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REMARKS

Dr. Woodrow Monte of Arizona State has an appointment to see this pltr. 9:30 August 2nd

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FAP 2A3661

SEARLE RESEARCH AND DEVELOPMENT

ASPARTAME FOR USE AS A SWEETENER IN CARBONATED BEVERAGES

PETITION CONTROL VOLUME 1 of 4

ASPARTAME IN CARBONATED BEVERAGES

I. INTRODUCTION

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a general purpose food additive that functions as a sweetener and flavor enhancer and was approved on July 24, 1981 (21 CFR 172.804). Aspartame (APM) is now proposed for use as a sweetener in liquid carbonated beverages in quantities necessary to achieve the intended effect. This petition will address the potential consumption and the functionality of APM in liquid food systems.

CONSUMPTION: In estimating APM consumption from the proposed extended use (carbonated beverages), an analysis is presented in Appendix 6 utilizing Market Research Corporation of America (MRCA) and US Department of Agriculture (USDA) 1977-78 data.

These estimates were derived in a manner similar to the March 1976 General Foods Corporation analyses of potential APM consumption that was submitted to the Hearing Clerk Docket No. 75-0355 Volume 103 and used by the Commissioner in his decision for the approval of APM.

Both analyses were based on MRCA data collected from actual dietary records kept by 4,000 households over a 2 week period staggered throughout the year and serving sizes based on food intake data from USDA Nationwide Food Survey. The data are presented by age groups and reported as percentiles to account for both the average and heavy user.

The projected consumption was calculated for all presently approved APM uses (21 CFR 172.804), for carbonated beverages alone, non-carbonated beverages and combinations of all categories with the assumption that all individuals would replace ALL of their present sweeteners and sweetened products with APM-containing products, and that ALL eatings in a particular food category would contain APM. As a result this assumption obviously greatly overstates the potential APM consumption.

If APM were to replace ALL sugar and saccharin in the several hundred products comprising the currently approved food categories, the mean potential exposure would range from 1.0 to 6.6 mg/kg/day, depending on age (the mean for all ages being 1.9 mg/kg/day), (Table 1, pg 10, Appendix 6). Heavy users of these foods (90th percentile of intake) might consume between 2.9 and 17 mg/kg/day (the level for all ages combined being 4.8 mg/kg/day), and the intake of very heavy users, consuming at the 99th percentile, might be between 6.5 and 38 mg/kg/day (the level for all ages being 17 mg/kg/day). The highest levels in relation to body weight are seen in the 2-4 years age-group, as is generally the case with any food.

If APM were also approved for use in carbonated beverages and if ALL sugar and ALL saccharin in these drinks were to be replaced by APM, such uses would add 0.95 mg/kg to the daily mean consumption of APM in the lowest intake age group (18 years +), and 3.3 mg/kg in the highest intake age group (2-4 years). In the heavy users (90th percentile) it would increase by 2.2 mg and 6 mg/kg respectively.

APM Consumption at 90th percentile

mg/kg/day

Age Group	currently approved uses	carbonated beverages plus currently approved uses
0-23 mos	11	15
2-4 yrs	17	23
5-6 yrs	13	17
7-8 yrs	11	15
9-12 yrs	7.5	11
13-17 yrs	4.9	8.0
18 yrs +	2.9	5.1
All ages	4.8	8.1

The very heavy user level (99th percentile) would increase by 3.5 mg/kg in the 18 years + group and by 6 mg/kg in the 2-4 years group (Table 1, pg 11, Appendix 6).

Although the projected amount of APM consumed by the heavy user exceeds the acceptable daily intake determined in 1973, data from human beings previously reviewed by the Agency and the Commissioner document that even at the projected unrealistically high intake levels, APM is safe. It has been established by clinical studies (L.D. Stegink et al, J. Nutr. 110 (1980) 2216) that even at abuse levels of 200 mg/kg, acute ingestion of APM results in blood levels of aspartate plus glutamate well below those (100 umol/dl) found acceptable by the Agency (FDA communication to Searle July 14, 1982) and "the remarkably low amount of amino acid intake which would result, from even the 99th percentile of estimated aspartame consumption, in relation to the prevalence of these same amino acids in common protein foods," (46FR 142:38287, July 24, 1981).

2 developed cancer during study

In chronic studies in normal adults and children, diabetics, obese, and PKU heterozygote adults, 1.8 gm APM/day were consumed for 13-21 weeks with no evidence of adverse effects. No clinically significant differences were noted in the clinical parameters measured: liver function, renal function, hematologic status including serum glucose and insulin or plasma levels of phenylalanine and tyrosine. No discernible effects of APM alone or in comparison to sucrose and/or placebo were reported from the 322 adults and children ingesting APM on a chronic basis (FAP 3A2885, Volumes E-60, 61, 64, 65 and 67).

Because many food additives have not been clinically studied prior to approval, the FDA has established a 100 fold safety factor below the "no harm" level in animals for projecting an acceptable intake by human beings (21 CFR 170.22).

The data from clinical studies of APM make the need for a 100 fold safety factor based on toxicity tests in animals less relevant. However, projected consumption of APM still falls within the 100 fold safety factor even at the overstated estimates at the 90th percentile of use.

Bureau of Foods Toxicology opinion states that "Conservatively we have taken the 2gm/kg test level in rat and dog as a 'no effect' level. However, APM at levels of 4 and 8gm/kg were fed to rats and up to 4gm/kg to dogs. The effects seen at these higher levels can be considered minimal..." (memorandum Dr. C. J. Kokoski, April 11, 1974 p.12). Additionally, the long-term animal studies of diketopiperazine were reviewed by the Bureau of Foods in 1975 and no-effect levels of 3 gm/kg (rat) and 1 gm/kg (mouse) were established.

As recognized by the Bureau of Foods Toxicology opinion the effects at 4 gm/kg/day in animal studies were minimal and without harm. This conclusion was arrived at independently by both the Joint FAO/WHO Expert Committee (JECFA) and the Canadian Health Protection Branch (HPB) in their reviews.

JECFA in its review of Searle's safety data assigned an acceptable daily intake level of 40 mg/kg based upon the two 104 week rat studies and the 110 week mouse study. This level was also accepted by the HPB which in its letter of intent to approve aspartame stated that "Based on the safety data available at that time an acceptable daily intake of 40 mg/kg body weight per day was established. This would permit all of the requested uses [including carbonated beverages] for which the Health Protection Branch had received a submission," (HPB I.L. 602, July 31, 1981) and "The data on the safety of Aspartame are the most comprehensive ever received by the Health Protection Branch in support of a food additive," (HPB I.L. 564, September 12, 1979).

In his decision on APM, the Commissioner, based on his review of the data, stated "Aspartame is being approved only because the available data establish that the maximum projected consumption of aspartame [34 mg/kg] is still far, far below any level even suspected of being toxic," (46 FR 142:38303, first column, 3rd paragraph).

Therefore, the expansion of APM uses to liquid food systems will not compromise the well-established safety margin as documented by human and animal studies.

Under conditions of normal use within the beverage industry, APM is and will be a functional sweetener.

