Original: Japanese
Provisional translation

Evaluation Report

Neotame

October 2006

Food Safety Commission

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Evaluation results about health risk assessment of Neotame in accordance with designation of additive

1. Introduction

Neotame has been developed as a sweetener with a high degree of sweetness and is obtained by *N*-alkylating aspartame, an existing sweetener. Its degree of sweetness varies according to the kinds of foods and blend composition and is 7,000 to 13,000 times and about 30 to 60 times sweeter than sugar and aspartame, respectively[1), 2)]. Neotame is said to have superior stability to existing sweeteners[3-6)] and not to release phenylalanine under normal storage conditions[7)].

In the United States, Neotame was permitted for use in the general food categories as a sweetener and a flavor potentiator in 2002[8)]. As of June 2004, it was permitted for use in 19 countries, including Australia and New Zealand, and has been used in foods, mainly beverages, as a sweetener and a flavor potentiator.

2. Background

The company requested the Ministry of Health, Labour and Welfare (MHLW) to designate Neotame as an additive. As the review of Neotame for designation as an additive was initiated, the MHLW requested the Food Safety Commission to assess the health risk of Neotame in accordance with the Food Safety Basic Law (receipt of related documents on January 31, 2005).

3. Outline of designation of additive

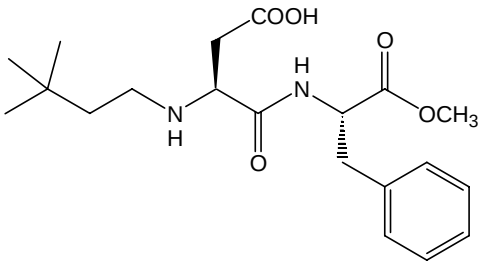
After the usage standard and specifications of Neotame used as a sweetener and a flavor potentiator are considered, Neotame will be designated as a new additive.

4. Outline of Neotame

Name: Neotame

Chemical name: *N*-[*N*-(3, 3-dimethylbutyl)-L-α-aspartyl]- L-phenylalanine 1-methyl ester

Chemical structure:



Chemical formula: $C_{20}H_{30}N_2O_5$

Relative molecular mass: 378.46

CAS number: 165450-17-9

Properties: Neotame is an odorless white to gray-white powder with a strong sweetness and is readily soluble in alcohols and slightly soluble in water. The 0.5% aqueous solution of Neotame is weakly acidic (pH 5.8).

5. Safety

(1) Absorption, distribution, metabolism, and excretion

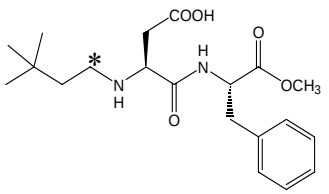
I. Nonclinical data

A. Absorption

(a) Single-dose study of ^{14}C-labeled Neotame in rats

The total plasma radioactivity and the change in plasma concentration were examined in male and female Sprague-Dawley (SD) rats after the single oral gavage administration of 15 (low dose) or 120 (high dose) mg ^{14}C-labeled Neotame/kg body weight and after the intravenous administration of 15 mg ^{14}C-labeled Neotame/kg body weight[*1]. The plasma concentration peaked 0.1 h after the intravenous dose and 0.25–0.75 h after the oral dose. The maximum plasma concentration (*C*max) and area under the plasma concentration versus time curve from 0 to 24 h (AUC_{24}) increased approximately corresponding to the increasing dose. These parameters were larger in males than in females. The unchanged substance was detected at a level slightly above the detection limit 0.25 h after the high oral dose in 50% of the rats, but was not detected after the low oral dose or the intravenous dose. Neotame was rapidly converted into NC-00751[*2] (80–90% of the total radioactivity) after administration, and its plasma concentration peaked 0.1 h after intravenous administration and 0.5–0.75 h after oral administration. The apparent half-life of NC-00751 was 0.3–0.6 h after an intravenous dose and 0.8–1.3 h after an oral dose. The *C*max and AUC_{24} of NC-00751 increased approximately corresponding to the increasing dose. These parameters were larger in males than in females. The absorption estimated from the urinary excretion of ^{14}C-labeled compound was 24–30% after oral administration. Little unchanged substance was detected in plasma after oral administration, and the bioavailability was extremely low. This may have been attributable to the fact that Neotame is fairly easily de-esterified in the body of rats[9), 10)].

[*1] The position of ^{14}C in ^{14}C-labeled Neotame.



*: ^{14}C-labeled position

[*2] See the table on page 24 for the chemical names (common names) and structures of Neotame-related compounds.

(b) Single-dose study of ^{14}C-labeled Neotame in dogs

The total plasma radioactivity and the change in plasma concentration were examined in male and female beagle dogs after the single oral gavage administration of 15 (low dose) or 120 (high dose) mg ^{14}C-labeled Neotame/kg body weight and after the intravenous administration of 15 mg ^{14}C-labeled Neotame/kg body weight. The plasma concentration peaked 0.03 h after the intravenous dose and 0.25–0.5 h after the oral dose. The *C*max and AUC_{24} increased at a rate slightly above the rate of the dose increase. Plasma Neotame decreased with a half-life of 0.2 to 0.3 h and 0.4 h after the oral low and high doses, respectively. Neotame was rapidly converted into NC-00751, and the plasma concentration peaked 0.25 h after intravenous administration and 0.75–1 h after oral administration. The apparent half-life of NC-00751 was 2.3 h after an intravenous dose and 3.4 h after an oral high dose. The *C*max and AUC_{24} of NC-00751 tended to increase above the rate of the dose increase. The systemic clearance and distribution volume of Neotame after intravenous administration were 26–32 mL/min/kg and approximately 1 L/kg, respectively. The systemic clearance that exceeded the hepatic plasma flow (18 mL/min/kg) suggested that Neotame was also metabolized other than in the liver. Furthermore, the distribution volume suggested that some of the Neotame distributed in the tissue. The estimated absorption from the urinary excretion of ^{14}C-labeled compound was 32–34% and about 47% after an oral low dose and an oral high dose, respectively. The bioavailability was estimated to be about 8% with a low dose and 19–32% with a high dose[11].

B. Distribution

(a) Organ and tissue concentrations

The tissue concentration was determined for 48 h in male colored rats after the single gavage administration of 15 mg ^{14}C-labeled Neotame/kg body weight. The radioactivity levels in the tissues other than the gastrointestinal tract, lymph node, prostate gland, and adrenal gland peaked 1 h after oral administration and those in the liver, kidney, and urinary bladder were higher than plasma radioactivity levels. The radioactivity levels in other tissues were substantially below their plasma radioactivity levels. The radioactivity levels in tissues other than the gastrointestinal tract rapidly decreased after that[12].

(b) Placental and fetal transfer

The placental to fetal transfer was examined with the use of whole-body autoradiography in pregnant rats after a single gavage dose of 15 mg ^{14}C-labeled Neotame/kg body weight on day 15 of gestation. The radioactivity level detected in the placenta 0.5 and 2 h after administration was low, similar to levels in other peripheral tissues and the blood vessel; however, no radioactivity transfer to the fetus was found. Twenty-four hours after administration, no radioactivity was detected in the fetuses, placenta or other tissues, and there was no tissue where the radioactivity specifically accumulated[13].

(c) Plasma protein binding

The *in vitro* protein binding rate in dog and human plasma was examined by centrifugal ultrafiltration with the use of ^{14}C-labeled Neotame (1–100 μg/mL in dogs and 10–1,000 ng/mL in humans). Neotame rapidly bound to plasma proteins to achieve equilibrium within 10 min. The plasma protein binding in dogs was 84–92% and 68–75% at concentrations of 1–10 and 100 μg Neotame/mL, respectively. In human plasma, protein binding was 94–98%, albumin binding was 80–81%, and α_1-acidic glycoprotein binding was 8–14%[14].

(d) Distribution into blood cells

The radioactivity level in blood cells was determined in rats and beagle dogs with the use of ^{14}C-labeled Neotame. The blood cells contained slightly more than 50% of the plasma radioactivity after intravenous administration (15 mg/kg body weight) and about 27% and about 40% of the plasma radioactivity after the low and high oral doses (15 and 120 mg/kg body weight), respectively, in dogs. In rats, the radioactivity level in red blood cells (RBCs) was generally low[11), 12)].

C. Metabolism

Neotame was primarily metabolized via de-esterification to methanol and NC-00751. NC-00751 was assumed to be partially metabolized via hydrolysis of peptide or amide binding to NC-00754 and also to be partially oxidized to undergo conjugation with glucuronic acid or carnitine (*see* Figure 1).

(a) Metabolism in rats administered ^{14}C-labeled Neotame

In plasma, a major metabolite was NC-00751, and little unchanged substance was found after oral administration[9)]. In urine, little unchanged substance was observed, and 5.0–7.0% of the oral dose, 31.1% of the intravenous dose in males, and 26.4% of the intravenous dose in females were found as a major metabolite, NC-00751. At least four metabolites, including G2 (glucuronic acid conjugate) and NC-00754, were detected in urine with 1.6% or less of the administered dose[10)]. NC-00784 (carnitine conjugate) was detected in the urine of female rats with two oral doses of 15 mg/kg body weight at an 8-h interval[15)]. In the feces, no unchanged substance was detected, and about 70–78% of the oral dose and about 51–52% of the intravenous dose were found as a major metabolite, NC-00751. Two metabolites of NC-00754 and Component 4 were also detected with 0.8–2.5% and 0.7–1.2% of the administered dose, respectively[10)]. The major metabolite of NC-00751 accounted for 92.9% of the radioactivity in the bile (4.7% of the dose)[16)].

