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August 16, 2000

VIA FEDERAL EXPRESS

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Re: Docket No. 99F-0187; FAP 9A4643; Neotame

Enclosed, in duplicate, are comments that a client of this firm has requested be filed in the above-captioned docket. The comments pertain to the adequacy of the safety data submitted in support of Neotame.

Very truly yours,

Richard H. Koby

RHK/im
Enc.

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99F-0187

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NEOTAME

Safety Data Review

August 2000

Neotame Safety Data Review

Summary

A food additive petition has been submitted to the FDA for the artificial sweetener neotame. In that petition, the sponsor claims the data presented demonstrate that the compound produces no adverse effects at a dose of 1000 mg/kg/day in the rat. The sponsor also claims that the product should be safe for patients with diabetes.

A review of the data submitted to the FDA does not support these conclusions. In fact, ***no*** safe human usage level can be determined based on the submitted data. The animal experimental evidence indicates a toxic effect on growth. The clinical evidence raises concerns about glucose control in patients with diabetes. Searches for an explanation resolving the adverse findings leaves no clear acceptable answers that would insure the safety of the public but does stimulate speculation on questions relating to possible liver effects.

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Neotame Issues

The following section presents those issues of concern in evaluating the toxicity of neotame and the understandings needed to approve a new food additive. Additional detail specifically dealing with; the determination of human exposure levels, the toxic effect on growth, the paucity of data eliminating toxicity as a possible cause of the noted effects, effect on patients with diabetes, and the possibility of liver involvement are presented in attached appendices. Those appendices include direct reference to the petition, tables and supplemental reports.

Toxic effect on growth:
A significant decrease in body weight gain compared to control animals is seen throughout the course of the two-year rat study that is not adequately explained. This effect not only occurs at the top dosage level (1000 mg/kg/day), but also at 500 and 50 mg/kg/day, the lowest, and most important, dose studied in the critical two-year study. The two explanations given by the sponsor for the adverse effect on growth are, 1) decreased food consumption and 2) diminished diet palatability, which is the cause for the decrease in food consumption. These explanations are not supported by the experimental data submitted. In fact, the limited data presented argues for quite the opposite conclusion, i.e., there is no decrease in food consumption capable of causing the dramatic decrease in body weight gain seen and any palatability issues that may exist are short-lived and quickly resolved. Studies designed to specifically investigate the possibility that toxicity and not diet induced palatability caused these effects were not presented. No studies were presented which investigated the relationship between food consumption and weight gain, such as paired feeding.

Diabetes study questions:
The sponsor claims that based on their interpretation of the results from a single study in persons with type 2 diabetes that the compound is safe for those individuals. Analysis of the data from that study indicates a quite different result, i.e., a possible adverse effect on glucose homeostasis, which is not explained.

Liver toxicity:
Lacking a clear explanation for the body weight gain decrement in rats and the apparent increases in glucose levels in patients with diabetes (possibly due to hepatic gluconeogenesis), the possibility of a common mechanism must be considered and investigated. Based on the structure of neotame one might be concerned about the formation of nitrosamine compounds by the gut microflora. Since these compounds are known hepatotoxins and because of the critical role the liver plays in growth and glucose metabolism, the associated data from multiple species were examined. Unfortunately no long-term gavage studies or pair feeding diet restriction studies are available to easily evaluate toxicity. Therefore, diet administration studies alone are available. A study in dogs shows a decrease in body weight gain without decreases in food consumption as was seen in the rat. Dogs also show elevations in alkaline phosphatase levels, indicative of liver toxicity. The petitioner demonstrated that the alkaline phosphatase found was indeed of hepatic origin. However, there were no special studies conducted to explain the findings and eliminate hepatotoxicity from consideration.

Credibility:
This combination of data misrepresentation and unsupportable assertion may be a cause for concern in its own right. Numerous places in the petition the sponsor addresses the body weight gain and the food consumption issue and regardless of how carefully the discussion is crafted, minimal dissection reveals serious inconsistencies. In any case, absent adequate explanation and experimental support to satisfactorily resolve these observations, it must be assumed that the test animals and human patients are experiencing a toxic insult. In the case of the test animals neotame prevents them from gaining weight consistent with control animals. The subjects with diabetes have either been inadequately tested or they are experiencing deleterious alterations in their glucose control consistent with hepatic glucose production.

Seeking approval without addressing the toxic signs:
Apparently the petitioner was concerned that regulators might note and be concerned by the toxicity observed at 1000, 500 and 50 mg/kg/day in the long term rat study. As an alternative to accepting the 1000 mg/ kg/day dose from the two-year study as the NOAEL, the sponsor also suggests using the 30 mg /kg /day dose from a one-year study as a NOEL. Apparently, if the regulators conclude that the depressed animal growth seen in the two-year study is a possible sign of toxicity they should accept the petitioner's reasons why the finding is of no concern. But, if the regulators discount the explanations presented by the sponsor as to why the toxicity should be disregarded then the regulators should ignore those explanations and that the doses in the two-year study were even tested. The regulators are also expected to accept the new contention that there is no toxicity seen at these levels (10 and 30 mg/kg/day), and none would have been detected if the study had been extended for the normal two-year period. This suggested failsafe is inappropriate since regulators around the world generally use data from the longer (two-year) study as the basis for ADI determination when such a choice is available. Toxic manifestations are more likely to be uncovered when higher doses are tested for longer periods. Using the longer study is specifically appropriate in this case since the observed toxicity progresses over the course of the two years, as is clearly demonstrated by both sexes at the 50 mg/kg/day level.

