



SECTION SIX

The history of use or other evidence of safety establishing that the dietary ingredient zinc carnosine when used under the conditions recommended or suggested in the labeling of dietary supplement products will reasonably be expected to be safe and which is the basis on which the distributor of zinc carnosine has determined that the use of zinc carnosine is reasonably expected to be safe. See 21 CFR § 190.6(b)(4).

INTRODUCTION

Zinc carnosine has been clinically evaluated in Japan for both safety and efficacy as a novel antiulcer agent. In the Japanese studies zinc carnosine is identified as Z-103 and/or polaprezinc. While zinc carnosine has been clinically evaluated in Japan for a therapeutic indication, this submission to the Food and Drug Administration (FDA) will only rely on the safety data in the enclosed clinical and scientific Japanese studies. In marketing zinc carnosine in the United States (U.S.) the distributor's intended use of the dietary ingredient is specifically for use in dietary supplement products as a new source of zinc and at a level of use of 75 mg per day. The dietary ingredient zinc carnosine will not be marketed by the distributor with any claims inconsistent with its status as a dietary ingredient for inclusion in lawfully marketed dietary supplement products.

The recommended level of use of 75 mg per day of zinc carnosine will result in a dietary use of 17-18 mg of zinc and 57 to 58 mg of L-carnosine. Zinc functions as a component of various enzymes in the maintenance of structural integrity of proteins and has a wide range of biological roles. Of identified biological systems, zinc has catalytic, structural or regulatory roles in more than 200 zinc metalloenzymes. Physiologically, zinc is needed for growth and development and may have both antioxidant and immunomodulatory activity. Zinc supplementation is reported to help restore impaired immune function in those with zinc deficiency. The recommended dietary allowance (RDA) for zinc for adults is 8 mg per day for women and 11 mg per day for men. The tolerable upper intake level for adults is 40 mg per day, a value based on reduction in erythrocyte copper-zinc superoxide dismutase activity.¹ See copy of Dietary Reference Intakes chart for zinc from Institute of Medicine, Food and Nutrition Board report on micronutrients as attachment 6(A).

¹ National Academy Press - Institution of Medicine Report "Dietary Reference Intakes For Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.(2001)

L-carnosine, the other component of zinc carnosine, is a small molecule composed of the amino acids histidine and B-alanine. It is found in relatively high concentrations in several body tissues - most notably in the skeletal muscle, heart muscle, and in the brain.² The exact biological role of carnosine is not completely known but it does possess antioxidant properties. Carnosine has been considered as a water soluble counterpart to Vitamin E in protecting membranes from oxidative damage.

L-carnosine has been included in some dietary supplement products currently being marketed in the U.S. at a level of use from 20-50 mg. See attachment 6(B) Twinlab Phos Fuel180 capsules and Endura High Magnesium Energy and Rehydration Drink. Therefore, both components of the dietary ingredient zinc carnosine have had a long use in the U.S. food supply and, at the level of recommended use of 75 mg, are at a very safe level of use for consumption as a dietary ingredient of zinc for use in dietary supplement products.

A full discussion of the scientific and clinical documentation providing a basis for the safety of zinc carnosine is provided in the expert review of Robert A. DiSilvestro, Ph.D. which is enclosed as Section 6(C). His curriculum vitae is enclosed as Section 6(D) and the scientific articles referenced by Dr. DiSilvestro are included as exhibits 6(E)(1) - 6(E)(26) as follows:

Review Articles

6(E)(1) Applicability of zinc complex of L-carnosine for medical use. *Biochemistry (Moscow)* 65:961-968, 2000.

² Quinn PJ, Boldyrev AA, Formazuyk VE, Carnosine: its properties, functions and potential therapeutic applications. *Mol Aspects Med* 1992; 13:379-444.

Bonfanti L, Peretto P, De Marchis S, Fasolo A. Carnosine-related dipeptides in the mammalian brain. *Prog Neurobiol* 1999; 59:333-53.

Klebanov GI, Teselkin YO, Babenkova IV. Effect of carnosine and its components on free-radical reactions. *Membr Cell Biol* 1998; 12:89-99.

Hipkiss AR, Preston JE, Himsworth DT. Pluripotent protective effects of carnosine, a naturally occurring dipeptide. *Ann NY Acad Sci* 1998;854:37-53.

- 6(E)(2) Metal complexes of carnosine. *Biochemistry (Moscow)* 65:928-927, 2000.
- 6(E)(3) Interactions between carnosine, zinc and copper: implications for neuromodulation and neuroprotection. *Biochemistry (Moscow)* 65:807-816, 2000.
- 6(E)(4) Endogenous mechanisms of neuroprotection: role of zinc, copper, and carnosine. *Brain Research* 852:56-61, 2000.
- 6(E)(5) Carnosine: physiological properties and therapeutic potential. *Age and Ageing* 29:207-210, 2000.
- 6(E)(6) Problems and perspectives in studying the biological role of carnosine. *Biochemistry (Moscow)* 65:751-756, 2000.