(b) Metabolism in dogs administered ^{14}C-labeled Neotame

In plasma, unchanged substance and NC-00751 were observed after oral administration. In urine, a

small amount of unchanged substance was detected. The major metabolite of NC-00751 was found with about 6–9% of the oral dose and with about 19–20% of the intravenous dose. Other metabolites were also detected, including G2 (with about 5% of the dose) and NC-00754 (with about 0.4–2% of the dose). In feces, no unchanged substance was detected, and the major metabolite, NC-00751, was found with about 62–74% of the oral dose and with about 42–43% of the intravenous dose[11].

(c) Xenobiotic-metabolising enzyme activities in rat liver

Male and female rats were given Neotame by gavage at doses of 0, 100, 300, and 1,000 mg/kg body weight/day or phenobarbital at a dose of 75 mg/kg body weight/day as a positive control for 14 days. In male rats, 1,000 mg/kg body weight/day decreased the activity of *p*-nitrophenol UDP-glucuronosil transferase (*p*-nitrophenol UDP-GT), but the decrease was within the range of the past background data in the control groups. There were also no effects of Neotame administration on the microsome protein content, cytochrome P450 content, cytoplasmic non-protein thiol content, or various enzyme activities[17]. These results indicated that Neotame does not have an important effect on the xenobiotic-metabolising enzyme system.

(d) Stability in simulated gastric juice and intestinal juice

The stability of Neotame was examined when the simulated gastric juice (with or without pepsin) and the simulated intestinal juice (with or without pancreatin) with addition of 50 μg Neotame/mL was incubated at 37°C for 120 min. Neotame was stable with or without pepsin but was fully hydrolyzed to NC-00751 within 15 min in the simulated intestinal juice with pancreatin. It was relatively stable in the simulated intestinal juice without pancreatin, and a very small amount of Neotame was hydrolyzed to NC-00751 with 1–2% of the total radioactivity after 120 min[18].

D. Excretion

(a) Excretion in rats

The excretion of Neotame in urine and feces was examined in male and female rats after oral administration (15 or 120 mg/kg body weight) or intravenous administration (15 mg/kg body weight) of ^{14}C-labeled Neotame. Urinary excretion 72 h after administration was 8.5–10.8% with an oral dose and 34.6% and 35.9% with an intravenous dose. Fecal excretion was 84.5–87.2% with an oral dose and 58.1% and 59.2% with an intravenous dose. No less than 90% of the administered Neotame was rapidly excreted within 48 h after administration, and no sex differences were observed[10]. Forty-eight hours after ^{14}C-labeled Neotame was given orally at a dose of 15 mg/kg body weight to male rats undergoing biliary cannulation, 6%, 5–9%, and 82–87% of the administered dose was excreted in the bile, urine, and feces, respectively[16].

(b) Excretion in dogs

The excretion of Neotame in urine and feces was examined in male and female dogs after oral administration (15 or 120 mg/kg body weight) or intravenous administration (15 mg/kg body weight) of ^{14}C-labeled Neotame. The urinary excretion 72 h after administration was 13–20% with an oral dose and 40–43% with an intravenous dose, whereas the fecal excretion was 72–83% with an oral dose and 53–54% with an intravenous dose. Not less than 80% of the administered dose was excreted in urine and feces within 48 h after administration[11].

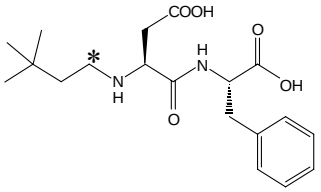
D. Others

The absorption, distribution, metabolism, and excretion of the major metabolite of Neotame—NC-00751—are addressed below (*see* Figure 1).

(a) Single-dose oral study of ^{14}C-labeled NC-00751 in rats

The absorption, distribution, metabolism, and excretion studies were conducted in male and female rats and in male rats undergoing biliary cannulation with a single gavage dose of 15 mg ^{14}C-labeled NC-00751/kg body weight*[3]. The plasma radioactivity level was low and peaked at about 0.1 μg NC-00751 equivalent/mL and fell below the detection limit (about 0.03 μg NC-00751 equivalent/mL) 4–8 h after administration. The main radioactive component in plasma 0.5 and 2 h after administration was NC-00751, which accounted for 59–78% of the total radioactivity. The main radioactive component in the bile, urine, and feces was also NC-00751, and radioactivities were equivalent to 1–2%, 0.8–0.9%, and 75–82% of the administration dose, respectively. The urinary and fecal excretions 72 h after administration were 1–2% and 99–101% of the administration dose in female and male rats, respectively. Almost all of the dose (100–103%) was excreted in urine and feces within 48 h after administration. In male rats undergoing biliary cannulation, the biliary, urinary, and fecal excretions of radioactivity were about 2%, about 2%, and about 92%, respectively[19].

*[3] The position of ^{14}C in ^{14}C-labeled NC-00751



*: ^{14}C-labeled position

(b) Plasma protein binding

The *in vitro* protein binding rate in dog and human plasma was examined by centrifugal ultrafiltration with the use of ^{14}C-labeled NC-00751 (100–10,000 ng/mL in rats; 1–100 μg/mL in dogs; and 50–5,000 ng/mL in humans). The plasma protein binding was 72–76% in rats and 46–54% in dogs. In human plasma, the protein binding was 85–91%, the albumin binding was 29–37%, and the α_1-acidic

glycoprotein binding was 0.2–9%[14].

(c) Stability in simulated gastric juice and intestinal juice

The stability of NC-00751 was examined when the simulated gastric juice (with or without pepsin) and the simulated intestinal juice (with or without pancreatin) with addition of 25 μg NC-00751/mL was incubated at 37°C for 120 min. NC-00751 was stable in the simulated gastric juice and intestinal juice with or without the enzymes [18].

II. Clinical data

A. Plasma concentration

(a) Single oral dose

The total plasma radioactivity and the changes in plasma concentrations of unchanged substance and NC-00751 were examined in healthy adult men after a single oral dose of about 0.25 mg ^{14}C-labeled Neotame/kg body weight. After administration, Neotame was rapidly absorbed; a *C*max of 95.7 ng/mL was reached at 0.4 h and was eliminated with a half-life of 0.6 h. The plasma concentration of NC-00751 reached a *C*max of 236 ng/mL 1 h after administration, and NC-00751 had a half-life of 1.5 h. NC-00751 and Neotame accounted for most of the plasma radioactivity—about 80% and about 8% of the $AUC_{(0\text{-}t)}$ of total radioactivity, respectively. The total radioactivity level in whole blood was lower than that in plasma. These values couple with hematocrit values indicated that most of the radioactivity was distributed in plasma rather than in cellular components[20].

The changes in plasma concentrations of unchanged substance and NC-00751 were examined in healthy adult men after administration of unlabeled Neotame at single oral doses of 0.1, 0.25, or 0.50 mg/kg body weight. The *C*max and AUC values of Neotame and NC-00751 showed almost linear behaviors at doses within the examined range of concentrations[21].

(b) Repeated eight oral doses

Changes in the concentrations of unchanged substance and NC-00751 were examined in healthy adult men repeatedly administered Neotame at an eight-time oral dose of 0.25 mg Neotame/kg body weight every hour. After the final dosing, the plasma concentration of Neotame reached a *C*max of 67.36 ng/mL at 0.35 h and then decreased, with a half-life of 0.88 h. The plasma concentration of NC-00751 reached a *C*max of 875.94 ng/mL at 0.69 h and then decreased in a biphasic manner, with an elimination half-life of 12.88 h[22].

(c) 14-day repeated oral dose

Neotame was given orally as a capsule to healthy adult men and women three times daily at a dose of 0.5 or 1.5 mg/kg body weight for 14 days. No Neotame could have been detected in plasma at the

trough. In contrast, the plasma concentration of NC-00751 was measurable and showed a linear behavior for the dose[23].

B. Metabolism

The metabolism of Neotame in humans had a main pathway of de-esterification and included metabolites similar to those observed in laboratory animals (*see* Figure 1). After oral administration of Neotame to healthy adult men, NC-00751 as a major metabolite and unchanged substance were found in plasma[20-22]. In healthy adult men administered ^{14}C-labeled Neotame at a single oral dose of about 0.25 mg/kg body weight, NC-00751 was found in urine as a major metabolite; 23.81% of the dose was found 72 h after administration. Unchanged substance (3.32% of the dose) and small amounts of NC-00754 and NC-00784 were also found in urine. Feces contained no unchanged substance and a major metabolite, NC-00751; 52.5% of the dose was found 96 h after administration. NC-00754 was also found (4.9% of the dose)[20]. Plasma, urine, and feces contained small amounts of unidentified metabolites, but NC-00784 was found in urine[20),24].

C. Excretion

(a) Single oral dose

After the oral administration of ^{14}C-labeled Neotame (about 0.25 mg/kg body weight) to healthy adult men, 34.3% and 63.7% of the administered radioactivity were excreted in urine and feces, respectively, by 168 h [20].

After a single oral dose of unlabeled Neotame (0.1, 0.25, or 0.50 mg/kg body weight), about 1% of the dose of Neotame was excreted in urine as unchanged substance and about 20% of the dose was excreted as NC-00751 by 84 h. The urinary excretion and renal clearance were constant regardless of the dose[21].

(b) Repeated eight oral doses

Healthy adult men were repeatedly administered Neotame at an eight-time oral dose of 0.25 mg/kg body weight every hour. About 3% of the total dose of Neotame was excreted in urine as unchanged substance, and about 23% of the total dose was excreted as NC-00751 by 168 h after the first dose[22].

