

CONSUMPTION OF DIKETOPIPERAZINE

The NutraSweet Company

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Aspartame is a high-intensity non-cariogenic sweetener that can be used to formulate highly palatable reduced-calorie products. The safety of aspartame in humans has been established not only from extensive safety assessment in animals but also from many clinical research studies including long-term high-dose tolerance studies. The human studies have also demonstrated it is safe and useful for diabetics and obese subjects in weight-loss regimens. The remarkable safety of aspartame is not surprising given its structure and metabolism. Aspartame (L-aspartyl-L-phenylalanine methyl ester) is rapidly and completely hydrolyzed in the gut to aspartic acid, phenylalanine and methanol, common dietary components. In fact, aspartame contributes only a small amount of these amino acids to that already consumed daily, even by heavy users of aspartame (e.g., 90th percentile, 14-day average).

The stability of aspartame in foods like any protein, peptide or peptide fragment, is dependent on the chemistry of the food (i.e., pH, water content, etc.) and its conditions of processing (e.g., heating). Peptides can undergo an intramolecular cyclization reaction to form diketopiperazines: they have been identified in a variety of food products ¹⁻⁴ and protein hydrolysates ⁵⁻⁸. Not surprisingly, aspartame was found to cyclize under conditions of use to cyclo (Asp-Phe), denoted herein as DKP.

DKP was subjected to extensive toxicity testing and ADI values for DKP of 7.5 and 30.0 mg/kg/day have been set by the Joint Expert Committee on Food Additives (JECFA)⁹ and the U.S. Food and Drug Administration (U.S. FDA)¹⁰, respectively. The purpose of this document is to discuss information

regarding the content of DKP in various foods and to estimate the overall intake of DKP in heavy users of aspartame-sweetened products. To estimate the consumption of DKP, it is necessary to determine: (i) the average amount of DKP (as a percent of APM initially present in the product) present in products in the marketplace or stored under conditions expected in the marketplace and (ii) the consumption rates of aspartame in the various food products in the U.S. Although this assessment will utilize data from the U.S., it is expected that it will be of value in evaluating the potential intake of APM in EEC countries. For example, very similar values for aspartame consumption have been recently obtained in a survey conducted by the Ministry of Agriculture, Fishing and Foods and the MRCA survey used in the U.S. to calculate these DKP intake rates. The amount of DKP in products has been obtained from studies conducted by NutraSweet using commercial products or prototype products. These studies include typical storage conditions as well as extreme conditions including temperature, storage time, pH, etc. The major food areas for which detailed information is available are summarized in this document and include: beverages (from samples obtained at retail), tabletop sweetener, baked goods, confections, frozen dessert, yogurts, and a broad category termed "others".

The levels of DKP present or expected at retail in the various food categories are summarized in Table 1. Based on a marketplace survey, the level of DKP in commercial carbonated beverages in the U.S. has been determined to be approximately 4.5% of APM initially present in the product. The details of this study are provided in Attachment A. As expected, the table top sweetener products contain low levels of DKP. The estimated quantity of DKP at retail for the other products is also summarized. It is important to note that the DKP concentrations shown are generally the average of the various prototypes tested at times and temperatures

generally encountered in the marketplace. The details of DKP estimation in foods are provided in Attachment A.

Using the DKP levels shown in Table 1, a conservative estimate of DKP consumption can be made using either current or projected consumption of APM. Table 2 summarizes the estimated consumption of DKP at the 90th percentile, 14-day average of APM consumption. The current consumption estimates for APM in the U.S. are from the July 1988 - June, 1989 annualized MRCA data for all ages, eaters only. Projected U.S. consumption for the baked goods and confections categories are from a comparable MRCA database (1984-1985) using the indicated level of APM. The consumption in the baked goods and confections categories are extreme overestimates because it assumes 100% market penetration of APM containing products.

