  $p < 0.05$ ) at 4 h post-treatment. At no time did the treated group's blood pressure surpass placebo values (Figure IV.B.1). This blood pressure response appears to be of no biological significance.

The effects of  $\beta$ -APM on the ECG are shown in Figure IV.B.2. An increase was noted for P-wave duration of 1.7 ms. Since the minimum measurable duration (data collection rate) for the microprocessor

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Figure IV.B.1



Effects of  $\beta$ -APM and placebo on mean arterial pressure (top) and heart rate (bottom). Compound or placebo (lactose) was administered orally after time 0. Each point represents the mean of 6 animals. Bars represent  $\pm 1$  S.E.

Searle Data Reference: RB 2604; ROZ5038  
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Table IV.B.1

Difference Between  $\beta$ -APM and Placebo Induced  
Changes from Pretreatment Values of Mean Arterial  
Blood Pressure (MAP)

Post-treatment Time Period	Change from Placebo <sup>a,b</sup> (mmHg)
LSD <sup>c</sup>	10.650
0.5 hours	- 4.3730
1.0 hours	1.6270
1.5 hours	0.3492
2.0 hours	1.7159
2.5 hours	0.5437
3.0 hours	9.1270
3.5 hours	9.7548
4.0 hours	13.7937 <sup>d</sup>

a The mean difference between placebo and  $\beta$ -APM treated groups of the changes from their respective pretreatment values.

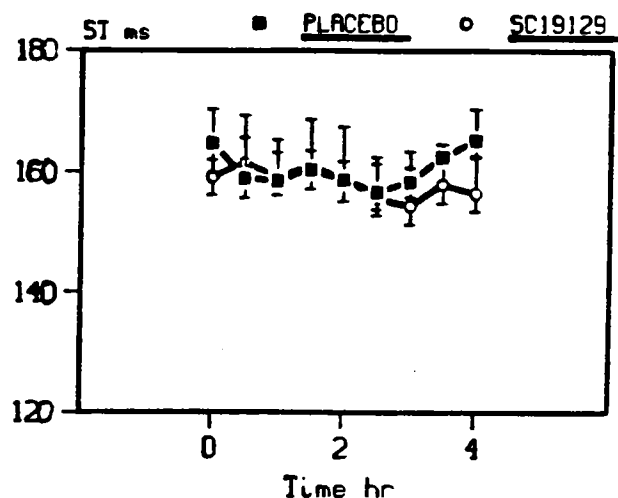
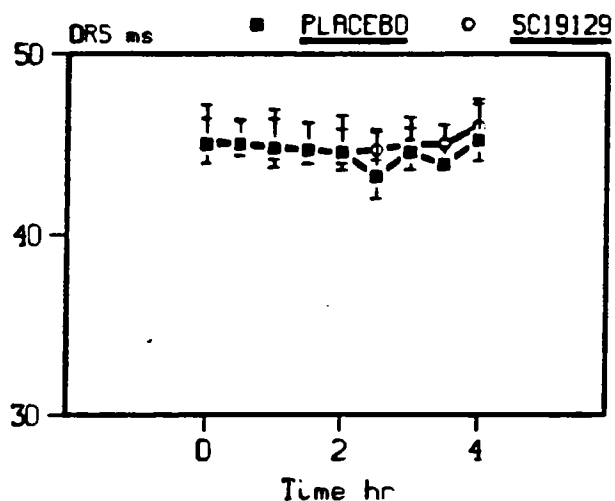
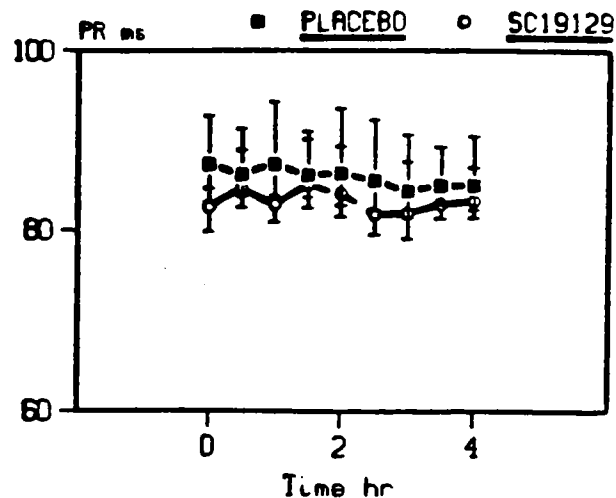
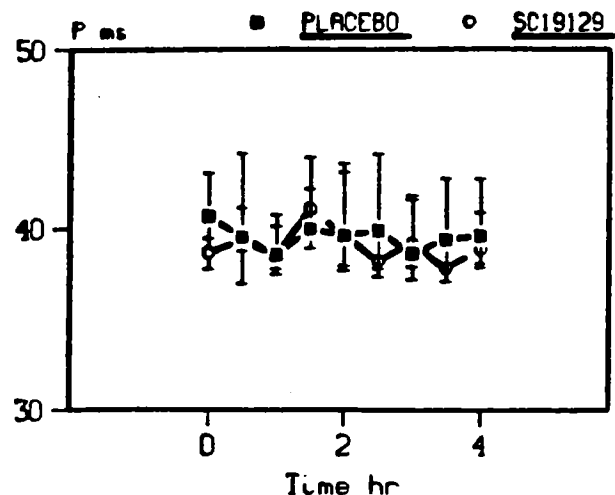
b n=6.

