GR

ORIGINAL SUBMISSION

000001

FLAMM ASSOCIATES
622 Beachland Blvd.
Vero Beach, FL 32963
Tel: 561-234-0096
Fax: 561-234-0026

November 19, 1998

Laura M. Tarautino, Ph.D.
Office of Pre-Market Approval (HFS-200), CFSAN
Food and Drug Administration
20 C St. S.W.
Washington, DC 20204

Dear Dr. Tarautino:

The undersigned, W.G. Flamm and G.A. Burdock, on behalf of Bonlac Foods Limited, submit this notification pursuant to the proposed rule entitled, "Notification of a claim for exemption based on a GRAS determination," 62 FR 18938-189, April 17, 1997. Attached hereto, in triplicate, is the information notifiers are to submit in accordance with the proposed regulation, 21 CFR 170.36(c).

Sincerely,

W.G. Flamm, Ph.D.

G.A. Burdock, Ph.D.
Burdock and Associates, Inc.
622 Beachland Blvd.
Vero Beach, FL 32963
Tel: 561-234-0053
Fax: 561-234-0096

1998 NOV 23 A 1
000002

NOTICE OF A CLAIM FOR EXEMPTION
BASED ON
A GRAS DETERMINATION FOR
CALCIUM CASEIN PEPTONE-CALCIUM PHOSPHATE

1. GRAS claim.

a. Notifier.

W. G. Flamm, Ph. D., F.A.C.T.
Flamm Associates
622 Beachland Blvd.
Vero Beach, FL 32963

and

G.A. Burdock, Ph.D., D.A.B.T.
Burdock and Associates, Inc.
622 Beachland Blvd.
Vero Beach, FL 32963

Agents for:

Bonlac Foods Limited
Level 7, 636 St. Kilda Road
Melbourne Vic., Australia 3004
PO Box 4313 Melbourne
Victoria, Australia 3001

b. Common name of the notified substance.

Calcium casein peptone-calcium phosphate.

c. The conditions of use.

The calcium casein peptone-calcium phosphate complexes would be used as texturizer added to chewing gum at a use level up to 5% wt/wt. The recognition of the use of casein as a texturizer in chewing gum dates back to the 1930's (Kleinfeld and Dunn, Trade Correspondence, 1938-1949, Vol. 1, Federal Food, Drug, and Cosmetic Act, pg. 596). Calcium phosphate (dibasic) is also recognized as a texturizer under the proposed GRAS affirmation regulation, 21 CFR 184.1214. A 3 gram portion of chewing gum would

provide up to150 mg of the calcium casein peptone-calcium phosphate complex or 2.14 mg/kg bw/serving for a 70 kg person.

d. The basis for the GRAS determination.

Calcium casein peptone-calcium phosphate has been determined GRAS on the basis of scientific procedures. (See attached - OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CALCIUM CASEIN PEPTONE-CALCIUM PHOSPHATE.)

e. Availability of information.

Data and information that are the basis for this GRAS determination are avaliable for the Food and Drug Administration's (FDA) review and copying at reasonable times at the offices of Burdock & Associates, Inc., 622 Beachland Blvd., Vero Beach, Florida, or will be sent to the FDA upon request.

2. Detailed information about the identity of the substance.

a. Identity

The common name (description) of this substance is: Calcium casein peptone with calcium phosphate. This substance is a fraction of a casein tryptic digest (casein peptone, CAS RN 977027-88-5) containing a mixture of casein phosphopeptides (CPP) in combination with amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2xH_2O$, CAS RN 7758-87-4).

CPP consists of a combination of four peptides and their truncated derivatives. The four primary CPP in the preparation are as follows:

Biochemical Name	Structure
α_{S1}-casein-5P(f59-79)	Gln-Met-Glu-Ala-Glu-Ser(*P*)-Ile-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ile-Val-Asn-Ser(*P*)-Val-Glu-Gln-Lys
β-casein-4P(f1-25)	Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(*P*)-Leu-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser-Ile-Thr-Arg
α_{S2}-casein-4P(f1-21)	Lys-Asn-Thr-Met-Glu-His-Val-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser-Ile-Ile-Ser(*P*)-Gln-Glu-Thr-Tyr-Lys
α_{S2}-casein-5P(f46-70)	Asn-Ala-Asn-Glu-Glu-Glu-Tyr-Ser(*P*)-Ile-Gly-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser(*P*)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys

The two major peptides of the preparation are: α_{s1}-casein-5P(f59-79) and β-casein-4P(f1-25)

b. Method of manufacture.

Calcium casein peptone-calcium phosphate is produced by the enzymatic hydrolysis of casein to form casein peptone, which is then complexed with amorphous calcium phosphate to form a calcium casein peptone-calcium phosphate complex as described in the following table.

