§ 140.100 Delegations of authority.

The Commission hereby re-delegates the delegations of authority made to the Directors of the Division of Trading and Markets and/or to the Division of Economic Analysis, and their respective designees, in all instances as they occur throughout this chapter, jointly to the respective Directors of the Division of Market Oversight and the Division of Clearing and Intermediary Oversight, and their respective designees.

Issued in Washington, DC, on July 2, 2002, by the Commission.

Jean A. Webb,

Secretary of the Commission.

[FR Doc. 02–17179 Filed 7–8–02; 8:45 am]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 172

[Docket Nos. 98F–0052 and 99F–0187]

Food Additives Permitted for Direct Addition to Food for Human Consumption; Neotame

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of neotame as a nonnutritive sweetener in food. This action is in response to two petitions filed by Monsanto Co., which subsequently sold the rights to the petitions to the NutraSweet Co.

DATES: This rule is effective July 9, 2002. Submit objections and requests for a hearing by August 8, 2002. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of a certain publication in 21 CFR 172.829, as of July 9, 2002.

ADDRESSES: Submit written objections and requests for a hearing to the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic objections to http://www.fda.gov/dockets/ecomments.

FOR FURTHER INFORMATION CONTACT: Blondell Anderson, Center for Food Safety and Applied Nutrition (HFS–265), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740–3835, 202–418–3106.

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I. Introduction

FDA published notices in the **Federal Register** on February 10, 1998, and February 8, 1999 (63 FR 6762 and 64 FR 6100, respectively), announcing that food additive petitions, FAP 8A4580 and FAP 9A4643, had been filed by Monsanto Co., Skokie, IL 60077. The petitions propose amending the food additive regulations to provide for the safe use of neotame as a nonnutritive sweetener for tabletop use (FAP 8A4580) and for general-purpose use in food (FAP 9A4643) where standards of identity do not preclude such use. Subsequently, the rights to the petitions were sold to the NutraSweet Co., 699 North Wheeling Rd., suite 103, Mount Prospect, IL 60056. This document grants the petitions via a regulation approving the general-purpose food use of neotame.

II. Safety Evaluation

A. Chemistry and Intake Considerations of Neotame

Neotame is the common or usual name for the chemical *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine-1-methyl ester (CAS Reg. No.165450–17–9). It is synthesized by reductive *N*-alkylation of L-phenylalanine-L-α-aspartyl methyl ester with 3,3-dimethylbutyraldehyde. According to the petitioner, neotame has a sweetening potency that is approximately 7,000 to 13,000 times that of sucrose, depending on its food application (Refs. 1 and 2).

The peptidyl linkage in neotame is stabilized by the *N*-alkyl substituent and is resistant to hydrolysis under typical use and storage conditions. Additionally, the *N*-alkyl substituent effectively prevents the common dipeptide cyclization reaction that results in the formation of a diketopiperazine derivative. The data from stability studies submitted by the petitioner show that the degradation of neotame in aqueous solutions is pH-, time-, and temperature-dependent. Based upon data from these stability studies on neotame, the agency concludes that minor decomposition of neotame could occur in neotame-containing foods only when stored under conditions that are not considered typical for a commercial product (Refs. 1 and 2).

The agency has determined the estimated daily intake (EDI) at the 90th percentile for neotame as a general-purpose sweetener to be 0.10 milligram per kilogram (mg/kg) body weight per day (bw/d) for consumers of all ages (eaters only) and 0.17 mg/kg bw/d for 2 to 5 year olds (eaters only). The corresponding mean intakes are 0.04 mg/kg bw/d and 0.05 mg/kg bw/d, respectively (Refs. 2 and 3).

B. Nature and Extent of Neotame Safety Studies Database

In support of the safety of neotame, the petitioner submitted, within the two petitions, a combined total of 113 preclinical, clinical, and special studies, plus an additional 32 exploratory and screening studies in Food Master File No. 575. All pivotal preclinical studies were conducted in compliance with FDA's ''good laboratory practice'' regulations in 21 CFR part 58.

The preclinical (animal) studies include short-term, subchronic, and chronic dietary toxicity tests in the rat, mouse, and dog; multi-generation

reproduction and developmental studies in the rat; teratology studies in the rat and rabbit; and lifetime/carcinogenicity studies in the rat and mouse. The genotoxicity of neotame, its metabolites, and decomposition products, are also evaluated in several tests using both in vitro and in vivo assay systems. Extensive metabolism and pharmacokinetic measurements were carried out in all animal species studied. The clinical (human) studies tested the response/acceptance to orally administered neotame in both men and women during short-term (e.g., acute, single-dosing) and longer-term (e.g., up to 13 weeks, repeat-dosing) periods. Pharmacokinetic (PK) measurements also were carried out in a number of these studies (Ref. 4).

Additionally, the petitioner provided three position papers in response to FDA questions. These position papers address: (1) The potential behavioral and neurotoxic effects of neotame, (2) the significance of elevated serum (hepatic) alkaline phosphatase activity in neotame-treated dogs as a measure of toxicity, and (3) body weight gain decrement in mice ingesting neotame. The key aspects of these position papers are discussed, as appropriate.

C. Toxicology/Safety Assessment of Neotame

1. Metabolism and Pharmacokinetics of Neotame

As a component of the toxicological testing program on neotame, the petitioner conducted an extensive series of metabolism and PK studies. These studies were designed to assess: (1) The absorption of neotame; (2) the elimination, distribution, and potential tissue accumulation of neotame; (3) the effects of neotame on drug metabolizing enzymes; and (4) the metabolites of neotame in rodents (rats and mice), dogs, rabbits, and humans.

a. *Absorption of neotame*. In all species studied, including humans, the agency finds that the absorption of ingested neotame occurs almost entirely in the small intestine. In the animal studies, the absorption of neotame was determined under fasting conditions using a dose level that was approximately 150 times greater than the 90th percentile estimated daily intake (EDI) of neotame for humans. Under these conditions, the amount of administered dose absorbed is reported to range from 18 to 38 percent in the rat, 15 to 44 percent in the rabbit, and 40 to 51 percent in the dog. These studies also indicate that, when mixed with the diet, the absorption of neotame is reduced. In the human clinical studies, the absorption of neotame approaches 100 percent in healthy male and female subjects when administered following an overnight fast and at dose levels ranging from one to five times the 90th percentile EDI. Individual absorption levels range from 68 to 126 percent (Ref. 5).

b. *Elimination, distribution, and potential tissue accumulation of neotame*. The agency estimates that approximately 40 percent of the systemic elimination of ingested neotame and metabolites occurs via the urine, and the remainder is eliminated via the fecal route. In a whole-body radiography study in the rat, following a gavaged dose of radiolabled neotame and serial sacrifice at timed intervals, post-dosing, the highest levels of radioactivity are associated with the intestinal tract, the liver, and the kidney. At final sampling, no residual radioactivity is detected in peripheral tissues, with some residual activity associated with the intestinal tract. No organs or tissues, including the brain, eye, and skin, concentrate or store radiolabled neotame or its metabolites.