c Least significant absolute difference at the 0.05 level. LSD was computed using a method of moments approximate t-test of the appropriate ANOVA contrast.

d Effect significant at the 0.05 level (i.e. greater in absolute value than the LSD).

Searle Data Reference: RB 2604

Figure IV.B.2



Effect of  $\beta$ -APM and placebo on P-wave duration, P-R interval, QRS duration and S-T segment length. Compound or placebo was administered orally after time 0. Each point represents the mean of 6 animals. Bars represent  $\pm 1$  S.E.

Searle Data Reference: RB 2604; ROZ5038

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system is 4 ms, the statistical significance is of doubtful biological relevance.

None of the other variables measured were significantly different from pretreatment or placebo values.

#### **IV.C. Effect on Renal Function in Unanesthetized Mongrel Dogs**

Methods: The effects of  $\beta$ -aspartame ( $\beta$ -APM, SC-19129) on renal function after intragastric administration to unanesthetized mongrel dogs were investigated.

Five female mongrel dogs with body weights ranging between 18.4 and 26.2 kg were randomly selected from the colony. Each test animal had been previously trained to accept intravenous catheterization, oral drug administration and bladder catheterization while standing in a suspension harness (Alice King Chatham - Medical Arts) on a raised platform. Animals were food deprived for 24 h, but had access to water ad libitum.

All dogs received  $\beta$ -APM or placebo intragastrically according to a random block design. A minimum 7 day interval was allowed between experiments for each dog.

Prior to dosing each dog was given an oral load of 35 ml/kg normal saline (0.9% Sodium Chloride-Travenol Labs) for volume expansion, which was followed by

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intravenous administration of 4 ml/min. isotonic saline infusion to achieve stabilization of renal hemodynamics. The sustaining infusion solution contained 0.45 g/dl inulin (Fisher Scientific Co.) for calculation of glomerular filtration rate (GFR) and 0.15 g/dl para-aminohippurate (PAH, Sigma Chemical Co.) for estimation of effective renal plasma flow (RPF). The infusion solution also contained 0.125 U/dl vasopressin (Pitressin®, Parke-Davis) to control for changes in renal function secondary to changes in pituitary release of ADH. A priming dose of inulin (5 ml, 10% solution) and PAH (2.5 ml, 20% solution) was given intravenously in order to achieve plasma saturation of clearance markers.

