

## SECTION H

**Environmental Assessment for  
N-[ N-(3,3 -dimethylbutyl) -L- $\alpha$ -aspartyl]-L-phenylalanine 1-methyl ester  
(NC-00723: Neotame)  
[21 CFR 25.31a]**

1. **Date:** December 17, 1998
2. **Name of Petitioner:** Monsanto Company
3. **Address:** 5200 Old Orchard Road, Tower 1  
Skokie, IL 60077
4. **Description of the proposed action:**
  - a. **Requested *approval*** Monsanto Company proposes a new direct food additive amendment to 21 CFR Part 172 for the approval of a new high intensity sweetener, N-[ N-(3,3 -dimethylbutyl) -L- $\alpha$ -aspartyl]-L-phenylalanine 1-methyl ester (NC-00723) as a sweetener and flavor enhancer in food under current good manufacturing practice.
  - b. ***Need for action:*** NC-00723 will function as a high intensity sweetener and flavor enhancer in food in accordance with current good manufacturing practice.
  - c. ***Location of Use:*** NC-00723 will be used as a sweetener at any location where food products containing these types of sweeteners are manufactured and will be available in such products at any location, such as homes, restaurants, or offices. This sweetener will be consumed as a component of the human diet in patterns corresponding to national population density similar to other currently regulated and/or generally recognized as safe non-nutritive and nutritive sweeteners.
  - d. ***Location of Disposal:*** Normal ingestion of this sweetener will result in excretion of the sweetener (approximately 5% of intake) and its major metabolite, N-[ N-(3,3-dimethylbutyl) -L- $\alpha$ -aspartyl]-L-phenylalanine (NC-00751 ) (approximately 80% of intake) through municipal waste water treatment plants and cesspools/septic tanks. Disposal of minor manufacturing site wastes will be through municipal wastewater treatment plants and/or solid waste treatment facilities or incineration. Disposal of minor consumer wastes from discarding the food products containing NC-00723 will be through solid waste treatment facilities or incineration.

5. **Identification of substances that are the subject of the proposed action:**

The physical and chemical properties of the proposed sweetener and its major metabolize are in Table 1; the chemical structures are in Table 3.

6. **Introduction of substances into the environment:**

a. *Manufacture:* There are no extraordinary circumstances that apply to the manufacture of NC-00723.

b. *Use:* NC-00723 is a high intensity sweetener (7,000 -13,000 times the sweetness of sugar) which will be used to replace other sweeteners and the proposed general use represents an extremely low exposure level (0.05 mg/kg). Even though this exposure is low, the animal metabolic data discussed in Section E of FAP 8A4580 demonstrate that about 5% of NC-00723 and about 80% of its metabolize, NC-00751, is available for excretion.

c. *Disposal* The normal ingestion of this proposed sweetener and minor wastes at various individual food manufacturing sites will result in excretion of the sweetener and its major metabolize through municipal waste water treatment plants and cesspools/septic tanks. Disposal of minor consumer wastes from discarding the food products containing NC-00723 will be through solid waste treatment facilities or incineration.

The estimates of the maximum yearly market volume of NC-00723 for the proposed use, and the calculated expected introduction concentrations (EIC) of NC-00723 and its main degradation product, NC-00751, to be present in wastewater effluents, in sewage sludge and in the atmosphere are provided in a separate table (Table 2) marked CONFIDENTIAL in accordance with 21 CFR 25.51 (a). From these calculations, it is apparent that the introduction of NC-00723 and NC-00751 will not represent a significant effect to the aquatic, terrestrial or atmospheric environments.

7. **Fate of substances released into the environment:**

See Table 1 for fate parameters.

8. **Environmental effects of released substances:**

NC-00723, N-[N-(3,3-dimethylbutyl)-L- $\alpha$ -aspartyl]-L-phenylalanine 1-methyl ester or neotame, is a high potency sweetener which is about 30-60 times sweeter than aspartame. Given its remarkable sweetness potency, NC-00723 will be used in much smaller quantities and lower concentrations

than other high intensity sweeteners. For general use, estimated daily consumer intakes at the mean and 90<sup>th</sup> percentile, users-only, are 0.02 and 0.05 mg/kg, respectively.