Further evidence for the lack of accumulation of neotame at expected levels of human intake is found in the analysis of PK parameters evaluated during a 13-week dog study. In dogs consuming dietary neotame at dose levels of 1,200 to 2,000 mg/kg bw/d, there is an indication of saturation of an elimination pathway that could lead to possible accumulation. However, these levels are at least 10,000 times greater than the 90th percentile EDI (0.1 mg/kg bw/d) of neotame for humans. This effect is not seen in dogs from the next lower treatment group (600 mg/kg bw/d), a level approximately 6,000 times above the 90th percentile EDI. Based on these findings, the agency concludes there is no concern for possible accumulation of neotame or its metabolites at expected human intake levels (Refs. 4 and 5).

c. *Effect of neotame on drug metabolizing enzymes*. The rat is generally considered an appropriate animal model to assess the effects of xenobiotics on phase I (i.e., cytochrome P–450 or mixed-function amine oxidase microsomal enzyme systems[1]) and phase II (i.e., conjugation or biotransformation reactions involving glucuronidation, sulfation, acetylation, or glutathione-S- transferase reactions) metabolism. Following a 14-day period during which dietary neotame was fed at 0 (control), 100, 300, or 1,000 mg/kg bw/d, rats were sacrificed and in vitro assays performed on isolated liver microsomal pellets. The agency concludes that, when compared against a positive control (phenobarbital, a known enzyme inducer), neotame does not induce P–450 microsomal mixed function oxidase metabolizing enzymes at any dose level administered during the in vivo phase of the study. In evaluating the effects of neotame on phase II metabolism, the agency notes that livers from rats in the 1,000 mg/kg bw/d treatment group show a statistically significant depression in phase II metabolism endpoints. However, at the next lower dose of 300 mg/kg bw/d, which is approximately 3,000 times the 90th percentile EDI for neotame for humans, there are no effects on these same endpoints (Ref. 5).

d. *Metabolites of neotame*. The initial step in the metabolism of neotame in rats, dogs, rabbits, and humans is de-esterification to *N*-[*N*-(3,3–dimethylbutyl)-L-α-aspartyl]-L-phenylalanine (DMB-Asp-Phe, coded in the petition as NC–00751) by Ca^{++}-dependent pancreatic esterases or after absorption by plasma esterases. De-esterification of neotame is similar in all species studied, including humans, although in the rat and rabbit this conversion occurs at a faster rate than in the dog and human. The de-esterified metabolite (NC–00751) is rapidly cleared from the plasma and excreted via the bile duct or in urine (Ref. 5). A small percentage of NC–00751 may undergo peptide-bond hydrolysis to form metabolites of dimethylbutylaspartate. The 3,3-dimethylbutyl portion of DMB-Asp-Phe is then oxidized to 3,3-dimethyl-butyric acid. This is followed by conjugation with glucuronic acid or with carnitine (a minor pathway).

Methanol release results from the de-esterification of neotame and occurs more rapidly in the rat and rabbit than in the dog and human. The agency concludes that at the 90th percentile EDI for neotame, exposure to resultant methanol will be insignificant, i.e., not more than 0.008 mg/kg bw/d. This exposure level is of no toxicological concern because humans are exposed to much greater levels of methanol intake from their daily diets (Refs. 4 and 5).

Based on neotame metabolism studies in the rat and dog, FDA concludes that some intestinal microvillar peptidase activity occurs in the gut, which results in the formation of other minor plasma metabolites of neotame, including phenylalanine (Ref. 5). Further review indicates that approximately 13 to 17

[1] Sipes, I. G. and Gandolfi, A. J., ''Biotransformation of Toxicants,'' chapter 4, pp. 88–109, in Casarett and Doull's Toxicology: *The Basic Science of Poisons*, 4th ed., edited by M. O. Amdur, J. Doul, and C. D. Klaassen, McGraw Hill, Inc., 1993.

percent of the total available phenylalanine in the ingested neotame is released into the plasma after absorption; the remainder is eliminated in feces and urine as DMB-Asp-Phe. The agency has estimated the amount of phenylalanine presented to the body from the ingestion of neotame. The phenylalanine content of neotame is 44 percent by weight. Given that the 90th percentile neotame EDI for a 60 kg adult is 0.10 mg/kg bw/d or 6 mg/d, and for a 2 to 5 year old (20 kg) child is 0.17 mg/kg bw/d or 3.4 mg/d, the estimated 90th percentile phenylalanine intake is 2.6 mg and 1.5 mg, respectively.

The agency notes that, for healthy adults, the daily dietary intake of phenylalanine may range from 2.5 to 10 grams per person per day (g/p/d), while that for a phenylketonuric (PKU) homozygous child (20 kg) may range from 0.4 to 0.6 g/p/d (Koch and Wenz[2]). Thus, the amount of phenylalanine from the 90th percentile intake of neotame is trivial compared to that from the normal adult diet. Even for the PKU homozygous child, the incremental amount of phenylalanine intake that can be expected from neotame is insignificant, i.e., equivalent to no more than 0.3 to 0.4 percent of the daily phenylalanine intake of the PKU homozygous child (Ref. 5). The agency concludes that the potential intake of phenylalanine that may result from use of neotame as a general-purpose sweetener does not pose any safety concern (Refs. 4 and 5).

Based on reviews of the metabolism and pharmacokinetic studies on neotame, the agency concludes that the metabolism of neotame is qualitatively similar across all species studied. Furthermore, there is no evidence that, at expected levels of intake, neotame or its metabolites will accumulate in the body or that ingestion of neotame will have any adverse effect in the body on Phase I and II metabolism. The metabolites of neotame are well characterized, and the potential intakes of metabolites, such as methanol and phenylalanine, are of no toxicological consequence. Therefore, the agency's review of the metabolism and pharmacokinetic studies of neotame does not raise any safety concerns (Refs. 4 and 5).

2. Critical Toxicology Studies and Issues

FDA reviewed all studies and supplemental information submitted by the petitioner. During its review, the agency determined that certain studies were more important than others to a regulatory decision on neotame. This determination was based on the nature of the endpoints investigated in these studies (i.e., reproductive and developmental effects, long-term exposure, chronic toxicity, carcinogenic potential, and human tolerance), and on specific issues presented by these studies. The critical studies and issues presented by the studies are: (1) The 2-generation reproduction study in rats—neurotoxicity and behavioral effects, (2) the chronic (52-week) dog study—toxicological significance of increased serum (hepatic) alkaline phosphatase levels, (3) the 104-week mouse carcinogenicity study—body weight gain decrement effect, (4) the 104-week rat carcinogenicity study—body weight gain decrement effect at all dose levels tested, (5) the chronic (52-week) rat feeding study—body weight gain decrement effect, and (6) the human clinical trials—human tolerance to neotame.

a. *A 2-generation reproduction study in the rat—neurotoxicity and behavioral effects.* Reproductive performance and fertility were assessed over two generations in CD (cesarean derived) rats fed diets containing neotame at levels of 0 (control), 100, 300, or 1,000 mg/kg bw/d. Each treatment group consisted of 28 males and 28 females. Animals were mated, the resultant offspring weaned, and the F1 generation animals selected and allowed to mature for 10 weeks and then mated. The F2 litters were terminated, post-weaning. Under the conditions of this study, the agency concludes that neotame has no effects on the reproduction or fertility of rats exposed to neotame at levels up to 1,000 mg/kg bw/d for two generations. Nor are there any treatment effects on measures of physical development, e.g., pinna unfolding, hair growth, tooth eruption, or eye opening (Refs. 4 and 6).

The 2-generation study included tests of motor activity and cognitive function. General motor activity was measured in F1 offspring by counting breaks in a pair of infrared light beams over a 12-hour period, while cognitive function was assessed by recording swim times up to 60 seconds maximally in six consecutive trials per animal in a water-filled Y-maze (Ref. 7). While the petitioner concludes there were no significant treatment effects on motor activity in F1 male and female offspring, the agency's analyses of pertinent data show a statistically significant reduction in motor activity among F1 males from the 1,000 mg/kg bw/d neotame treatment group. No effects are noted on motor activity in F1 females at any dose level.

With regard to results from the swim-maze tests that were conducted in F1 offspring at approximately 24 to 28 days of age, both the petitioner and the agency conclude that there is a statistically significant increase in mean swimming time (an indicator of reduced performance) to the ''correct'' arm of the Y-maze in F1 males from the 1,000 mg/kg bw/d group. Specifically, this increased swim time is noted in two of six trials in the F1 males from the high dose group. While an increase in swim time is also noted for one of six trials in F1 males from the 300 mg/kg bw/d dose group, this singular observation is not accompanied by any other indication of treatment-related behavioral changes and therefore is not considered to be indicative of a biologically relevant effect. As with motor activity, there are no effects on cognitive performance (as measured by swim maze times) noted in F1 female offspring from any treatment group.

The F1 offspring from the 2-generation reproduction study also were subjected to specific tests that measured the development of auditory and visual responses. The agency's evaluation of results on auditory startle, pupil closure, and visual placing show no treatment-related effects in F1 males or females at any level of neotame tested.