The urinary bladder was catheterized (Bardex Foley Catheter - C.R. Bard, Inc.) and washed with 25 ml sterile water rinse. Two 10-minute urine samples were collected and an 8.5 ml blood sample was obtained at the midpoint of each clearance period in order to determine the baseline measurements of renal function parameters.

Each test animal received  $\beta$ -APM and placebo on separate days. The test compound was given 60 mg/kg intragastrically in a gelatin capsule. Placebo animals received gelatin capsule only. Treatment was followed by 6 more 10-minute clearance periods. Urine

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samples were obtained during each clearance period in order to determine urine volume excretion and inulin, PAH and electrolyte concentrations in the urine. The inulin and PAH concentrations in urine and plasma were determined by a colorimetric reaction method on the Autoanalyzer. Sodium and potassium concentrations were determined by an ion-selective electrode method, performed by ASTRA-7 (Automated Stat/Routine Analyzer) system.

The renal function parameters measured were: urine volume, glomerular filtration rate (GFR), renal plasma flow (RPF) and renal blood flow (RBF). In addition absolute and fractional excretions of sodium and potassium were also calculated.

The maximum response ( $C_{\max}$ ) and the area under the curve (AUC) over the six post-treatment clearance time periods were computed separately for each renal function variable. Multivariate analyses of variance were performed on  $C_{\max}$  and AUC separately for each renal function variable. The model included an effect due to treatment (either placebo control or  $\beta$ -APM at 60 mpk i.g.) and an effect due to dog ( $n=5$ ), leaving the dog by treatment interaction error term as the residual. The multivariate hypothesis of no overall treatment effect on  $C_{\max}$  and AUC was tested jointly with an exact F-test using Wilk's criterion. All

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tests were performed at the 5% level of significance.

Results: Statistical examination of the test data demonstrated that there were no significant differences between the urine output of  $\beta$ -APM and placebo groups (Table IV.C.1).  $C_{\max}$  and AUC for absolute and fractional excretion of sodium and potassium in the  $\beta$ -APM group were not significantly different from those in the placebo control group ( $p>0.05$  for all multivariate F-tests,  $df=2,3$ ). There were no significant differences between renal hemodynamic parameters (GFR, RPF and RBF) of  $\beta$ -APM and placebo groups (Table IV.C.2).

In conclusion,  $\beta$ -APM as compared to placebo did not exhibit significant effects on  $C_{\max}$  and AUC in any of the renal functions examined ( $p>0.05$  for all multivariate F-tests,  $df=2,3$ ) following intragastric administration to unanesthetized mongrel dogs.

#### **IV.D. Inhibition of Angiotensin I Converting Enzyme**

Inhibitors of angiotensin I converting enzyme (ACE) in vitro possess potent anti-hypertensive activity in man and animals.  $\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated to determine its effects on this system (screening test protocol 16a11).

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Table IV.C.1

Urine Electrolyte Excretion After Intragastric  
Administration of Placebo and  $\beta$ -APM to  
Unanesthetized Dogs

Renal Function Parameter	Mean $C_{\max}$ $\pm$ SE <sup>a</sup>		Mean AUC $\pm$ SE <sup>b</sup>		F (2,3) <sup>c</sup>
	Placebo	$\beta$ -APM	Placebo	$\beta$ -APM	p-value
Urine Volume (ml)	49.2 $\pm$ 3.3	45.4 $\pm$ 2.5	2308 $\pm$ 152	2163 $\pm$ 125	0.24
Na <sup>+</sup> Excretion (mcEq)	965.8 $\pm$ 102.9	921.7 $\pm$ 45.8	38751 $\pm$ 4299	40609 $\pm$ 2787	0.39
K <sup>+</sup> Excretion (mcEq)	63.2 $\pm$ 4.1	56.2 $\pm$ 9.2	2552 $\pm$ 199	2206 $\pm$ 380	0.76
Fractional Na <sup>+</sup> Excretion	0.136 $\pm$ 0.028	0.108 $\pm$ 0.010	4.89 $\pm$ 0.96	4.44 $\pm$ 0.52	0.67
Fractional K <sup>+</sup> Excretion	0.502 $\pm$ 0.152	0.316 $\pm$ 0.046	15.9 $\pm$ 3.6	10.9 $\pm$ 1.1	0.55

a  $C_{\max}$  denotes maximum response.

b AUC denotes area under curve.

c Multivariate ANOVA F-test of hypothesis of no overall effect of treatment on  $C_{\max}$  and AUC.