Using the current APM consumption of 2.2 mg/kg body weight/day and a DKP level of 5% of the APM initially present, the estimated consumption of DKP is 0.11 mg/kg/day. The 5% level was utilized as representative of all foods currently consumed in the U.S. because the largest current component of APM consumption in the U.S. (>80%) is the beverage category. The estimated consumptions of DKP in baked goods and soft and hard confections are 0.28, 0.13 and 0.03 mg/kg/day, respectively. A summation of these DKP consumption estimates yields a value of 0.56 mg/kg/day at the 90th percentile, 14-day average for all ages, eaters only. (The intake of DKP is estimated to be 0.15 mg/kg/day at the 50th percentile, 14-day average for all ages, eaters only.) This combined value is the best available estimate of DKP consumption; however, it is an overestimate because 90th percentile intake rates when summed from individual categories are routinely much higher than estimation of the 90th percentile of use in all foods. Even this overestimate of DKP consumption is significantly lower than the ADI levels

for DKP of 7.5 mg/kg/day by JECFA and 30 mg/kg/day by the U.S. FDA.

In summary:

- ♦ The level of DKP has been estimated in the following categories: beverages, tabletop sweetener, baked goods, confections, frozen dessert, yogurts, and other foods. DKP levels in food were based on levels present in commercially available products (i.e., beverages) or in prototype products stored under relevant conditions.
- ♦ DKP consumption estimates (90th percentile, 14-day average for all ages, eaters only) were made using current APM consumption and anticipated APM use in remaining categories assuming a 100% market penetration. .
- ♦ The estimates of DKP consumption of 0.56 mg/kg/day at the 90th percentile, 14-day average of all age groups, eaters only, is significantly below the ADI levels set by JECFA and by the U.S. FDA for DKP of 7.5 mg/kg/day and 30.0 mg/kg/day, respectively.

TABLE 1

FOOD CATEGORY	DKP LEVEL AT RETAIL (% of aspartame initially present)
♦ Beverages	
Carbonated	4.5
Juices	
Teas	
Wines	
♦ Tabletop Sweetener	<0.3 ^a
♦ Baked Goods	9.9 ^a
♦ Confections	
Soft Candy	8.9 ^a
Hard Candy	5.6 ^a
♦ Frozen Dessert	3.5 ^a
♦ Yogurts	9.2 ^a
♦ Others ^b	14.0 ^c

^a Based on prototype products stored under relevant marketplace conditions.

^b Other categories include: Breakfast cereals, chewing gum, dry mixes, chewable vitamins, cookie fillings, frozen cheesecake, frozen fruit, frozen fruit toppings, frozen dairy and non-dairy toppings, frosting and fillings, fruit spreads, fruit toppings, and fruit syrups.

^c Worst-case estimate based on range of DKP concentrations on representative prototype in the other category (1.6-13.7%).

TABLE 2
APM AND DKP CONSUMPTION ESTIMATES

	DKP CONTENT ^a (% of aspartame initially present)	APM CONSUMPTION ^b (mg/kg/day)	DKP CONSUMPTION (mg/kg/day)
CURRENT APM CONSUMPTION IN THE U.S.			
	5%	2.2	0.11
PROJECTED U.S. CONSUMPTION OF:			
♦ Baked Goods	10%	2.8 ^c	0.28
♦ Confections			
Soft Candy	9%	1.4 ^c	0.13
Hard Candy	6%	0.6 ^c	0.04
		TOTAL	0.56^d

^a These values are provided as projected estimates of DKP content at retail of all the foods as categorized. The 5% value of DKP for current APM consumption in the US was chosen because of the large contribution that beverages make to the overall current consumption of aspartame in the U.S. (>80%).

^b Annualized MRCA survey data July 1988 - June 1989, 90th percentile, 14-day average for all ages, eaters only.

^c Estimated intake values are based on average use levels of aspartame of 2,000 ppm for baked goods, 2,500 ppm soft candy and 3,000 ppm for hard candy. These values are provided as estimates of consumption based on current consumption of applicable foods as determined by the MRCA Survey data base. These estimates are overestimates as they assume 100% market penetration of aspartame-containing products.

^d The total value represents the sum of the 90th percentile, 14-day average values for current use and of the projected uses of baked and confectionery products. This value is an overestimate as it is unlikely that the 90th percentile user of beverages will also be a 90th percentile user of baked goods and confections.

ATTACHMENT A

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Beverages

In a study of commercially available carbonated beverages, samples of various beverages were obtained from retail outlets in four U.S. cities and the amount of APM in each type of soft drink (20 brands total) was measured. The quantity of DKP in these beverages can be estimated by knowing the extent to which the APM had degraded. These estimates are derived from data in a beverage study (partial presentation, ref. 11) that examined the stability of APM under a number of pH and temperature conditions approximating retail market environments.