Chemical stability studies and functionality studies conclusively show that NC-00723 is fully functional under general use conditions. NC-00723 is stable as a dry powder, and in various food applications under relevant conditions of use. The methyl ester moiety of NC-00723 is very slowly hydrolyzed, depending on pH and temperature, to form NC-00751 in aqueous solution.

Neotame has an excellent metabolic and pharmacokinetic profile. Neotame is rapidly but incompletely absorbed in all species. It is completely eliminated with recovery of a dose of radiolabeled neotame in urine and feces exceeding 98% in the human and greater than 93% in the rat and the dog. Neotame is readily de-esterified to NC-00751; approximately 80% of a neotame dose is excreted as NC-00751 in all species tested. Neotame and NC-00751 have short plasma half-lives with rapid and complete elimination. All human metabolites detected in amounts of approximately 1% or greater have been identified and shown to be present in the toxicology species. In human pharmacokinetic studies, plasma concentrations of neotame and NC-00751 increase proportionally with dose, and there is no evidence of accumulation. Neotame does not induce liver microsomal enzymes. A comparison of the metabolism and pharmacokinetic data in humans with those from animals used in the toxicology studies indicates that the animal species are relevant for predicting human safety.

Toxicity studies were done with neotame at dose levels 10,000-80,000 times the 90<sup>th</sup> percentile estimated human consumption for general use in food. These included chronic toxicity (with in utero exposure) and carcinogenicity (with in utero exposure) studies in the rat, a carcinogenicity study in the mouse, a chronic toxicity study in the dog, in vitro and in vivo genotoxicity assays, a two-generation reproduction study in the rat and teratology studies in the rat and rabbit. In addition, auto-exposure to NC-00751, the major metabolite and major hydrolysis product under conditions of use, occurred in all toxicology species.

There is no evidence of target organ toxicity in subchronic studies in rats, mice and dogs or chronic toxicity studies in rats (with in utero exposure) and dogs. Neotame is not mutagenic or clastogenic. Furthermore, neotame is not teratogenic in either the rat or rabbit. Similarly, no effects are observed on reproductive parameters or offspring development in a rat two-generation reproduction study. Neotame is not carcinogenic in either the rat with in utero exposure or in the mouse.

As addressed and concluded previously in FAP 8A4580, the only noteworthy finding, aside from palatability-related reductions in food consumption and body weight in rodents, is an increase in serum hepatic alkaline phosphatase activity (AP) in dogs only at the highest dosage in the one-year study. The increase in AP is rapidly reversible and not associated with any evidence of target organ toxicity. The increase in AP is a species-specific physiological response in dogs that is not considered adverse.

As previously discussed and concluded in FAP 8A4580, the reductions in food consumption and body weight observed in rodents are also not adverse; rather, a diet preference study in rats establishes that decreased diet palatability occurs at concentrations associated with weight reduction.

The lack of toxicity or other adverse effects at high dosages and the low estimated intake combine to provide remarkably large safety margins for neotame relative to other sweeteners. Even at the estimated 90<sup>th</sup> percentile consumption for general use in foods, the margins of safety are at least 10,000 to 80,000-fold based on no-observed-adverse-effect levels (NOAELs).

In addition to demonstrating the safety of neotame for use as a general purpose sweetener, the safety of degradants formed under conditions of intended use was also assessed. The close structural similarity of neotame to aspartame and the understanding of degradation pathways of aspartame contributed to the complete characterization of neotame degradation.

As mentioned previously, soft drink beverages are the largest use category for high intensity sweeteners and present food chemistry conditions likely to promote degradation of neotame. Therefore, a mock beverage stability study was done to develop data relevant to consumer exposure to degradants. This mock beverage stability study used either phosphate or citrate solutions buffered at low pH to simulate either cola- or citrus-based soft drinks, respectively. Excellent mass balance in mock beverages of approximately 97-101 mole percent was achieved for neotame and its degradation products at all conditions of pH, temperature and storage duration.

Stability data reveal that NC-00751 is the major degradant formed in aqueous solution by slow hydrolysis of the methyl ester of neotame. NC-00751 is also the major metabolite of neotame in animals and humans; thus, all preclinical and clinical safety studies done with neotame include auto-exposure to NC-00751. This and additional safety studies clearly establish the safety of NC-00751 .