Further, there is a serious inconsistency between the two-year and one-year studies and questions about the results reported. Of particular concern are the results from animals at 10 and 30 mg/kg/day in the one-year study and what those data would have looked like if the one-year study had been extended for a longer period. Rats consuming 50 mg/kg/day throughout the two-year study experienced a statistically significant 19% decrease in body weight gain with no difference in food consumption compared to controls. Six percent of the weight gain effect occurred in the second year of the study. During the course of that two-year study, after it was clear that neotame was having an adverse effect on growth unrelated to food consumption, the sponsor initiated a one-year study. Inexplicably, the sponsor included three dose levels, which were clearly shown in the ongoing study to be toxic and did not repeat the low dose from the ongoing study to aid comparison. In what must have been a surprise the dose closest to the 50 mg/kg/day dose, i.e., 30 mg/kg/day was reported to exhibit ***no*** significant difference in body weight gain compared to control animals, this finding is an intellectual challenge. Based on the magnitude of the effect in the two-year study, the fact that the effect becomes significant in all groups within 3 months of testing, and that these findings are progressive between one and two years at the 50 mg/kg/day dose level. It is biologically reasonable to expect ***some*** continuation of the decrease in body weight gain

effect in the females in the one-year study at such a similar dose. Finally, the fact that the males in the 30 mg/kg/day group and the females in the 10 mg/kg/day group show decreases in body weight gain over the course of the 1 year study while their food consumption is for the most part greater than controls begs the questions. What would their growth and food consumption curves have looked like if the study had been continued for two years? What caused the toxic effect on growth since food consumption does not appear to be the cause?

Importance of a mechanism and an explanation:
A plausible explanation, supported by literature and experimental data, explaining why the animals failed to gain comparable weight when they ate the same amount of food is critical. Similarly, an explanation as to how such similar doses can have such different results is very important. The sponsor and regulators should understand these effects before the public is consuming this product. Controversial products like artificial sweeteners are watched carefully by consumer interest groups and academic scientists. Skeptics among these groups will focus on the claim that a toxic effect has inexplicably disappeared after what appears to be an insignificant decrease in dose. The most effective response to their concern is an explanation supported by data and literature addressing the inconsistency.

Without a defendable mechanism for a significant toxic event it is not possible to identify and examine the organs and systems most susceptible to the toxicity in detail. It may be that the wrong species is being used as the most appropriate model for man. Additionally, toxic events often initiate an attempt at detoxification. Understanding this process allows for the careful evaluation of those particular systems involved in detoxification, as any impairment of their functionality could be disastrous.

The only explanation given for the adverse effect on growth and a rationale as to why the finding should be ignored is the sponsor's insistence that a palatability-induced decrease in food consumption capable of causing the sizable decrease in body weight gain does exist. As noted, the available data do not support this conclusion. Therefore, of utmost importance would be any data from safety related studies, whether they be from GLP studies or not, even studies which have not been "completed", which specifically evaluated the possibility of toxicity. Undoubtedly such studies, if not conducted were considered, and the rationale and discussions concerning them would be invaluable in the determination of toxicity and possible target tissues. Unfortunately we are left with no reported data or discussion to help our investigator. If the sponsor has additional information and is confident and correct that neotame significantly reduces the palatability of diet and causes reductions in food consumption responsible for the depression in growth, then the presented data are in error. The most likely conclusion would then be that the food consumption values presented for the one and two year study along with the palatability study are seriously inaccurate. Consequently, what confidence would the public have in the entire database if such important parameters were not accurately measured and recorded in the most important studies?

Conclusions:

- The data indicate that neotame is producing adverse effects on animal growth.
- The data indicate that neotame may be producing adverse effects on glucose control in individuals with type 2 diabetes.
- The explanations presented by the sponsor designed to eliminate concern about the observed toxicity do not hold up to scrutiny and defy logic.
- Absent an acceptable mechanism for the observed toxicity the sponsor has failed to adequately evaluate the liver as a possible target organ for toxicity.
- No safe dose of neotame has been adequately tested to establish either a NOAEL or a NOEL.