(c) Bioequivalence of preparations

The pharmacokinetic parameters were determined in plasma concentrations from human administered Neotame at a 2-time dose of 10 mg as a capsule or a dose of 20 mg as solution. The bioavailabilities of Neotame and NC-00751 in the capsule preparation were the same as and higher than those in the solution preparation[25].

(2) Toxicity

I. Repeated-dose toxicity study

A. 13-week dietary administration study in mice

ICR mice (20 males and 20 females in a group) were fed diets containing 0, 100, 1,000, 4,000, or 8,000 mg Neotame/kg body weight/day for 13 weeks. The mean corpuscular volumes (MCV) significantly decreased in females at 4,000 and 8,000 mg/kg body weight/day. Because the variation in MCVs was small and was not associated with alterations in other RBC parameters, the decrease could not have resulted from Neotame administration. The relative liver weight increased in both sexes at 4,000 and 8,000 mg/kg body weight/day, and the absolute liver weight increased in both sexes at 8,000 mg/kg body weight/day[26].

On the basis of the increase in relative liver weight in both sexes at doses of 4,000 mg/kg body weight/day and higher, the no observed adverse effect level (NOAEL) was considered to be 1,003 mg/kg body weight/day*[4].

B. 13-week dietary administration study in rats followed by a 4-week reversibility period

SD rats (20 or 25 males and females in a group) were fed diets containing 0, 100, 300, 1,000, or 3,000 mg Neotame/kg body weight/day for 13 weeks. Additionally 5 males and 5 females in the groups that received 0, 1,000, and 3,000 mg/kg body weight/day underwent a 4-week recovery period to evaluate the reversibility of toxicity. Final body weight, body weight gain, and food consumption decreased in males at 3,000 mg/kg body weight/day. The decreases in final body weight and body weight gain were accompanied by a decrease in food consumption, probably resulting from reduced cravings for the food containing a high concentration of Neotame. Alkaline phosphatase (ALP) increased in males at 1,000 mg/kg body weight/day and in both sexes at 3,000 mg/kg body weight/day. In males, at 3,000 mg/kg body weight/day, there were decreases in organ weights (liver, kidney, adrenal gland, heart, spleen, thymus, and prostate gland) and in relative spleen weight and increases in relative brain weight and relative testis weight, which were considered to be accompanied by decreases in body weight gain. Calcification in the renal corticomedullary junction was observed in females at 1,000 and 3,000 mg/kg body weight/day. This lesion observed frequently in female rats in association with sexual maturation was not found at the end of the 4-week recovery period[27].

On the basis of the increase in ALP in both sexes at doses of 1,000 mg/kg body weight and higher, the NOAEL was considered to be 293 mg/kg body weight/day*[4].

C. 13-week dietary administration study in dogs followed by a 4-week reversibility period

Beagle dogs (4 or 6 males and females in a group) were fed diets containing 0, 60, 200, 600, or

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*4 The actual consumption of Neotame calculated from food consumption.

2,000/1,200 mg Neotame/kg body weight/day for 13 weeks (2,000 mg/kg body weight/day until day 14 followed by 1,200 mg/kg body weight/day thereafter) and then underwent a 4-week recovery period to evaluate the reversibility of toxicity. Body weight gain and food consumption decreased at 2,000/1,200 mg/kg body weight/day. The decrease in body weight gain was accompanied by a decrease in food consumption, *i.e.*, avoidance of the diet containing the high concentration of Neotame. The RBC count, hemoglobin concentration, and hematocrit decreased in both sexes at 2,000/1,200 mg/kg body weight/day. ALP increased in females at 200 and 600 mg/kg body weight/day and in both sexes at 2,000/1,200 mg/kg body weight/day. The elevation in ALP, however, was low in both sexes at 200 mg/kg body weight/day after 52 weeks followed by a 4-week reversibility period (*see* Section E below). Relative liver weight increased in females at 600 mg/kg body weight/day and in both sexes at 2,000/1,200 mg/kg body weight/day. Relative adrenal gland weight increased and lung weight and epididymis weight decreased in males at 2,000/1,200 mg/kg body weight/day, which may have resulted from the change in body weight associated with the decrease in food consumption. An increase in glycogen in hepatocytes was seen in both sexes at 600 and 2,000/1,200 mg/kg body weight/day. This result may indicate little toxicologic significance[28].

On the basis of the elevation in ALP in both sexes at doses of 200 mg/kg body weight/day and higher, the NOAEL was considered to be 59.7 mg/kg body weight/day*[4].

D. 52-week dietary administration study in offspring of exposed female rats followed by a 4-week reversibility period

SD rats (25 males and 25 females in a group) were fed diets containing Neotame at 0, 10, 30, 100, 300, or 1,000 mg/kg body weight/day for 4 weeks before mating and during mating; the female rats continued to receive the diets from gestation and lactation through day 21 postpartum (weaning). Dams allocated to the dose of 1,000 mg/kg body weight/day were fed diets containing Neotame at 300 mg/kg body weight/day from days 14 to 21 postpartum, and F_1 offspring were also fed the same dose of Neotame (300 mg/kg body weight/day) from the start of weaning through days 26 to 28 after weaning (start of 52-week toxicity study). The F_1 offspring (20 males and 20 females in a group) were then fed diets containing 0, 10, 30, 100, 300, or 1,000 mg Neotame/kg body weight/day for 52 weeks. Additionally, 10 males and 10 females in each group underwent a 4-week recovery period at 0, 100, 300, or 1,000 mg/kg body weight/day to evaluate the reversibility of toxicity.

A behavior which showed slight excitement was seen more frequently in males administered doses at 300 mg/kg body weight/day and higher than in control animals. This was not due to Neotame administration because they are generally observed in elderly male rats and the similar behaviors were not seen in the long-term (104 weeks) carcinogenicity study. Decreases in body weight and food consumption were observed in females at doses of 100 mg/kg body weight/day and higher and a decrease in body weight gain was seen in females at doses of 300 mg/kg body weight/day and higher.

These changes indicate a reduced craving for food. In females at doses of 100 mg/kg body weight/day and higher, heart weights were lower than those of the control animals and relative heart weights were similar. Histopathological examinations showed a significant, dose-independent increase in the incidence of pituitary adenoma in females at 30 mg/kg body weight/day, but the incidence fell within the background data[29].

On the basis of these results, no finding suggestive of toxicity was observed, and the NOAEL was considered to be 1,006 mg/kg body weight/day and higher*[4].

E. 52-week dietary administration study in dogs followed by a 4-week reversibility period

Beagle dogs (4 or 6 males and females in a group) were fed diets containing 0, 20, 60, 200, or 800 mg Neotame/kg body weight/day for 52 weeks; additionally 2 males and 2 females in each group underwent a 4-week recovery period at 0, 200, or 800 mg/kg body weight/day to evaluate the reversibility of toxicity. Food consumption decreased in the dogs administered 800 mg/kg body weight/day, and ALP increased significantly during the treatment period. The isozyme of ALP was derived from the liver, and an increase in ALP was reversible and returned to the normal concentration during the recovery period. The liver weight and results of the macroscopic and histopathological examinations were normal after treatment with Neotame[30].

On the basis of the elevation in ALP in both sexes at 800 mg/kg body weight/day, the NOAEL was considered to be 197 mg/kg body weight/day*[4].

II. Reproductive toxicity study

A two-generation reproductive toxicity study was conducted in SD rats (28 males and 28 females in a group). Diets containing 0, 100, 300, or 1,000 mg Neotame/kg body weight/day were fed to F_0 males for a total of 14 weeks from week 10 before mating, to F_0 females for 10–11 weeks from week 4 before mating through weaning of F_1 offspring, to F_1 males for 15–16 weeks from weaning, and to F_1 females for 17–20 weeks from weaning through weaning of F_2 offspring.

The feed efficiency was reduced in parental animals (F_0 and F_1) at 1,000 mg/kg body weight/day during the premating period. At 1,000 mg/kg body weight/day, low body weight and a decrease in body weight gain were found in males during the premating period and in females during the premating and gestation periods. Decreases in the absolute weights of various organs and increases in the relative weights of various organs were found at 1,000 mg/kg body weight/day. These changes may be associated with low body weight. The treatment with Neotame also had no effect on estrous cycle, mating performance, fertility, gestation length, parturition, or gestation index.

In offspring (F_1 and F_2), low body weight was seen in F_1 males at 300 mg/kg body weight/day and at 1,000 mg/kg body weight/day on postnatal day (PND) 1; in F_1 animals at doses of 300 mg/kg body weight/day and higher; and in F_2 animals at 1,000 mg/kg body weight/day on PND 21. The learning ability of F_1 animals was assessed with the use of a water maze; swimming time was significantly

prolonged in F_1 males at 1,000 mg/kg body weight/day. The variation in swimming time, however, was small and fell within the range of the background data. The treatment with Neotame also had no effect on health condition, number of pups born, fetal survival rate, sex ratio, physical development, or functional development[31].

On the basis of the low body weight in F_1 animals at doses of 300 mg/kg body weight/day and higher on PND 1, Neotame did not affect reproduction, and the NOAEL was considered to be 96.5 mg/kg body weight/day*[4]. (The NOAEL was considered to be 299 mg/kg body weight/day*[4] for general toxicity in parental animals and 96.5 mg/kg body weight/day*[4] for reproductive and developmental toxicity. There was no effect on reproductive parameters.)