Searle Data Reference: RB 2578/94-113

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Table IV.C.2

Renal Hemodynamics After Intragastric  
Administration of Placebo and  $\beta$ -APM to  
Unanesthetized Dogs

Renal Function Parameter	Mean $C_{\max}$ $\pm$ SE <sup>a</sup>		Mean AUC $\pm$ SE <sup>b</sup>		F (2,3) <sup>c</sup>
	Placebo	$\beta$ -APM	Placebo	$\beta$ -APM	p-value
GFR (ml/min.)	82.2 $\pm$ 12.7	90.8 $\pm$ 18.1	3115 $\pm$ 514	3288 $\pm$ 492	0.37
RPF (ml/min.)	191.4 $\pm$ 25.3	193.4 $\pm$ 25.9	6833 $\pm$ 1080	7067 $\pm$ 771	0.92
RBF (ml/min.)	319.4 $\pm$ 46.1	314.3 $\pm$ 40.9	11362 $\pm$ 1950	11476 $\pm$ 1161	0.95

a  $C_{\max}$  denotes maximum response.

b AUC denotes area under curve.

c Multivariate ANOVA F-test of hypothesis of no overall effect of treatment on  $C_{\max}$  and AUC.

Searle Data Reference: RB 2578/94-113

Methods: ACE was purified from hog lung tissue homogenates to about two units per mg of protein. The enzyme inhibition assay consisted of a thirty-minute incubation at 37°C of the following final concentrations of reagents (total volume 0.25 ml): Five milliunits ACE, two mM substrate Hippuryl-L-Histidyl-L-Leucine (HHL), and  $5 \times 10^{-5} \text{M}$   $\beta$ -APM in 100 mM  $\text{K}_2\text{HPO}_4$ -300 mM NaCl buffer, pH 8.3. The reaction was terminated by acidification with 0.25 ml 1 N HCl and the hippuric acid generated by the action of ACE extracted and determined spectrophotometrically at 228 m $\mu$ . Compounds that inhibit ACE activity by 50% or more at the initial screening concentration are considered active.

Results:  $\beta$ -APM did not inhibit ACE activity at the screening concentration of  $5 \times 10^{-5} \text{M}$ .

Searle Data Reference: RB 2208/147

#### **IV.E. Inhibition of Human Renin, In Vitro**

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated for its ability to inhibit human renin activity in vitro (screening test protocol 16a13).

Methods: The international reference standard for human renin was used as the enzyme preparation while human blood plasma served as the angiotensinogen substrate source. The enzyme inhibition assay consisted of a two-hour incubation at

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37°C of the following final concentrations of reagents (total volume of 0.25 ml): 0.1 mG units/ml human renin, 0.05 ml human plasma,  $\beta$ -APM  $10^{-4}$ M, 6 mM  $\text{Na}_2\text{EDTA}$ , 2.4 mM PMSF, 1.5 mM 8-hydroxyquinoline, 0.4 mg/ml BSA, 0.024 mg/ml neomycin sulfate in a 100 mM Tris Acetate buffer, pH 7.5. All assays were carried out in duplicate. The reaction was terminated by boiling for 10 min. and the angiotensin I produced was determined by radioimmunoassay. Compounds that inhibit renin activity by 20% or more at the initial screening concentration of  $10^{-4}$ M are considered active.

Results: As  $\beta$ -APM inhibited human renin activity in vitro by only 9% at the screening concentration of  $10^{-4}$ M it therefore has no activity in this assay.

