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Long-term food consumption and body weight changes in neotame safety studies are consistent with the allometric relationship observed for other sweeteners and during dietary restrictions

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Abstract

In long-term safety studies with neotame, a new high-intensity sweetener 7000–13,000 times sweeter than sucrose, the percent changes (%Δ) in body weight gain (BWG) in Sprague–Dawley rats were several-fold greater than the %Δ in overall food consumption (FC). This study investigates the question of whether the changes in BWG were adverse or secondary to small, long-term decrements in FC. The hypothesis tested in Sprague–Dawley rats was that the relationship between long-term %Δ in FC and %Δ in BWG is linear and in a ratio of 1:1. The %Δ in FC were compared to %Δ in BWG after 52 weeks on study in one saccharin (825 rats), two sucralose (480 rats), two neotame (630 rats), and five dietary restriction (>1000 rats) studies. Non-transformed plotting of data points demonstrated an absence of linearity between %Δ in FC and %Δ in BWG; however, log–log evaluation demonstrated a robust ($R^2 = 0.97$) linear relationship between %Δ in FC and %Δ in BWG. This relationship followed the well-known allometric equation, $y = bx^a$ where x is %ΔFC, y is %ΔBWG, b is %ΔBWG when ΔFC = 1, and a is the log–log slope. Thus, in Sprague–Dawley rats at week 52, the long-term relationship between %Δ in FC and %Δ in BWG was determined to be: $\%\Delta BWG = 3.45(\%\Delta FC^{0.74})$ for males and $\%\Delta BWG = 5.28(\%\Delta FC^{0.68})$ for females. Sexes were statistically different but study types, i.e., the high-intensity sweeteners saccharin and sucralose versus dietary restriction, were not. The %Δ in BWG are allometrically consistent with the observed %Δ in FC for these high-intensity sweeteners, including neotame. BW parameters are not appropriate endpoints for setting no-observed-effect levels (NOELs) when materials with intense taste are admixed into food. An approach using objective criteria is proposed to delineate BW changes due to toxicity from those secondary to reduced FC.

Keywords: Sweetener; Neotame; Palatability; Preference; Rat; Sprague–Dawley; Food consumption; Allometry; Allometric growth; Dietary restriction; Body weight gain

1. Introduction

Neotame is a dipeptide methyl ester derivative of aspartame with a sweetness potency in humans 7000–13,000 times that of sugar. The safety of neotame was assessed in subchronic, chronic, carcinogenicity, reproduction, teratology and in utero/postnatal evaluations at doses in definitive studies up to 40,000 times 90th percentile estimated human exposure (FDA, 2002). Regulatory review of neotame safety studies has been completed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and in Australia, New Zealand and the US (ANZFA, 2001; FDA, 2002; WHO, 2003) and other

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reviews are ongoing. Neotame was well tolerated in toxicology species including Sprague–Dawley CD rats, CD-1 mice, beagle dogs, and New Zealand rabbits.

The most consistent findings in toxicology species from neotame safety studies were reductions in food consumption (FC), body weight (BW), and body weight gain (BWG) compared to controls at doses requiring high dietary concentrations (Mayhew et al., 2003). Typically, when a test material having intense taste is admixed into diets at high concentrations, toxicology species exhibit a preference for basal diet. The maximum tolerable doses (MTDs) of materials with intense taste are typically limited by reduced palatability of diets containing high concentrations of that compound. The operational definitions for terms including taste, preference, and palatability are summarized in Table 1. For neotame, the MTDs from subchronic studies were determined by doses where decrements in BWG exceeded 10% of control, a standard guideline for dose setting, e.g., US FDA 'Redbook' Guidelines (FDA, 1982, 1993). BWG was reduced more than 10% compared to controls in rats, mice, and dogs when neotame concentrations exceeded approximately 35,000 ppm in food (Mayhew et al., 2003).

Consequently, concentrations greater than 35,000 ppm were not used during definitive safety studies with neotame. Thus, the MTDs for neotame were determined by poor palatability of neotame-containing diets and were not associated with toxicity in any species tested (Mayhew et al., 2003). The MTD for substances with intense taste has been described as the 'maximum palatable dose' (MPD); that is, the highest dose at which animals will eat and drink sufficient quantities to remain healthy (Chowaniec and Hicks, 1979).

Regulatory agencies reviewing neotame safety data have alternately concluded either that the changes in BW and BWG were due to a palatability related decrease in FC (ANZFA, 2001; WHO, 2003) or that changes in BW and BWG may not be fully explained by the observed decreases in food intake (FDA, 2002). In addition to the example cited for neotame, regulatory agencies previously have arrived at different conclusions regarding the relationship between BW and FC from evaluations of the same safety data for other high-intensity sweeteners (FDA, 1988, 1998; WHO, 1983, 1991).

The percent changes (%Δ) in BWG in rats were several-fold greater than the observed %Δ in overall FC in long-term studies with neotame. In this report, this effect is evaluated in terms of the relationship between long-term changes in FC in Sprague–Dawley rats and resultant changes in BW and BWG. Specifically, the hypothesis tested is that the relationship between long-term %Δ in FC and BWG in Sprague–Dawley rats is linear and related 1:1. The relationship between FC and BWG was evaluated by comparing results after 52 weeks on study for available saccharin, sucralose, neotame, and dietary restriction studies in Sprague–Dawley rats.

In safety studies, a change in BW parameters can be the result of either an adverse toxic effect of the compound or an effect secondary to non-adverse reductions in FC. However, the distinction between adverse effects on BW and effects secondary to reduced FC, such as from reduced palatability of diets, often can be established from careful evaluation of data from safety studies. An algorithm is proposed using objective criteria to make critical distinctions between adverse and non-adverse effects on BW and BWG in long-term safety studies.