III. Developmental toxicity study

A. Developmental toxicity study in rats

SD rats (24 females in a group) were fed diets containing 0, 100, 300, or 1,000 mg Neotame/kg body weight/day for 28 days before mating and from the beginning of the mating period through day 20 of gestation. Pregnant rats underwent cesarean section on day 20 of gestation to examine the fetuses. Low body weight and decreases in the total food consumption and body weight gain were observed in dams at 1,000 mg/kg body weight/day during the first week of treatment; however, these changes were due to poor palatability of the test compound given in diet. The treatment with Neotame had no effect on the pregnancy rate, numbers of corpora lutea and fetuses, fetal survival rate, and pre- or postimplantation loss. The administration of Neotame also had no effect on placental weight, viable fetal weight, or morphologic findings of external, skeletal, and visceral structures [32].

On the basis of these results, the NOAEL was considered to be 964 mg/kg body weight/day and higher*[4] for dams and fetuses. No teratogenicity of Neotame was found.

B. Developmental toxicity study in rabbits

New Zealand white rabbits (20–25 females in a group) were given Neotame by gavage at 0, 50, 150, or 500 mg/kg body weight/day for 14 days from days 6 to 19 of gestation. Pregnant rabbits underwent cesarean section on day 29 of gestation to examine the fetuses.

Total litter losses resulted in one dam each from the control and 500 mg/kg body weight/day groups. In the dam of the 500 mg/kg body weight/day group, only one embryo was implanted, but it was dead. One death and two abortions were seen in the 500 mg/kg body weight/day group. These changes may have resulted from considerable decreases in food consumption and subsequent decreases in body weight, which were observed in all of the pregnant rabbits. The food consumption and body weight gain in the treatment groups did not differ significantly from those in the control group. The treatment with Neotame had no effect on the number of fetuses, fetal survival rate, and pre- or postimplantation loss. The administration of Neotame also had no effect on placental weight, viable fetal weight, and morphologic findings of external, skeletal, and visceral structures [33].

On the basis of these results, the NOAEL was considered to be 150 mg/kg body weight/day*[4] for dams (based on the deaths and abortions observed in the 500 mg/kg body weight/day group) and 500 mg/kg body weight/day and higher for fetuses. No teratogenicity of Neotame was found.

IV. Carcinogenicity study

A. 104-week carcinogenicity study in mice

ICR mice (140 males and 140 females in a control group and 70 males and 70 females in treatment groups) were fed diets containing 0, 50, 400, 2,000, or 4,000 mg Neotame/kg body weight/day for 104 weeks. In both sexes, body weight and food consumption were lower in the groups that received doses of 400 mg/kg body weight/day than in the control group. Heart weight decreased in females at 4,000 mg/kg body weight/day, but the macroscopic examinations showed no effect due to treatment with Neotame. At 4,000 mg/kg body weight/day, the incidences of hepatocellular adenomas in males and the incidence of bronchiolo-alveolar adenocarcinomas in females tended to increase without any significant differences[34)].

On the basis of these results, Neotame showed no carcinogenicity.

B. 104-week carcinogenicity study in rats with exposure *in utero*

SD rats (F_0) (170 males and 170 females in a control group and 85 males and 85 females in treatment groups) were fed diets containing 0, 50, 500, or 1,000 mg Neotame/kg body weight/day; males were fed for 4 weeks before mating and during the mating period, and females were fed for 4 weeks before mating and from the mating period to day 21 postpartum. F_1 offspring born to the F_0 animals (147 males and 147 females in a control group and 73–75 males and 73–75 females in treatment groups) were fed diets containing Neotame at the same doses for 104 weeks. Decreases in body weight gain and food consumption were observed in the F_1 treatment groups. Histopathological examinations showed a significant increase in the incidence of renal adenomas in males receiving 50 mg/kg body weight/day. Because the increase was independent of the dose, this change was considered to be incidental[35)].

On the basis of these results, Neotame showed no carcinogenicity.

V. Antigenicity study

The dermal sensitization potential of Neotame was examined in guinea pigs [Crl:(HA)BR] (5 males and 5 females in a control group and 10 males and 10 females in a treatment group). The animals were first sensitized by applying adhesive patches coated with Neotame (0 or 0.4 g/animal) to the skin for 6 hours per week for 3 weeks (three times) and then underwent challenge exposure after 2 weeks. No skin reaction to Neotame was observed in any of the groups[36)].

No findings suggestive of allergies to Neotame were found in any studies in any animals or humans.

VI. Genotoxicity study

A. Bacterial reverse mutation assay

In bacterial reverse mutation assays using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* WP2*uvrA* at concentrations of 312 to 10,000 μg/plate, neotame was negative results with or without S9mix[37].

B. An *in vitro* mouse lymphoma tk assay

The *in vitro* mammalian gene mutation was evaluated using L5178Y mouse lymphoma cells at concentrations of 100 to 1,000 μg/mL. It did not induce mutants with or without S9mix[38].

C. Chromosome aberration test in Chinese hamster ovary (CHO) cells

Neotame induced neither structural nor numerical chromosomal aberration in Chinese hamster ovary (CHO) cells with S9mix at concentrations of 250 to 1,000 μg/mL or without S9mix at concentrations of 62.5 to 250 μg/mL[39].

D. Micronucleus assay in ICR male and female mice

Neotame was negative in the *in vivo* micronucleus assay using ICR mice (10 males and 10 females in a group) at 500, 1,000, and 2,000 mg/kg body weight by gavage[40].

From these results, we considered that Neotame was not genotoxic.

VII. General pharmacology study

A. Effects on general symptoms and behaviors

After dietary treatment with Neotame in rats and dogs for 13 weeks, no effects on general symptoms or on behavior were observed[27), 28)] (*see* "I. Repeated-dose toxicity study").

B. Effects on the central nervous system

(a) Effects on spontaneous motor activity

Effects of Neotame on spontaneous motor activity were examined in a reproductive study using rats. Neotame did not affect spontaneous motor activity[31)] (*see* "II. Reproductive study").

(b) Effects on anesthetic action

SD rats (5 males and 5 females in a group) were orally administered Neotame at a dose of 5 or 15 mg/kg body weight plus 150 mg hexobarbital/kg body weight (males) or 100 mg hexobarbital/kg body weight (females) intraperitoneally 30 min after treatment with Neotame. No effect on hexobarbital-induced sleeping time was found[41].

(c) Other effects

Dietary administration of Neotame in rats and dogs for 13 weeks showed no effect on the central nervous system[27), 28)] or on body temperature in dogs (*see* "I. Repeated-dose toxicity study").

C. Effects on the autonomic nervous system

Effects of Neotame on reactions to constrictors, including acetylcholine and histamine, were examined in the isolated ileum from male Dunkin-Hartley guinea pigs exposed to Neotame (0, 20, 60, or 200 ng/mL) or NC-00751 (60, 200, or 600 ng/mL). Neotame did not affect the ileum contraction induced by various constrictors or the ileum tonus. No activation action, cooperative action, or antagonistic action to the examined receptor system were observed[42)].

D. Effects on the cardiovascular and respiratory systems and renal function

Beagle dogs (6 males in a group) were intraduodenally administered 5 or 15 mg Neotame/kg body weight. Blood pressure, heart rate, respiratory rate, tidal volume, and urinary concentrations of sodium and protein were recorded. No effects on the cardiovascular, respiratory, or renal parameters were observed[43)].

E. Effects on the digestive system

SD rats (10 males in a group) were orally administered 5 or 15 mg Neotame/kg body weight and were orally administered a charcoal-water suspension (5%, weight:volume) about 30 min after treatment with Neotame. Neotame did not affect the migration distance of charcoal between the pyloric sphincter and the cecum[44)].

VIII. Dietary preference study in rats

Dietary preference was assessed in SD rats (14 males and 14 females in a group) allowed to access diets containing Neotame (0, 50, 150, 500, 1,500, 5,000, or 15,000 ppm) ad libitum. All animals, except male rats administered 50 ppm, showed a reduced craving for the food containing Neotame, and animals administered doses of 5,000 ppm and higher completely avoided eating these foods[45)].

IX. Safety study of Neotame degradation products

Studies of Neotame degradation products were conducted in various animals. The degradation pathway under severe conditions is shown in Figure 2 for reference.

A. Single-dose toxicity study

SD rats (10 males and 10 females in a group) were given NC-00764, NC-00777, or NC-00779 by gavage at doses of 0, 0.6, 2.0, or 6.0 mg/kg body weight; 0, 0.6, 2.0, or 6.0 mg/kg body weight; or 0, 0.3, 1.0, or 3.0 mg/kg body weight, respectively, and were monitored for 14 days. No effects due to

treatment with NC-00764, NC-00777, or NC-00779 were found[46-48].

B. Repeated-dose toxicity study

SD rats (15 males and 15 females in a group) were fed diets containing NC-00764, NC-00777, and NC-00779 with a mixing ratio of 0.2/0.2/0.1, 0.6/0.6/0.3, 2.0/2.0/1.0, or 6.0/6.0/3.0 mg/kg body weight/day, respectively, for 4 weeks. No effects due to administration of the mixture of NC-00764, NC-00777, and NC-00779 were found[49].

C. Genotoxicity study

(a) Bacterial reverse mutation assay

In bacterial reverse mutation assays using *Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537 with NC-00751 or NC-00764 at concentrations of 50 to 5,000 μg/plate, NC-00751 and NC-00764 were negative with or without S9mix[50), 51)].

NC-00777 or NC-00779 were also negative in bacterial reverse mutation assays using *Salmonella typhimurium* TA97a, TA98, TA100, TA102, and TA1535 at concentrations of 10 to 5,000 μg/plate with or without S9mix[52), 53)].