The finding of statistically significant effects on two separate behavioral tests (i.e., motor activity and swim maze times) in F1 males from the 1,000 mg/kg bw/d dose group supports the conclusion that this dose is an effect level. Based on the findings from the studies of motor activity and cognitive function, the agency considers the 300 mg/kg bw/d dose to be a no observed adverse effect level (NOAEL) for these endpoints (Refs. 4 and 7).

Early in its evaluation of the neotame safety database, the agency determined that the petitioner should provide a more specific assessment addressing the potential neurotoxicity and behavioral effects of neotame. In response to the agency's request, the petitioner submitted a position paper entitled ''Neotame Does Not Cause Any Behavioral or Neurotoxic Effects'' (Ref. 8). This document contains summaries and discussions of data and information from two principal sources. The first involves several ''key'' preclinical studies (12 in all) and 4 clinical studies from the neotame studies database. The second source of information discussed in the position paper is a series of 20 publications that are primarily related to aspartame. Collectively, these 20 publications provide little information

[2] Koch, R. and Wenz E. J., ''Aspartame Ingestion by Phenylketonuric Heterozygous and Homozygous Individuals,'' chapter 30, pp. 593–603, in *Physiology and Biochemistry*, edited by Stegink, L. D. and L. J. Filer, Jr., 1984.

that is relevant to the agency's overall safety assessment of neotame and are not discussed further.

With regard to the ''key'' animal studies, the petitioner states in its position paper that these studies incorporated clinical observations/testing enhancements as ''effective procedures for detecting neurotoxic effects.'' During the ante mortem phase of the animal studies, these enhancements included detailed physical, behavioral, and clinical observations to detect signs of neurological disorder, behavioral abnormality, physiological dysfunction, and other signs of nervous system toxicity. Post mortem enhancements included extensive histopathological evaluations of brain, spinal cord, and peripheral nerves.

FDA has reviewed thoroughly all of the preclinical and clinical studies discussed in the position paper. With the exception of the 2-generation rat reproduction study in which statistically significant decreases in motor activity and statistically significant increases in swim times are observed in F1 offspring males at 1,000 mg/kg bw/d, the preclinical studies do not show behavioral or neurotoxic effects associated with the ingestion of neotame.

Based on available preclinical and clinical information from the neotame studies database, the agency concludes that there is no concern for potential neurotoxic or behavioral effects in humans from the ingestion of neotame as a general-purpose sweetener in foods. This conclusion is reinforced further by the NOAEL of 300 mg/kg bw/d established for motor activity and cognitive performance in F1 males from the 2-generation reproduction study, a dose level that is at least 3,000 times greater than the 90th percentile EDI of 0.1 mg/kg bw/d (Refs. 4 and 7).

b. *Chronic (52-week) dog study—toxicological significance of elevated serum (hepatic) alkaline phosphatase.* Beagle dogs were fed diets containing neotame at levels of 0 (control), 20, 60, 200, or 800 mg/kg bw/d over a 52-week period. Detailed data were collected on animal survival, growth, food intake, clinical chemistries, hematology, urinalyses, and gross organ pathology and histopathology. At the conclusion of the study, a limited number of dogs from the neotame treatment groups were placed on a control diet for an additional 4-week ''reversibility period.'' During the agency's review of this study, a question arose about the toxicological significance of increased serum alkaline phosphatase (ALP) levels (of hepatic origin) noted in female dogs from the 200 mg/kg bw/d dose group and in both sexes at the 800 mg/kg bw/d dose group. Other effects noted were statistically significant dose-related increases in absolute liver weights and in relative liver weights (liver to brain weight ratio) in female dogs in the 200 and 800 mg/kg bw/d dose groups. There was no evidence of histopathological changes in the liver, brain, sciatic nerve, and spinal cord or in other organs or tissues examined from neotame-treated dogs.

Because elevated serum ALP levels had also been observed in shorter duration studies (2-week and 13-week) in dogs ingesting neotame containing diets, the agency requested that the petitioner provide further clarification on this matter. In its response, the petitioner submitted a position paper entitled ''Increases in Serum Alkaline Phosphatase in the Dog Are Not Associated with Target Organ Toxicity,'' together with several publications related to hepatotoxicity and serum ALP activity (Ref. 9). In this position paper, the petitioner reasons that the increased serum ALP levels observed in neotame-treated dogs are not due to a hepatotoxic response, but to a ''nonspecific, physiological response'' to the high doses of neotame.

FDA conducted further statistical analyses on the liver weight parameters mentioned previously. Based on these analyses, the agency concludes that the means for these liver effects from the 200 and 800 mg/kg bw/d dose groups are statistically significantly higher than the means for the 0 (control), 20, and 60 mg/kg bw/d treatment groups. Furthermore, there are no statistically significant differences between the 0 (control), 20, and 60 mg/kg bw/d dose group means for any of the liver weight parameters that were evaluated.

From the review of the data from the 52-week dog study and the supplemental information submitted by the petitioner in its position paper, the agency concludes that the changes in serum ALP levels are most likely due to a nontoxic response to the higher levels (200 and 800 mg/kg bw/d) of administered neotame. This conclusion is based on the following: (1) There are no significant effects from neotame on other liver enzymes (e.g., alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase), (2) serum albumin levels are not decreased in neotame-treated dogs (a decrease would have been an indicator of chronic liver toxicity), (3) serum bilirubin levels are normal in both sexes at high doses of neotame (an increase would have been seen if cholestasis was occurring), and (4) the liver in both sexes and at all dose levels appears normal on histopathological examination. In this 52-week dog study, FDA establishes a no observed effect level (NOEL) of 60 mg/kg bw/d, based on liver effects (e.g., serum (hepatic) alkaline phosphatase and relative liver weights) as the most sensitive endpoints (Refs. 4 and 10).

c. *A 104-week mouse carcinogenicity study—body weight gain decrement effect.* CD–1 mice were fed neotame-containing diets for 104 weeks at levels of 0 (control), 50, 400, 2,000, or 4,000 mg/kg bw/d. Based on an evaluation of the histopathological data from this carcinogenicity study, FDA concludes that, under the conditions of the study, doses of neotame up to 4,000 mg/kg bw/d administered to male and female CD–1 mice for up to 2 years did not induce neoplastic lesions (Ref. 11).

Although there was no evidence of carcinogenicity in mice exposed to neotame for 104 weeks, during the agency's review of other endpoints, we noted negative effects on body weight gain (and thus body weight) in both sexes. In light of only small decreases in cumulative food consumption, the agency was concerned about the potential toxicological significance of the decrease in body weight gain. In response to the agency's request for further clarification on this issue, the petitioner submitted a position paper entitled ''In the Mouse Carcinogenicity Study With Neotame Small Changes in Body Weight Gain at Some Intervals in Female Mice at 50 mg/kg bw Relative to Controls Are Due to a Decrease in Food Consumption'' (Ref. 12). In its analysis, the petitioner states that the mouse is not a reliable model for determining the relationship between body weight gain and food consumption. Reasons cited include the small differences in body weight gain over a lifetime in mice, both in absolute terms and in proportion to initial body weights at the start of a study, and well-known difficulties in obtaining accurate measures of food intake for mice (e.g., mice frequently spill food from their food cups and contaminate their food with feces and urine). The petitioner reiterated its belief that the body weight gain decrements noted in mice during the 104-week study were due to a small but consistent reduction in food consumption which is attributable to poor diet palatability and should not be viewed as a toxicological response to neotame.

In further evaluation of this body gain weight decrement issue, FDA subjected the data on body weight, body weight gain, and adjusted (for neotame content)

food intake to extensive statistical evaluation. Using an analysis of covariance model and pair-wise dose comparisons of body weights and body weight gain, the agency notes statistically significant effects for the 400, 2,000, and 4,000 mg/kg bw/d dose groups. Based on these analyses, the agency concludes that the body weight gain decrement effect in both male and female mice in the three highest dose groups is not accounted for by the small decreases in food consumption. However, in the 50 mg/kg bw/d treatment group, the effects on body weight and body weight gain are not statistically different from controls. Based on the detailed statistical evaluation of data pertinent to the body weight gain decrement noted in the 104-week dietary carcinogenicity study in mice, the agency establishes a NOEL of 50 mg/kg bw/d for this endpoint (Refs. 4 and 10).

d. *A 104-week rat carcinogenicity study—body weight gain decrement effect at all dose levels tested.* A 104-week rat carcinogenicity study (with an in utero phase) was conducted during which neotame was fed at 0 (control), 50, 500, or 1,000 mg/kg bw/d. Based on a thorough evaluation of the histopathological data from this carcinogenicity study, FDA concludes there is no evidence of neotame-induced neoplastic lesions in rats ingesting diets containing neotame at levels up to 1,000 mg/kg bw/d for 104 weeks (Ref. 11).