Table 1
Operational definitions

Taste is an inherent property of a material in solution resulting from contact with sensory receptors. Perception of taste occurs within the first few minutes following ingestion
Palatability is the sensory and physical attributes of a food that determine whether and how much will be eaten when no other choice is available
Preference is the selection of one food over another when more than one food choice is available
Accuracy is used to mean the nearness of a measurement to the actual value of the parameter being measured
Precision is used to mean the variability inherent in the measurement of a parameter, such as variability introduced when repeated measurements are required to approximate a parameter

2. Methods

2.1. BWG and FC in Sprague–Dawley rats during neotame safety studies

Neotame was subjected to extensive investigations in toxicological studies following established protocols. Neotame was administered in the diet because this route of exposure is the most relevant to human consumption. Diets were formulated and concentrations adjusted weekly or biweekly in long-term studies to provide constant dosages in mg/kg bw/day rather than as fixed percentages of neotame in the diet. Neotame studies included design elements to enhance the accuracy of FC data collection. These considerations included single-housing of the test animals (with the exception of breeding pairs and littered pups), assessment of spilled diet, and specially designed food containers to minimize spillage or contamination. FC calculations for individual intervals and individual animals were summed consecutively to calculate overall FC where appropriate. The neotame studies most pertinent to evaluating the relationship between long-term FC and BW parameters in Sprague–Dawley rats were the one-year chronic study with in utero exposure and the two-year carcinogenicity study with in utero exposure. More complete methodology for these studies is presented elsewhere (Mayhew et al., 2003).

Reported FC values were adjusted for non-caloric neotame content. FC calculations for individual intervals

and individual animals were summed for consecutive intervals to calculate overall FC where appropriate. For example, to calculate overall FC in long-term rat studies, FC data are summed from a large number of individual weekly/biweekly intervals for each animal, each interval having a number of repeated measures.

2.2. Comparison of FC and BWG data in other long-term studies

From the published literature, studies were identified that utilized dietary dosing of Sprague–Dawley rats for 26 weeks or longer with high-intensity sweeteners and/or dietary restriction paradigms. FC and BWG data were extracted for weeks 26, 52, and 78, where applicable, from saccharin (Schoenig et al., 1985), sucralose (FDA, 1998; Mann et al., 2000), and neotame (Mayhew et al., 2003) studies and a number of dietary restriction and or dietary optimization studies involving restrained eating in Sprague–Dawley rats (Christian et al., 1998; Duffy et al., 2001; Duffy et al., 2002; Hubert et al., 2000; Keenan et al., 1994; Keenan et al., 1996; Laroque et al., 1997; Nolen, 1972). FC was adjusted for content of non-nutritive materials. From these data, the %Δ in FC and the %Δ in BWG were calculated for each of these time points. The week 52 time point was selected for further analysis in that it was representative of "long-term" dosing and was generally available from both chronic toxicity and carcinogenicity study designs.

Resultant week 52 data points were plotted as %ΔBWG versus %ΔFC and as log%ΔBWG versus log%ΔFC. A regression was done on the non-neotame log%ΔBWG versus log%ΔFC data in order to test for differences in intercepts and slopes between the sexes (male and female), study type (dietary restriction, sucralose, saccharin), and the interaction between sex and type of study. Where there were no significant differences between sexes, types of study, or a significant interaction (all at $p < 0.05$), then a single model was used to fit the data.

Depending on the results from the above analysis, the data were evaluated for goodness of fit to the allometric model $y = bx^a$ where x is %ΔFC, y is %ΔBWG, b is %ΔBWG when %FC = 1, and the exponent a is the slope of the straight line when the data are plotted log–log. Subsequent to the first stage of analysis, separate models were used where the log–log regression indicated significantly different intercepts and slopes.

3. Results

3.1. BWG and FC in Sprague–Dawley rats during neotame safety studies

In the one- and two-year neotame studies in rats, both with in utero exposures, overall FC was reduced in treated groups but was within 5% of FC by controls (Table 2). Although FC was measured throughout dosing in the two-year study, FC beyond Week 78 was not analyzed due to confounding by inter-current morbidity and mortality in aging rats. Reductions in BW and BWG in the one- and two-year rat studies were quantitatively similar in all affected dose groups with no dose response in either sex over a combined 20-fold range of doses (50–1000 mg/kg bw/day).

3.2. Comparison of FC and BWG data in other long-term studies

The percent reduction in FC resulted in a correspondingly greater percent reduction in BWG after 26, 52, or 78 weeks of dosing with saccharin, sucralose, neotame, or various degrees of dietary restriction in all studies available for evaluation (Table 2). The greatest number of data points were available at the 52-week interval; thus, this interval was selected from Table 2 for further analysis. These %ΔFC and %ΔBWG data were plotted for males and females (Figs. 1 and 2, respectively). A one-to-one relationship between %ΔFC and %ΔBWG would be indicated by the dotted line in Figs. 1 and 2. In no case was the relationship between %ΔFC and %ΔBWG one-to-one. The percent reductions in BWG were greater than percent reductions in FC for all 52-week data points.

When 52-week data were plotted log–log as in Figs. 3 and 4 for males and females, respectively, the 52-week %Δ data points assumed linearity on a log–log scale. Excluding the 52-week neotame %Δ data, the log–log linearity of %Δ data points demonstrated a goodness of fit of $R^2 = 0.97$, confirming an apparent allometric relationship between %Δ in BWG and %Δ in FC for these data from a number of different studies. Statistically significant differences in the %Δ data occurred between sexes but not between the type of study, i.e., sweetener or dietary restriction.