(b) Gene mutation assay in cultured AS52/XPRT mammalian cells

NC-00751 and NC-00764 showed negative results with or without S9mix in gene mutation assays using AS52/XPRT mammalian cells at concentrations of 625 to 5,000 μg/mL[54), 55)]. NC-00777 and NC-00779 at concentrations of 100 to 390 μg/mL and 313 to 5,000 μg/mL, respectively, did not induce gene mutation in the same assay system mentioned above with or without S9mix[56), 57)].

(c) Micronucleus assay in ICR mice

NC-00764, NC-00777 and NC-00779 were tested *in vivo* micronucleus assay using ICR mice (10 males and 10 females in a group) at dose-levels of 500, 1,000, or 2,000 mg/kg body weight, all were negative[58)59), 60)].

On the basis of these results, Neotame degradation products (NC-00751, NC-00764, NC-00777, and NC-00779) were considered to have no genotoxicity.

X. Findings in humans

A. Single-dose study

Healthy men (6 men in each group) were administered Neotame dissolved in mineral water in single oral doses of 0.1, 0.25, or 0.5 mg/kg body weight. No abnormality due to treatment with Neotame was found[61].

B. 2-week administration study

Healthy men and women (12 men and 12 women in a group) were administered Neotame at a dose of 0, 0.5, or 1.5 mg/kg body weight/day three times daily repeatedly for 2 weeks. No abnormality due to treatment with Neotame was found[23].

C. 13-week administration study

Neotame was administered orally three times daily at a dose of 0 or 0.5 mg/kg body weight/day (24 healthy men and 24 healthy women) or 1.5 mg/kg body weight/day (23 healthy men and 23 healthy women) repeatedly for 13 weeks. No abnormalities due to treatment with Neotame were found[62].

D. 2-week three-way crossover study in patients with non-insulin dependent diabetes mellitus

A 2-week three-way crossover study was conducted in patients (17 men and 17 women) with non-insulin dependent diabetes mellitus (NIDDM). The patients were orally administered Neotame three times daily at a dose of 0, 0.5, or 1.5 mg Neotame/kg body weight/day repeatedly. No abnormality due to treatment with Neotame was found, and multiple doses of Neotame did not affect plasma glucose and insulin concentrations[63].

XI. Assessment of aspartame

In 2005 and 2006, an Italian foundation—European Foundation of Oncology and Environmental Sciences "B. Ramazzini"—released their study results and reported that administration of aspartame led to increases in the incidences of leukemia and other tumors in rats[64), 65)]. Because the structure of aspartame is similar to that of Neotame, the data from the study on aspartame were evaluated.[66)]

SD rats (100–150 males and 100–150 females in a group) were administered diets containing 0, 80, 400, 2,000, 10,000, 50,000, and 100,000 ppm aspartame equivalent to 4, 20, 100, 500, 2,500, and 5,000 mg/kg body weight/day*[5], respectively, until the animals died spontaneously. The total incidences of lymphoma and leukemia significantly increased in female rats at doses of 400 ppm and higher, but were not dose-related. When compared with the background data, the increases could be falsely significant due to the low incidence accidentally found in the female control group. In addition when lymphomas or leukemias were classified into subtypes, no significant differences were observed. Bronchopneumonia was observed in nearly 100% of the control animals (93.3% of the males and 96.7% of the females), and brain abscess, meningitis, pleurisy, pericarditis, liver abscess, pyelonephritis,

*[5] Estimation of food consumption with the reduced values used by the Joint Expert Committee on Food Additives[67)]

Species	Final body weight (kg)	Food consumption (g/animal/day)	Food consumption (g/kg body weight/day)
Rat	0.4	20	50

and peritonitis were prevalent. Thus, it should be taken into account in this study that tumor development was associated with lymphocytic hyperplasia due to inflammation; therefore, the increase in the total incidence of lymphoma and leukemia in the females may not have been related to the administration of aspartame.

The significant increase in the occurrence of cancer of the renal pelvis and ureter was observed in females at 100,000 ppm. Calcification induced by irritating substances was specific to rats and cannot be extrapolated to humans.

Malignant schwannoma was observed in the groups given aspartame, but its incidence was low and did not differ significantly from that observed in the control group.

On the basis of these results, it was concluded that aspartame does not induce any specific tumors.

The European Food Safety Authority (EFSA) also assessed the results of this study and concluded that the slight increase in the total incidences of lymphoma and leukemia did not correlate with dose, and that chronic inflammation observed in the lungs was the major cause. The changes in proliferation found mainly in the kidney, ureter, and urinary bladder of females were not specific to the treatment with aspartame, but were typical of rats administered high doses of the chemical substance, which cause calcification in the renal pelvis as a result of imbalances in calcium metabolism. The number of occurrences of malignant schwannoma was also small and did not correlate with dose; the diagnosis of tumors was uncertain. The EFSA, therefore, concluded that the results of this study did not suggest the carcinogenic potential of aspartame, and there is no reason to review the previous SCF opinion on aspartame[68].

6. Assessments by international organizations

(1) Assessments in the Australia New Zealand Food Authority (ANZFA) in 2001[69]

Assessments of studies in various animals and human administered Neotame concluded that Neotame showed good tolerability at every dose used in all studies. Decreases in body weight gain were found at high doses, but the decreases did not suggest toxicity because this finding was associated with a decrease in food consumption due to reduced cravings for the diets containing Neotame. The elevated serum ALP concentration observed in dogs given repeated doses of Neotame for 52 weeks was the only finding suggestive of toxicity.

Toxicologic significance was unclear, but the effect level was determined as the dose at which an elevated ALP concentration was found in a 52-week repeated-dose toxicity study in dogs. The acceptable daily intake (ADI) was established to be 2.0 mg/kg body weight/day on the basis of a no observed effect level (NOEL) of 200 mg/kg body weight/day and a safety factor of 100.

(2) Assessments in the Food and Drug Administration (FDA) in 2002[8]

The assessments of studies in various animals and humans administered Neotame concluded that no toxicologic finding was seen in any studies.

The effect level was determined as the dose at which a decrease in body weight gain was found in a 52-week repeated-dose toxicity study in rats. The ADI was established to be 0.3 mg/kg body weight/day on the basis of a NOEL of 30 mg/kg body weight/day and a safety factor of 100.

(3) Assessments in the French Food Safety Agency (AFSSA:Agence Française de Sécurité Sanitaire des Aliments) in 2004[70)]

The assessments of studies of various animals and humans administered Neotame concluded that Neotame caused no toxicity in any of the studies.

The effect level was determined as the dose at which an increase in plasma ALP was found in 13- and 52-week repeated-dose toxicity studies in dogs. The provisional ADI*[6] was established to be 0.6 mg/kg body weight/day on the basis of a NOEL of 60 mg/kg body weight/day and a safety factor of 100.

(4) Assessments in JECFA in 2003[71-73)]

The assessments of studies in various animals and humans administered Neotame reported that Neotame caused no toxicity in any of the studies.

The elevated serum ALP concentration in 13- and 52-week repeated-dose toxicity studies in dogs was the only finding suggestive of toxicity. This slow, reversible elevation in ALP did not suggest hepatotoxicity. However, because the elevation was reproducible and dose-dependent and indicated a statistically significant difference, the effect level was determined as the dose at which an elevated ALP concentration was found in 52-week repeated-dose toxicity studies in dogs. The ADI was established to be 0–2 mg/kg body weight/day on the basis of a NOEL of 200 mg/kg body weight/day and a safety factor of 100.

7. Estimation of daily consumption

Neotame is expected to be used as a sweetener in various foods.

On the assumption that sugar contained in foods is replaced by Neotame based on food consumption (total number) derived from the food categories provided by the National Nutrition Survey (NNS) 2001[74)], estimated Neotame consumption is calculated on the basis of food consumption and on the amount of Neotame added to food. Thus, Neotame consumption*[7] in humans is estimated to be 3.84

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*[6] ADI was established to be 0.6 mg/kg bw/day by applying a safety factor of 100 to NOEL of 60 mg/kg bw/day when the elevation in serum ALP level was observed in 13- and 52-week repeated-dose toxicity studies in dogs. Since further study may remove concerns about effects on the liver of which the elevated serum ALP activity was suggestive, the ADI was temporarily established with a two-year deadline in expectation of submission of further study data.

*[7] The amount of Neotame addition is calculated according to the foods expected to use Neotame, using the reference amounts of Neotame addition (calculated from sensory evaluation of sweetness degree), based on the estimated food-category-based sugar consumption from the food consumption (total number) according to the food category of the 2001 NNS data. The amounts of added Neotame degradation products are calculated by multiplying the calculated amount of Neotame addition by a production rate of each degradation product.

mg/human/day (0.0769 mg/kg body weight/day for a person weighing 50 kg). Similarly, Neotame consumption is estimated to be 3.54 mg/human/day in children aged 1–6 years (0.225 mg/kg body weight/day) and 4.45 mg/human/day in persons aged 7–14 years (0.118 mg/kg body weight/day) on the basis of food consumption according to the food category by age. The daily intakes*[7] of NC-00777, NC-00764, and NC-00779, which are degradation products associated with Neotame intake, are estimated to be 0.042, 0.136, and 0.021 μg/kg body weight/day, respectively[7), 75-91)].

However, on the assumption that aspartame is replaced by Neotame based on data from the food consumption survey for eight sweeteners by the market basket method in fiscal 2002[92)], food consumption is divided by 40 of a sweetness ratio of Neotame to aspartame*[8]. As a result, Neotame consumption is estimated to be 0.146 mg/human/day (0.00292 mg/kg body weight/day). Similarly, when calculated as a sweetness ratio of 31*[9] on the basis of mean values and 90th percentiles*[10] of aspartame consumption in the United Kingdom[93)] and the United States[94)], the mean Neotame consumption and 90th percentile of Neotame consumption were estimated to be 0.01 and 0.05 mg/kg body weight/day in the United Kingdom and 0.04 and 0.10 mg/kg body weight/day in the United States, respectively.