Data from beverage industry sources indicate that a typical carbonated beverage sweetened with either sucrose or saccharin is consumed within three months of manufacture. Typically, the unsweetened beverage concentrate is prepared by a supplier. The bottling company carbonates, adds the sweetener and packages in one day. Normal warehouse inventory is turned over every two weeks with delivery to the retail stores weekly. Storage and delivery are conducted at ambient temperatures.

Fountain syrups are formulated with the basic beverage base and the sweetener at syrup branches and packaged immediately for distribution. All storage and delivery are performed at ambient temperatures. Ultimate consumption usually occurs within two to three months of manufacture.

As described below, APM-sweetened beverages remain acceptable over a range of APM concentrations and storage conditions.

II. APM STABILITY - SENSORY EVALUATION

The satisfactory stability of APM (as the neat chemical and in dry food systems) has been documented (volume A-2 of FAP 3A2885). When added to liquid systems, APM undergoes hydrolysis and cyclization at a rate that is dependent on pH and temperature.

To methanol

To evaluate the rate of APM degradation and its effect on functionality the following studies were conducted in 1981-2: 1) a sensory evaluation and chemical stability study of APM in carbonated beverages and 2) a chemical stability study of APM in syrups. These studies are summarized below and the full reports are contained in the Appendices. In 1975 a sensory evaluation of the acceptance of varying concentrations of APM was performed and is summarized in Part III. Concomitant chemical stability analyses were not conducted.

1.a. CHEMICAL STABILITY METHODS

CARBONATED BEVERAGES: Four different flavors of ready-to-drink carbonated beverages sweetened only with APM and one with both APM and saccharin were studied. The beverages contained the following APM and saccharin concencentrations at the start of the study:

Flavor	APM (mg/100 ml)	12oz Serving	Saccharin (mg/100 ml)	pH
Cola	57.7	197 mg	-	3.05
Lemon-Lime	50.1	171 mg	-	3.72
Orange	92.6	316 mg	-	3.49
Root Beer	60.5	206 mg	-	4.59
Cola	13.7	46 mg	14.5	2.83

These levels were chosen through the use of sensory screening panels and were determined as having acceptable sweetness. APM or APM plus saccharin was added by commercial bottling firms to the flavor formulations in lieu of sucrose. The beverages were packaged in standard glass bottles with either crown or screw caps.

Bottles of each carbonated flavor were stored at 55°, 40°, 30°, 20° and 5°C with the exception of the cola containing APM plus saccharin which was stored at 55°, 40° and 20°C.

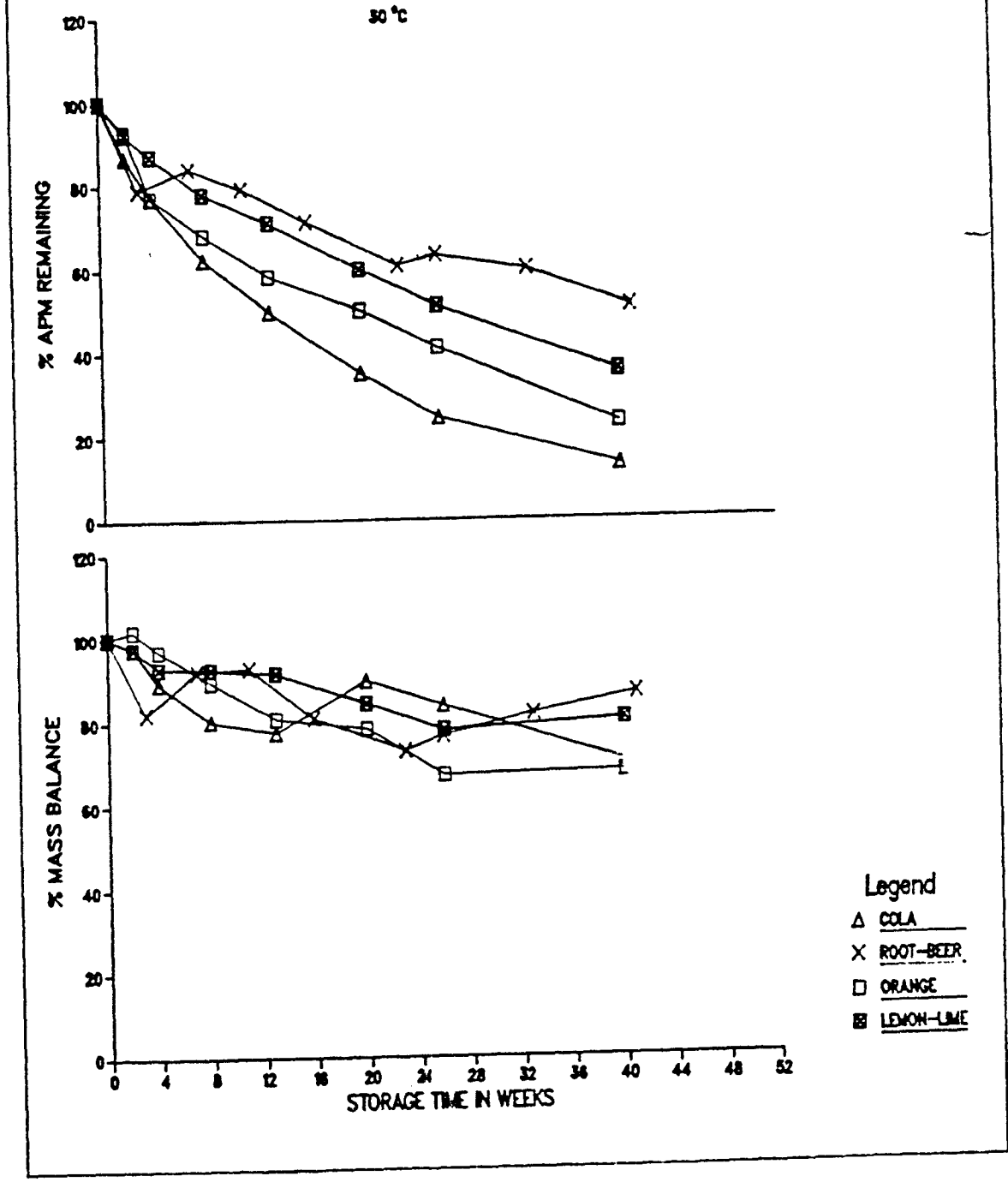
Samples for chemical stability were analyzed at the same time points as specified in the sensory evaluation protocols.

The 55° and 40°C temperature samples were utilized for identification of degradation products and were not included in the sensory evaluation protocol. For products stored at 30°C, analyses were performed through 20 weeks for orange, lemon-lime, and cola and through 23 weeks for the root beer. Chemical analyses were conducted through 40 weeks for orange and lemon-lime, 41 weeks for root beer and 52 weeks for cola beverages stored at 20° and 5°C.