Upon formation of NC-00751, a negligible quantity of methanol is also released. This amount of methanol is about 2,000 times less than the level the FDA has stated is safe. Three minor non-functional degradants can also develop in beverages subjected to relevant storage conditions (i.e., pH 3.2,

20°C for 8 weeks). These substances arise through pH-dependent and temperature-dependent cyclization of neotame to NC-00777, beta-rearrangement from the cyclized product to NC-00764, and hydrolysis of the methyl ester of the cyclized product to NC-00779. Under relevant storage conditions, the levels of these degradants are <1% of the initial concentration of neotame in soft drink beverages. Based on degradant exposure estimates of <0.4 µg/kg, these levels equate to a dietary concentration of less than 10 ppb of each minor degradant at the estimated 90<sup>th</sup> percentile consumption level of neotame.

The properties of these minor degradants of neotame share a number of similarities with indirect food additives from the perspective of consumer exposure. Neither indirect additives nor the minor degradants of neotame are present or detectable at the time the food or beverage is packaged. The concentrations of indirect food additives and degradants of neotame increase over time. Consumer exposure to indirect food additives and non-functional degradants, therefore, is determined by the length of time the product is stored. Consequently, as with indirect additives, exposure to the minor degradants of neotame is variable and not subject to self-selection or abuse exposures by the consumer. The consumer does not intentionally select those products with the highest concentration of degradant any more than the consumer would intentionally select products with the highest concentration of indirect additives. Thus, studies were done with these minor degradants following FDA's policy for indirect additives occurring at dietary concentrations less than 50 ppb.

NC-00764, NC-00777 and NC-00779 were examined individually for genotoxicity and acute toxicity. In addition to FDA's guidance, a 4-week dietary safety study was done in rats with these degradants combined in proportion to estimated exposure to assess the effects of degradants, potential interactions and synergistic effects. There was no evidence of toxicity observed in these studies. This establishes the safety of the degradants under the intended conditions of use of neotame. The highest dosages of the 4-week study demonstrate a margin of safety exceeding 15,000 times the estimated 90<sup>th</sup> percentile of consumer exposure to each of these degradants.

Although not required by FDA's 1982 Redbook guidelines, clinical studies were done to further confirm the safety of neotame in humans. Clinical studies have evaluated the safety of neotame when administered in single and multiple doses ranging up to 13 weeks in healthy populations including male and female subjects. In addition, neotame was evaluated in male and female individuals with non-insulin dependent diabetes mellitus. Studies in humans have included assessments of adverse experiences, clinical well-being, physical examinations, clinical chemistry, hematology, urinalysis, electrocardiograms, and ophthalmologic evaluations. Results from these

studies demonstrate that neotame is safe and well tolerated in humans. The results of the study in diabetics also demonstrate that neotame is well tolerated and confirm that neotame does not affect glycemic control in a non-insulin dependent diabetic population.

The metabolism and pharmacokinetics of neotame have also been evaluated as part of the human safety studies. The results from these studies confirm the conclusions from the animal studies that neotame metabolism is simple and well characterized. Neotame is rapidly absorbed and rapidly eliminated with the major metabolic pathway being de-esterification to NC-00751. The pharmacokinetics and metabolism of neotame in humans are also dose proportional over a large range of single and multiple doses, which further support the desirable metabolic profile of this sweetener. Neither neotame nor its de-esterified metabolite NC-00751 accumulate following doses of up to 40 times the 90<sup>th</sup> percentile of estimated consumption. Furthermore, all neotame metabolites in humans present at greater than 1 % of the dose have been shown to be present in the toxicology species, thus confirming the safety of these metabolites through auto-exposure.

The human studies included single doses ranging from 0.1 to 0.5 mg/kg body weight, repeated doses of 0.25 mg/kg hourly for eight hours totaling 2 mg/kg/day, and daily doses of 1.5 mg/kg for up to 13 weeks. To put these doses into perspective, the 0.25 mg/kg hourly dose in the repeated dose study is equivalent to consuming about 1 liter of beverage sweetened with neotame in a bolus dose every hour for an 8 hour period. The high dose of 1.5 mg/kg/day in the 13-week study is equivalent to consuming about 6 liters of beverage sweetened with NC-00723 daily for 13 weeks. Thus, the results of the clinical studies with neotame, using doses up to 40 times the projected 90<sup>th</sup> percentile consumption level, clearly confirm the safety of neotame for use by the general population.