During its review of the 104-week rat carcinogenicity study, the agency noted effects on body weight gain (and thus body weight) in both sexes of neotame-treated rats at all dose levels tested. Statistically significant decreases in cumulative body weight gains were observed at various intervals throughout the study. At week interval 0 to 52, cumulative body weight gains were 9 to 11 percent less and 13 to 19 percent less, respectively, in neotame-treated male and female rats, than in control animals. Similar effects were noted at week intervals 0 to 78 and 0 to 104, i.e, cumulative body weight gains ranging from 10 to 13 percent less in treated males and 17 to 20 percent less in treated females. In reporting this information, the petitioner suggests that the lower body weights and lower body weight gains among neotame-treated rats can be attributed to reduced food intake due to reduced palatability of the diets containing neotame.

The agency, however, based on an analysis of the food intake data, concludes that the decreases in adjusted (for neotame content) food intake among the neotame-treated rats are small and do not fully explain the magnitude of the differences in body weight and body weight gain observed in these animals at week 52 and thereafter up to week 104. In view of the significant body weight gain decrement effect observed in all neotame treatment groups during the 104-week rat carcinogenicity study, a NOEL cannot be established. Lacking a suitable explanation for this effect based on decreased food intake (as argued by the petitioner), the agency considered the body weight gain decrement effect unresolved by the 104-week rat study (Refs. 4 and 10).

e. *Chronic (52-week) rat feeding study—body weight gain decrement effect.* In order to resolve the body weight gain decrement issue in rats, the agency carried out a thorough analysis of data from a 52-week rat feeding study. This study employed a wide range of neotame dose levels, two of which were below the lowest dose tested in the 104-week rat carcinogenicity study (as discussed in section II.C.2.d of this document). The results of this analysis are presented in the following paragraphs.

In the chronic (52-week) rat feeding study (with an in utero phase) rats received neotame at 0 (control), 10, 30, 100, 300, or 1,000 mg/kg bw/d. Except for body weight and body weight gain, there were no statistically significant treatment-related effects of neotame during this 52-week feeding study. With respect to both body weight and body weight gain, female rats appear to be more sensitive than males.

In regard to body weight, at the end of the 52-week study, body weights in females from the 100, 300, and 1,000 mg/kg bw/d groups were statistically significantly lower than those of control female rats. However, the body weights of females from the 10 and 30 mg/kg bw/d groups were not statistically different from control females. Among males, only the 100 mg/kg bw/d group had statistically significant body weight differences from control male rats.

As for cumulative body weight gains during the 0 to 52-week interval, statistically significant decreases are noted in treated females, compared to controls, only from the 300 and 1,000 mg/kg bw/d treatment groups. While the body weight gains in females from the 100 mg/kg bw/d are lower than in control female rats, this difference is not statistically significant. Compared with controls, there are no significant differences in cumulative body weight gains in females from the two lowest treatment groups (10 and 30 mg/kg bw/d) for the 0 to 52-week interval. Cumulative body weight gains in male rats from the 30, 100, 300, and 1,000 mg/kg bw/d neotame treatment groups, while somewhat lower than controls, are not statistically different. As noted in the 104-week carcinogenicity study, female rats in the 52-week dietary study were more sensitive to body weight gain decrement effects than males.

FDA performed a detailed analysis of the results from the 52-week dietary rat study and concludes that this study provides an adequate basis to assess the body weight gain decrement effect noted in the 104-week carcinogenicity rat study for four reasons. First, the range of neotame dose levels studied in the 52-week study is comparable to the doses tested in the 104-week study. Second, in each study, the female rat is more sensitive. Third, a parallel comparison of the 52-week study and the first 52 weeks of the carcinogenicity study shows that the body weight gain decrement effect was of a similar order of magnitude in both studies. Fourth, the magnitude of decrease in body weight gain occurring during week interval 0 to 52 in the 104-week study does not worsen during the last half of the study. These observations add strength to the utility of the 52-week dietary rat study in resolving any concern about the body weight gain decrement effect and in establishing a NOEL of 30 mg/kg bw/d for this endpoint (Refs. 4 and 10).

f. *Clinical studies assessments—human tolerance to neotame.* The petitioner submitted the results of six human clinical trials that investigated the ingestion of neotame under varied conditions, including acute-single exposure, acute-repeat exposure, and short-term (2-week) and longer-term (13-week) daily exposure. Five of these trials employed healthy adult subjects, while one trial evaluated non-insulin dependent diabetes mellitus (Type II diabetic) adult subjects. In each of these trials, subject tolerance to neotame intake was determined by physical examinations, vital signs, electrocardiograms, routine clinical laboratory measurements (e.g., hematology, clinical chemistries, and urinalysis), and self-assessments of adverse experiences.

The levels of neotame administered in these clinical trials ranged from 1 to 15 times the 90th percentile EDI level of 0.1 mg/kg bw/d or 6 mg per person per day (mg/p/d).

The agency concludes that in all six trials there are no treatment-related effects reported for any of the parameters examined. Although headache was the most frequently noted adverse experience, the incidence of

headache is comparable for the treated and control groups and is not considered to be associated with neotame intake. Results from ancillary pharmacokinetic measurements in several of the clinical trials do not raise any safety concerns. In the trial with Type II diabetic subjects, no adverse effects are noted in any of the subjects. Under the conditions of that trial, the agency concludes that the ingestion of neotame at levels up to 1.5 mg/kg bw/d does not produce significant changes in either fasting-state glucose or insulin levels in Type II diabetic subjects.

Based on reviews of these clinical trials, the agency concludes that the ingestion of neotame at levels up to 1.5 mg/kg bw/d (15 times the 90th percentile EDI) for a period as long as 13 weeks is well tolerated by healthy male and female subjects. The agency also concludes that in the study with Type II diabetic subjects, the intake of neotame at levels up to 1.5 mg/kg bw/d does not have significant effects on fasting plasma glucose or insulin levels in study subjects (Refs. 4 and 13).

D. Estimating an Acceptable Daily Intake for Neotame

In determining an acceptable daily intake (ADI) for a new food additive, the agency relies on a comprehensive evaluation of all relevant studies and information submitted by the petitioner. As the agency's evaluation of the neotame safety studies database progressed, four studies with attendant issues emerged as having the greatest impact in reaching a safety decision; these studies are highlighted in table 1 of this document.

TABLE 1.—SUMMARY OF STUDY DATA PERTINENT TO ESTABLISHING AN ACCEPTABLE DAILY INTAKE VALUE FOR NEOTAME

Study Information	Pivotal Endpoint	NOEL (mg/kg bw/d)	Safety Factor[A]	ADI (mg/p/d)
2-Generation Reproduction (Rat)	Motor Activity and Cognitive Function in F1 Males	(300)[B]	1,000	18
52-week (Dog)	Serum (Hepatic) ALP Levels and Relative Liver Weights in Females	60	100	36
104-week (Mouse)	Body Weight Gain Decrement in Both Sexes	50	100	30
52-week (Rat)	Body Weight Gain Decrement in Females	30	100	18

[A] Safety factors typically applied by the agency in establishing an ADI based on effects from a reproductive toxicity study or from a chronic study are 1000 and 100, respectively.
[B] The value reported is the NOAEL as discussed in Section II.C.2.a of this document.

Based on the NOAEL or NOEL identified for the most sensitive endpoint in each of the four studies, ADI values were determined ranging from a high of 36 mg/p/d to a low of 18 mg/p/d. In taking a conservative approach, the agency concludes that the appropriate ADI for neotame is 18 mg/p/d (Ref. 4). This level is three times higher than the 90th percentile EDI for neotame of 6 mg/p/d.