Because of the extremely small percentage changes in FC in neotame studies and the inherent variability within data for long-term measures of overall FC, 'goodness of fit' was not a meaningful parameter for neotame data alone. Nonetheless, when %Δ in FC and %Δ in BWG from the 12 applicable data points from the one- and two-year rat studies with neotame were included, the overall goodness of fit for the 52-week data points for all the sweeteners including neotame and dietary restrictions did not change ($R^2 = 0.97$). Therefore, the neotame %Δ data points were included with other available %Δ data points in solving the antilog transformation for the linear log–log relationship between 52-week %Δ in BWG and %Δ in FC as,

$$\%\Delta BWG = 3.45(\%\Delta FC^{0.74}) \text{ for male rats and}$$

$$\%\Delta BWG = 5.28(\%\Delta FC^{0.68}) \text{ for female rats.}$$

Table 2
Percent changes (%Δ) in BWG and overall FC at Weeks 26, 52, and 78 in long-term studies with Sprague–Dawley rats

Treatment/ intervention	Treatment group	Sex	Week 26		Week 52		Week 78		References
			% ↓ Overall FC	% ↓ BWG	% ↓ Overall FC	% ↓ BWG	%↓ Overall FC	% ↓ BWG	
Saccharin[c,e]	1% Diet	M	1	3.6	1	3.7	1	4.9	Text and extrapolated Fig. 1, p. 479; Schoenig et al. (1985) ($N = 700$)
Saccharin[c,e]	7.5% Diet	M	6	13.3	6	17.8	6	20.3	Text and extrapolated Fig. 1, p. 479; Schoenig et al. (1985) ($N = 125$)
Neotame[d,c,e]	50 mg/kg bw/day Diet	M	0.1	7.5	0.9	8.9	1.3	11.2	Tables 4 and 5; Mayhew et al. (2003); US FDA Docket No. 98F-0052, two-year study, PCR1000 ($N = 75$)
Neotame[d,c,e]	100 mg/kg bw/day Diet	M	2.5	6.4	3.3	9.7	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)
Neotame[d,c,e]	300 mg/kg bw/day Diet	M	0.5	5.2	2.3	8.0	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)
Neotame[d,c,e]	500 mg/kg bw/day Diet	M	3.2	10.5	2.9	11.4	2.3	12.8	Tables 4 and 5; Mayhew et al. (2003), US FDA Docket No. 98F-0052; two-year study, PCR1000 ($N = 75$)
Neotame[d,c,e]	1000 mg/kg bw/day Diet	M	1	2.8	1	4.5	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)
Neotame[d,c,e]	1000 mg/kg bw/day Diet	M	2.2	7.7	1.6	10.4	1.8	11.0	Tables 4 and 5; Mayhew et al. (2003); US FDA Docket No. 98F-0052, two-year study, PCR1000 ($N = 75$)
Neotame[d,c,e]	50 mg/kg bw/day Diet	F	(+0.3)	7.9	2.4	13.0	0.7	13.4	Tables 4 and 5; Mayhew et al. (2003); US FDA Docket No. 98F-0052, two-year study, PCR1000 ($N = 75$)
Neotame[d,c,e]	100 mg/kg bw/day Diet	F	2.9	7.0	3.8	10.8	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)
Neotame[d,c,e]	300 mg/kg bw/day Diet	F	1.2	8.4	3.2	14.4	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)
Neotame[d,c,e]	500 mg/kg bw/day Diet	F	0.4	9.0	2.7	18.5	3.2	19.3	Tables 4 and 5; Mayhew et al. (2003); US FDA Docket No. 98F-0052, two-year study, PCR1000 ($N = 75$)
Neotame[d,c,e]	1000 mg/kg bw/day Diet	F	3.4	7.0	4.3	15.8	Not applicable	Not applicable	Tables 2 and 3; Mayhew et al. (2003); US FDA Docket No. 98F-0052, one-year study, PCR1011 ($N = 30$)

Table 2 (*continued*)

Treatment/ intervention	Treatment group	Sex	Week 26		Week 52		Week 78		References
			% ↓ Overall FC	% ↓ BWG	% ↓ Overall FC	% ↓ BWG	%↓ Overall FC	% ↓ BWG	
Neotame[d,c,e]	1000 mg/kg bw/day Diet	F	3.5	8.6	5.3	16.7	3.8	16.9	Tables 4 and 5; Mayhew et al. (2003); US FDA Docket No. 98F-0052, two-year study, PCR1000 ($N = 75$)
Sucralose/Dietary restriction[d,e]	5% Dietary restriction "Group 2"	M	8.9	11.0	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,e]	10% Dietary restriction "Group 3"	M	13.9	19.2	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,e]	15% Dietary restriction "Group 4"	M	17.1	20.2	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	1% Sucralose in Diet, ad lib "Group 5"	M	8.8	13.6	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	3% Sucralose in Diet, ad lib "Group 6"	M	8.7	16.7	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	1% Sucralose in Diet, 90% Dietary restriction "Group 7"	M	15.5	19.6	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	3% Sucralose in Diet, 90% Dietary restriction "Group 8"	M	14.1	21.3	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,e]	5% Dietary restriction "Group 2"	F	11.2	16.6	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,e]	10% Dietary restriction "Group 3"	F	11.7	19.8	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,e]	15% Dietary restriction "Group 4"	F	15.3	24.5	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	1% Sucralose in Diet, ad lib "Group 5"	F	7.3	14.7	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	3% Sucralose in Diet, ad lib "Group 6"	F	9.1	20.6	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"