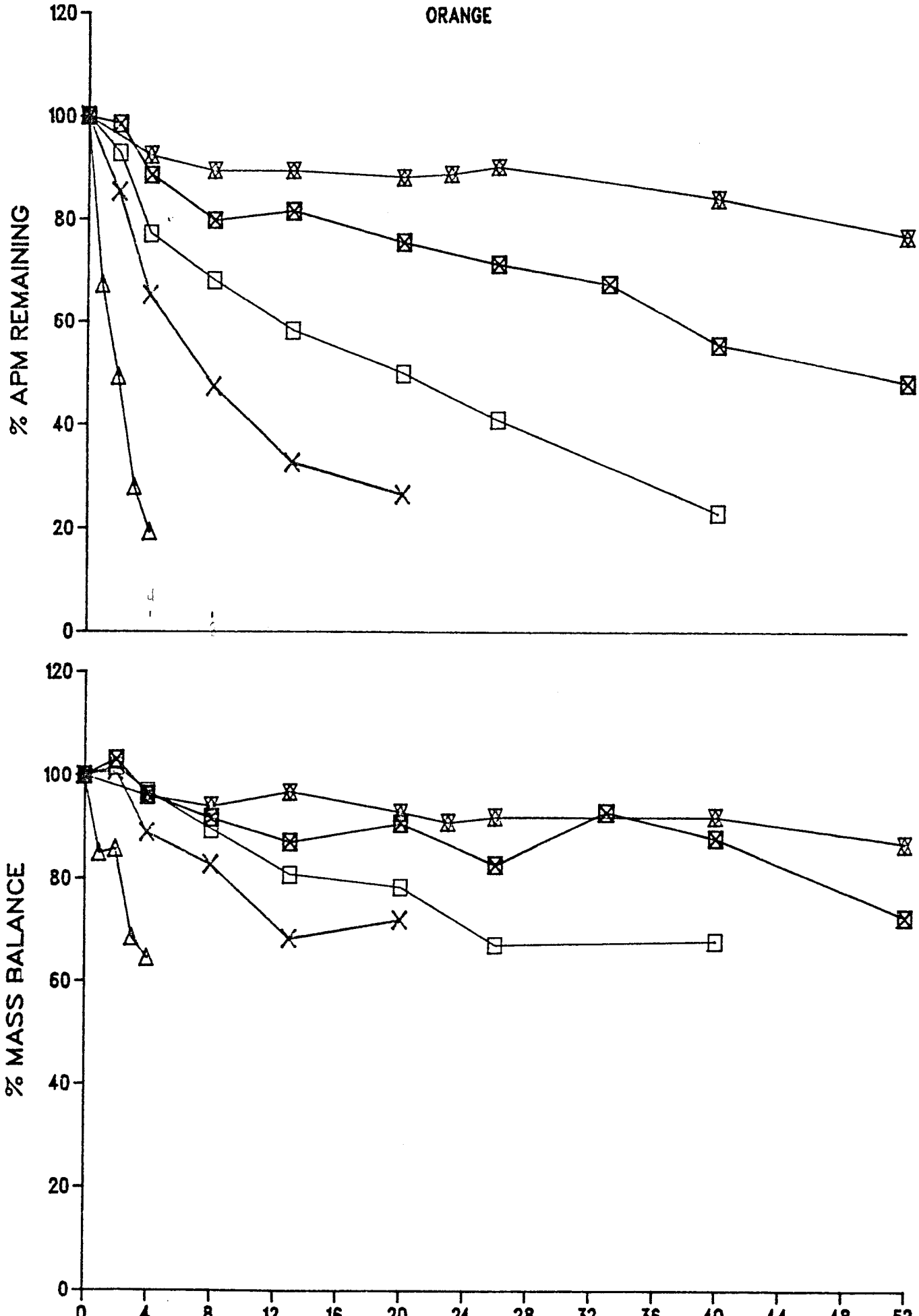
With the exception of the APM plus saccharin sweeten cola, the mass balance was calculated at each study point as a percentage of the initial quantity of APM Since PM and beta- APM were not identified and therefore not quantified until later in the study, t percent recovery in some cases is lower for the earl time periods. The percent recovery in relation to t percent of APM remaining for all four carbonated beverages at 30°C storage temperature is illustrated Figure 4.

FIGURE 4

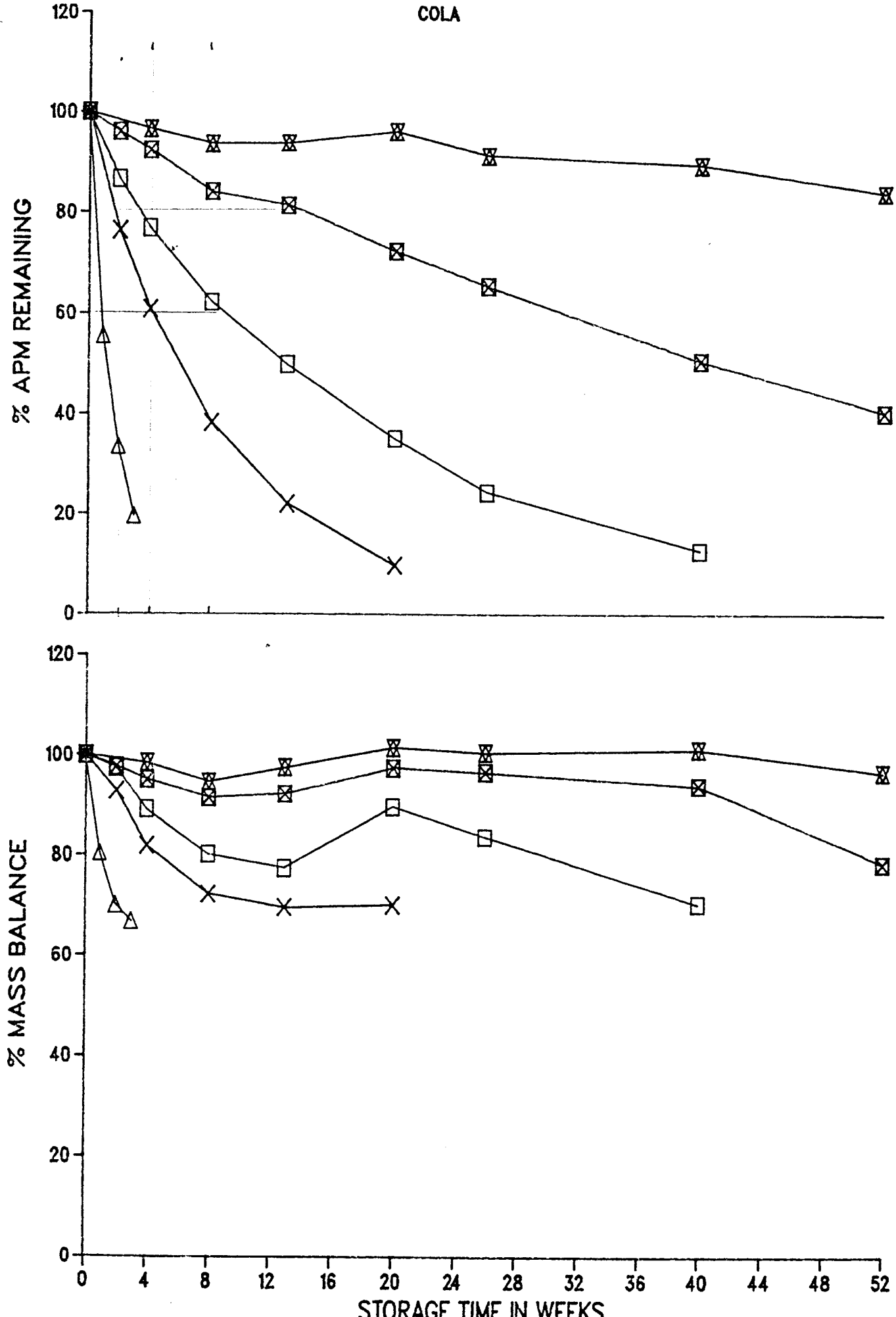
APM STABILITY IN CARBONATED BEVERAGES



APM STABILITY IN CARBONATED BEVERAGES



APM STABILITY IN CARBONATED BEVERAGES



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SEARLE RESEARCH AND DEVELOPMENT