III. Comments

Thirty comments were submitted to FDA's Dockets Management Branch in response to the filing of the two neotame food additive petitions (25 for FAP 8A4580 and 5 for FAP 9A4643). The issues raised in the comments are identified and grouped into the following subject categories.

Aspartame

The majority of the comments compared neotame to aspartame. In these comparisons, the comments assumed that neotame produces the same metabolic breakdown products as aspartame and thus would be responsible for the same health effects they allege to be associated with aspartame, which is the subject of a food additive regulation (21 CFR 172.804). In response to these comments, FDA points out that neotame is chemically and metabolically different (see section II.A of this document and Ref. 1, and section II.C.1 of this document, respectively) from aspartame even though they are structurally related. Therefore, the comments' assertions about neotame are without basis. Because the comments do not provide the agency with any information regarding the safety of neotame, they will not be discussed further.

Estimated Daily Intake

Several comments objected to the tabletop use petition on the basis that the petitioner's EDI for neotame is inaccurate, implying that it is too low. In determining an EDI, FDA makes projections based on the amount of the additive proposed for use in particular foods and on data regarding the consumption levels of these particular foods, commonly using the 90th percentile as a measure of high chronic exposure. The agency concludes that the 90th percentile EDI calculated for neotame, as discussed in section II.A of this document, accurately reflects the exposure to neotame as a general-purpose sweetener in all foods (except for meats and poultry), including tabletop use (Ref. 2).

One comment noted that the petitioner assumes that neotame will replace 50 percent of aspartame's current applications and argued that this assumption may be limited unduly and not sufficiently conservative. FDA agrees with the comment on this point, and disagrees with the petitioner's use of the 50 percent replacement factor in their estimation of exposure to neotame. The agency conservatively assumes that this new sweetener will replace all existing uses of aspartame (Ref. 14) and uses this estimate in its safety evaluation.

No Observed Effect Level, Body Weight, and Body Weight Gain Effects

One comment stated that there is no NOEL established by the 104-week rat carcinogenicity study for neotame, because all doses show adverse effects on growth. The comment also asserted that the data contained in this study do not support the petitioner's explanation that decreases in body weights in the treated rats are due to reduced palatability of the neotame-containing diets. In addition, the comment indicated that the petitioner did not supply any gavage, pair-feeding, or dietary restriction studies to prove that the body weight gain decrements are due to palatability and not toxicity. The comment also claimed that a safe usage level for neotame cannot be determined from the safety database provided in the neotame food additive petitions.

FDA agrees that a NOEL cannot be established based on the 104-week rat carcinogenicity study, in view of the body weight gain (decrement) effect. The agency also notes that, while neotame may have had some influence

on diet palatability, the decreases in food intake (adjusted for neotame content) among neotame-treated rats of both sexes in the 104-week study are too small to explain the magnitude of the body weight gain decrement that occurred in rats from the neotame treatment groups (see section II.C.2.d of this document and Refs. 4 and 10). FDA disagrees, however, about the necessity for additional testing requested by the comment to resolve the body weight gain decrement issue. While the proposed studies might address mechanistic relationships between food consumption and weight gain, the agency believes that they will not provide meaningful data to explain the magnitude of differences in body weight and body weight gain in neotame-treated rats from the 104-week study in view of the small decreases in food consumption noted in these animals. In addition, FDA believes that a safe usage level for neotame can be established from the database provided by the petitioner. As discussed in section II.C.2.e of this document, the results in the 52-week rat dietary toxicity study provide a strong scientific basis to resolve concerns over the body weight gain decrement effect (Refs. 4, 10, and 15). Based on the 52-week rat study and using body weight gain decrement as the most sensitive endpoint for toxicity, the agency is able to establish a NOEL for neotame of 30 mg/kg bw/d. From this NOEL, FDA derives an ADI for neotame of 18 mg/p/d (see table 1 in section II.D of this document and Ref. 4).

Serum Alkaline Phosphatase and Liver Toxicity

Several comments expressed concerns regarding potentially adverse effects of neotame based on changes observed in serum ALP levels in dogs consuming high doses of neotame (i.e., 200 mg/kg bw/d and higher) in both 13-week and 52-week feeding studies. Additional comments suggested that neotame is hepatotoxic, as evidenced by effects on other endpoints, such as changes in absolute and/or relative liver weight, changes in serum cholesterol and triglycerides, and neotame-related cholestasis.

The agency notes that most of these comments focused on effects observed in the 13-week dog study. In its review of the subchronic (13-week) dog study, the agency observed the liver effects referenced in the comments (Ref. 16). Ordinarily, in the absence of a longer duration study, the agency would have given more weight to the results of the 13-week dog study. However, a chronic (52-week) dog study was also submitted in support of the safety of neotame, and that study provides for a more complete manifestation of the target organ toxicity in neotame-treated dogs.

While the agency considers the 13-week dog study useful for obtaining preliminary toxicological information (i.e., identification of target organs) and for determining the appropriate range of doses of neotame that would be fed in the 52-week dog study, the 52-week study provides a stronger basis for assessing the potential chronic toxicity of neotame in the dog. Because the results from this longer-term study supersede those of the 13-week study and because all of the effects noted in the shorter-term study occurred at levels of exposure well above the NOEL established by the 52-week study, the agency concludes that no further discussion is needed in response to issues raised in comments concerning the 13-week dog study.

Several comments asserted that elevated serum ALP levels observed in the neotame-treated dogs in the 52-week dog study indicate liver toxicity. As discussed in section II.C.2.b of this document, FDA recognizes that in the 52-week dog study elevated serum ALP levels are observed in both sexes of dogs from as early as 13 weeks until the end of the study at neotame dose levels of 200 and 800 mg/kg bw/d. However, the agency disagrees with comments that these elevated serum ALP levels are evidence of hepatic toxicity. While an increase in serum ALP may be an indicator of liver toxicity, such a conclusion cannot be substantiated in the absence of additional corroborative changes. Specifically, hepatic damage may result in increased levels of other liver enzymes, such as alanine aminotransferase, aspartate aminotransferase, or gamma glutamyl transferase. None of these liver enzymes was elevated in the neotame-treated dogs. Also, a decrease in blood albumin levels may indicate chronic liver toxicity. Blood albumin levels in dogs from all neotame dose groups were normal and comparable to control values. Furthermore, an elevation in serum bilirubin indicates cholestasis; serum bilirubin levels were unaffected by neotame treatment.

Increased cholesterol levels are another indication of altered liver function. Plasma cholesterol and triglyceride levels in dogs from the 52-week study, although somewhat variable, were well within the normal range for dogs and unaffected by neotame treatment. Additionally, histopathological examinations of livers from dogs from the neotame-treated groups did not reveal any evidence of necrosis, blockage of bile flow, or any other abnormalities that were not detected in control animals. Collectively, these observations support the agency's conclusion that data from the 52-week study do not show evidence of hepatic toxicity in dogs administered neotame (Refs. 4, 17, and 18).

Several comments asserted that neotame-related liver toxicity is not reversible, as is implied by the petitioner, based primarily on the increases in both serum ALP levels and relative liver weights in the dog studies. The agency concludes that the reversibility of these effects is not relevant to a safety decision regarding chronic ingestion of neotame. While FDA agrees, as noted in section II.C.2.b of this document, that increases in serum ALP levels and relative liver weights occur in dogs from the 200 and 800 mg/kg bw/d neotame groups in the 52-week study, neither of these parameters is affected at the lower levels tested (20 or 60 mg/kg bw/d). By considering serum ALP and relative liver weights as the most sensitive endpoints of potential neotame toxicity, the agency determines for the 52-week dog study that 60 mg/kg bw/d is an appropriate NOEL (Refs. 4, 10, and 17).

Liver as a Target Organ for Neotame Toxicity

One comment emphasized the importance of the liver in animal growth and glucose homeostasis. This comment asserted, based on analyses of the neotame safety studies database, that neotame affects growth in both rats and dogs, and appears to affect glucose homeostasis in persons with diabetes. Based upon these findings, along with the elevated serum ALP levels in neotame-treated dogs and the structure of neotame, the comment concluded that it was important to rule out the liver as a target organ.