Sucralose/Dietary restriction[d,c,e]	1% Sucralose in Diet, 90% Dietary restriction "Group 7"	F	13.0	24.5	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose/Dietary restriction[d,c,e]	3% Sucralose in Diet, 90% Dietary restriction "Group 8"	F	14.0	28.4	Not applicable	Not applicable	Not applicable	Not applicable	Sucralose study E-160 ($N = 20$); Barton memo (FDA, 1998), US FDA Docket No. 87F-0086, Fed Reg. 63(64) "Ref. 33"
Sucralose[f,c]	0.3% Diet	M	4.3	9.8	4.5	10.3	6.3	13.8	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	0.3% Diet	M	4.3	7.7	4.7	8.7	4.9	8.7	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	1% Diet	M	4.7	11.7	6.1	16.2	8.1	19.1	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	1% Diet	M	6.4	11.0	6.9	13.8	7.2	15.3	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6B and 1.5A; US FDA Docket No. 87F-0086
Sucralose[f,c]	3% Diet	M	5.7	13.6	6.0	15.8	7.6	18.8	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	3% Diet	M	7.7	12.2	8.0	15.5	8.4	17.3	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6B and 1.5A; US FDA Docket No. 87F-0086
Sucralose[f,c]	0.3% Diet	F	4.4	11.6	5.9	21.2	7.5	23.6	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	0.3% Diet	F	4.8	13.9	5.1	17.3	5.7	18.3	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6B and 1.5A; US FDA Docket No. 87F-0086
Sucralose[f,c]	1% Diet	F	6.3	14.8	7.8	21.1	10.8	28.7	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	1% Diet	F	7.9	14.9	8.4	20.6	8.8	20.4	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6B and 1.5A; US FDA Docket No. 87F-0086
Sucralose[f,c]	3% Diet	F	6.6	11.6	8.2	20.6	9.8	20.4	Sucralose study E-057 Tox Group ($N = 30$), Tables 1.6E and 1.5B; US FDA Docket No. 87F-0086
Sucralose[f,c]	3% Diet	F	8.0	13.9	9.2	21.3	10.0	24.4	Sucralose study E-057 Onco Group ($N = 50$), Tables 1.6B and 1.5A; US FDA Docket No. 87F-0086
FDA Dietary Restriction[d,e]	10% Diet Restriction	M	10	13	10	16	10	17	($N = 40$) Duffy et al. (2001), "6 and 12 month" data extrapolated from Fig. 2
FDA Dietary Restriction[d,e]	25% Diet Restriction	M	25	26	25	30	25	34	($N = 40$) Duffy et al. (2001), "6 and 12 month" data extrapolated from Fig. 2

Table 2 (*continued*)

Treatment/ intervention	Treatment group	Sex	Week 26		Week 52		Week 78		References
			% ↓ Overall FC	% ↓ BWG	% ↓ Overall FC	% ↓ BWG	%↓ Overall FC	% ↓ BWG	
FDA Dietary Restriction[d,e]	40% Diet Restriction	M	40	41	40	47	40	49	($N = 40$) Duffy et al. (2001), "6 and 12 month" data from Fig 2
FDA Dietary Restriction[d,e]	31% Diet Restriction	M	31	34	31	44	31	43	SD Rat fed AIN-93M diet, ($N = 60$) Duffy et al. (2002), extrapolated from Fig. 3
Dietary Restriction[e]	"Optimized Diet"	M	15–25	34	15–25	34	15–25	38	($N = 70$) Christian et al. (1998), extrapolated from Fig. 3
Dietary Restriction[e]	"Optimized Diet"	F	15–25	26	15–25	36	15–25	46	($N = 70$) Christian et al. (1998), extrapolated from Fig. 3
Dietary Restriction[e]	25% Dietary Restriction	M	25	34	25	34	25	32	($N = 120$) Hubert et al. (2000), Tables 1 and 4, extrapolated from Fig. 3
Dietary Restriction[e]	25% Dietary Restriction	F	22	40	22	47	22	51	($N = 120$) Hubert et al. (2000), Tables 1 and 4, extrapolated from Fig. 4
Dietary Restriction[e]	50% Dietary Restriction	M	56	76	56	74	56	76	($N = 120$) Hubert et al. (2000), Tables 1 and 4, extrapolated from Fig. 3
Dietary Restriction[e]	50% Dietary Restriction	F	54	88	54	83	54	82	($N = 120$) Hubert et al. (2000), Tables 1 and 4, extrapolated from Fig. 4
Dietary Restriction[e]	25% Dietary Restriction	M	26	53	26	41	26	35	($N = 100$–134) Laroque et al. (1997), Table 1, Fig. 1
Dietary Restriction[e]	25% Dietary Restriction	F	22	40	22	39	22	49	($N = 100$–134) Laroque et al. (1997), Table 1, Fig. 2

[a]Group housed animals, 5/cage. FC determined intermittently for cages during study.
[b]No apparent differences in FC measured for groups of rats. Decrements in FC represent % adjustment for non-nutritive material in diet.
[c]FC adjusted for amount of non-nutritive material in diet.
[d]Spilled diet weighed, anti-spill feed jars. Overall adjusted FC and/or percent dietary restriction reported.
[e]Individually housed animals. FC determined regularly for individual rats throughout study.
[f]Group housed animals, 5/cage. FC determined regularly for cages throughout study.