Neotame is proposed as a high potency sweetener and flavor enhancer which is functional in a wide variety of food applications. The functional properties, low consumer exposure, stability, and safety of neotame make it an ideal sweetener for general use applications. This petition conclusively establishes that even at the highest dose levels administered in safety studies, neotame and its degradation products do not produce toxicity or adverse effects. Neotame is not carcinogenic, mutagenic, teratogenic or associated with any reproductive toxicity, or general toxicity. The no-adverse-effect-levels for neotame are at least 1,000 mg/kg in rats, 4,000 mg/kg in mice, 800 mg/kg in dogs, and 500 mg/kg in rabbits. Consumer exposure for general use in food at the 90<sup>th</sup> percentile is estimated to be 0.05 mg/kg/day; thus margins of safety for neotame in test species are > 20,000-fold in rats, > 80,000-fold in mice, > 16,000-fold in dogs, and

> 10,000-fold in rabbits. The safety of the major degradation product under conditions of use, NC-00751, has been established through auto-exposure as it is also the major metabolite of neotame in all toxicology species. The minor degradants would not be detectable at actual use concentrations of neotame in soft drinks under relevant conditions of use. Nonetheless, results of genotoxicity tests, acute and 4-week studies in the rat with the minor degradants establish they are not mutagenic or clastogenic, and there is no evidence of toxicity with large margins of safety.

Thus, neotame is safe and well tolerated in male and female human subjects in amounts up to 40 times the projected 90<sup>th</sup> percentile of use. In addition, neotame is well tolerated and does not alter glycemic control in a population with non-insulin dependent diabetes mellitus. In conclusion, this petition clearly establishes neotame as a general use high potency sweetener and flavor enhancer with excellent functionality and extremely large margins of safety for the general population.

**9. Use of resources and energy:**

NC-00723, a high intensity sweetener, will provide an alternative choice for consumers to products currently containing sucrose, aspartame, acesulfame potassium, sucralose, and saccharin. The manufacturing process for NC-00723 uses less energy than is required for the production of aspartame and much less than is required for the production of sucrose. Therefore, there is no effect on the use of natural resources and energy when the proposed sweetener is produced, transported, used, and/or the wastes generated from the production, use and/or disposal.

**10. Mitigation measures:**

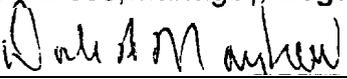
There are no adverse environmental effects identified in the production, transportation, use and disposal of NC-00723; therefore, no mitigation measures are needed.

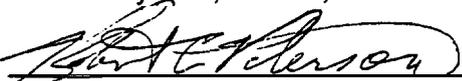
**11. Alternative to the proposed action:**

There are no potential adverse environmental impacts identified in the proposed action of introducing NC-00723, a high intensity sweetener for general use and, therefore, there is no need to consider alternatives to the proposed action.

**12. List of Preparers:**

  
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Sue Andress, Manager, Regulatory Affairs

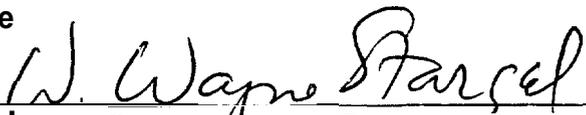
  
\_\_\_\_\_  
Dale Mayhew, Ph.D., %od Safety Director

  
\_\_\_\_\_  
Robert C. Peterson, Director, Regulatory Compliance

**13. Certification:**

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Monsanto Company.

12 / 17 / 98  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
W. Wayne Stargel, Pharm.D.  
Vice President, Nutrition and Consumer Regulatory Affairs