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Appendix I

POINTS TO CONSIDER WHEN EVALUATING THE TOXICITY OF NEOTAME

Product Background

Neotame (NC-00723) is a new artificial sweetener being developed by J.W. Childs Equity Partners II, L.P. who recently purchased the product from the Monsanto Company. To date neotame has not been approved anywhere in the world. The chemical structure of neotame, N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester, is similar to aspartame, but its potency is 30-60 times greater than that of aspartame. In December 1997, Monsanto submitted a food additive petition (FAP 8A4580) to the FDA for use of neotame as a tabletop sweetener. However, the submission did not include the detailed histopathology data required for a complete toxicological review. During the review process for the first petition Monsanto submitted a second food additive petition in January 1999 (9A4643), requesting expanded use for neotame as a general use sweetener in food and included the histopathology findings previously unavailable. However, no additional histopathology or microscopic data of specific organ systems (ie. liver) were submitted by the sponsor as a result of their initial database review in an effort to explain any findings from the two submissions. Additionally, it is not clear if any regulatory agencies have requested additional histopathology to address their findings to date.

Human Exposure Estimation:

The sponsor claims that neotame has a clean, sweet taste with a superior taste and stability profile and requires no special labeling, compared to aspartame (Nutrasweet®). However, the sponsor based its consumer exposure intake estimates on assuming only a 50% replacement of the current uses of aspartame. Such an assumption may be unduly limited and not reasonably conservative, if the sponsor's claim is correct that Neotame is superior to aspartame and also a high quality safe artificial sweetener. Therefore, the projected intake levels may be significantly low compared to eventual use levels. At the mean and 90th percentile consumption for eaters-only these intake estimates based on the aforementioned assumptions were 0.02 and 0.05 mg/kg/day, respectively. However, the margin of safety will depend upon both an accurate estimation of intake and the no-observed-effect level (NOEL) determined from animal toxicology data. Establishing the correct Estimated Daily Intake (EDI) is critical to assuring a safe use level.

Toxicology:
A number of key points relative to a toxicological review of the existing data and the determination of an appropriate NOEL have been developed and are presented below.

Key Points

Overall Assessment:

1. There is no NOEL established in the longest rat dietary study (104-Weeks). All of the dose groups showed adverse effects on growth with no adequate explanation. The claim by the sponsor that the NOAEL is the top dose of 1000 mg/kg/day is not supported by the data. The explanation the sponsor supplied as to why an apparent adverse effect on growth should be disregarded is absolutely indefensible. In fact the explanation the sponsor provides is so incompatible with the data one wonders what reason could be given for the sponsor promoting such an error? No safe level of neotame consumption by humans can be determined from the existing data and explanations.

Toxic Effect on Growth

2. The data show a decrease in body weight gain of 7-20% in all dose groups compared to the controls in the two year rat carcinogenicity study. The decrease is statistically significant in five of the six groups tested. See Body Weight Table Appendix II.

3. The first part of a two part explanation given as to why this adverse effect on growth should be ignored is that the treated animals consumed less food then the control animals in amounts responsible for the dramatic decreases in body weight gain. That position is not supported by the data (See Food Consumption Table Appendix III) . There was no statistically significant decrease in total food consumption as a percent of control in the neotame two year study after 104 weeks of treatment. The male animals in the 50 mg/kg/day group showed a 13% decrease in body weight gain compared to concurrent controls over the course of the study while consuming more food than the control group. The female example is even more extreme.

4. The second part of the explanation as to why the toxic effect on growth should be ignored focuses on why the animals (according to the sponsor but not the data) consumed less food. The sponsor argues that there **is** a decrease in food consumption of sufficient magnitude to account for the large decrease in body weight gain and that the decrease in food consumption is caused by decreased diet palatability and not toxicity. As noted, a decrease in food consumption sufficient to cause even a slight decrease in body weight gain was not found. Most importantly, the claim that the "phantom" drop in food consumption is caused by a change in diet palatability is a direct contradiction to the results found in the petition. The single study that investigates diet palatability

concluded that there was no lasting effect, and that the palatability effects are reversed within 2-3 days. (See Palatability Study Quote Appendix IV). Why the sponsor would make such an unsupportable claim that is so crucial to an accurate assessment of the data is perplexing. The only conclusion that can be reached from the data and discussion is that: No experimental results have been presented or scientific literature quoted which would support the claims made by the sponsor concerning the amount of food consumed and the relationship between that level of food consumption and the measured decreases in body weight gain observed. The data presented are at such odds with the sponsors claims with regard to the relationship between body weight gain and food consumption that the absence of at least a paired feeding study is more than obvious. Similarly no data demonstrating a significant persistent effect of neotame on diet palatability altering food consumption to an extent capable of causing a 7-20% decrease in body weight gain have been presented. The sponsor presents a fall back position concerning the establishment of a NOEL by suggesting that the one year study be used instead. The body weight gain and food consumption data from that study (Appendix V) do not support the explanation put forward for the body weight gain deficit and are at odds with the two year study. It is not possible from these data to predict the body weight gain or food consumption values which would be present if the study were to be extended for two years.