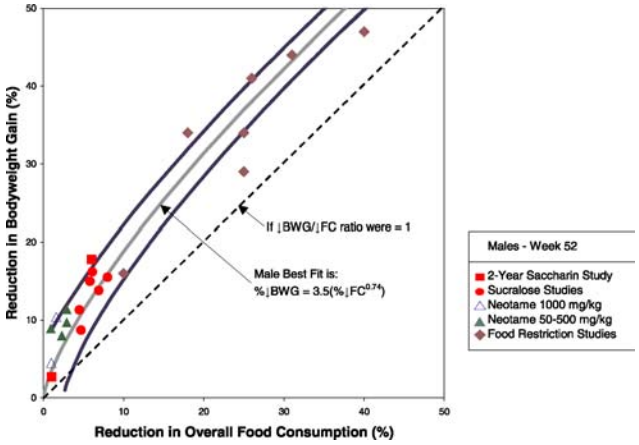


Fig. 1. Reductions in BWG (%) and overall FC (%) for male Sprague–Dawley rats at 52 weeks in long-term studies. Dotted line indicates a theoretical 'one-to-one' relationship between changes in FC and BWG. Centerline indicates "best fit" of data according to the allometric formula, $\%\Delta BWG = 3.5(\%\Delta FC^{0.74})$. Outer lines indicate range of "best fit" ±2.5% ΔFC; the precision of FC measurements in long-term rodent studies is assumed to be within 5% of actual FC.

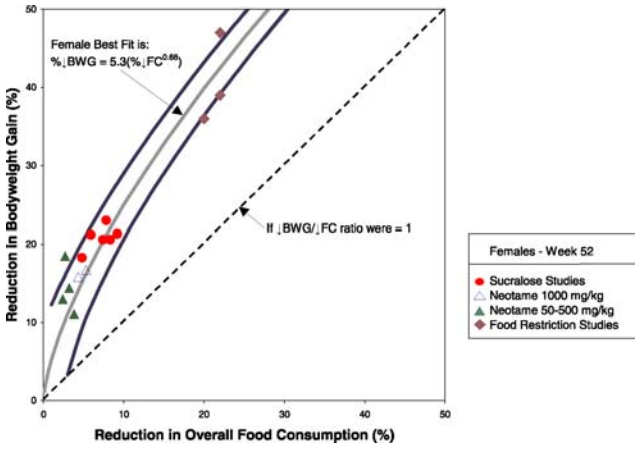


Fig. 2. Reductions in BWG (%) and overall FC (%) in female Sprague–Dawley rats at 52 weeks in long-term studies. Dotted line indicates a theoretical 'one-to-one' relationship between changes in FC and BWG. Centerline indicates "best fit" of data according to the allometric formula, $\%\Delta BWG = 5.3(\%\Delta FC^{0.68})$. Outer lines indicate range of "best fit" ±2.5% ΔFC; the precision of FC measurements in long-term rodent studies is assumed to be within 5% of actual FC.

4. Discussion

4.1. BWG and FC in Sprague–Dawley rats during neotame safety studies

In the one- and two-year neotame studies, FC, BW, and BWG are lower than controls in groups dosed with neotame at 50–1000 mg/kg bw/day. Dietary concentrations of neotame in dose groups showing changes in BWG range from approximately 930–24,000 ppm during the latter parts of these studies. The long-term %Δ in

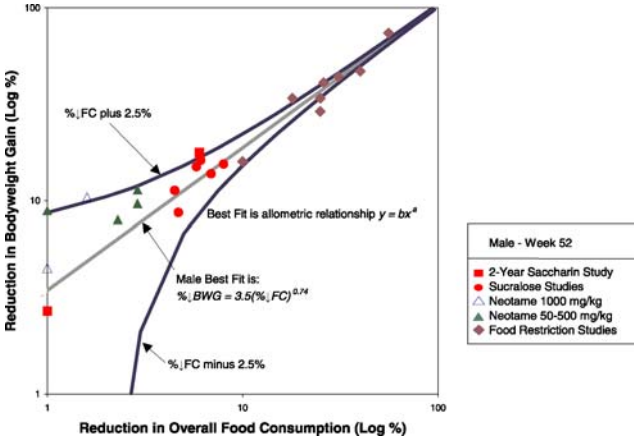


Fig. 3. Reductions in BWG (log %) and overall FC (log %) in male Sprague–Dawley rats after 52 weeks in long-term studies. Linear centerline describes allometric "best fit" ($R^2 = 0.97$) of data, $\%\Delta BWG = 3.5(\%\Delta FC^{0.74})$. Curved outer lines plot "best fit" for $\Delta FC \pm 2.5\%$; the precision of FC measurements in long-term rodent studies is assumed to be within 5% of actual FC. Log–log scale for $\Delta FC \pm 2.5\%$ demonstrates the physiological allometric principle that small initial changes of a variable result in larger relative responses than do progressively greater changes.

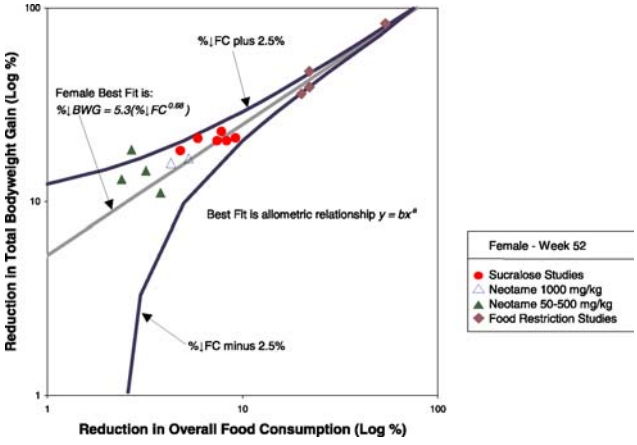


Fig. 4. Reductions in BWG (log %) and overall FC (log %) in female Sprague–Dawley rats after 52 weeks in long-term studies. Linear centerline describes allometric "best fit" ($R^2 = 0.97$) of data, $\%\Delta BWG = 5.3(\%\Delta FC^{0.68})$. Curved outer lines plot "best fit" for $\Delta FC \pm 2.5\%$; the precision of FC measurements in long-term rodent studies is assumed to be within 5% of actual FC. Log–log scale for $\Delta FC \pm 2.5\%$ demonstrates the physiological allometric principle that small initial changes of a variable result in larger relative responses than do progressively greater changes.