Studies in Cell Cultures and Other Studies In Vitro

- 6(E)(7) Hydrogen peroxide-mediated Cu,Zn-superoxide dismutase fragmentation: protection by carnosine, homocarnosine and anserine. *Biochimica et Biophysica Acta* 1472:651-657, 1999.
- 6(E)(8) Polaprezinc protects gastric mucosal cells from noxious agents through antioxidant properties in vitro. *Alimentary Pharmacology & Therapeutics* 13:261-269, 1999.
- 6(E)(9) Polaprezinc down-regulates proinflammatory cytokine-induced nuclear factor-kappaB activation and interleukin-8 expression in gastric epithelial cells. *Journal of Pharmacology & Experimental Therapeutics* 291:345-352, 1999.
- 6(E)(10) Effect of polaprezinc (N-(3-aminopropionyl)-L-histidinato zinc), a novel antiulcer agent containing zinc, on cellular proliferation: role of insulin-like growth factor I. *Biochemical Pharmacology* 58:245-250, 1999.
- 6(E)(11) Insulin-like growth factor I plays a role in gastric wound healing: evidence using a zinc derivative, polaprezinc, and an in vitro rabbit wound repair model. *Alimentary Pharmacology & Therapeutics* 12:1131-1138, 1998.

Studies In Vivo-Nonhuman Species

- 6(E)(12) Toxicity of the novel anti-peptic ulcer agent polaprezinc in beagle dogs. *Arzneimittel Forschung (Drug Research)* 45:52-60, 1995.
- 6(E)(13) Changes in tissue contents of zinc, copper and iron in rats and beagle dogs treated with polaprezinc. *Journal of Toxicological Sciences* 21:177-187, 1996.
- 6(E)(14) High levels of dietary carnosine are associated with increased concentrations of carnosine and histidine in rat soleus muscle *Journal of Nutrition* 131:287-90, 2001.
- 6(E)(15) Tissue distribution of polaprezinc in rats determined by the double tracer method. *Journal of Pharmaceutical & Biomedical Analysis* 19:453-461, 1999.
- 6(E)(16) Effects of polaprezinc on lipid peroxidation, neutrophil accumulation, and TNF-alpha expression in rats with aspirin-induced gastric mucosal injury. *Digestive Diseases & Sciences* 46:845-851, 2001.
- 6(E)(17) Irritant action of monochloramine in rat stomachs: effects of zinc L-carnosine (polaprezinc). *General Pharmacology* 29:713-718, 1997.
- 6(E)(18) Mucosal ulcerogenic action of monochloramine in rat stomachs: effects of polaprezinc and sucralfate. *Digestive Diseases & Sciences* 42:2156-2163, 1997.

Series of papers translated from Japanese

- 6(E)(19) Clinical Evaluation of Z-103 on Gastric Ulcer. *Japanese Pharmacology & Therapeutics* 20, 1992.
- 6(E)(20) Clinical Evaluation of Z-103 on Gastric Ulcer-A Multicenter Double-Blind Comparative Study with Cetraxate Hydrochloride. *Japanese Pharmacology & Therapeutics* 20, 1992.
- 6(E)(21) Clinical Evaluation of Z-103 in the Treatment of Gastric Ulcer-A Multicenter Double-Blind Dose Finding Study. *Japanese Pharmacology & Therapeutics* 20(1):181-197, 1992.
- 6(E)(22) Clinical Evaluation of Z-103 on Gastric Ulcer-Preliminary Evaluation for Dosage. *Japanese Pharmacology & Therapeutics* 20, 1992.

- 6(E)(23) Clinical Study of Z-103-Clinical Effects on Gastric Ulcer and Influence on Endocrine Function. Japanese Pharmacology & Therapeutics 20(1):245-254, 1992.
- 6(E)(24) Clinical Evaluation of Z-103 on Gastric Ulcer. Japanese Pharmacology & Therapeutics 20, 1992.
- 6(E)(25) Clinical Evaluation of Z-103 on Gastric Ulcer-Results of phase III general clinical trial. Japanese Pharmacology & Therapeutics 20, 1992.
- 6(E)(26) Clinical Phase I Study of Z-103. Clinical Pharmacology 20(1):149-63, 1992.
- 6(E)(27) Toxic appearance and changes of zinc and copper content in rats by long-term oral dosing of Z-103. Biomed. Res. Trace Elem 4(2):61-2, 1993.

Other Human Clinical Studies

- 6(E)(28) Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. Journal of Viral Hepatitis 8:367-371, 2001.