Elimination of Toxicity as a Cause for the Decrease in Growth

5. The petitioner has not supplied any gavage, pair feeding, or dietary restriction data to prove that the body weight gain decrement is due to palatability and not due to toxicity. If all of the decrease in body weight gain could be proven to be due to palatability induced decreases in food consumption and a no-observed-adverse-effect level (NOAEL) in dietary studies established, then an ADI could be calculated. However, it is clear that this case can not be made with the available data. The presented data reveal significant compound related toxicity at 50 mg/kg/day which extends over two years and no adequate explanation for the observation. It is conceivable that the sponsor could do a series of studies, which would identify a dose where no effect on body weight gain were seen. However, reviewing toxicologists would look with extreme skepticism at such studies if they miraculously presented a dose, close to the toxic level of 50 mg/kg/day, which showed no effects. The skeptical toxicologist would require accompanying explanation for the dramatic effects at a slightly higher dose but conveniently absent at a quite similar dose which supplied an ADI that allowed for the products use. This is precisely the case with neotame. If the effects on body weight gain and food consumption is not shown to be due to palatability, it must be assumed to be due to toxicity and a mechanism of action as well as a no-observed-effect level (NOEL) determined. As a minimum a gavage study in rats with doses ranging very widely should be conducted. Such a study would eliminate the issue of palatability induced decreases in food consumption from the list of variables under consideration as a cause of the toxic effect on growth. If indeed a gavage study establishes that the animal's growth rates are similar in control and treated animals then additional

experiments could establish a true dietary NOEL. On the other hand if growth rates are retarded in some groups of the treated animals it should be possible to focus in on the mechanisms of that toxicity.

6. Lacking a clear explanation for the body weight gain decrement in rats, one could consider the possibility that unreported/under-investigated toxicity might be present. Without knowing the true explanation for the body weight decrement, it is difficult to focus attention on the proper target organ(s) of toxicity. The liver could be a target organ as there are some limited data pointing in that direction, but insufficient additional data is available for analysis.

Diabetes Study Questions

7. The sponsor supplied a study in people with Type 2 diabetes which is purportedly supportive of the safety of neotame in that sub-population. A review of the study is presented as Appendix VI. The study design was such that only very large changes in the primary and secondary endpoints could be statistically identified. Despite the design limitations there appear to be indications of adverse effects on glycemic control in the study. Specifically, 1) dose-related higher glucose AUEC $_{(0\text{-}180\ min)}$ values in the treatment groups compared to the placebo group 2) dose related increases in glucose E_{max} and 3) dose-related higher glucose E_{min} values. These findings could be caused by hepatic gluconeogenesis. However, there simply is not enough data in the study to make a reasonable conclusion.

Are There Common Threads to the Signs of Toxicity?

8. In the 52-week dog study elevated plasma alkaline phosphatase levels indicative of liver toxicity is apparent in as early at 13 weeks at doses of 200 mg/kg/day and higher (Appendix VII). Interestingly, in the 13-week dog study there is a 15% and 24% body weight gain decrement in the male and female dogs, respectively at 600 mg/kg/day, with no associated decrease in food consumption, reminiscent of the finding in rats. Unfortunately, there is just not enough data presented to determine if this observation is related to the unexplained toxicity in the rat?

9. The structure of Neotame suggests that metabolic formation of nitrosamine compounds by the gut microflora is possible as well as formation in some food products. The extent of this metabolic conversion is unknown and maybe small. However, it is well known that nitrosamines are potent hepatotoxins and hepatocarcinogens (Casarett and Doull's, 1996).

10. Note: The importance of the liver in animal growth and glucose homeostasis is clearly understood. The fact that neotame appears to have effects on both these systems has been shown. Neotame effects growth in both rats and dogs and appears to impact glucose homeostasis in persons with diabetes. Those findings along with its structure and the alterations seen in canine hepatic alkaline phosphatase makes it important to absolutely rule out the liver as a target organ.

Casarett and Doull's Toxicology: The Basis Science of Poisons. 5th edition, McGraw-Hill, New York, 1996.

APPENDIX II

Neotame 104 Week Carcinogenicity Study in Rats

BWG % of Control

Males

Weeks

Dose mg/kg/day	0 - 4	0 - 13	0 - 26	0 - 52	0 - 78	0 – 104
50	102	94 c	93 c	91 c	89 c	87 b
500	98	95 a	89 c	89 c	87 c	90 a
1000	99	97	92 c	90 c	89 c	93

Females

Weeks

Dose mg/kg/day	0 – 4	0 - 13	0 – 26	0 – 52	0 – 78	0 –104
50	100	96	92 c	87 c	87 c	81 c
500	98	94 b	91 c	81 c	81 c	80 c
1000	96	95 a	91 c	83 c	83 c	83 b

a = $p < 0.05$; b = $p < 0.01$; c = $p<0.001$

Weeks 1 - 104 show a 7 –20% decrease in BGW

Derived from Table 4B Cumulative bodyweight gain - group mean values. Neotame FAP8A4580 Study PCR 1000: NC-00723 Oncogenicity study by dietary administration to CD rats with exposure in utero. Page APP-6701.