ASPARTAME FOR USE AS A SWEETENER IN CARBONATED BEVERAGES

PETITION CONTROL VOLUME 2 of 4

Table I--Daily Potential Aspartame Exposure in Milligrams per Kilogram Body Weight

(Based on Total Sample Within Each Age Group)

	Group A Currently Approved Foods			Group B All Carbonated Soft Drinks			Group C Non-Carbonated Soft Drinks		
Age Group	Mean	90-PCTL	99-PCTL	Mean	90-PCTL	99-PCTL	Mean	90-PCTL	99-PCTL
0-23 mos.	3.4	11	29	1.3	6.3	19	0.60	-	15
2- 4 yrs.	6.6	17	38	3.3	10	25	1.1	-	20
5- 6 yrs.	5.0	13	24	2.9	8.5	20	0.68	-	13
7- 8 yrs.	4.4	11	24	2.4	7.5	18	0.57	-	12
9-12 yrs.	2.9	7.5	15	1.9	6.0	14	0.37	-	7.6
13-17 yrs.	1.8	4.9	10	1.5	4.8	11	0.26	-	6.1
18 yrs. +	1.0	2.9	6.5	0.95	3.2	8.1	0.11	-	3.8
All Ages	1.9	4.8	17	1.3	4.5	13	0.24	-	6.7

Table III--Potential Consumption of Aspartame From Group A Foods as Obtained by General Foods (1976) and Present Report (1982), in Milligrams per Kilogram Body Weight

(Based on Total Sample Within Each Age Group)

	General Foods		Present Report	
Age Group	Mean	90-PCTL	Mean	90-PCTL
0-23 mos.	-	-	3.4	11
Under 2 yrs.	2.9	9.6	-	-
2- 4 yrs.	-	-	6.6	17
2- 5 yrs.	4.9	12.9	-	-
5- 6 yrs.	-	-	5.0	13
7- 8 yrs.	-	-	4.4	11
9-12 yrs.	-	-	2.9	7.5
6-12 yrs.	2.9	7.7	-	-
13-17 yrs.	1.5	4.2	1.8	4.9
18-24 yrs.	1.1	3.2	-	-
18 yrs. +	-	-	1.0	2.9
25 yrs. +	1.0	2.9	-	-
All Ages	-	-	1.9	4.8

the potential aspartame consumption levels accordingly. Thus, whether the apparent increase in aspartame consumption, small though it appears to be, is real or not remains questionable.

Potential Aspartame Consumption versus Dietary Intake of Aspartame Amino Acid Constituents

Aspartame is comprised of approximately 50% phenylalanine (Phe) and 40% aspartic acid (Asp), the remainder being a methyl ester, moisture, etc. These same two amino acids occur in ordinary protein consumed each day by the U.S. population.

The earlier GF report (p. 8) presented data showing estimates of the Phe and Asp contents in the diets of different age groups, based upon average daily protein intake estimates published by the U.S. Department of Agriculture (1965). The USDA-derived data presented by GF are reproduced below in Table IV.

Table IV--Estimate* of Dietary Phe and Asp, Based upon Average Daily Protein Intake, in Milligrams per Kilogram Body Weight

Age	Weight	Phe	Asp
8 month old baby	8 kg	229	395
4 year old infant	16 kg	206	316
9 year old youth	28 kg	144	219
15 year old boy	50 kg	100	170
34 year old woman	60 kg	52	80

*Estimate based upon a representative diet delivering the total average protein consumed by people in the age/weight categories shown in the left column. Source: "Food Intake and Nutritive Value of U.S. Diets, Spring 1965," (USDA Study).

average amounts per eating or drinking occasion, in grams, for all FDA nutrition categories, from which appropriate products were selected to correspond with those needed for the present study. These are identified by the Searle Codes shown in Annex B of this Appendix. The portion-size data were correlated with the food and beverage products for which MRCA had tabulated the data on frequency of eating/drinking occasions (see Annexes A and B).

Detailed data on food/beverage portion sizes by age of respondent are presented in Annex D.

FORMULAS (AMOUNT OF ASPARTAME IN A GIVEN FOOD OR BEVERAGE)

With few exceptions, the prototype formulations developed by General Foods' Central Research were employed in the present study. The exceptions concerned levels of aspartame which were found to be optimum in certain soft drinks, as discussed on page 7 of this report, and the addition of a new product segment for "international" flavored coffee dry mix.

Listed below (pp. TA-7 and 8) are the levels of aspartame assigned to product types in Groups A, B, and C, as used in this study.

As pointed out earlier by GF, the levels shown do not necessarily represent those occurring in finished commercial products. Obviously, such formulas would vary from product to product. It is believed, however, that the levels indicated

do, in fact, approximate the sweetness necessary to replace sugar or saccharin in the 31 commercial product segments analyzed in this study.

Milligrams Aspartame (APM) per Gram Serving Weight for Each of 31 Product Segments

	Product Segments	mg APM Per Gram Eaten*	Sugar Sweetness Index**
	Group A -- Approved Products		
1.	Iced Tea - Dry Mixes	0.528	178
2.	Powdered Soft Drinks - Dry Mixes	0.546	194
3.	Juice Drinks ("Tang," etc.) - Dry Mixes	0.630	180
4.	Alcoholic Beverages - Dry Mixes	0.345	179
5.	Instant Breakfasts - Powder (Liq. Basis)	0.459	179
6.	Dietary Weight Control, Other Meal Replacement Products - Powdered	0.335	181
7.	Nutritional/Dietary Supplements - Powdered (Dry Basis)	2.820	180
8.	Flavored Milk Drinks - Powder/Mix	0.445	180
9.	Sugar (Table Top Uses Only)	5.500	180
10.	Sugar Substitutes (Table Top Uses Only)	5.500	-
11.	Gelatin - Dry Mixes	0.823	187
12.	Puddings - Dry Mixes	0.595	224
13.	Non-Dairy Toppings - Powdered/Dry	0.800	174
14.	Ready-to-eat Cereals - Presweetened (Dry Basis)	1.950	185
15.	Ready-to-eat Cereals - Regular (Dry Basis)	0.650	-
16.	Chewing Gum	6.275	125
17.	"International" Flavored Coffee - Dry Mixes	0.528	178