Neotame is expected to be used as a flavor potentiator (flavoring) in various foods at low concentrations [threshold level (4.1 ppm) or lower], where the sweetness concentration is not given. The amount of Neotame used as a flavoring is assumed to be very small relative to the amount used as a sweetener. It is also thought that Neotame is not used as a flavoring in foods in which it has already been used as a sweetener. The above estimated daily food intakes, therefore, are thought to include those foods in which Neotame is used as a flavoring.

8. Discussion about phenylalanine consumption

Considering that Neotame dose not release phenylalanine under normal storage conditions[7)], the risks of phenylalanine from Neotame intake can be disregarded.

On the assumption that all Neotame is converted to phenylalanine, the estimated phenylalanine consumption in Japan is calculated from the estimated daily Neotame consumption provided in the NNS[74)]. The phenylalanine consumption is estimated to be 1.68 mg/human/day (0.034 mg/kg body weight/day) in adults and 1.55 mg/human/day (0.098 mg/kg body weight/day) in children aged 1–6 years. These intakes correspond to 0.7% or lower of the reference consumptions[95)] in patients with

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*[8] The value (8000/200=40) is calculated based on the sweetness of aspartame used for general foods (200 times sweeter than sugar) and the sweetness of Neotame (about 8,000 times sweeter than sugar).

*[9] The ratio (550/17=31) is calculated from the ratio of average amounts added in carbonated beverages, in which aspartame and Neotame are most frequently used, (aspartame: 550 to 600ppm; Neotame: 17 ppm), based on the daily aspartame consumptions in the U.S. and the UK.

*[10] The *n*th percentile is a value that is located on *n*% of the observations in ascending order. ex) The 10-percentile in 1,000 observations is a value that is located on 10% (100th) of the observations in ascending order.

phenylketonuria (20–40 mg/kg body weight/day in patients aged 1–3 years; 15–35 mg/kg body weight/day in patients aged 3 years or older).

When similarly estimated from the 90th percentile of daily Neotame consumption in the United States, the phenylalanine exposure level is 2.64 mg/human/day in adults. When compared with the phenylalanine intake from diets in healthy persons (2.5–10 g/day), the phenylalanine exposure estimated from Neotame consumption is thought to be minimal. The phenylalanine exposure level in children with a body weight of 20 kg is 1.50 mg/human/day, which corresponds to 0.4% or less of the daily phenylalanine consumption in children with phenylketonuria with a body weight of 20 kg (0.4–0.6 g/day)[8]. On the basis of these comparisons, the US FDA concluded that the phenylalanine consumption derived from Neotame intake is acceptable in terms of safety.

9. Evaluation results

The evaluation of the data from studies in various animals and humans administered Neotame concluded that Neotame showed no teratogenicity, genotoxicity, or carcinogenicity and that the main effects due to Neotame consumption are a decrease in body weight gain and an elevation in serum ALP with high doses. The elevation in ALP found in dogs and rats did not lead to any changes in other enzyme activities, and none of the findings from histopathological and other examinations suggested any effects due to treatment with Neotame. However, the possibility that this elevation in ALP could be an adverse health effect could not be discounted conclusively; therefore, it was considered a toxic effect. In the 13-week study of the dietary administration of Neotame in dogs, the NOAEL of 59.7 mg/kg body weight/day was determined on the basis of the elevation in ALP observed at doses of 200 mg/kg body weight/day and higher. However, in the 52-week study, where the methods were the same as those of the 13-week study (except for the longer dosing period), no elevation in ALP was found at a dose of 200 mg/kg body weight/day. Furthermore, Neotame did not accumulate. Accordingly, it was determined that the elevation in ALP observed with the dose of 200 mg/kg body weight/day in the 13-week study in dogs was a transient symptom, and the NOAEL determined in this study was not used when establishing the ADI.

In contrast, it was determined that the decrease in body weight gain resulted from the decrease in food consumption due to a reduction in the craving for diets containing high concentrations of Neotame; therefore, this symptom was not considered to be a toxic effect. However, the low body weight observed in F_1 offspring during the early lactation period in the two-generation reproductive study in rats was considered to be a toxic effect because the craving for food was not reduced in parental animals, and the growth of newborns depended on maternal milk.

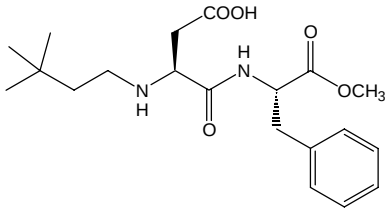
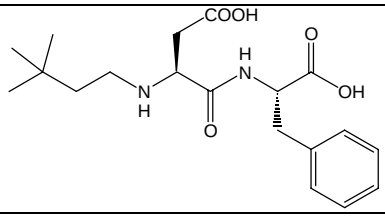
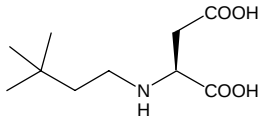
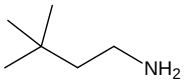
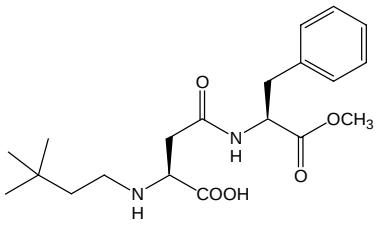
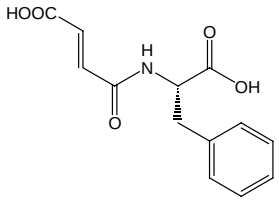
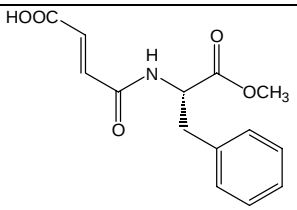
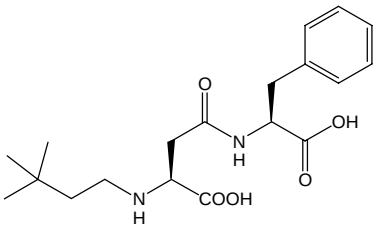
Because the NOAEL of Neotame is considered to be 96.5 mg/kg body weight/day based on the low body weight observed in F_1 offspring animals in the two-generation reproductive study in rats, the ADI of Neotame was determined to be 1.0 mg/kg body weight/day on the basis of a safety factor of 100.

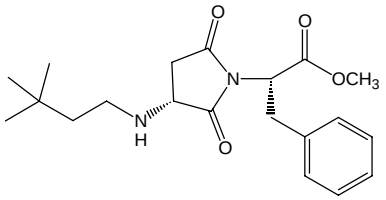
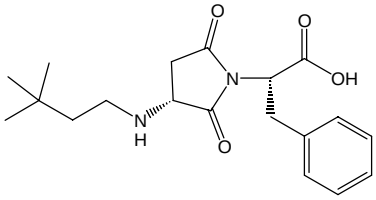
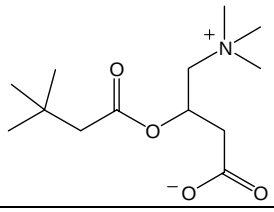
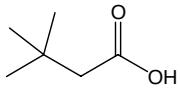
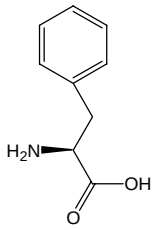
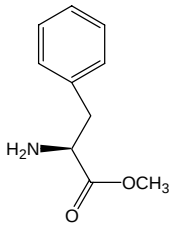
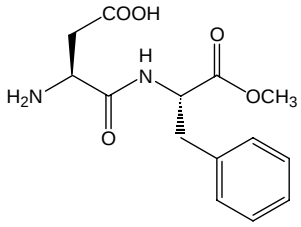
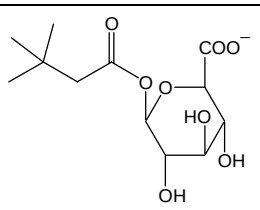
Although the data are limited, the results indicate that the degradation products of Neotame did not

have any particular adverse effects on the body.