Searle Data Reference: RB 2379/087

#### **IV.F. Summary of Cardiovascular and Renal Effects**

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a series of tests to determine potential effects on the cardiovascular and renal systems. These tests included evaluations of autonomic function in conscious rats, hemodynamic effects in the normotensive dog, evaluation of renal function in unanesthetized dogs, effects on the inhibition of angiotensin I converting enzyme and effects on the in vitro inhibition of human

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renin. It was concluded that  $\beta$ -aspartame did not possess any biologically significant activity in any of these tests.

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## **V. Evaluation of the Effects of $\beta$ -Aspartame on Additional Pharmacological Parameters**

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$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a series of screening tests to determine its potential effects on various pharmacological systems. These tests included an evaluation of the inhibitory effects of  $\beta$ -APM on adenosine diphosphate (ADP)-induced platelet aggregation in human and non-human blood platelets; effects on leukotriene D<sub>4</sub> antagonism of guinea pig ileum contractions; effects on the inhibition of pancreatic lipase; effects on the inhibition of sheep seminal vesicle microsome cyclooxygenase, slow reacting substance biosynthesis and 5-lipoxygenase activity; and effects on the in vivo antagonism of leukotriene D<sub>4</sub>-induced bronchoconstriction in the guinea pig.

### **V.A. Inhibition of ADP-Induced Platelet Aggregation in Human and Non-Human Blood Platelets**

Methods:  $\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in two screening tests to determine its potential effects on the inhibition of ADP-induced platelet aggregation in human (screening test protocol 11F01A) and rat (screening test protocol 11F01) blood platelets. The addition of ADP to platelet-rich

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plasma induces platelet aggregation. A compound is rated active in these procedures if, after three separate incubations with platelet rich plasma at the initial screening concentration of  $10^{-4}\text{M}$ , the mean ADP-induced response is reduced by 50% or more.

Results: The results of  $\beta$ -APM's effects on the inhibition of ADP-induced platelet aggregation are summarized in Table V.A.1.  $\beta$ -APM produced a 7% inhibition of both human and rat platelet aggregation and was therefore below criterion response at  $10^{-4}\text{M}$  concentration.

**V.B. Effects on Leukotriene  $\text{D}_4$  ( $\text{LTD}_4$ ) Antagonism of Guinea Pig Ileum Contractions**

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a screening procedure to determine its effects on  $\text{LTD}_4$  antagonism of guinea pig ileum contractions (screening test protocol 7a15A).

Methods: Segments of guinea pig ilea were mounted in 2 ml tissue baths containing modified Tyrode solution with 0.5% dimethylsulfoxide. Contractions between 20% and 80% of maximum were produced by adding 2 concentrations of  $\text{LTD}_4$  to the bathing solution. A solution containing  $10^{-6}\text{M}$   $\beta$ -APM was then substituted for the control bathing solution, and doses of  $\text{LTD}_4$  that produced contractions nearly equal to the control

Table V.A.1  
Antiplatelet Activity of  $\beta$ -APM<sup>a</sup>

	Concentration(M)	% Inhibition
Human Platelets <sup>b</sup>	10 <sup>-4</sup>	7 d
Rat Platelets <sup>c</sup>	10 <sup>-4</sup>	7 e

a  $\beta$ -APM was evaluated to determine its effects on ADP-induced platelet aggregation.

b Screening protocol 11F01A.

c Screening protocol 11F01.

d Searle data reference: RB 2608/131,179

e Searle data reference: RB 2608/131

contractions were determined. Dose ratios (the ratio of LTD<sub>4</sub> doses in the presence and absence of  $\beta$ -APM which produce equal responses) were determined.

Results: The effects of  $\beta$ -APM on LTD<sub>4</sub> antagonism of guinea pig ileum contractions is summarized in Table V.B.1. The mean dose ratio produced in two tissues with  $10^{-6}$ M  $\beta$ -APM was 0.63. This was not significantly different ( $P > 0.05$  by Student's t-test) from the value of 0.71 obtained in control experiments.