bwgneotameoncorat8.7.2000

APPENDIX III

Neotame 104 Week Carcinogenicity Study in Rats

FC % of Control

Males

Weeks

Dose mg/kg/day	1 - 4	1 - 13	1 - 26	1 - 52	1 - 78	1 – 104
50	103	99	100	99	99	101
500	101	96 b	97 a	97	98	101
1000	101	97 a	98	98	98	99

Females

Weeks

Dose mg/kg/day	1 – 4	1 - 13	1 – 26	1 – 52	1 – 78	1 –104
50	101	101	100	98	99	99
500	103	101	100	97	97	97
1000	96 b	97 a	96 a	95 c	96 a	99

a = $p < 0.05$; b = $p < 0.01$

Weeks 1 - 104 show a 1 – 3% decrease in FC

Derived from Table 5E Cumulative food consumption - group mean adjusted values. Neotame FAP8A4580 Study PCR 1000: NC-00723 Oncogenicity study by dietary administration to CD rats with exposure in utero. Page APP-6719.

fcneotameoncorat8.7.2000

APPENDIX IV

NC-00723: Dietary Preference Study

FINAL REPORT

The purpose of this study was to assess the effects of NC-00723 on the palatability of diets to rats. Six groups of 14 male and 14 female rats of the Crl:CD® (SD) BR VAF PLUS™ strain were provided diets with NC-00723 at concentrations of 50, 150, 500, 1500, 5000 or 15000 ppm in RM1 (E) SQC FG diet. A similarly constituted group received basal diet only. The study had four consecutive phases of evaluation in which food consumption was measured each day for five days as follows:

Pre-treatment Phase (Days -5 to -1) to acclimatise rats to a two jar feeding regimen and provide baseline data, with basal diet only,

Preference Phase 1 (Days 1 to 5) in which rats were provided randomly placed jars of basal diet and diet containing specified NC-00723 concentrations,

Treatment Phase (Days 6 to 10) in which NC-00723-containing diet was provided in both jars and,

Preference Phase 2 (Days 11 to 15) which used the same design and dietary concentrations as the first preference phase.

Duplicated from Neotame FAP 8A4580 Study PCR 1150: NC-00723 Dietary preference study (rat). Page APP 3141.

NC-00723: Dietary Preference Study

FINAL REPORT

The diet jars were changed daily throughout the study. During Preference Phase 1 and 2 the jars were placed randomly between two positions in each cage according to a computer-generated plan.

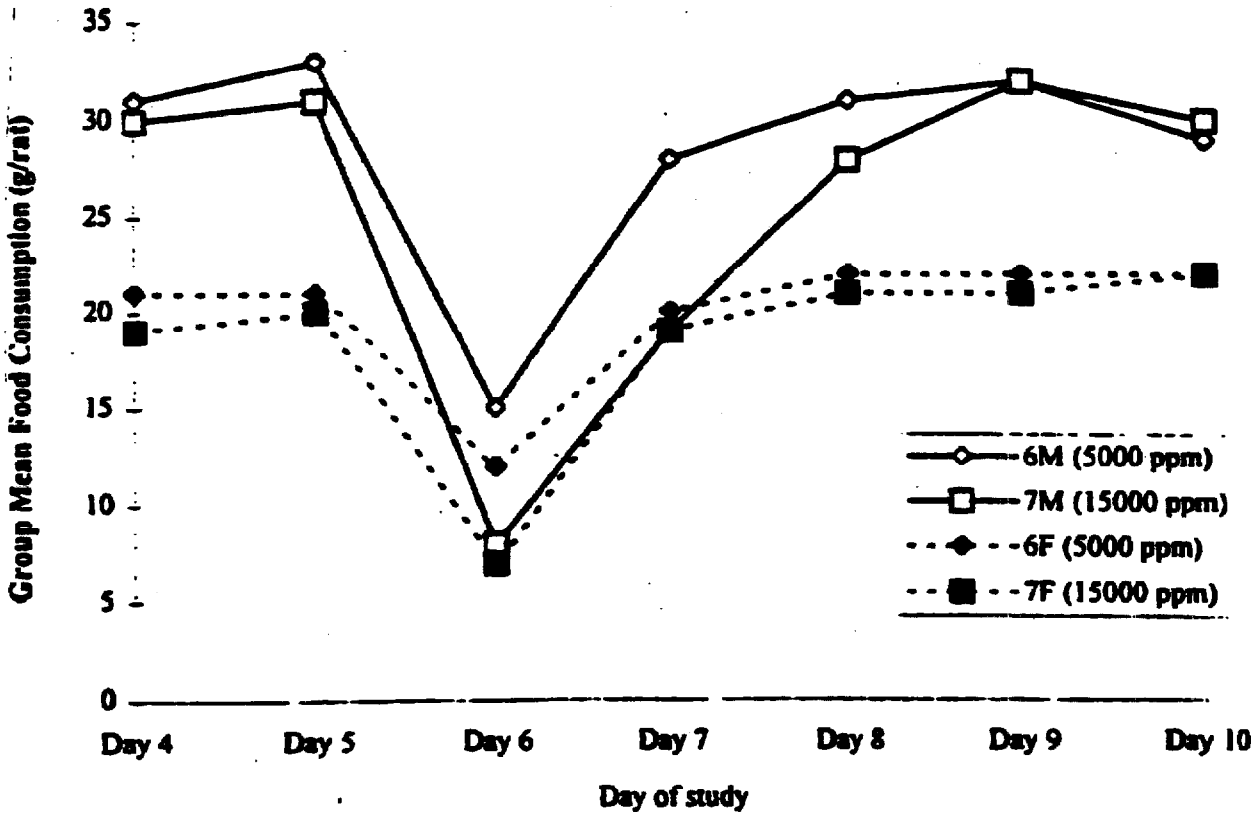
As expected, no preference for either jar position was observed in the Pre-treatment Phase when both jars contained basal diet. The addition of NC-00723 diets greatly increased the preference for rats to consume basal diet during Preference Phase 1. Clear preference was observed at NC-00723 concentrations as low as 150-500 ppm; complete preference for basal diet was observed at NC-00723 concentrations greater than or equal to 5000 ppm. Addition of NC-00723 to diets provided in both jars produced an immediate, but transient decrease in total food intake. On the first day of the Treatment Phase diets containing NC-00723 concentrations greater than or equal to 5000 ppm were refused. Food consumption for these animals returned to near Control values by the second or third day of the Treatment Phase. Finally, rats that had accommodated to NC-00723 continued to prefer basal diet in Preference Phase 2 at dietary concentrations of 50 to 150 ppm or greater.