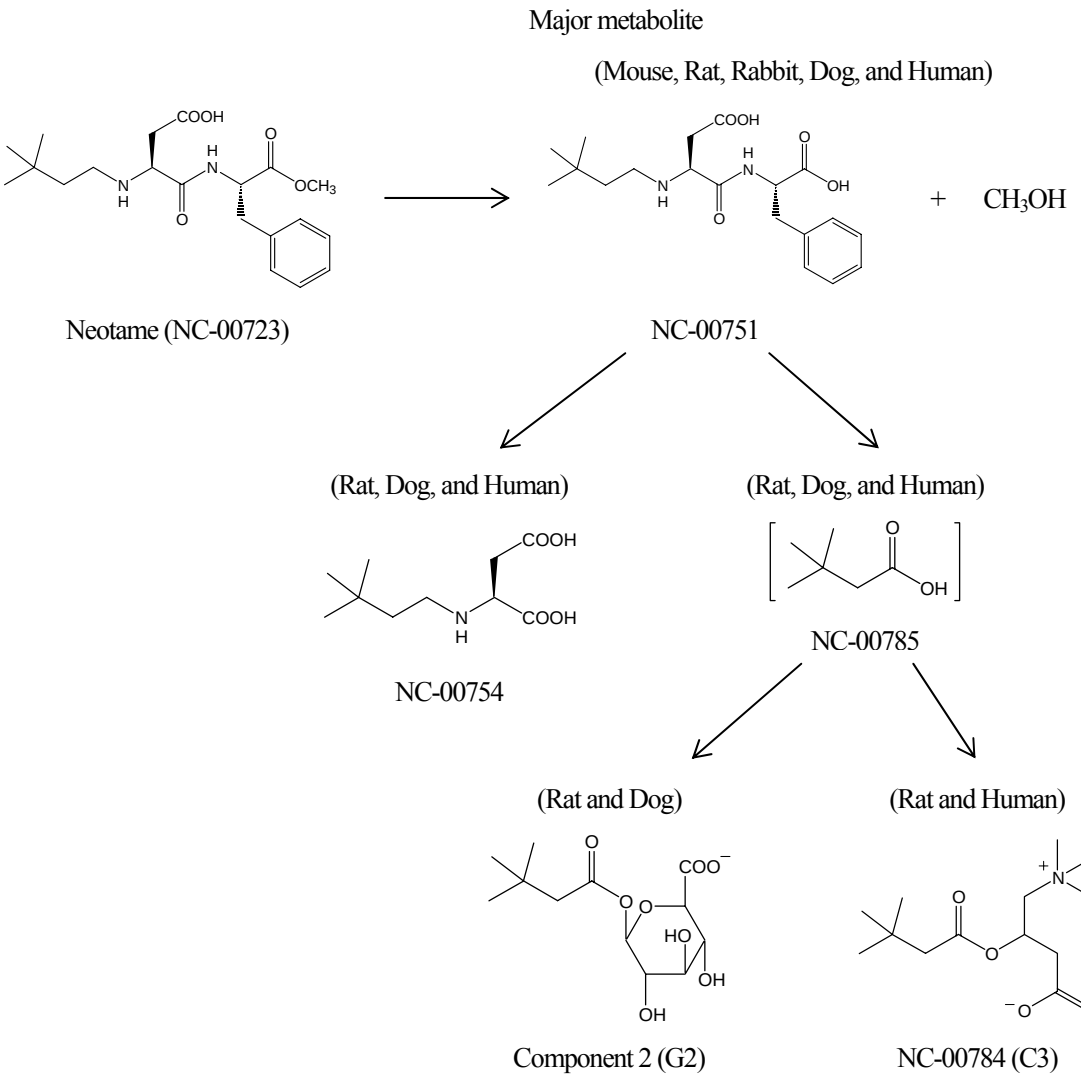
ADI: 1.0 mg/kg body weight/day
(Referred data for ADI): Two-generation reproductive study
(Laboratory animal tested): Rats
(Administration route): Mixed feeds
(Referred findings for NOAEL): Low body weight of F_1 offspring animals
(NOAEL): 96.5 mg/kg body weight/day
(Safety factor): 100

[Table List of related compounds of Neotame]

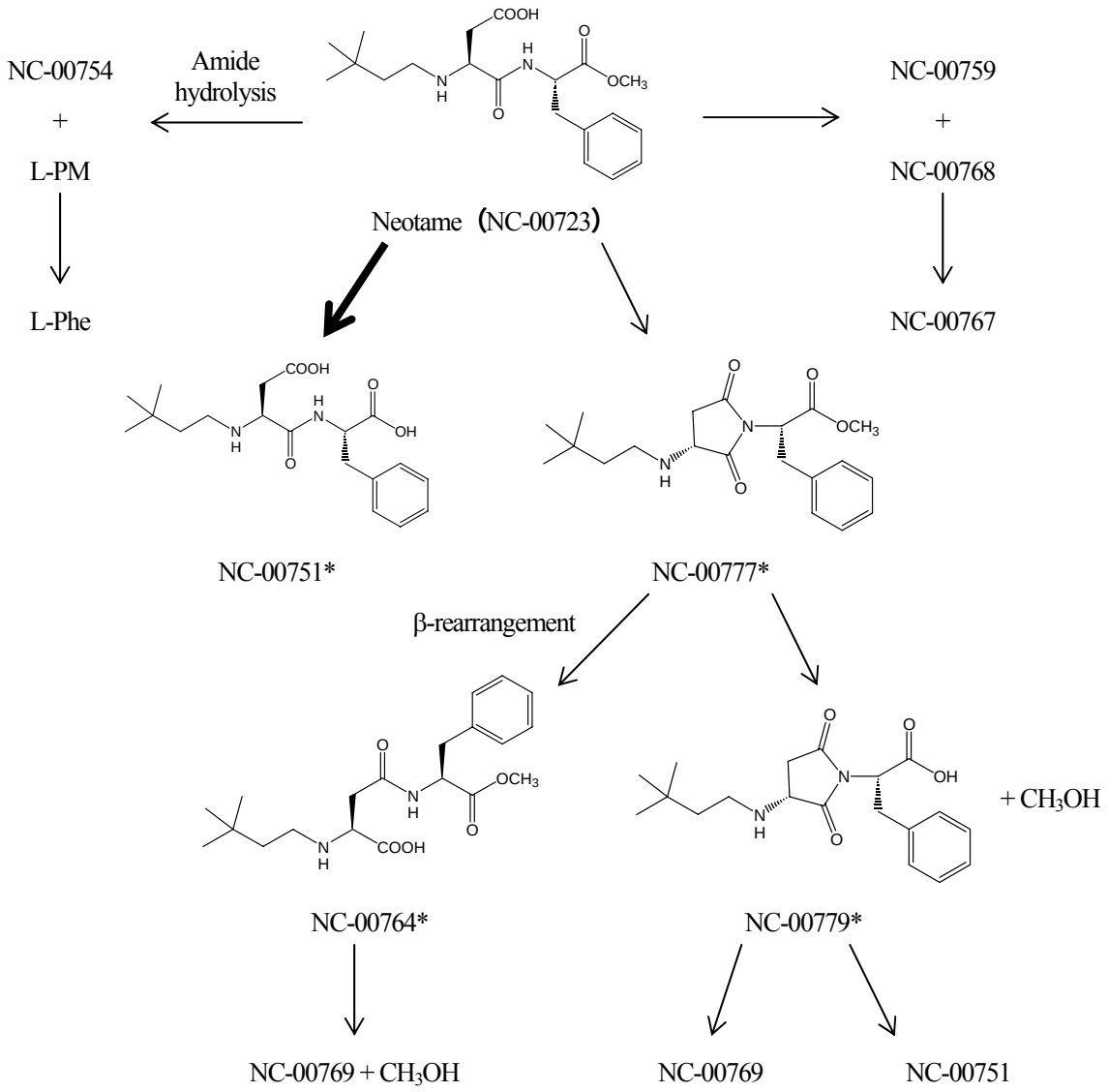
Name	Chemical name (Common name)	Chemical structure
Neotame (NC-00723)	Neotame *N*-[*N*-(3,3,-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester	
NC-00751	*N*-[*N*-(3,3,-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine	
NC-00754	*N*-(3,3,-dimethylbutyl)-L-α-aspartic acid	
NC-00759	3,3,-dimethylbutylamine	
NC-00764	*N*-[*N*-(3,3,-dimethylbutyl)-L-ß-aspartyl]-L-phenylalanine 1-methyl ester	
NC-00767	*N*-fumaryl-L-phenylalanine	
NC-00768	*N*-fumaryl-L-phenylalanine 1-methyl ester	
NC-00769	*N*-[*N*-(3,3,-dimethylbutyl)-L-ß-aspartyl]-*L*-phenylalanine	

Name	Chemical name (Common name)	Chemical structure
NC-00777	*N*-[*N*-(3,3,-dimethylbutyl)-L-aspartimide]-L-phenylalanine 1-methyl ester	
NC-00779	N-[N-(3,3,-dimethylbutyl)-L-aspartimide]-L-phenylalanine	
NC-00784 (C3)	3,3-dimethylbutanoyl-L-carnitine	
NC-00785	3,3-dimethylbutanoic acid	
L-Phe	L-phenylalanine	
L-PM	L-phenylalanine methyl ester	
Aspartame (APM)	Aspartame α-L-aspartyl-L-phenylalanine methyl ester	
Component 2 (G2)	β-glucuronide 3,3-dimethylbutanoic acid (Glucuronic acid conjugate)	

[Figure 1 Estimated metabolic pathway of Neotame][10), 11), 15), 20), 24), 33), 34)]



[Figure 2 Degradation pathway of Neotame (under severe conditions)] [7)]



* Degradation products under practical storage conditions (pH: 3.2; 20°C; 8 w)

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*: "Unpublished report" in this evaluation report is an unpublished paper.