BWG, generally ranging from 5 to 20%, are several-fold greater than the small %Δ in FC, generally ranging from 1 to 5%. Although there is evidence of reduced FC, the question is whether very small long-term reductions in FC can result in relatively larger long-term changes in BWG.

The small reductions in FC are due to reduced palatability of diets at concentrations of neotame required for dosing these groups (Mayhew et al., 2003). The changes in FC, BW, and BWG are not dose-related over this 20-fold range of doses. The absence of dose–response over a large range of doses demonstrates a change is not due to either pharmacologic or toxic effects. Dose–response is an inherent characteristic of toxicological and pharmacological responses; that is, severity of response increases with increasing dose (Eaton and Klaassen, 2001).

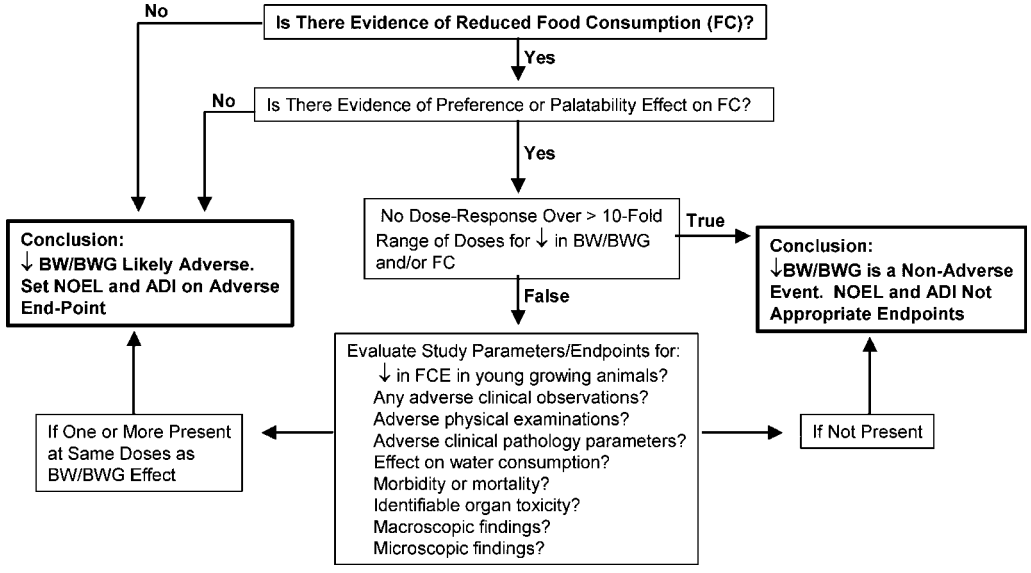
Further supporting the conclusion that changes in BW and BWG are not adverse in neotame safety studies is that food assimilation and utilization, as assessed by food conversion efficiency (FCE) calculations, are not altered during Weeks 1–13 of dosing, the period of active growth in rats (Mayhew et al., 2003). Thus, neotame does not affect the ability of animals to utilize energy from the diets. Careful evaluation of all study parameters and endpoints, including the absence of adverse findings from clinical observations and physical examinations, in clinical pathology parameters, on morbidity and mortality, and the lack of identifiable organ toxicity and absence of adverse macroscopic or microscopic findings, all further confirm the conclusion that the changes in BW and BWG in long-term safety studies with neotame are not adverse (Mayhew et al., 2003). Based upon these considerations for neotame, a conservative approach using objective criteria is proposed to delineate changes in BW and BWG observed in safety studies as due to toxicity or secondary to non-adverse changes in FC (Table 3).

4.2. Comparison of FC and BWG data in other long-term studies

When comparing changes in FC and BWG across long-term studies for high-intensity sweeteners and dietary restriction paradigms, statistically significant differences occur between sexes but not between the study types, i.e., sweetener or dietary restriction. The relatively smaller changes in FC and BWG observed from reduced palatability of diets containing high concentrations of high-intensity sweeteners are allometrically equivalent to the much larger changes in FC and BWG observed after marked dietary restriction. These relationships between changes in FC and BWG are clearly consistent throughout a wide range of reduced FC with the well-known allometric model of $y = bx^a$ where x is %ΔFC, y is %ΔBWG, b is %ΔBWG when $\Delta FC = 1$, and the exponent a is the slope of the straight line when the data are plotted log–log. Specifically, the very small long-term reductions in FC observed in Sprague–Dawley rats in neotame safety studies allometrically account for the several-fold greater reductions in BWG.

A common misconception is the assumption that reductions in FC result in linear reductions in BWG on a "one-to-one" quantitative basis. However, in no case from the long-term neotame safety studies or from the other available long-term studies in Sprague–Dawley rats is the relationship between %Δ in FC and %Δ in BWG 'one-to-one.' Percent reductions in BWG are always greater than the percent reductions in FC. This observation is in contrast with the stated position of some regulatory agencies including the FDA (1998, 2002).

Table 3
Algorithm to establish body weight effects as not adverse[a]



[a] MTD for long-term studies from subchronic studies (up to 5% in diet (FDA) or ↓BWG not more than 10%).