	Product Segments	mg APM Per Gram Eaten*	Sugar Sweetness Index**
	Group B -- Carbonated Soft Drinks		
18.	Cola, Regular (Sugar-sweetened)	0.550	181
19.	Orange/Fruit, Regular	0.950	181
20.	Fruit/Not Orange, Regular	0.550	181
21.	Non-Fruit/Non-Cola, Regular	0.550	181
22.	Quinine/Tonic/Carbonated Water, Regular	0.550	181
23.	Cola, Diet (Saccharin-Sweetened)	0.550	181
24.	Orange/Fruit, Diet	0.950	181
25.	Fruit/Not Orange, Diet	0.550	181
26.	Non-Fruit/Non-Cola, Diet	0.550	181
27.	Quinine/Tonic/Carbonated Water, Diet	0.550	181
	Group C -- Non-Carbonated Soft Drinks		
28.	Fruit Drink, Excluding Orange	0.550	181
29.	Orange Fruit Drink	0.950	181
30.	Soft Drink, Excluding Orange	0.550	181
31.	Orange Soft Drink	0.950	181

*The values in this column are based on the mg of aspartame per gram of food or beverage product as consumed, except for Group A Items 7, 14, and 15, which are stated on the dry basis.

**The Sugar Sweetness Index expresses the relative sweetness of aspartame to that of sugar for the particular product indicated. For example, in Iced Tea - Dry Mixes, aspartame is approximately 178 times as sweet as sugar.

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SEARLE RESEARCH AND DEVELOPMENT

ASPARTAME FOR USE AS A SWEETENER IN CARBONATED BEVERAGES

PETITION CONTROL VOLUME 3 of 4

STABILITY OF

ASPARTAME

REVISED 1974

ANALYTICAL RESEARCH LABORATORY

QUALITY CONTROL DEPARTMENT

SEARLE LABORATORIES

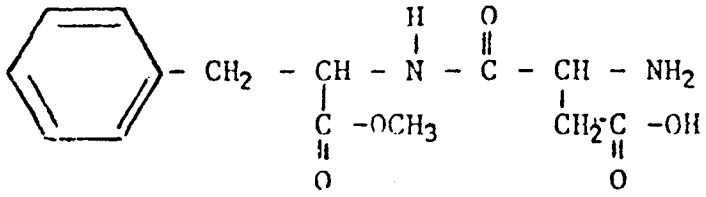
DIVISION OF G. D. SEARLE AND COMPANY

1972

GLOSSARY OF TERMS
FOR ASPARTAME
AND ITS DIKETOPIPERAZINE

A. aspartame

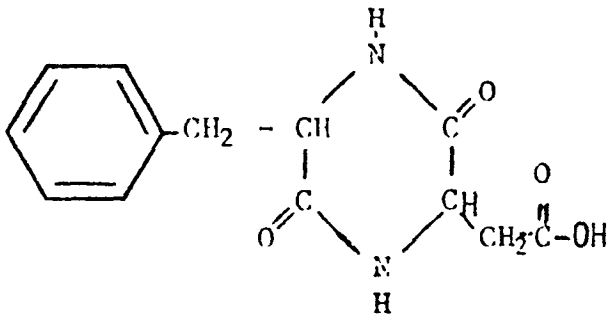
1. SC-18862



2. APM
3. Protid
4. aspartyl phenylalanine
5. L-aspartyl-L-phenylalanine methyl ester — $C_{14}H_{18}N_2O_5$, MW 294.30
6. 3-amino-N-(α-carboxy phenethyl) succinamic acid methyl ester

B. diketopiperazine of aspartame

1. SC-19192
2. DKP
3. diketopiperazine
4. 5 - benzyl - 3,6 - dioxo - 2 - piperazineacetic acid



$C_{13}H_{14}N_2O_4$

MW 262.258

STBAILITY OF ASPARTAME

ABSTRACT

This report contains data on the stability of aspartame in soluti in solid form stored in a glass vial, when containing various amounts water, and at an extremely high temperature. Data on the stability of aspartame in the spoon-for-spoon formulation in a marketed package and in a number of representative food preparations are also included.

In the study of the stability of aspartame in solution, buffered solutions of the substance were prepared at pH's between one and eight order to determine the rate of conversion as a function of pH. This study was carried out at four temperatures: 32°C, 40°C, 55°C, and 68° The method used for analysis of the extent of transformation was the ga liquid chromatographic detection of the methanol which would be formed any of the conversion reactions of aspartame. The rate of hydrolysis a the above temperatures appear to be pseudo first-order with respect to concentration of the compound. The optimal stability of the solution was found to be at pH 4.5 at the temperatures studied. The effect of temperature was demonstrated and the energy of activation was found to range from 14K Cal/mole to 21K Cal/mole depending on the pH. The half-lives of the conversion as functions of pH and temperature were reporte The stability at any pH and temperature can be calculated from the data given.

The stability of aspartame chemical (SC-18862) was evaluated by thin layer chromatography. The chemical was applied to a silica gel G plate along with appropriate amounts of diketopiperazine, the major conversion product. After elution with a solvent system of chloroform: methanol:water:acetic acid, 64:30:4:2 and detection with t-butyl hypochlorite and KI-starch, the quantity of diketopiperazine was determined by comparison to the amounts applied and other conversion products were noted.