Table A summarizes the results of the Commercial Carbonated Beverage Study. Samples were obtained for analysis on three separate occasions: July, September, and November 1985. The average amount of APM remaining in these beverages was constant at 87-88% over the sampling period.

- (i) the geographical locations where the samples were taken (Chicago, Los Angeles, Miami and Phoenix) and the time of year of sampling would increase the probability that these samples were exposed to higher than US average ambient temperatures,
- (ii) the percent APM remaining assumes an industry average starting level of 500ppm APM,
- (iii) the data presented in Table A do not include those beverages that are blends with saccharin.

The average amount of DKP in these beverages, as estimated using the results of the beverage stability study, is 4.5%,

Table B. Given that the beverage study did not have a sample that had degraded exactly 88% it was necessary to interpolate what the concentrations of the DKP would be. The above percentages represent the mean of the values that were immediately higher and lower than the 88% APM remaining level, Table B. The data for the beverage stability study were obtained on the pH 3.19 solutions buffered with phosphoric acid and sodium citrate because of its similarity to the majority of the carbonated beverages examined.

Tabletop Sweeteners

The level of DKP in aspartame is generally 0.3% or lower.

Baked Goods

The level of DKP (% of aspartame initially present) in aspartame containing baked goods is 9.9%. This represents a mean value for five prototype baked good products and they were determined in the storage conditions indicated.

Product	DKP (% of aspartame initially present)	Time	Temperature
Cheesecake	3.4	9 days	70°F
Yellow Cake	12.5	8 days	70°F
Chocolate Cake	28.2	10 days	70°F
Cookie	3.6	12 weeks	70°F
Fig Bar	1.8	16 weeks	70°F
Mean 9.9			

Confections

Soft Candy: The level of DKP (% of aspartame initially present) in aspartame containing soft candy is 8.9%. This represents a mean value for four prototype soft candy products determined after storage in the conditions indicated.

Product	DKP (% of aspartame initially present)	Time	Temperature
Gelled Candy	9.5	8 weeks	70°F
Nougat	16.6	7 weeks	70°F
Caramel	5.3	9 weeks	70°F
Candy Coating	4.3	9 weeks	70°F
Mean		8.9	

Hard Candy: The level of DKP (% of aspartame initially present) in aspartame containing hard candy is 5.6%. This is the value determined after storing a prototype hard candy for twenty-six weeks at 70°F and 40-50% relative humidity.

Frozen Dessert

The level of DKP (% of aspartame initially present) in aspartame containing frozen yogurt is 3.5%. This value was determined after storage for 8.5 months at -20°F.

Yogurt

The level of DKP (% of aspartame initially present) in aspartame containing yogurt is 9.2%. This represents a mean value for three prototype yogurt products and they were determined in the storage conditions indicated.

Product	DKP (% of Aspartame initially present)	Time	Temperature
Yogurt-type	11.5	4 weeks	40°F
Raspberry swiss -style	12.0	4 weeks	40°F
Raspberry sundae -style	4.2	4 weeks	40°F
Mean 9.2			

Other Categories

This group includes: breakfast cereal, chewing gum, dry mixes, chewable vitamins, cookie fillings, frozen cheesecake, frozen fruit, frozen fruit toppings, frosting and fillings, fruit spreads, fruit toppings and fruit syrups. The level of DKP (% of aspartame) in representative categories includes a low of 1.6% in dry mixes to a high 13.7% in fruit spreads. The level of DKP in four dry mixes was determined after storage of 20-24 weeks at 70°F and 50% relative humidity. The level of DKP in fruit spreads was determined after 3 months at 70-75°F.

TABLE A

SUMMARY OF COMMERCIAL CARBONATED BEVERAGE STUDY^a

SAMPLING DATE	% OF APM REMAINING
7/85	87 ± 11
9/85	88 ± 12
11/85	88 ± 12

^a Values represent mean ± SD of 51-56 samples.

TABLE B

SUMMARY OF MOCK BEVERAGE STUDY^a

PERCENT PRESENT ^b	
APM	DKP
88.9	3.2
84.0	5.8
(Mean)	4.5

^a Initial pH = 3.19, with preservative (.2000 g/l), buffered with phosphoric acid (.8300 g/l), sodium citrate (.4600 g/l). Reference 11.

^b Expressed as percent of APM initially present.

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