In regard to the effect of neotame on body weight gain in the rat, the agency has established a NOEL of 30 mg/kg bw/d, based on the 52-week rat feeding study, as summarized in section II.C.2.e of this document. We discuss our analyses of the 52-week rat feeding study and our resolution of the body weight gain effect in more detail in Refs. 10 and 15.

In regard to the effect of neotame on body weight and body weight gain in the 52-week dog feeding study, the effect occurred only in male dogs and only in the highest neotame dose group (i.e., 800 mg/kg bw/d) during weeks 1 to 5 and 7 to 8 (Ref. 18). At all other dose levels tested (i.e., 20, 60, and 200 mg/kg bw/d), there were no statistically significant effects on body weight or body weight gain in either sex. Furthermore, as discussed in

section II.C.2.b of this document, the agency relies on more sensitive endpoints, i.e., serum ALP levels and relative liver weights, for establishing a NOEL for neotame from the 52-week dog study.

The agency also disagrees with the comment's assertion that neotame appears to affect glucose homeostasis in persons with diabetes. We explain our basis for concluding that neotame does not appear to affect glucose homeostasis in persons with diabetes later in this document, in the discussion entitled ''Type II Diabetes Study.''

As for changes in serum ALP levels, the agency does not consider these to be a manifestation of hepatic toxicity in the 52-week dog study. Our reasons for discounting the toxicological significance of the changes in serum ALP are discussed previously (see section II.C.2.b of this document and the fourth subject category in section III ''Serum Alkaline Phosphatase and Liver Toxicity'').

The comment asserted that ''[t]he structure of neotame suggests that the metabolic formation of nitrosamines by gut microflora is possible as well as formation in some food products.'' The agency acknowledges that a number of nitrosamine compounds are potent hepatotoxins and hepatocarcinogens. The agency also recognizes that neotame contains a secondary amine that could hypothetically form nitrosoneotame in the presence of a nitrosating agent. However, there is no scientific evidence presented in this comment to demonstrate that the presence of neotame in food leads to the formation of nitrosoneotame either through chemical reaction in food products or by metabolic processes in the gut upon ingestion (Ref. 14). Furthermore, the petitioner addressed this issue using many maximizing assumptions concerning the formation and potency of the hypothetical nitrosoneotame. In particular, the petitioner assumed that nitrosoneotame would be formed and that it would be as potent a carcinogen as dimethylnitrosamine. Based on this scenario, the petitioner concluded that the amounts of nitrosamine that could be formed would be extremely small, that any hypothetical risk would be trivial, and that additional analyses were not necessary. After evaluating the petitioner's reasoning, FDA agrees with this conclusion (Refs. 1 and 14). Furthermore, as noted in sections II.C.2.c and II.C.2.d of this document, there is no evidence of chronic liver toxicity or pre-neoplastic or neoplastic liver lesions in lifetime carcinogenicity feeding studies in rats and mice ingesting neotame in amounts up to 1,000 mg/kg bw/d and 4,000 mg/kg bw/d, respectively. Thus, the agency concludes that the hypothetical formation of nitrosamine compounds from neotame poses no safety concerns.

Finally, the agency recognizes that one cannot absolutely rule out the liver as a target organ for the toxic effects of neotame when it is ingested at exaggerated dose levels. However, as discussed in the agency's response to this comment, and elsewhere in this document, the agency concludes that at expected levels of dietary intake of neotame there is no concern for potential toxic effects to the liver.

Systemic Exposure/Body Weight Gain

One comment stated that ''[t]he long-term studies conducted in the dog species show definite signs of toxicity which, through close inspection of the pharmacokinetic data generated in the study and specific PK metabolism studies, is shown to be related to systemic exposure of the parent compound.'' Subsequently, the comment referred to ''a non-linear increase in systemic exposure of the parent compound and its metabolite over the dose range studied.'' The comment asserted that this nonlinear increase in systemic exposure to the parent compound and its metabolite is related to decreases in body weight gain in the dog.

In response, the agency notes that the analysis of PK parameters (i.e., area under the curve, and maximum concentration) discussed in the comment is based on data from the 13-week dog study, which the agency does not consider to be a long-term study as claimed in the comment. In the agency's review of this study (Ref. 16), decreased body weight gains were observed in dogs of both sexes at dietary neotame intakes of 600 and 2,000 mg/kg bw/d (the 2,000 mg/kg bw/d dose level was reduced on day 15 to 1,200 mg/kg bw/d for the remainder of the 13-week study). These extremely high dose levels are 6,000 to 20,000 times greater than the 90th percentile EDI for neotame. At lower levels of neotame intake (i.e., 60 and 200 mg/kg bw/d), there were no effects on body weight gain in either sex. In considering the PK parameters derived from blood concentration data from the dogs fed these lower levels of neotame, the agency concludes (Ref. 19) that there was no evidence of increased systemic exposure to neotame or its metabolites. (It should be noted that PK measurements in the dog were evaluated only in the 13-week subchronic study.)

Moreover, as mentioned in Refs. 4, 10, and 17, a chronic (52-week) neotame dog feeding study was conducted. Because of its longer duration, the 52-week study is more definitive than the subchronic (13-week) dog study for assessing the toxicity of neotame. In the 52-week dog study, decreased body weight gains were noted only at the highest dose tested (800 mg/kg bw/d) and not at any of the lower dose levels (20, 60, and 200 mg/kg bw/d).

Bile Salt Metabolism and Excretion

One comment pointed out that neotame produced discolored feces (white and gray) at the highest doses tested (200 and 800 mg/kg bw/d) in the 52-week dog study. This comment suggested that the change in fecal color was due to neotame's effect on bile salt metabolism and excretion. The agency agrees that dogs from the 800 mg/kg bw/d treatment group frequently excreted gray or white feces. However, there were only two incidences of gray feces from animals in the 200 mg/kg bw/d treatment group (a female on day 322 and a male on day 328), and no changes in appearance of feces from dogs in the 20 or 60 mg/kg bw/d treatment groups. There was also one incident of white feces observed for a female in the control group on day 70 of the study. Based on this evidence, as well as information in section II.C.2.e of this document, the agency concludes that there is no evidence to support a correlation between fecal color and liver toxicity in dogs fed neotame-containing diets during the 52-week study (Ref. 20).

Developmental (Teratology) Studies

One comment claimed that the dose levels of neotame tested in the definitive rabbit developmental (teratology) study were too low. The agency disagrees. FDA's evaluation of this study shows that there are statistically significant decreases in feed consumption and maternal body weights during the gestation period. Thus, the highest dose in the study (500 mg/kg bw/d) was sufficient to achieve maternal toxicity (Refs. 4 and 6). In addition, FDA notes that this study satisfies dose selection criteria recommended in the agency's Redbook guidelines (Ref. 21).

Another comment raised concern over post-implantation effects of neotame based on a maternal toxicity range-finding study in the rabbit. Because of the study's limitations, the agency does not share this concern. While a range-finding study may aid in identifying a compound's potential target organ effects, the primary objective of such a study is to establish appropriate dose levels to be further evaluated in a more definitive toxicity study. In the study in question, the agency notes that only six animals were used in each dose group, too few for an adequate assessment of

the developmental (teratogenic) potential of a compound (Ref. 21). In the definitive rabbit developmental (teratology) study, a total of 25 mated females were assigned to the control and high-dose groups, and 20 each in the low- and mid-dose groups (Ref. 6). This larger number of animals allows for a more accurate assessment of the teratogenic potential of neotame in the rabbit as well as increasing the statistical power of the study. In the definitive rabbit teratology study, there were no significant dose-dependent, post-implantation effects due to neotame treatment.

One comment argued that neotame-induced effects on post-implantation loss, fetal size, and limb development in rabbits in the teratology study may be masked by the quality of the study and the high background incidence of these effects. The comment disagreed with the petitioner's interpretation of the data on post-implantation and other fetal observations. In particular, the comment asserted that the petitioner's interpretation of data was scientifically flawed because the petitioner made comparisons between treatment groups and the concurrent control group whose incidence percentages, according to the comment, were higher than those incidence percentages typically seen in historical control data.