Evidence that the higher concentrations of neotame used in safety studies reduce palatability of food is clear (Mayhew et al., 2003). This is also the case for high concentrations of other intense sweeteners such as sucralose (Mann et al., 2000) and saccharin (Chowaniec and Hicks, 1979; Schoenig et al., 1985). The concentrations of high-intensity sweeteners, including neotame, used in animal studies are many 100–1000-fold greater than those intended for human use, thus specific qualities of taste and palatability at such high concentrations are not relevant to human exposures. Nonetheless, palatability of diets containing the very high concentrations required for dosing in safety studies is critical to study outcome when the material administered has intense taste.

This analysis of long-term FC and BWG data in Sprague–Dawley rats demonstrates that the relationship between changes in FC and changes in BWG follows an allometric relationship. Allometric relationships are common to a wide variety of physiological responses including metabolic activity, biological growth within the individual, changes in size across a population, and comparisons of size and body surface area across species (Andresen et al., 2002; Blaxter, 1986; Huxley, 1932; Paxton, 1995; Reeve and Huxley, 1945). Along an allometric curve, a physiological variable (x) and a physiological response y can appear to approximate 1:1 linearity, but only *after* large changes in the response (y) have already occurred. However, the small initial changes in a variable x result in much larger relative changes in the response y. As a corollary, the smallest change that can be precisely and accurately measured in variable x will always result in the largest relative response y that will be observed. This paradoxical relationship is predicted by the allometric model for relating changes in biological size, $y = bx^a$, where b is the value of y when $x = 1$, and a is the slope of the data plotted log–log (Huxley, 1932; Reeve and Huxley, 1945).

The allometric relationship observed between %ΔBWG and %ΔFC in Sprague–Dawley rats at week-52 is $\%\Delta BWG = 3.45(\%\Delta FC^{0.74})$ for males and $\%\Delta BWG = 5.28(\%\Delta FC^{0.68})$ for females. Consequently, these formulae predict a long-term decrement of 1% in FC in females would result in a 5-fold greater decrement in BWG of 5.28%; a long-term decrement in FC of 10% in these same animals would result in a 2.5-fold greater decrement in BWG of 25%. However, it is not methodologically possible to accurately or precisely measure a 1% change in FC over a long period of time in a safety study. Nonetheless, a 1% change in FC would theoretically result in a 5% change in BWG that could be measured accurately and precisely. It is interesting to note sexual dimorphism in allometric growth has been previously reported for Sprague–Dawley rats (Stewart and German, 1999). These authors attributed the adult dimorphism to consistently different patterns of growth between the sexes. Males had a higher rate of growth that occurred later in time than did growth in females, and males also had a longer duration of growth (Stewart and German, 1999).

The slopes of 0.74 for male rats and 0.68 for female rats reflected by the allometric rate exponents for %Δ in BWG and %Δ in FC are consistent with the accepted allometric scale of $W^{3/4}$ which relates body mass to basal metabolic rate (Brown et al., 2000). This slope constant of $W^{3/4}$ relating body mass and basal metabolic rate has been characterized as one of the few well-established universal principles of biology (Shiner and Uehlinger, 2001). Other allometric slope constants approximating the 3/4 power include pharmacokinetic dosing between pediatrics and adults (Anderson et al., 1997) and physiological volume rates such as cardiac output, ventilation and oxygen uptake (Lindstedt and Schaeffer, 2002). The allometric rate constants found relating %Δ in BWG and %Δ in FC appear to have a robust physiological basis.

In safety studies, BW can be measured with great accuracy and precision. The calculated mean for overall BWG has good accuracy and minimal variability; thus, differences in BWG of less than 5% will likely be statistically significant. FC is generally determined for either weekly or biweekly intervals depending on study duration and requires a number of measurements and calculations for each interval. The precision of the FC measurement is subject to the variability contributed to the data by repeated weighings and calculations required to derive FC for an interval. Thus, the accuracy and precision of FC data are never as great as that for BWG data. Consequently, a 5% difference in FC between dose groups for any particular interval is not likely to be statistically significant. Overall FC is the best available indication of how much food the animals have consumed over a long period of time. However, considering the effect on accuracy and precision from summing FC for individual intervals, over the long-term, the numbers derived for overall FC are likely only approximations of *actual* FC within 5%, i.e., ±2.5% (Flamm, 2002). Nonetheless, it is important to evaluate overall FC in long-term safety studies when determining whether lower FC is the cause of observed changes in BWG.

The magnitude of the small changes in FC observed in the long-term neotame safety studies adequately explains the relatively larger changes in BWG in these studies. No neotame dose group had more than a 5% reduction in overall FC from that of controls. The long-term neotame studies in rats included design considerations to enhance the accuracy of weekly or biweekly FC measurements, such as, single-housing of animals, assessment of spilled diet, and specially designed food containers to minimize spillage or contamination. Consequently, the small magnitude of change in overall FC observed in neotame rodent studies is likely the

smallest that can be measured during long-term safety studies with accuracy or precision.

The approach proposed in Table 3 uses objective criteria to differentiate changes in BW parameters due to toxicity from those secondary to reduced FC in safety studies. As is illustrated with the example of neotame, the data required to determine the relationship between BWG and FC are available from the safety studies. Due to the lack of appreciation for the allometric relationship between FC and BWG, the resolution of questions regarding long-term changes in BW parameters in dietary safety studies has traditionally involved more data collection from additional studies of shorter duration. Typically, the additional studies have followed gavage or pair-feeding dosing paradigms; both of these approaches have the limitation of being less predictive of human exposure than the ad libitum dietary administrations in long-term safety studies.

Gavage administration of large amounts of test material is not physiologically equivalent to dietary administration of test materials, nor is the duration of typical gavage studies representative of long-term dietary dosing. Bolus dosing contributes confounding variables to the study design that are not found in dietary studies, such as, differences in pharmacokinetics, metabolism, fluid balance, water consumption and hydration, potential osmotic changes, and changes in gastric transit time. Further, gavage dosing is generally more physically stressful and contributes additional morbidity and mortality due to accidental dosing errors.