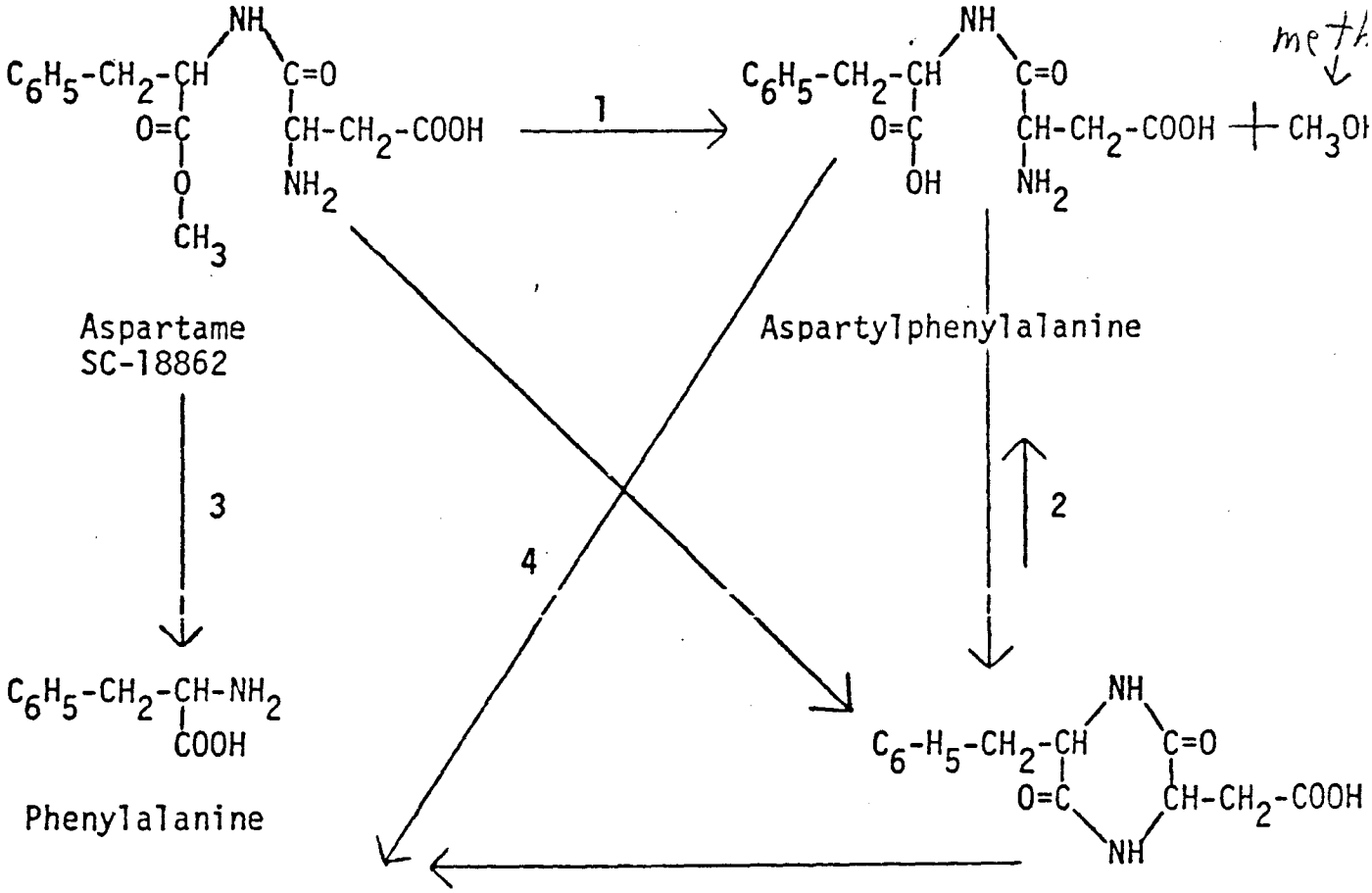
The studies conducted on aspartame chemical demonstrate the required stability as little conversion occurs at room temperature or under accelerated storage conditions.

The stability of aspartame of various water contents was evaluated in a similar manner. No significant degradent pattern was detected as a function of water content. These studies demonstrate the relat stability of aspartame through 8% water content.

Solid conversion at extreme conditions of high temperature was carried out, and the amount of conversion product (SC-19192) was determined by gas chromatography. It is concluded that the food additive is very stable even at temperatures as high as 105°C.

STABILITY OF ASPARTAME IN SOLUTION

The transformation of aspartame in aqueous solution is due mainly to the hydrolysis of the methyl ester linkage of the carboxylic compound. Although splitting of the amide linkages may occur, the rate of hydrolysis is much slower than that of the methyl ester. The hydrolysis may proceed as follows; with route 1 being the predominant mechanism.



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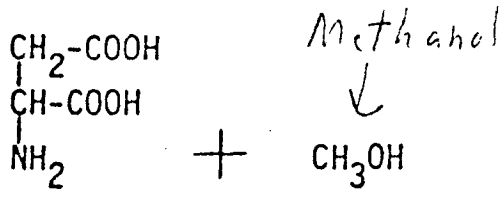

Aspartic Acid

Methanol

Analytical Method

Regardless of what pathway the hydrolysis may follow, methanol will be formed on the splitting of the methyl ester linkage. Following the formation of methanol will thus be the same as following the transformation of the Aspartame itself. Since the analysis of methanol in the reaction mixture is much more convenient it was decided that the kinetics of hydrolysis will be calculated based on methanol formation.

Gas chromatography was selected as the method of determination of methanol. In order to prevent the formation of more methanol from Aspartame during the high temperature gas chromatographic analysis, Amberlite IR-120 ion exchange resin was used to remove the Aspartame, DKP and other hydrolysis products. A typical chromatogram is shown in Figure 1.

Analytical Procedure

At periodic intervals 0.5 ml. aliquots were removed from the reaction tube and injected into a 5 ml. vial containing approximately 1 ml. of Amberlite IR-120 ion exchange resin. The resin absorbed excess SC-18862 to prevent interference with the gas chromatographic analysis. Methanol concentration was determined using a Varian 1800 series gas chromatograph. The sample (5.0 mcl.) was injected directly onto a 6 ft. x 1/4 in. o.d. teflon lined aluminum column packed with Chromosorb 102, 100/120 mesh. Injector temperature was approximately 180°C and column temperature 105°C. Detection was by flame ionization.

Normally each sample was injected into the gas chromatograph within 5 minutes after being taken from the reaction tube. If this was not possible the vial with the sample and the ion exchange resin was kept in ice. In no case did the delay exceed 20 minutes. For each group of samples analyzed, at least 3 methanol standards were used to determine a standard curve. These standard solutions were prepared fresh weekly. However, comparison of old standards with new indicated that the standards were good for at least 6 weeks.

To evaluate the stability of the SC-18862 in a vial with amberlite IR-120, two vials were injected with the same sample. One vial was placed in ice; the other left at room temperature. The following results were obtained:

continued

LONG-TERM TOLERANCE OF ASPARTAME

BY NORMAL ADULTS

The primary objective of this study was to study the effects of aspartame on normal volunteers when administered on a long-term basis. The quantity of aspartame ingested each twenty-four hour period was maintained at a constant level (1.8 gm) equivalent to approximately three times the normally expected adult daily consumption of aspartame when used as a sweetener. 1.3 grams in 1 Liter of Orange Soda

The study was conducted by Gunther H. Frey, M.D., of Hill Top Research, Inc., Miamiville, Ohio.

Materials and Methods

The study population consisted of two groups: (1) Subjects who had completed participation in the previously described short-term study of the tolerance of aspartame by normal adults and who were willing to continue without interruption in the long-term study for an additional 21 weeks; and (2) subjects who would follow the same study design, but who had not participated in the initial 6-week short-term study. All members of the latter group were screened in the same manner as those previously enrolled in the short-term study and were required to fulfill the same criteria for admission. Subjects were between the ages of 21 and 45, in apparent good health, and with baseline plasma phenylalanine levels that showed no evidence of a defect in phenylalanine metabolism of the phenylketonuric type.

Group I — participants in the 21-week extension of the short-term study without interruption — consisted of 18 males and 32 females. The members of this group continued to take the same preparation (aspartame or placebo capsules) to which they had originally been randomly assigned on a double-blind basis.

Group II — participants in the 21-week long-term study only — consisted of 12 males and 5 females. All members of this group took aspartame.

Each participant was instructed to take two capsules three times daily with meals for 21 weeks. Each capsule of aspartame contained 300 mg of the substance. All individuals continued their customary regular diet.