In conclusion, the results show that NC-00723 decreases the palatability of diets to rats at relatively low concentrations. The results of this study are important in interpreting food consumption data from rat toxicology studies where dietary NC-00723 concentrations are adjusted during growth to provide a nominal mg/kg/day dosage.

Duplicated from Neotame FAP 8A4580 Study PCR 1150: NC-00723 Dietary preference study (rat). Page APP 3141.

Palatability of Diets Containing Neotame

Text-figure 2 Food consumption during Days 4 to 10 of the study for animals receiving 5000 or 15000 ppm



Duplicated from Neotame FAP 8A4580 Study PCR 1150: NC-00723 Dietary preference study (rat). Page APP 3151.

APPENDIX V

Neotame 52-Week Toxicity Study in Rats

Body Weight Gain % of Controls

Males
Weeks

Dose mg/kg/day	0-4	0-13	0-26	0-52	(R0-R4)
10	100	103	105	106	-
30	98	98	99	96	-
100*	93	94	94	90	(182)
300	98	93 a	95	92	(188)
1000	96	94	97	95	(95)

Females
Weeks

Dose Mg/kg/day	0-4	0-13	0-26	0-52	(R0-R4)
10	103	100	97	96	-
30	103	104	101	102	-
100*	100	97	93	89	(181)
300	101	97	92 a	86 b	(168)
1000	98	97	93	84 b	(249)

- The 100 mg/kg/day group started at 87% and 85% of control for males and females , respectively. P<0.001. Weekly body weight was significant at most weeks.

- Week 1-52: 2-4% Decrease in Food Consumption
  4-16% Decrease in Body Weight Gain

Derived from Table 2B Cumulative bodyweight gain - group mean values. Neotame FAP8A4580 Study PCR 1011: NC-00723 52-week toxicity study by dietary administration to CD rats with exposure in utero and followed by a 4-week reversibility period. Page APP-5113-5114.

Neotame 52-Week Toxicity Study in Rats

Food Consumption % of Controls

Males
Weeks

Dose mg/kg/day	1-4	1-13	1-26	1-52	(R0-R4)
10	104	104	104	104	-
30	101	101	102	100	-
100*	95 a	96	98	97	(101)
300	102	99	100	98	(102)
1000	99	97	99	100	(106)

Females
Weeks

Dose Mg/kg/day	1-4	1-13	1-26	1-52	(R0-R4)
10	100	99	100	103	-
30	105	101	102	102	-
100*	100	97	97	96	(99)
300	103	98	99	97	(101)
1000	102	97	97	96	(111)

- The 100 mg/kg/day group started at 87% and 85% of control for males and females , respectively. P<0.001. Weekly body weight was significant at most weeks during the study.

Derived from Table 3D Cumulative food consumption - group mean adjusted values. Neotame FAP 8A4580 Study PCR 1011: NC-00723 52-week toxicity study by dietary administration to CD rats with exposure in utero and followed by a 4-week reversibility period. Page APP-5144 and 5146.

APPENDIX VI

Review of Neotame Study in Persons with Diabetes

Title of Study: Multiple Dose Study of NC-00723 Versus Placebo Administered in NIDDM Subjects – PCR 1115

Background:

This randomized, double-blind, placebo-controlled, cross-over study was to determine the safety and pharmacodynamic effects of NC-00723 (neotame) on glucose homeostasis in people with Type 2 diabetes mellitus. Eligible subjects in this study were treated during three 15-day periods with placebo, 60 mg/day neotame or 150 mg/day neotame (in 3 divided doses) for 15 days. (Based on average body weight, the average mg/kg neotame doses were 0, 0.6 and 1.5, respectively.) Evaluable subjects for efficacy were those who met the criteria for completers, defined as those who ingested 90% of the prescribed doses and completed all testing.