**14. References:**

Not applicable

**15. Attachments:**

- Table 1
- Table 2- Confidential
- Table 3

**Table 1**  
**Chemical Characterization of NC-00723 and NC-00751**

<b>NC-00723; N-[N-(3,3-dimethylbutyl)-L-<math>\alpha</math>-aspartyl]-L-phenylalanine 1-methyl ester (lot# 96NK008-8)</b>																											
<b>NC-00751: N-[N-(3,3-dimethylbutyl)-L-<math>\alpha</math>-aspartyl]-L-phenylalanine (lot# 1P-1 123-27-5)</b>																											
<b>Parameters</b>	<b>NC-00723</b>	<b>NC-00751</b>																									
Empirical formula	<b>C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub></b>	<b>C<sub>19</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub></b>																									
Structural formula	<b>C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub></b>	<b>C<sub>19</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub></b>																									
Molecular weight	<b>378</b>	<b>364</b>																									
CAS registry #	<b>165450-17-9</b>	<b>190910-14-6</b>																									
Assay (wt/wt%) <sup>[1]</sup>	<b>100.9</b>	<b>99.24 (area%)</b>																									
Water content	<b>4.5%</b>	<b>0.14%</b>																									
Other related substances <sup>[1]</sup>	<b>None <math>\geq</math> 0.05</b>	<b>One impurity 0.69 area% L-phenylalanine 0.03 wt/wt%</b>																									
pH <sup>[2]</sup>	<b>5.80 (0.5% aqueous)</b>	<sup>[3]</sup>																									
Melting point <sup>[2]</sup>	<b>80.9 -83.4 °C</b>	<b>178- 180 °C</b>																									
Solution density <sup>[2]</sup>	<b>0.997 g/mL (0.5% aqueous)</b>	<sup>[3]</sup>																									
Specific gravity <sup>[2]</sup>	<b>1.00 (0.5% aqueous)</b>	<sup>[3]</sup>																									
Volubility in various liquids <sup>[2]</sup>	<table border="0"> <tr> <td></td> <td><u>25 °C</u></td> <td><u>40 °C</u></td> <td></td> </tr> <tr> <td>Water:</td> <td>1.26%</td> <td>1.80%</td> <td>Water: &lt;0.1%</td> </tr> <tr> <td>Ethanol:</td> <td>&gt;100%</td> <td>&gt;100%</td> <td>Ethanol: &lt;0.1 %</td> </tr> <tr> <td>Ethyl Acetate:</td> <td>7.700%</td> <td>23.89%</td> <td></td> </tr> </table>		<u>25 °C</u>	<u>40 °C</u>		Water:	1.26%	1.80%	Water: <0.1%	Ethanol:	>100%	>100%	Ethanol: <0.1 %	Ethyl Acetate:	7.700%	23.89%		<table border="0"> <tr> <td></td> <td><u>25 °C</u></td> <td><u>40 °C</u></td> </tr> <tr> <td>Water:</td> <td>&lt;0.1%</td> <td>&lt;0.1%</td> </tr> <tr> <td>Ethanol:</td> <td>&lt;0.1 %</td> <td>&lt;0.1 %</td> </tr> </table>		<u>25 °C</u>	<u>40 °C</u>	Water:	<0.1%	<0.1%	Ethanol:	<0.1 %	<0.1 %
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Ethanol:	>100%	>100%	Ethanol: <0.1 %																								
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Water:	<0.1%	<0.1%																									
Ethanol:	<0.1 %	<0.1 %																									
Refractive index <sup>[2]</sup>	<b>1.3338 (0.5% aqueous)</b>	<sup>[3]</sup>																									
Octanol/water partition coefficient (log <sub>10</sub> P) <sup>[2]</sup>	<b>0.917</b> <b>1.70<sup>[5]</sup></b>	<b>0.23 (Lot# IP-1 281-47-1 )<sup>[4]</sup></b> <b>-1.52<sup>[5]</sup></b>																									
Elemental analysis <sup>[2]</sup>	<b>C 61.9% H 8.05%</b> <b>N 7.21 % O 22.8%</b>	<b>C 62.18% H 7.74%</b> <b>N 7.66% O 22.42%</b>																									
Residue on ignition <sup>[1]</sup>	<b>0.06%</b>	<b>Ash: 0.7%</b>																									
DSC <sup>[2]</sup>	<b>transition at 83.4 °C</b>	<b>transition at 188.8 °C</b>																									
Specific rotation <sup>[1]</sup>	<b>-41.5°</b>	<b>-14.20°<sup>[6]</sup></b>																									

<sup>[1]</sup> NC-00723 data obtained from specification testing

<sup>[2]</sup> NC-00723 data obtained from physicochemical properties testing

<sup>[3]</sup> Insufficient volubility to acquire data

<sup>[4]</sup> pH about 5.3

<sup>[5]</sup> pH 7.3

<sup>[6]</sup> Sample solvent is glacial acetic acid