

## Neotame as a sweetener and flavour enhancer<sup>1</sup>

### Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2003-137)

Adopted on 27 September 2007

#### PANEL MEMBERS

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#### SUMMARY

Following a request from the European Commission, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was asked to deliver a scientific opinion on the safety of neotame as a sweetener and flavour enhancer.

Neotame is a dipeptide methyl ester derivate. Its chemical structure is N-[N-(3,3-dimethylbutyl)-L- $\alpha$ -aspartyl]-L-phenylalanine 1-methyl ester. It is intended for use in food as a sweetener and flavour enhancer. Neotame has a sweetness factor approximately 7000 to 13000 times greater than that of sucrose and approximately 30 to 60 times greater than that of aspartame, depending upon the food application.

Neotame is manufactured by the reaction of aspartame and 3,3-dimethylbutyraldehyde, followed by purification, drying, and milling. Neotame is generally stable under conditions of intended use as a sweetener across a wide range of food and beverage applications. Neotame degrades slowly in aqueous conditions such as those in carbonated soft drinks. The hydrolysis of neotame results in equimolar amounts of N-[N-(3,3-dimethylbutyl)-L- $\alpha$ -aspartyl]-L-phenylalanine (NC-00751) and methanol. Three other minor degradation products, N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine 1-methyl ester (NC-00777) formed by cyclisation, N-[N-(3,3-dimethylbutyl)-L- $\beta$ -aspartyl]-L-phenylalanine 1-methyl ester (NC-00764) formed by  $\beta$ -rearrangement of neotame, and N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine (NC-00779) formed by methyl ester hydrolysis of NC-00777,

<sup>1</sup> For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from European Commission on Neotame as a sweetener and flavour enhancer. *The EFSA Journal* (2007) 581, 1-3

are not detected at the anticipated concentrations and conditions of neotame use in carbonated soft drinks.

At least 30% of neotame ingested is rapidly absorbed in all species. The major metabolic pathway is de-esterification by non-specific esterases to NC-00751, which accounts for approximately 80% of the neotame dose as NC-00751 in all species tested. Peak plasma concentrations of neotame and NC-00751 are observed at approximately 0.5 hours and within 1 hour, respectively. Neotame is completely eliminated from the body with recovery in urine and faeces exceeding 98% in the human and greater than 93% in the rat and the dog within 72 hours. The majority of radioactivity is excreted in faeces in all species. The major component in the faeces is NC-00751.

Studies with radiolabelled neotame given orally to rats indicate no accumulation in tissues. The highest radioactivity is associated with the contents of the gastrointestinal tract and organs of metabolism and excretion (liver, kidney, urinary bladder). In whole body autoradiography studies with pregnant rats no radioactivity has been reported in the fetus.

The safety of neotame has been investigated in *in vitro* studies and in short and long-term studies in mice, rats, rabbits and dogs. The results indicate that neotame is not genotoxic, carcinogenic, teratogenic or associated with any reproductive/developmental toxicity. The consistent findings in animal studies were reduced feed consumption, body weight and body weight gain relative to that of controls, with no clear dose response. These effects are considered not adverse or indicative of toxicity but a consequence of reduced palatability of the neotame-containing diets. Therefore body weight parameters were not considered appropriate endpoints for setting no-observed-adverse-effect levels (NOAELs) in these studies.

A consistent finding was an increased activity of alkaline phosphatase (AP) of hepatic origin in dogs in the 13-week study at doses of 600 and 1200 mg/kg bw and in the 52-week study at 800 mg/kg bw. In the latter study AP activity was also increased compared to controls at 200 mg/kg bw in females in weeks 26 and 52. The Panel noted that the mean baseline value in this dose group was 20% greater than that of controls and AP was not significantly increased at any time point compared to controls by analysis of covariance for repeated measures using predosing AP activity as the covariate. Although the increase in serum AP seen at the two highest dose levels in both dog studies was not accompanied by any other indication of hepatotoxicity, the Panel considered the increase in AP to be the critical endpoint and established a NOAEL of 200 mg/kg neotame/kg bw/day for setting an ADI.

The major degradation product and metabolite NC-00751 will have been present in both the human and animal studies. Its safety has also been established by *in vitro* genotoxicity studies. The three minor degradation products (NC-00764, NC-00777 and NC-00779) have been shown to have a low acute toxicity and are not genotoxic. No treatment-related adverse effects were observed in a 4-week dietary study in rats with a mixture of the three minor degradation products. Based on the above, the dietary exposure to degradation products is not considered to pose any safety concern.

The results of human studies demonstrated that neotame was well tolerated by healthy and diabetic human subjects at dose levels up to 1.5 mg/kg bw/day (the highest dose tested).

The exposure to methanol, which may result from ingestion of neotame-containing foods and beverages is considered negligible compared to that from other dietary sources and as such of no concern from the safety point of view.

The Panel noted that the additional phenylalanine intake expected from ingestion of neotame as a general purpose sweetener represents a relatively small increment in the exposure to phenylalanine of the phenylketonuric homozygous child.

The hypothetical formation of nitrosamines in the gastrointestinal tract from reaction of nitrite with neotame and its major degradation product and/or metabolite NC-00751 has been considered by the Panel. No nitrosated neotame (N-nitroso-(3,3-dimethyl)-L-aspartyl-L-phenylalanine methyl ester, N-nitrosoneotame; NC-00799) and no nitrosated de-esterified neotame (N-nitroso-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine, NC- 00800) could be detected under simulated gastric juice conditions. Furthermore, both compounds were synthesised and shown to be without mutagenic activity in the Ames test. In view of the high sensitivity of the Ames test to genotoxic nitrosocompounds, the Panel considered that nitrosation of neotame, should it occur, is not a matter of safety concern.

After considering all the data on stability, degradation products and toxicology, the Panel concluded that neotame is not of safety concern with respect to the proposed uses as a sweetener and flavour enhancer.

The Panel established an Acceptable Daily Intake (ADI) of 0-2 mg/kg bw/day based on the application of a 100-fold safety factor to the NOAEL of 200 mg/kg bw from a 52-week dog study.

Conservative estimates of dietary exposure both in adults and children suggest that it is very unlikely that the ADI would be exceeded at the proposed use levels.

The Panel recommends that the limit for lead in the specifications should not be higher than 1 mg/kg.

**Key words:**

Neotame, E 961, CAS N° 165450-17-9, L-phenylalanine, N-[N-(3,3-dimethylbutyl)-L- $\alpha$ -aspartyl]-,1-methyl ester artificial sweetener, intense sweetener, food additive.