#### **V.C. Inhibition of Pancreatic Lipase**

Methods:  $\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated at  $1.25 \times 10^{-3}$ M to determine its effects on the hydrolysis of triglycerides by crystalline pancreatic lipase (screening test protocol 23c02). The amount of titratable free fatty acid appearing after incubation provides a measure of the rate of hydrolysis.

Results:  $\beta$ -APM did not show any biologically significant inhibition of the hydrolysis of triglycerides by crystalline pancreatic lipase.

#### **V.D. Inhibition of Sheep Seminal Vesicle Microsome Cyclooxygenase, Slow Reacting Substance Biosynthesis and 5-Lipoxygenase Activity**

Methods:  $\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in screening procedures for its

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Table V.B.1

The Effects of  $\beta$ -APM on LTD<sub>4</sub> Antagonism  
of Guinea Pig Ileum Contractions

	Concentration (M)	N	Dose Ratio <sup>a</sup>
Control	--	8	.71
$\beta$ -APM	10 <sup>-6</sup>	2	.63 <sup>c</sup>

- a The ratio LTD<sub>4</sub> doses in the presence and absence of  $\beta$ -APM which produce equal responses.
- b Control experiments conducted on 4 tissues before  $\beta$ -APM and 4 tissues after  $\beta$ -APM.
- c No significant difference from control value (Student's t-test,  $P > 0.05$ ).

Searle data reference: RB 2615/137, 176, 181

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inhibitory effects against 5-lipoxygenase (5-LO), cyclooxygenase and slow reacting substance (SRS).

In the 5-lipoxygenase inhibition assay (screening test protocol 27D7) radiolabeled arachidonic acid is oxygenated by Rat Basophilic Leukemia Cell (RBL-1) 5-lipoxygenase in the presence and absence of test compounds. The product, 5-hydroxy-eicosatetraenoic acid (5-HETE), is quantitated and per cent inhibition determined.

In the cyclooxygenase inhibition assay (screening test protocol 27A12) arachidonic acid is oxygenated by sheep seminal vesicle microsomal cyclooxygenase in the presence and absence of test compounds. The initial rate of oxygen-uptake is monitored with an oxygen electrode.

In the SRS-biosynthesis inhibition assay (screening test protocol 27D3) intact RBL-1 cells are stimulated to synthesize SRS in the presence and absence of test compounds. SRS released is quantitated by guinea pig ileum SRS bioassay.

Results: The results of the effects of  $\beta$ -APM on 5-LO inhibition, cyclooxygenase inhibition, and SRS biosynthesis are summarized in Table V.D.1. At the screening concentration of  $10^{-4}$ M  $\beta$ -APM did not exhibit any biologically relevant effects.

Table V.D.1

Inhibitory Effects of  $\beta$ -APM Against  
5-Lipoxygenase<sup>a</sup>, Cyclooxygenase<sup>b</sup>, and  
SRS-Biosynthesis<sup>c</sup>

Assay	$\beta$ -APM Concentration <sup>d</sup> (M)	% Inhibition <sup>e</sup>
5-Lipoxygenase	10 <sup>-4</sup>	<50% <sup>f</sup>
Cyclooxygenase	10 <sup>-4</sup>	<50% <sup>g</sup>
SRS-Biosynthesis	10 <sup>-4</sup>	<50% <sup>h</sup>

a 27d07 Screening Protocol

b 27a12 Screening Protocol

c 27d3 Screening Protocol

d Final assay concentration

e A test compound needs to exhibit >50% at the  
screening concentration to be evaluated as active.

f Searle data reference: RB 2482/171

g Searle data reference: RB 2452/169

h Searle data reference: RB 2469/068

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**V.E. Effects on the In Vivo Antagonism of  
Leukotriene D<sub>4</sub>-Induced Bronchoconstriction  
in the Guinea Pig**