It is not experimentally possible to replicate *small* changes in FC in pairedfeeding studies. Inherent to FC measurements is variability that compromises the precision of overall FC data. A targeted reduction in FC of less than 10% is difficult to achieve in a paired-feeding experimental design. For example, as is illustrated in Table 2, in a recent well-designed paired-feeding study with the high-intensity sweetener, sucralose, the pair-fed group targeting a 5% dietary restriction consumed 9 and 11% less food than did controls (males and females, respectively), and the pair-fed group targeting a 10% dietary restriction consumed 12 and 14% less food than did controls (females and males, respectively). Consequently, the traditional regulatory approach of requesting additional gavage or pair-feeding data may prove more confounding to the distinctions between adverse and non-adverse effects on BW parameters than does following the objective criteria proposed here.

4.3. Regulatory considerations

In contrast to the findings presented here, the stated position of US FDA is that a unit decrement in BW should be accompanied by an equivalent unit decrement in FC to resolve concerns over changes in BW parameters (FDA, 1998; FDA, 2002). However, other regulatory agencies have not considered changes in BWG to be adverse or of concern when associated with lower FC, such as those accompanying reduced palatability of diets. Thus, changes in BWG due to lower FC have not been used to establish no-observed-effect-levels (NOELs) by other international agencies such as the JECFA. For example, JECFA did not use lower BWG when setting the acceptable daily intake (ADI) for high-intensity sweeteners, including neotame, aspartame, sucralose, and acesulfame-potassium.

Specifically, JECFA (WHO, 1987) states,

> When analysing a toxicological study and setting a no-observed-effect level, a distinction must be drawn between reversible changes that are due entirely to normal physiological processes or homeostasis-maintaining mechanisms, and to toxic responses themselves... Examples of the former include... decreased growth rate and food consumption related to the dietary administration of an unpalatable substance. And,
>
> The procedures followed by JECFA for determining an ADI demand that a no-observed-effect level should be established. For this level to be established, it is necessary to establish an effect level and, when all else has failed, a generalized decrement in weight gain has been used for this purpose, provided reduced food intake is not the obvious cause... And,
>
> The determination of an adverse effect in a particular study depends on... the ability to distinguish between real adverse effects and false positives ... In addition, a reduction in bodyweight gain coupled with decreased food consumption is difficult to interpret as an adverse effect, because palatability of the chow (food) might be affected by the presence of high levels of the test compound.

For these reasons, in the absence of toxicity, JECFA has concluded that changes in BWG are not appropriate for establishing NOELs when they are associated with lower FC. It is the normal practice of JECFA to recognize when BW is affected by reduced palatability of food containing high concentrations of test material. For example, JECFA noted that lower BWG for sucralose at the high dose in the long-term rat study (1500 mg/kg bw/day) was due to poor palatability of diet and did not consider this finding adverse when setting the NOEL and an ADI of 0–15 mg/kg bw/day (WHO, 1991). Similarly, the highest dose of acesulfame-potassium, 1500 mg/kg bw/day, was likewise associated with lower BWG. JECFA did not consider this an adverse finding when establishing an ADI for acesulfame-potassium of 0–15 mg/kg bw/day (WHO, 1991). The applicable changes in FC and BWG for sucralose are included in the data plotted in Figs. 1–4; the long-term studies with acesulfame-potassium were not done using the Sprague–Dawley strain of rats.

After a thorough review of safety data for neotame, the Australia New Zealand Food Authority (ANZFA, 2001) in their approval of neotame agreed with the general principles set forth by JECFA. ANZFA concluded that changes in BW and BWG are the result of poor palatability of food containing high concentrations of neotame and, thus, are not adverse,

> . . .Neotame is well tolerated in all species (rats, mice and dogs) with little evidence of treatment-related adverse effects. The most significant finding in these animal species was a decrease in bodyweight and bodyweight gain at the higher dose levels that is accompanied by a decrease in food consumption. These findings are considered to be related to decreased palatability of the neotame-containing diet rather than to toxicity . . .

Thus, the ADI for neotame set by ANZFA is not based on changes in BW or BWG.

In the absence of any toxicological or adverse findings over at least a 20-fold range of doses in neotame safety studies, the effect on BW and BWG can clearly be considered a non-adverse effect secondary to reduced palatability of food containing high concentrations of neotame. Careful consideration of the prudent criteria outlined in Table 3 further confirms the conclusion that the lower BW and BWG observed in neotame studies are not due to toxicity. The magnitude of lower FC in long-term neotame safety studies adequately explains the changes in BWG relative to controls observed in neotame safety studies. Thus, BW and BWG in animal safety studies are not relevant for setting NOELs for high-intensity sweeteners, including neotame; neither are BW parameters relevant to human exposure when establishing an ADI.

5. Conclusion

Lower BW and BWG were the only consistent findings in neotame safety studies. These changes are consistent in magnitude with small reductions in FC during long-term neotame safety studies according to the allometric relationship between changes in BWG and changes in FC previously reported for other high-intensity sweeteners and dietary restriction studies in the same sex and strain of rats. The relationship between long-term changes in FC and BWG is not one-to-one as is the stated position of some regulatory agencies. Rather, changes in BWG after changes in FC follow an established allometric relationship, $\%\Delta BWG = b(\%\Delta FC^{a})$. The relatively small changes in FC and BWG observed in neotame safety studies fit the allometric model for larger changes in FC and BWG previously reported for other high-intensity sweeteners and dietary restrictions. Consequently, BW parameters are not appropriate endpoints for setting either NOELs or ADIs for high-intensity sweeteners, including neotame.

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