The following laboratory tests were done initially and 6, 12, 20, and 21 weeks after capsule-taking began:

Complete blood count (CBC)
Urinalysis, complete
PTT (partial thromboplastin)
Prothrombin time
BUN

SERUM:

Thyroxine (T_4)
Bilirubin (direct and indirect)
SGOT
Alkaline phosphatase
Uric acid
Creatinine
Cholesterol (total)
Triglycerides

Serum insulin and glucose levels were determined after a 4-hour fast (for baseline values) and repeated 30 minutes after loading each subject with 100 gm glucose orally. These tests were repeated 12, 16, 20, and 21 weeks later.

Eye examinations. — Pre- and post-test data for visual acuity, fundoscopic, and slit lamp examinations were received for all 67 participants; no abnormalities were noted. Data from tonometry examinations on a similar basis were received for 66 subjects and were within normal limits. Only post-test values which were not abnormal were available for the remaining individual. One male participant taking aspartame had a tonsillectomy performed during the study and complained of a "spot in his left eye" a month following the surgery. Examination by a consultant ophthalmologist revealed no abnormality, and the complaint ceased within 4 weeks. The consultant did not believe this complaint to be product-related.

Complaints.--In general, the complaints listed by those who completed both the short- and long-term study (50 subjects) paralleled the 17 subjects who participated in the long-term study only. Therefore, the complaint data from the two groups have been pooled because of their similarity. The most frequent complaints are listed in Table 12.

Table 12

COMPLAINT	ASPARTAME GROUP			PLACEBO GROUP		
	Male	Female		Male	Female	
(No. of subjects)	(23)	(24)		(7)	(13)	
Appetite decreased	1	1	(2)	2	0	(2)
Appetite increased	1	2	(3)	0	0	(0)
Constipation	3	3	(6)	1	0	(1)
Cramps, menstrual	0	6	(6)	0	1	(1)
Depression, mental	0	2	(2)	0	1	(1)
Diarrhea	1	1	(2)	0	0	(0)
Headache	2	3	(5)	0	0	(0)
Menses, early onset	0	4	(4)	0	1	(1)
Menses, less flow	0	3	(3)	0	0	(0)
Menses, greater flow	0	6	(6)	0	2	(2)
Spotting, vaginal	0	3	(3)	0	1	(1)
Stools, loose	3	1	(4)	0	1	(1)
Tired	0	4	(4)	1	0	(1)
Swelling, general	0	3	(3)	0	0	(0)
Weight gain	1	4	(5)	0	1	(1)
			58/47			12/20

Phenylpyruvic acid could not be detected before, during, or at the completion of capsule-taking. Tests for pregnancy using the urinary gonadotropin method were consistently negative throughout and at the end of the study for all women.

The data show no significant variation between Group I, participants in both the short-term and long-term studies without interruption, and Group II, those who completed the long-term study only.

No product-related side effects were reported.

FAP 2A3661

SEARLE RESEARCH AND DEVELOPMENT

ASPARTAME FOR USE AS A SWEETENER IN CARBONATED BEVERAGES

PETITION CONTROL VOLUME 4 of 4

TOLERANCE OF ASPARTAME BY DIABETIC SUBJECTS

The nutritive sweetening agent aspartame (aspartyl-phenylalanine-methylester) is approximately 180 times as sweet as sugar with 0.5% the caloric value for an equivalent amount of sweetening. It is anticipated that aspartame might therefore become widely used as a sugar substitite, of special value to persons with metabolic disorders that limit the use of sucrose.

The investigations described here were part of a program of studies to demonstrate the safety of aspartame for various types of prospective users. The present studies were designed to determine whether diabetic subjects -- both insulin-dependent and non-insulin-dependent -- can consume 1.8 gm aspartame daily for 90 days without signs or symptoms of intolerance and without elevation of the plasma phenylalanine level. This intake is about three times the expected adult daily consumption of aspartame when used as a sweetener.

The studies were conducted by Dr. Sheldon J. Bleicher of Roslyn Heights, N.Y. and Dr. Sol B. Stern of New Orleans, La. Their studies of the two types of diabetic patients will be discussed separately.

I. INSULIN-DEPENDENT DIABETIC SUBJECTS

Material and Methods

The subjects involved in this study were adults between the ages of 21 and 70 years who were dependent on insulin for the control of diabetes. They were presumed to have a normal ability to metabolize phenylalanine if their noon 4-hour fasting PA level did not exceed 4 mg/100 ml. There were no weight or blood pressure restrictions in enrolling subjects, but pregnancy, excessive alcohol consumption, and drug addiction or habituation were reasons for exclusion.

Prior to acceptance into the study, each subject was given a complete physical examination with the requirement that his results be within normal range on the following laboratory tests:

Complete blood count (CBC)
Partial thromboplastin time
Prothrombin time
BUN
Creatinine
T_4
Bilirubin (total, direct & indirect)

The following tests were also done at screening, but elevated levels did not exclude the subject:

SGOT
SGPT
LDH
Alkaline phosphatase
Glucose (fasting blood sugar)
Uric acid
Cholesterol (total & esters)
Triglycerides

Complete eye examinations, including tonometry, fundoscopic, and slit lamp examination, were done prior to and on completion of the study.

The study design was double blind, with the subjects randomly assigned to receive aspartame or matching placebo capsules. Participants were instructed to continue their customary diet and to take two capsules of the assigned study preparation three times daily with meals.

The study involved three periods: an initial week of screening; 13 consecutive weeks of capsule-taking; and a final week of follow-up tests.

Results

Seventy-seven of the insulin-dependent subjects completed the study. Table 1 shows the distribution of the study population.

TABLE 1

Study Population: Insulin Dependent Subjects

	Aspartame		Placebo		
Study of:	Male	Female	Male	Female	Total
Dr. Bleicher	9	25	10	25	69
Dr. Stern	5	0	3	0	8
	14	25	13	25	77

A second Stern patient (MRB#7) was forced to discontinue therapy at the end of week 11 when a left mastectomy was performed for adenocarcinoma. In view of information showing no evidence of carcinogenesis in animals who received large amounts of Aspartame over a prolonged period coupled with pathologist opinions regarding this type of tumor, the investigator postulated that the relationship between aspartame and the carcinoma was coincidental. (See appendix)

In Dr. Bleicher's study a 72-year-old female with prior atherosclerotic heart disease, suffered a stroke during her 8th week on aspartame. Careful review of the patient revealed that her FBS at entry was 193 mg% and her post-prandial blood sugar was 354 mg%. Serial fasting blood sugar values at 2-week intervals were 180, 120, 268, and 293 mg%. The patient remained stable during her hospitalization and was transferred to a nursing home where she later expired.

One Bleicher subject in the placebo group became pregnant and was dropped from the study. She elected to have an abortion, was re-enrolled, became pregnant a second time, was dropped again, and had a second abortion.

The most common complaints among the insulin-dependent subjects were directly related to their disease: insulin reaction, nervousness, and dizziness after insulin injection, and mild hypoglycemia (six aspartame and six placebo subjects). Skin rashes occurred in two placebo and one aspartame subject. Two other participants in the aspartame group complained of mild gastrointestinal distress.