FDA disagrees with this assessment. By using concurrent control animals, the study avoided the inherent variability that may be introduced into data analyses when historical control data are used in place of concurrent control data. Potential sources of variability from the use of historical control data include: (1) Differences in animal husbandry and animal room environment, (2) differences in diet compositions, (3) differences in times of study conduct, (4) differences in the sources of nutrients in animal diets, (5) differences in skills and experience of technicians or scientists, and (6) genetic drifts, as discussed in Haseman et al., 1989[3] and Roe, 1994.[4] Therefore, the agency concludes that, within the definitive rabbit study, in the absence of compelling evidence to the contrary, it is more appropriate to compare results between treated and concurrent control animals than to compare results between treated animals and historical control data. The agency also notes that the study followed the Redbook guidelines. Additionally, the agency finds no dose-dependent effects on post-implantation data when this study's treated and concurrent control groups are compared (Refs. 6 and 21).

In further response to this comment, the agency concludes that the manner in which the comment has analyzed the data from the rabbit developmental study is incorrect. More specifically, the comment compared control and treated groups on a per-fetus, rather than on a per-litter incidence basis. As recognized by authoritative sources[5 6 7] the maternal animal, not the developing organism, is randomly and independently assigned to control and treatment groups during the gestation period. Therefore, the analyses of effects should be reported as incidence-per-litter or as number and percent of litters with particular endpoints. Because the comment's analysis is based on inappropriate per-fetus comparisons, its conclusions are inherently flawed. Furthermore, the agency finds that the comparisons between the concurrent control and treated groups, on a percent per-litter basis, show no treatment-related effects on the litter incidence of any fetal endpoint examined in the rabbit developmental (teratology) study (Refs. 6 and 21).

One comment focused on the dosimetric and pharmacokinetic aspects of the rabbit developmental (teratology) study. The comment asserted that if a higher dose level, e.g., 1,000 mg/kg bw/d, rather than 500 mg/kg bw/d, had been used as the top dose in this definitive study, higher systemic exposure and greater toxicity would have occurred in the neotame-treated rabbits. As noted earlier with regard to the levels of neotame tested in this study, the agency finds that overall study design and dose selection were sufficient to achieve maternal toxicity. FDA believes that it is irrelevant if greater toxicity were to occur at a higher dose level than the highest dose used in the rabbit developmental (teratology) study. The highest dose used was sufficient to achieve maternal toxicity, based on statistically significant decreases in both feed intake and body weight gain, at the 500 mg/kg bw/d dose level. Furthermore, there is an appropriate NOEL for these effects (Refs. 6 and 21).

This comment also suggested that decreases in food intake and maternal body weight gain noted in the dams from the 500 mg/kg bw/d dose group were due to (tissue) accumulation of neotame. Based on a review of the PK data from the definitive rabbit developmental study, the agency concludes that these data do not suggest that bioaccumulation of neotame or its metabolites would occur even at a dose level of 500 mg/kg bw/d (Ref. 22). With regard to a possible relationship between (tissue) accumulation of neotame and decreases in feed intake and maternal body weight gain, the agency finds that a mechanistic explanation is unnecessary for an adequate evaluation of the study because the agency has determined an appropriate NOEL for these effects. As noted previously in section II.C.1.b of this document, based on the evaluation of other neotame feeding studies in the rat and dog, FDA concludes that there is no concern for the potential bioaccumulation of neotame or its metabolites at expected human intake levels.

Type II Diabetes Study

One comment criticized several aspects of the Type II diabetes study. The comment stated that the design of this study was not adequate to detect small differences resulting from neotame treatment in the parameters examined. It cited the following inadequacies: Limited statistical power, parameters measured only under the quiescent metabolic condition of extended fasting, short duration, and no meal test. Despite these deficiencies, the comment recommended inclusion of the Type II diabetes study in the safety evaluation, because no other studies in the neotame safety database investigated the effects of neotame on glucose homeostasis in patients or animals with diabetes. Finally, the comment concluded that results from the Type II diabetes study were strongly suggestive of a treatment-related effect of neotame on fasting glucose control.

FDA agrees that although the experimental design of the Type II diabetes study limits its utility for assessing the potential effects of neotame on glucose homeostasis in Type II diabetics, it should be included in the safety evaluation of neotame (Ref. 23). Based on findings obtained during a directed clinical investigator site inspection and audit of study records at the facility responsible for this clinical

[3] Haseman, J. K., Huff, J. E., Rao, G. N., and Eustis, S. I., "Sources of Variability in Rodent Carcinogencity," *Fundamental and Applied Toxicology*, vol. 12(4), pp. 793–804, 1989.

[4] Roe, F. J. C., "Historical Histopathological Control Data for Laboratory Rodents: Valuable Treasure or Worthless Trash?" *Laboratory Animals,* vol. 28(2), pp. 148–154, (London), 1994.

[5] FDA, "Guidelines for Developmental Toxicity Studies," chapter IV.C.b, section III.D, *Redbook 2000 Toxicology Principles for the Safety of Food Ingredients* (http://www.cfsan.fda.gov/~redbook/redivc96.html).

[6] Tyl, R. W. and M. C. Merr, "Developmental Toxicity Testing-Methodology," chapter 7, pp. 217, *Handbook of Developmental Toxicology*, edited by R. D. Hood, CRC Press, New York, NY, 1997.

[7] Kimmel, C. A. and G. I. Kimmel, "Principles of Developmental Toxicity Risk Assessment," chapter 21, pp. 671–672, *Handbook of Developmental Toxicology*, edited by R. D. Hood, CRC Press, New York, NY, 1997.

trial, FDA concludes that the study was well-executed, irrespective of previously noted design limitations (Ref. 23).

The agency disagrees with the comment's conclusion that results from the trial with Type II diabetic subjects are strongly suggestive of a treatment-related effect of neotame on glucose control. FDA performed a detailed evaluation of the study data on fasting glucose pharmacodynamic parameters including: (1) Area under the effect curve, (2) area under curve, (3) percent perturbation, and (4) normal variations in glucose concentrations. Based on these analyses, the agency finds that under the conditions of the study, there were no significant changes in these parameters in study subjects that are attributable to neotame (Ref. 23). Overall, FDA concludes that under the conditions of the Type II diabetic study, blood glucose concentrations in Type II diabetic subjects following neotame treatment (at levels ranging from 5 to 15 times the 90th percentile EDI of 0.1 mg/kg bw/d) are comparable to those in the same subjects when given a placebo, and that any changes noted are within the normal range of variation and not the result of neotame treatment (Ref. 23).

Methanol and Phenylalanine Formation

Several comments expressed concern that harmful levels of methanol and phenylalanine may result from ingesting neotame-containing foods and beverages. FDA disagrees with these comments. Methanol release results from the de-esterification of neotame, which occurs more rapidly in the rat and rabbit than in the dog and human (see section II.C.1.d of this document). The agency concludes that, at the 90th percentile EDI of neotame, the resultant exposure to methanol would be extremely low, approximately 0.008 mg/kg bw/d (Ref. 5). Humans are exposed to much higher levels of methanol intake from their daily diet. For example, the methanol content of fruit juices ranges from 64 mg/liter (L) in orange juice to 326 mg/L in apricot juice. In contrast, the methanol content of neotame-sweetened carbonated beverages is estimated to be 1.37 mg/L.

Similarly, FDA concludes that the potential intake of phenylalanine from the use of neotame will be extremely low in comparison to that present in the daily diet. Based upon data cited by Koch and Wenz, 1984 (see footnote 2 in section II.C.1.d of this document), the agency notes that the daily dietary intake of phenylalanine for a healthy individual may range from 2.5 to 10 g/p/d. The daily intake of phenylalanine for a PKU homozygous child with a body weight of 20 kg is reported to range from 0.4 to 0.6 g/p/d or 400 to 600 mg/p/d (Ref. 5).