Metabolic testing was conducted in the controlled setting of an inpatient unit. Testing was a three-hour (180 min) treatment challenge test during which glucose and insulin levels were measured at defined intervals. The tests were conducted on Day 15 of each treatment period, after an 8-hour fast. Fasting was continued during the 3-hour test, although water was permitted ad lib one hour before and one hour after testing was started. The evening prior to testing, all subjects were given a low-fat meal and snack. On the morning of Day 15, all concurrent medications, including antidiabetic medications, were witheld until after the completion of the metabolic tests.

Glucose and insulin pharmacodynamic parameters calculated from the results of the treatment challenge tests were used to assess the possible effect of neotame on glucose control. These parameters included: $Effect_{min}$ or E_{min} (concentration at the 180 min time point minus the baseline or time 0 concentration), E_{max} (maximum concentration minus minimum concentration during the 180 min test), T_{Emax} (time of observed E_{max}), and AUEC (AUC_{0-180} minus 180 x time 0 concentration).

Based on the above endpoints, the study investigators concluded that there were no significant clinical effects on glucose or insulin levels.

Analysis:

This study had very little power (5% to 33%) to detect the differences seen in its primary and secondary endpoints for the assessment of effects on glucose control (i.e., the pharmacodynamic measures of glucose and insulin). One could argue that this is because small differences were seen. However, the study design was such that only large differences (42% to 100% of placebo values) were detectable. Such large differences could not, realistically, be expected under the conditions of this study, that is when subjects are studied under the quiescent metabolic conditions of extended fasting. Therefore, the study was designed to **not** detect differences that could be realistically expected, and the study design biased towards the outcomes reported. Additionally, since the study was significantly underpowered, there can be no conclusions drawn from the statistical analyses conducted. To properly assess the meaningfulness of the differences seen, a much larger study, in at least 200 patients, would be necessary.

The results of the study as a whole, however, should not be discounted. Persons with diabetes are expected to have higher than average low-calorie sweetener consumption and more consistent consumption over time compared to non-diabetic consumers[1,2]. Further, this is the only clinical trial to investigate the effect of neotame in persons with diabetes. No studies in laboratory animals with diabetes were conducted, nor are there studies in non-diabetic humans or laboratory animals that specifically investigated the potential of neotame to affect glucose homeostasis. Thus, it is the only study that evaluates the safety of neotame in a potentially high-use population subgroup with fundamental differences in metabolism, medical risk, and expected drug uses as compared to generally healthy non-diabetic subjects.

On the whole, the pharmacodynamic measures of glucose in this study suggested an adverse effect on glycemic control. This is demonstrated by:

1) Dose-related higher glucose $AUEC_{(0\text{-}180\ min)}$ values in the treatment groups compared to the placebo group.

2) Dose-related increases in glucose E_{max}.

3) Dose-related higher glucose E_{min} values.

The study investigators reported these parameters as percent of placebo. This leads to a confusing picture, because placebo values were sometimes negative values. On the surface, this representation of the data makes it appear as if the total AUEC and E_{min} values are **less** than the respective placebo values, when the absolute values, i.e., the actual blood glucose levels, are higher than the placebo's.

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[1]Toeller, M., 1993, Diabetes Metab. Rev. 9:93-108.

[2]**market research – need to get appropriate reference**

For example, when the neotame low dose group glucose AUEC value (-774.4 mg°min/dl) is divided by the placebo group glucose AUEC value (-973.0 mg°min/dl), the resulting ratio is 0.768 or 76.8%. This representation makes it appear that, compared to placebo, the area under the blood glucose effect vs. time curve is less for the treated group. However, the treatment group's AUEC is actually **higher** than the placebo group's AUEC by a difference of 199.4.

A more representative picture, then, is one that looks at the % change from placebo, as shown in the following table:

Glucose Pharmacodynamic Parameters	Placebo Arithmetic mean (SD)	Treatment A Arithmetic mean (SD)	% Change from Placebo	Treatment B Arithmetic mean (SD)	% Change from Placebo
$AUEC_{(0\text{-}180\ min)}$	-973.0 (1826)	-774.4 (2055)	+20%	-663.4 (2420)	+32%
E_{max}	4.59 (7.29)	5.63 (11.8)	+29%	6.90 (12.2)	+59%
E_{min}	-16.1 (16.9)	-13.0 (20.6)	+19%	-11.0 (22.7)	+31%

Treatment A = 60 mg/day neotame. Treatment B = 150 mg/day neotame.

The standardized study conditions, e.g., standardized meals, time of fasting, inpatient admission, etc., lend credence to the reliability of this data. The standardized low-fat meal and overnight and post-dose fasting would also probably keep glucose and insulin levels and variability low. Glucose levels would be expected to decline with these conditions, and that expectation is verified by the negative $AUEC_{(0\text{-}180\ min)}$ and E_{min} values for all treatment groups. Under the conditions of test, then, a potential explanation for the higher glucose pharmacodynamic levels with neotame treatment is a treatment-induced increase in hepatic glucose production. The long-term consequences of such an effect cannot be determined from this study.