3 months, and the mental development of such an infant will be evaluated at 6-month intervals until its third birthday.

One of Dr. Stern's non-insulin dependent patients in the aspartame group (LAD#62) was dropped from the study in the 11th week when she underwent surgery to remove a reticulosarcoma of the stomach. Both the investigator and a consultant pathologist (Dr. Bernard M. Wagner of Beekman Downtown Hospital, New York City) are of the opinion that detection of the gastric lymphoma was fortuitous and coincidental. Dr. Wagner reviewed slides of stomach sections and regional lymph drainage from rats who had consumed large amounts of aspartame for two years. He found no evidence of adverse effect on the gastric mucosa or other gastric tissues and no evidence of reticuloendothelial cell hyperplasia in the lymphoid aggregates adjacent to the stomach, reinforcing his opinion that the occurrence was unrelated to aspartame ingestion. (See appendix)

The most common complaints among the non-insulin-dependent diabetics were mild gastrointestinal discomforts as listed in Table 7.

TABLE 7

Complaints of Non-Insulin-Dependent Diabetics

Symptom	Incidence of Symptom	
	Aspartame Group (36 subjects)	Placebo Group (33 subjects)
Cramps	0	2
Nausea	1	1
Constipation	2	5
Diarrhea	3	0
Loss of appetite	1	1
Nervous	2	2

Overall Summary

Two studies have been described in which aspartame and placebo were administered over a period of 13 weeks to randomly assigned insulin-dependent diabetic volunteers and non-insulin-dependent diabetic volunteers. There was no indication that either aspartame or the placebo produced change or deterioration in the degree of diabetic control of any of the subjects in either study.

Prior to and at the end of the study the patients were subjected to a battery of laboratory determinations and other measurements, including weight, blood pressure, and pulse, ophthalmologic examinations, ECGs, plasma phenylalanine, and tyrosine. No discernible effect of aspartame or placebo was seen in either type of diabetic patient in any of the functions studied.

In summary, aspartame appears to be well tolerated by diabetic subjects as a safe sugar substitute.

March 6, 1973

Fred J. McIlreath, Ph.D.
Searle Laboratories Box 5110
Chicago, Illinois 60680

Dear Doctor McIlreath:

Enclosed are my detailed observations on the tissues studied. All microscopic slides were approximately 10-15 microns thick and stained with hematoxylin and eosin.

I. Patient M.R.B Female Age 50

Gross: Mass from left breast for frozen section.
Left mastectomy specimen including axillary contents

Microscopic: Slides (9) labelled S-72-7421. There are ductular epithelial cells arranged in small acini to large nests demonstrating prominent nucleoli and mitotic figures. These cells infiltrate the stroma and lymphatic invasion is present. An intense stromal hyalinization is noted throughout with little mesenchymal cell reaction. Metastatic cells are noted in lymph nodes.

Diagnosis: Adenocarcinoma of the breast, scirrhous type with axillary node involvement.

Comment: This lesion is an extremely common type of female breast carcinoma. It is difficult, if not impossible to determine the natural history of this tumor. However, the intense stromal hyalinization in the relative absence of fibroblastic proliferation, suggest a progressive sclerosis of some duration. Multiple, sequential biopsies of the skin and subcutaneous tissues from patients with generalized scleroderma (progressive system sclerosis), show that the process of collagen deposition-hyalinization takes many months. One may speculate that this pathologic process in association with cancer also requires many months.

II. Patient L.A.D. Female Age 50

Gross: Portion of stomach representing a subtotal resection. A large fungating mass arising from the mucosal surface is noted. The mass is 10.0 cm. in diameter. Also submitted (a) sections from proximal and distal edges of resection (b) pieces of attached adipose tissue (c) sections of omentum and (d) adipose tissue with lymph node.

Microscopic: Slides (13) labelled S-72-7035. The main tumor mass is characterized by an extensive pleomorphic cellular infiltrate of the gastric mucosa. Numerous abnormal mitotic figures are present and the proliferative process extends as sheets of cells forming a luminal mass. There is also invasion of the gastric wall involving the muscularis. Numerous plasma cells, mature lymphocytes and eosinophiles are intermingled with the large, mononuclear tumor cells.

One section of stomach (fundus) shows no evidence of tumor. All of the lymph nodes examined show varying degrees of reticuloendothelial (RE) cell hyperplasia. The RE cells are mononuclear, large with prominent nucleoli but abnormal mitoses are not present. Chronic inflammatory cells are present in the sinuses and medullary areas of the nodes.

Diagnosis: Malignant lymphoma, stomach, reticulum cell type (reticulum cell sarcoma)
Chronic lymphadenitis with RE cell hyperplasia

Comment: Primary lymphomas of the stomach are generally associated with a favorable prognosis as compared to lymphomas arising at other sites. One theory suggests that the pathogenesis is multifocal and that a "pre-malignant" phase is highly probable. The presence of extensive RE cell hyperplasia with cellular atypia in regional lymph nodes would tend to support this hypothesis. Etiology is unknown but by virtue of retrospective studies, patients may be in the "pre-malignant" phase for months to years.

III. Discussion

These two cases of malignancy, occurring in females who were involved in the clinical trial of Aspartame, are of the type that occur spontaneously in the population. Our current understanding of the natural

history of carcinoma of the female breast would not relate its etiology and pathogenesis to the compound under study. It is quite possible that the tumor was present before the study. In my opinion, the detection of breast cancer in this patient (M.R.B.) during the course of the study was coincidental and is not in any way related to the Aspartame study.

The reticulum cell sarcoma or malignant lymphoma occurring in patient L.A.D. is of the type frequently associated with a so-called "pre-malignant" phase of undetermined duration. In addition, not every atypical hyperplasia of reticuloendothelial need progress to malignant transformation. In my opinion, the detection of this malignancy during the Aspartame study was fortuitous and coincidental. There is no obvious relationship between the compound and the emergence of the tumor.

However, since RE hyperplasia was observed in the lymph nodes, a review of all of the stomach sections from the long-term rats wa

IV. Review of stomach sections from rats

Enclosed are the lists of slide numbers for each rat and group designation. The terminology of the original work sheets has been followed. A total of 60 female and 58 male controls were reviewed and all stomach sections were within normal limits. The following rats were studied:

Group	Male	Female
2	40	39
3	40	34
4	38	36
5	39	39
Total	157	148

Special attention was directed to mucosal alterations and lympho-reticuloendothelial cells. Wherever lymphoid aggregates were present, they were considered to be within normal limits. There were no significant pathological changes in the gastric mucosa or submucosa. It is obvious that under the conditions of the toxicity study, Aspartame was not toxic for the stomach.

V. Conclusions

1. Malignant tumors arising in 2 patients while receiving Aspartame were concluded to be coincidental and unrelated to the compound.

2. Aspartame did not induce lympho-reticuloendothelial cell hyperplasia and/or infiltration of the rat gastric wall under the conditions of the toxicity study.

Sincerely yours,

Bernard M. Wagner

Bernard M. Wagner, M.D.
Director of Laboratories,
Clinical Professor of Pathology
College of Physicians and Surgeons

BMW/mz