Methods:  $\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated for its ability to antagonize leukotriene D<sub>4</sub> (LTD<sub>4</sub>)-induced bronchoconstriction in the guinea pig (screening test protocol 27D05). Male Hartley guinea pigs were pretreated with pyrilamine maleate (5 mg/kg, i.p., 2 hrs), propranolol HCl (2 mg/kg, i.p., 2 hrs) and indomethacin (100 mg/kg, i.g., 1 hr) prior to  $\beta$ -APM (60 mg/kg, i.g.). One hr after  $\beta$ -APM the animals were challenged with LTD<sub>4</sub> (200 ng, i.v.). A compound is considered active if it caused a significant ( $P < 0.05$ ) reduction in intratracheal insufflation pressure induced by LTD<sub>4</sub>.

Results: The results of the effects of  $\beta$ -APM on the antagonism of LTD<sub>4</sub>-induced bronchoconstriction are summarized in Table V.E.1. No significant differences ( $P > 0.05$ , Student's t-test) were noted between the control and  $\beta$ -APM test groups.

**V.F. Summary of the Additional Pharmacology**

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) was evaluated in a series of screening tests to determine its potential effects on various pharmacological systems. These tests included an evaluation of

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Table V.E.1

Effects of  $\beta$ -APM on the In Vivo Antagonism  
of LTD<sub>4</sub>-Induced Bronchoconstriction  
in the Guinea Pig<sup>a</sup>

Treatment	Dose (mg/kg) $\beta$ -APM	Route	N	Bronchoconstriction (mmHg) $\pm$ S.E.M.
Vehicle Control <sup>b</sup>	--	i.g.	6	18.2 $\pm$ 1.5
$\beta$ -APM	60	i.g.	5	21.8 $\pm$ 1.2 <sup>c</sup>

a  $\beta$ -APM was administered 1 hr prior to LTD<sub>4</sub> (200 ng/  
i.v.) challenge.

b DMSO:PEG (20:80; v:v)

c No significant difference from vehicle control  
(Student's t-test,  $p > 0.05$ ).

Searle data reference: PDF/RLA-310

inhibitory effects of  $\beta$ -APM on ADP-induced platelet aggregation in human and non-human blood platelets; effects on leukotriene D<sub>4</sub> antagonism of guinea pig ileum contractions; effects on the inhibition of pancreatic lipase; effects on the inhibition of sheep seminal vesicle microsome cyclooxygenase, slow reacting substance biosynthesis and 5-lipoxygenase activity; and effects on the in vivo antagonism of leukotriene D<sub>4</sub>-induced bronchoconstriction in the guinea pig.

In this series of tests no significant biological effects of  $\beta$ -APM were noted.

## VI. CONCLUSIONS

$\beta$ -Aspartame ( $\beta$ -APM, SC-19129) is a minor constituent of aspartame ( $\beta$ -APM) (Figure I.1).  $\beta$ -APM may also be formed from aspartame under certain storage conditions. A series of studies was undertaken to evaluate the potential effects of  $\beta$ -APM on various organ systems and pharmacological parameters.

Studies were conducted to determine the potential effects of  $\beta$ -APM on the central nervous system, cardiovascular and renal systems, gastrointestinal system, and additional pharmacological systems.

In initial testing for proconvulsant activity 2/10 mice evaluated at 60 mg/kg, i.g. potentiated PTZ-induced convulsions. The procedure was repeated using doses of 30, 60, and 90 mg/kg, i.g., with 1/10, 1/10, and 0/10 mice convulsing at each respective dose. Because no dose response increase in convulsive activity was noted,  $\beta$ -APM was concluded to be devoid of proconvulsant activity.

When evaluated for hemodynamic effects in the normotensive dog,  $\beta$ -APM produced a slight but significant increase in blood pressure at 4 h post-treatment compared to pretreatment values. At no time did the  $\beta$ -APM group's blood pressure surpass placebo values. This blood pressure response appears to be of

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no biological significance.

In summary, all studies conducted  $\beta$ -APM did not exhibit any significant biological activity.

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