Using a conservative approach (Refs. 4 and 5), the agency calculates that the amount of phenylalanine exposure expected from the 90th percentile intake (0.1 mg/kg bw/d) of neotame (Ref. 2) by a 60 kg adult is 2.64 mg/p/d. FDA finds this amount of exposure trivial in contrast to that expected from the normal adult diet. For the PKU homozygous child, the additional phenylalanine intake expected from the 90th percentile ingestion of neotame (i.e., 0.17 mg/kg bw/d) (Ref. 3) by a 20 kg individual is 1.50 mg/p/d, an incremental amount that is equivalent to no more than 0.3 to 0.4 percent of the PKU homozygous child's normal daily phenylalanine intake. From these conservative estimates, the agency concludes that the potential intake of phenylalanine that may result from use of neotame as a general-purpose sweetener does not pose any safety concern (Refs. 4 and 5).

IV. Conclusion

The agency has evaluated all the data and other information submitted by the petitioner in support of the safe use of neotame as a general-purpose sweetener and concludes that there is a reasonable certainty that no harm will result from the use of neotame as proposed. In accordance with a memorandum of understanding (MOU) between the Food Safety and Inspection Service (FSIS), United States Department of Agriculture, and FDA (65 FR 51758, August 25, 2000), a restriction from use ''in meat and poultry'' appears in the neotame regulation. This restriction is required when the petitioner does not specify whether the food additive is intended for such use. At this time, FSIS has not made a determination on the use of neotame in or on meat or poultry. Therefore, FDA concludes that the food additive regulations should be amended as set forth in this document.

In accordance with § 171.1(h) (21 CFR 171.1(h)), the petitions and the documents that FDA considered and relied upon in reaching its decision to approve the petitions are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person. As provided in § 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

V. Environmental Effects

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (see **ADDRESSES**) between 9 a.m. and 4 p.m., Monday through Friday.

VI. Paperwork Reduction Act of 1995

This final rule contains no collection of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

VII. References

The following references have been placed on display in the Dockets Management Branch (see **ADDRESSES**), and you may review them between 9 a.m. and 4 p.m., Monday through Friday.

1. Memorandum from DiNovi, Division of Product Manufacture and Use, Chemistry Review Team, to Anderson, Division of Product Policy, March 31, 1998.

2. Memorandum from DiNovi, Division of Product Manufacture and Use, Chemistry Review Team, to Anderson, Division of Product Policy, August 12, 1999; addendum memorandum to the August 12, 1999, memorandum from DiNovi, Division of Biotechnology and GRAS Notification Review, to Anderson, Division of Petition Review, February 28, 2002.

3. Memorandum from DiNovi, Division of Product Manufacture and Use, Chemistry Review Team, to Anderson, Division of Product Policy, December 14, 2000.

4. Memorandum from Biddle, Lin, Whiteside, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, January 31, 2001; addendum memorandum to the January 31, 2001, memorandum from Whiteside, Division of Petition Review, to Anderson, Division of Petition Review, February 28, 2002.

5. Memorandum from Bleiberg, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, January 31, 2001; addendum memorandum to the January 31, 2001, memorandum from Biddle, Division of Petition Review, to Anderson, Division of Petition Review, February 28, 2002.

6. Memorandum from Welsh, Scientific Support Branch, to Anderson, Division of Product Policy, January 31, 2001.

7. Memorandum from Mattia, Scientific Support Branch, to Anderson, Division of Product Policy, January 31, 2001; addendum memorandum to the January 31, 2001, memorandum from Biddle, Division of Petition Review, to Anderson, Division of Petition Review, April 12, 2002.

8. Position paper from The NutraSweet Co., ''Neotame Does Not Cause Any Behavioral or Neurotoxic Effects.''

9. Position paper from The NutraSweet Co., ''Increases in Serum Alkaline Phosphatase in

the Dog Are Not Associated With Target Organ Toxicity.''

10. Memorandum from Whiteside, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, January 21, 2001.

11. Memorandum of Conference from the Center for Food Safety and Applied Nutrition—Cancer Assessment Committee, August 16, 2000.

12. Position paper from The NutraSweet Co., '' In the Mouse Carcinogenicity Study with Neotame Small Changes in Body Weight Gain at Some Intervals in Female Mice at 50 mg/kg bw Relative to Controls are Due to a Decrease in Food Consumption.''

13. Memorandum from Chen, Scientific Support Branch, to Anderson, Division of Product Policy, July 19, 2000.

14. Memorandum from DiNovi, Division of Product Manufacture and Use, Chemistry Review Team, to Anderson, Division of Product Policy, January 10, 2001.

15. Memorandum from Whiteside, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, January 31, 2001.

16. Memorandum from Ikeda, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, May 28, 1999.

17. Memorandum from Ikeda, Division of Health Effects Evaluation, to Biddle, Division of Health Effects Evaluation, January 31, 2001.

18. Memorandum from Ikeda, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, June 16, 2000; addendum memorandum to the June 16, 2000, memorandum from Whiteside, Division of Petition Review, to Anderson, Division of Petition Review, February 28, 2002.

19. Memorandum from Bleiberg, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, February 5, 2001.

20. Memorandum from Ikeda, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, February 5, 2001.

21. Memorandum from Shackleford, Division of Heath Effects Evaluation, to Anderson, Division of Product Policy, February 12, 2001.

22. Memorandum from Roth, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, February 28, 2001.

23. Memorandum from Park, Roth, and Klontz, Division of Health Effects Evaluation, to Anderson, Division of Product Policy, January 30, 2001.

VIII. Objections

Any person who will be adversely affected by this regulation may at any time file with the Dockets Management Branch (see **ADDRESSES**) written objections by August 8, 2002. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents are to be submitted and are to be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

List of Subjects in 21 CFR Part 172

Food additives, Incorporation by reference, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 172 is amended as follows:

PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 172 continues to read as follows:

Authority: 21 U.S.C. 321, 341, 342, 348, 371, 379e.

2. Section 172.829 is added to subpart I to read as follows:

§ 172.829 Neotame.

(a) Neotame is the chemical *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine-1-methyl ester (CAS Reg. No. 165450–17–9).

(b) Neotame meets the following specifications when it is tested according to the methods described or referenced in the document entitled ''Specifications and Analytical Methods for Neotame'' dated April 3, 2001, by the NutraSweet Co., 699 North Wheeling Rd., Mount Prospect, IL 60056. The Director of the Office of the Federal Register has approved the incorporation by reference of this material in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the Office of Food Additive Safety (HFS–200), Center for Food Safety and Applied Nutrition, 5100 Paint Branch Pkwy., College Park, MD 20740. Copies may be examined at the Center for Food Safety and Applied Nutrition's Library, 5100 Paint Branch Pkwy., rm. 1C–100, College Park, MD 20740, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC 20001.

(1) Assay for neotame, not less than 97.0 percent and not more than 102.0 percent on a dry basis.

(2) Free dipeptide acid (*N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine), not more than 1.5 percent.

(3) Other related substances, not more than 2.0 percent.

(4) Lead, not more than 2.0 milligrams per kilogram.

(5) Water, not more than 5.0 percent.

(6) Residue on ignition, not more than 0.2 percent

(7) Specific rotation, determined at 20 °C $[\alpha]_D$: -40.0° to 43.4° calculated on a dry basis.

(c) The food additive neotame may be safely used as a sweetening agent and flavor enhancer in foods generally, except in meat and poultry, in accordance with current good manufacturing practice, in an amount not to exceed that reasonably required to accomplish the intended technical effect, in foods for which standards of identity established under section 401 of the Federal Food, Drug, and Cosmetic Act do not preclude such use.

(d) When neotame is used as a sugar substitute tablet, L-leucine may be used as a lubricant in the manufacture of tablets at a level not to exceed 3.5 percent of the weight of the tablet.

(e) If the food containing the additive purports to be or is represented to be for special dietary use, it shall be labeled in compliance with part 105 of this chapter.

Dated: July 2, 2002.

Margaret M. Dotzel,

Associate Commissioner for Policy.

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DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Parts 1 and 602

[TD 8997]

RIN 1545–BA76

Carryback of Consolidated Net Operating Losses To Separate Return Years; Correction

AGENCY: Internal Revenue Service (IRS), Treasury.

ACTION: Correction to temporary regulations.

SUMMARY: This document contains corrections to temporary regulations