Results of safety studies for Neotame and related compounds

Study category	Animal	Study	Administration route	Number of animals/group	Test compound	Dose	Results <NOAEL>	Reference
Repeated-dose toxicity	Mouse	13-week dietary administration study	Dietary	20 males and 20 females	Neotame	0, 100, 1,000, 4,000, and 8,000 (mg/kg bw/day)	The relative liver weight increased at 4,000 mg/kg bw/day and higher. The absolute liver weight increased at 8,000 mg/kg bw/day. <1,003 mg/kg bw/day>	26
	Rat	13-week dietary administration study followed by a 4-week reversibility period	Dietary	20 or 25 each of males and females	Neotame	0, 100, 300, 1,000, and 3,000 (mg/kg bw/day)	ALP increased in males at 1,000 mg/kg bw/day and at 3,000 mg/kg bw/day. <293 mg/kg bw/day>	27
	Dog	13-week dietary administration study followed by a 4-week reversibility period	Dietary	4 or 6 each of males and females	Neotame	0, 60, 200, 600, and 2,000/1,200 (mg/kg bw/day)	ALP increased in females at 200 and 600 mg/kg bw/day and at 2,000/1,200 mg/kg bw/day. RBC, Hb, and Hct decreased in the 2,000/1,200 mg/kg bw/day group. <59.7 mg/kg bw/day>	28
	Rat	*in utero* exposure /52-week dietary administration study followed by a 4-week reversibility period	*in utero*/dietary	Parent: 25 males and 25 females F_1: 20 males and 20 females	Neotame	0, 10, 30, 100, 300, and 1,000 (mg/kg bw/day)	No effect <1,006 mg/kg bw/day and higher>	29
	Dog	52-week dietary administration study followed by a 4-week reversibility period	Dietary	4 or 6 each of males and females	Neotame	0, 20, 60, 200, and 800 (mg/kg bw/day)	ALP increased at 800 mg/kg bw/day <197 mg/kg bw/day>	30
Reproductive toxicity	Rat	Reproductive toxicity study(Note 1)	Dietary	28 males and 28 females	Neotame	0, 100, 300, and 1,000 (mg/kg bw/day)	Parental animals (F_0, F_1): The feed efficiency decreased at 1,000 mg/kg bw/day during the pre-mating period. Offspring (F_1, F_2): Low body weight was seen in F_1 males at 300 mg/kg bw/day and at 1,000 mg/kg bw/day on Postnatal day (PND) 1, and F_1 in 300 mg/kg bw/day and higher and F_2 at 1,000 mg/kg bw/day on PND 21. <299 mg/kg bw/day for general toxicity in parental animals,: 96.5 mg/kg bw/day for reproductive and developmental toxicity>	31
Developmental toxicity	Rat	Developmental toxicity study(Note 2)	Dietary	24 females	Neotame	0, 100, 300, and 1,000 (mg/kg bw/day)	No teratogenicity was found. <964 mg/kg bw/day and higher>	32

Note 1 F_0: male: 14 weeks from pre-mating to F_1 birth; female: 10 to 11 weeks from pre-mating to F_1 weaning
F_1: male: 15 to 16 weeks from weaning to F_2 birth; female: 17 to 20 weeks from weaning to F_2 weaning
Note 2 Day 28 pre-mating to Day 20 post-mating

Study category	Animal	Study	Administration route	Number of animals/group	Test compound	Dose	Results <NOAEL>	Reference
	Rabbit (pregnant)	Developmental toxicity study(Note 3)	Oral gavage	20 - 25 animals	Neotame	0, 50, 150, and 500 (mg/kg bw/day)	No teratogenicity was found. Dam: One death and two abortions in the 500 mg/kg bw/day group. <150 mg/kg bw/day for dams; 500 mg/kg bw/day and higher for fetuses>	33
Carcinogenicity	Mouse	104-week carcinogenicity study	Dietary	Control group: 140 each of males and females Treatment group: 70 each of males and females	Neotame	0, 50, 400, 2,000, and 4,000 (mg/kg bw/day)	No carcinogenicity was found.	34
	Rat	*in utero* exposure /104-week carcinogenicity study	*in utero*/dietary	(F_0) Control group: 170 each of males and females Treatment group: 85 each of males and females (F_1) Control group: 147 each of males and females Treatment group: 73 - 75 each of males and females	Neotame	0, 50, 500, and 1,000 (mg/kg bw/day)	No carcinogenicity was found.	35
Antigenicity	Guinea pig	Dermal sensitization study	Closed patch	Control group: 5 each of males and females Treatment group: 10 each of males and females	Neotame	0, and 0.4 (g/animal)	No skin reaction was observed.	36
Genotoxicity	*in vitro*	Reverse mutation assay (+/-S9mix)	TA98, TA100, TA1535, TA1537, TA1538, and WP2*uvrA*		Neotame	312-10,000 (μg/plate)	Negative	37
		Gene mutation assay (+/-S9mix)	L5178Y mouse lymphoma cells		Neotame	100-1,000 (μg/mL)	Negative	38
		Chromosome aberration test (+/-S9mix)	Chinese hamster ovary (CHO) cells		Neotame	With S9mix: 250-1,000 (μg/mL) Without S9mix: 62.5-500 (μg/mL)	Negative	39
	in vivo	Mouse micronucleus assay	Oral gavage	10 males and 10 females	Neotame	500, 1,000, and 2,000 (mg/kg bw)	Negative	40
General pharmacology	Rat	General symptoms and behaviors Central nervous system	Dietary	20 or 25 each of males and females	Neotame	0, 100, 300, 1,000, and 3,000 (mg/kg bw/day)	No effect	27
	Dog	General symptoms and behaviors Central nervous system	Dietary	4 or 6 each of males and females	Neotame	0, 60, 200, 600, and 2,000/1,200 (mg/kg bw/day)	No effect	28
	Rat	Central nervous system (spontaneous motor activity)	Dietary	28 each of males and females	Neotame	0, 100, 300, and 1,000 (mg/kg bw/day)	No effect	31

Note 3 14 days from Days 6 to 19 of gestation

Study category	Animal	Study	Administration route	Number of animals/group	Test compound	Dose	Results <NOAEL>	Reference
	Rat	Central nervous system (anesthetic action)	Oral	5 each of males and females	Neotame	5, and 15 (mg/kg bw)	No effect	41
	Isolated male-guinea-pig ileum	Autonomic nervous system	Exposure		Neotame	0, 20, 60, and 200 (ng/mL)	No effect	42
					NC -00751	60, 200, and 600 (ng/mL)		
	Dog	Cardiovascular and respiratory systems and renal function	Intraduodenal	6 males	Neotame	5, and 15 (mg/kg bw)	No effect	43
	Rat	Digestive system (small intestine propulsion of charcoal)	Oral	10 males	Neotame	5,and15 (mg/kg bw)	No effect	44
Preference	Rat	Preference study	Dietary	14 males and 14 females	Neotame	0, 50, 150, 500, 1,500, 5,000, and 15,000 (ppm)	Craving for diets containing Neotame was reduced in all rats except for males of the 50 ppm group. Rats administered at 5,000 ppm and higher completely avoided eating the diet.	45
Single-dose toxicity	Rat	Single-dose toxicity study	Oral gavage	10 males and 10 females	NC- 00764	0, 0.6, 2.0, and 6.0 (mg/kg bw)	No effect	46
	Rat	Single-dose toxicity study	Oral gavage	10 males and 10 females	NC- 00777	0, 0.6, 2.0, and 6.0 (mg/kg bw)	No effect	47
	Rat	Single-dose toxicity study	Oral gavage	10 males and 10 females	NC- 00779	0, 0.3, 1.0, and 3.0 (mg/kg bw)	No effect	48
Repeated-dose toxicity	Rat	4-week dietary administration study	Dietary	15 males and 15 females	Mixture of NC- 00764, NC- 00777 and NC- 00779	NC-00764/NC-00777/NC-00779:0.2/0.2/0.1, 0.6/0.6/0.3, 2.0/2.0/1.0, and 6.0/6.0/3.0 (mg/kg bw/day)	No effect	49
Genotoxicity	*in vitro*	Reverse mutation assay (+/-S9mix)	TA98, TA100, TA102, TA1535, and TA1537		NC- 00751	50-5,000 (μg/plate)	Negative	50
		Gene mutation assay (+/-S9mix)	AS52/XPRT cells			625-5,000 (μg /mL)	Negative	54
		Reverse mutation assay (+/-S9mix)	TA98, TA100, TA102, TA1535, and TA1537		NC- 00764	50-5,000 (μg/plate)	Negative	51
		Gene mutation assay (+/-S9mix)	AS52/XPRT cells			625-5,000 (μg /mL)	Negative	55
	in vivo	Mouse micronucleus assay	Oral	10 males and 10 females		500, 1,000, and 2,000 (mg/kg bw)	Negative	58
	in vitro	Reverse mutation assay (+/-S9mix)	TA97, TA98, TA100, TA102, and TA1535		NC- 00777	10-5,000 (μg/plate)	Negative	52
		Gene mutation assay (+/-S9mix)	AS52/XPRT cells			100-390 (μg g/mL)	Negative	56
	in vivo	Mouse micronucleus assay	Oral	10 males and 10 females		500, 1,000, and 2,000 (mg/kg bw)	Negative	59
	in vitro	Reverse mutation assay (+/-S9mix)	TA97a, TA98, TA100, TA102, and TA1535		NC- 00777	10-5,000 (μg/plate)	Negative	53
		Gene mutation assay (+/-S9mix)	AS52/XPRT cells			313-5,000 (μg g/mL)	Negative	57
	in vivo	Mouse micronucleus assay	Oral	10 males and 10 females		500, 1,000, and 2,000 (mg/kg bw)	Negative	60
Findings in humans	Healthy adult man	Single dose study	Oral	6 men in each group	Neotame	0.1, 0.25, and 0.5 (mg/kg bw)	No effect	61
	Healthy adult	2-week administration study	Oral	12 men and 12 women	Neotame	0.5, and 1.5 (mg/kg bw/day)	No effect	23

Study category	Animal	Study	Administration route	Number of animals/group	Test compound	Dose	Results <NOAEL>	Reference
	Healthy adult	13-week administration study	Oral	24 each of men and women in the placebo group and 0.5 mg/kg bw/day group; 23 each of men and women in the 1.5 mg/kg bw/day group.	Neotame	0.5, and 1.5 (mg/kg bw/day)	No effect	62
	NIDDM patient	2-week theree-way crossover study	Oral	17 patients in a group	Neotame	0.5, and 1.5 (mg/kg bw/day)	No effect	63