The other measures of metabolic control in this study were the insulin pharmacodynamic parameters. Treatment group differences in these parameters were not consistently increased or decreased. Although there was no consistent trend across the insulin variables, a significant change in insulin level might not be expected in a study such as this. The study duration was short and the treatment challenge tests were conducted in fasted patients who continued to fast during the three hour test. Meal tests, which stimulate insulin secretion, were not performed in this study. Thus, under the fasted conditions of the test, differences in the pharmocodynamic insulin parameters would be expected to be minimal and between-group differences are of questionable clinical significance.

In sum, the pharmacodynamic glucose responses to the challenge treatment tests are important data and the most important data of the study. This is because the total amount of glycemia and time of exposure to hyperglycemia is probably what has the negative biological effects that ultimately leads to diabetic complications.

Three of these pharmacodynamic measures, $AUEC_{(0\text{-}180\ min)}$, E_{max}, and E_{min}, all direct measures of glucose, strongly suggest a potential effect of neotame on glycemic control in persons with Type 2 diabetes. For each of these measures, the treatment group results were higher than placebo group results, based on absolute values, and the changes were dose-related (higher with increased dose). The results of the statistical analyses conducted in this study cannot rule out that these changes may be real, since the study had little power to detect the changes seen. Moreover, any change, under the fasting conditions of this study, deserves serious consideration. Therefore it is quite worrisome that there is a consistent dose-related pattern of higher (albeit non-significantly higher) -than-placebo values for each of the actual glucose measures performed in this test, i.e., $AUEC_{(0\text{-}180\ min)}$, E_{max}, and E_{min}. Again, these results are strongly suggestive of an effect on glucose control. In light of these findings, a much larger and longer study is necessary and warranted to assess the true effect of neotame on glucose homeostasis in persons with diabetes.

the reversibility period and were not associated with any effects on the gastrointestinal tract. The fecal discoloration (whitening) was considered to be related to fecal excretion of NC-00723 and its metabolite, NC-00751, both of which are white.

There were no test article-related effects on final body weights or overall body weight gains. Furthermore, there were no effects on weekly body weights, weekly cumulative body weight gains, or food consumption at 20, 60 or 200 mg/kg. There were immediate decreases in food consumption in both sexes at 800 mg/kg that generally persisted for the first 2 weeks. As expected, weekly cumulative body weight gains were decreased during the first few weeks for males and the body weight gains for females were also lower (not statistically) during this period. The transient decreases in food consumption and body weight gains at 800 mg/kg were most likely related to an initial decreased palatability of diets containing high concentrations of NC-00723.

The only clinical chemistry finding was increased serum ALP activity at 800 mg/kg. ALP isoenzyme analyses established that the increased activity was of hepatic origin and not the bone or glucocorticoid-inducible form. There was a pronounced decrease in serum ALP activity during the 4-week reversibility period demonstrating reversal of the effect with time. There were no other clinical pathology changes, no organ weight changes, and no macroscopic findings in the liver or any organ. Thus, the increased ALP activity was considered physiologic and not adverse.

CONCLUSIONS

There were no test article-related effects in dogs at dosages up to 200 mg/kg. The only consistent effects observed at 800 mg/kg were gray feces and increased serum hepatic ALP activity. Both effects were reversible, not associated with any toxicity, and not considered adverse. Therefore, there were no adverse effects at dosages up to 800 mg/kg.

Text Table 1

Mean Alkaline Phosphatase Activities

Males

Dose (mg/kg/day)	Week -2	Week 13	Week 26	Week 39	Week 52
0	156	108	80	79	72
20	160	102	73	64	58
60	179	125	90	82	78
200	150	125	92	70	71
800	159	247***	255***	240***	273***

Females

Dose (mg/kg/day	Week -2	Week 13	Week 26	Week 39	Week 52
0	167	122	83	78	76
20	142	100	71	68	62
60	167	124	86	79	78
200	202	168	119*	115	113*
800	160	328***	315***	342***	356***

* $p \leq 0.05$, Dunnett's t-test.
** $p \leq 0.01$, Dunnett's t-test.
*** $p \leq 0.001$, Dunnett's t-test.

There were relatively few statistically significant or otherwise notable differences for other clinical pathology test results, and none of these differences were considered to be effects of the test article. None of these differences were consistent over time or between sexes, and several exhibited no relationship to dose (e.g., the high-dose group was unaffected).

Weeks 55 and 56 (Reversibility Animals). Findings at Weeks 55 and 56 indicated that the effect on serum alkaline phosphatase activity was reversible. Mean serum alkaline phosphatase activity for the high-dose recovery males decreased from 306 IU/L at Week 52 to 117 IU/L at Week 55 and 94 IU/L at Week 56. Mean activity for the

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