Method of Manufacture – Calcium casein peptone-calcium phosphate		
Sequence number and operation	Narrative	Materials and additives*
1 – Preparation of casein peptone.	The casein is dissolved in deionized water at 10% w/v and hydrolyzed by the addition of trypsin at 0.05% w/v at 50°C for 2 hours at pH 8.0.	Deionized water, casein, trypsin.
2 - pH adjustment of the casein peptone solution.	The pH of the casein peptone solution is adjusted to 9 0 by the addition of sodium hydroxide.	Sodium hydroxide
3 – Formation of calcium casein peptone-calcium phosphate complexes.	The calcium casein peptone-calcium phosphate complexes are formed by the addition of calcium chloride and disodium hydrogen phosphate. Sodium hydroxide is added as necessary to maintain the pH at 9.0.	Calcium chloride, disodium hydrogen phosphate, sodium hydroxide.
4 – Purification of the calcium casein peptone-calcium phosphate complexes.	The calcium casein peptone-calcium phosphate clusters that form are then purified by diafiltration with three to five volumes of deionized water.	Diatomaceous earth, deionized water.
*All materials used are foods or ingredients cited in 21 CFR for the purposes employed.		

c. Specifications for food grade material.

Item	Specifications
Appearance	White powder
Moisture	<5% w/w
CPP content	40-60% w/w
Calcium	13-20% w/w
Inorganic phosphate (PO4)	20-30% w/w
The pH of aqueous solution	7.0-9.0
Foreign taste and odor	Free
Objectional matter	Free
Standard plate count	<5000 cfu/g
Molds & yeasts	<100 cfu/g
Coliforms	Negative cfu/g
Salmonella	Negative in 25 g
Arsenic, As	<1 ppm
Cadmium, Cd	<1 ppm
Lead, Pb	<1 ppm
Heavy Metals	<15 ppm

3. Information on any self-limiting levels of use.

Limited by virtue of use in chewing gum only.

4. The determination that calcium casein peptone-calcium phosphate is GRAS is on the basis of scientific procedures.

See attached - OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CALCIUM CASEIN PEPTONE-CALCIUM PHOSPHATE.

5. Signatures of agents for the notifier.

W.G. Flamm, Ph.D., F.A.C.T.
Flamm Associates

11/19/98
Date:

G.A. Burdock, Ph.D., D.A.B.T.
Burdock and Associates, Inc.

19 Nov 98
Date

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CALCIUM CASEIN PEPTONE-CALCIUM PHOSPHATE

Introduction

The undersigned, an independent panel of recognized experts (hereinafter referred to as Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Bonlac Foods Limited, to determine the Generally Recognized as Safe (GRAS) status of calcium casein peptone-calcium phosphate for use in chewing gum. A comprehensive search of the scientific literature for safety and toxicity information on calcium casein peptone-calcium phosphate was conducted through November 1997, and was made available to the Expert Panel. In addition, the Expert Panel reviewed materials submitted by the sponsor and other materials deemed appropriate or necessary. The members of the Expert Panel independently evaluated these materials, conferred by telephone and unanimously agreed to the decision described herein.

Data and information that are the basis for this GRAS determination are available for review and copying at reasonable times at Burdock & Associates, Inc; 622 Beachland Boulevard, Vero Beach, FL 32963.

The common name (description) of this substance is: Calcium casein peptone-calcium phosphate. This substance is a fraction of a casein tryptic digest (casein peptone, CAS RN 977-27-88-5, 21 CFR 184.1553) containing a mixture of casein phosphopeptides (CPP) in combination with amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2xH_2O$, CAS RN 7758-87-4, 21 CFR 182.1217).

CPP consists of a combination of four peptides and their truncated derivatives. The four primary CPP in the preparation are as follows:

Biochemical Name	Structure
α_{S1}-casein-5P(f59-79)	Gln-Met-Glu-Ala-Glu-Ser(*P*)-Ile-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ile-Val-Pro-Asn-Ser(*P*)-Val-Glu-Gln-Lys
β-casein-4P(f1-25)	Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(*P*)-Leu-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser-Ile-Thr-Arg
α_{S2}-casein-4P(f1-21)	Lys-Asn-Thr-Met-Glu-His-Val-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser-Ile-Ile-Ser(*P*)-Gln-Glu-Thr-Tyr-Lys
α_{S2}-casein-5P(f46-70)	Asn-Ala-Asn-Glu-Glu-Glu-Tyr-Ser(*P*)-Ile-Gly-Ser(*P*)-Ser(*P*)-Ser(*P*)-Glu-Glu-Ser(*P*)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys

The two major peptides of the preparation are: α_{S1}-casein-5P(f59-79) and β-casein-4P(f1-25). Minor modifications of the peptides can occur such as slight truncation, deamidation and oxidation.

[1]Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (*curriculum vitae)* documenting the expertise of the Panel members.

Specifications are as follows:

Item	Specifications
Appearance	White powder
Moisture	<5% w/w
CPP content	40-60% w/w
Calcium	13-20% w/w
Inorganic phosphate (PO4)	20-30% w/w
PH of aqueous solution	7.0-9.0
Foreign taste and odor	Free
Objectional matter	Free
Standard plate count	<5000 cfu/g
Molds & yeasts	<100 cfu/g
Coliforms	Negative cfu/g
Salmonella	Negative in 25 g
Arsenic, As	<1 ppm
Cadmium, Cd	<1 ppm
Lead, Pb	<1 ppm
Heavy Metals	<15 ppm

Calcium casein peptone-calcium phosphate is manufactured from food grade casein using the food grade enzyme trypsin, pharmaceutical grade calcium chloride and pharmaceutical grade disodium hydrogen phosphate. The casein is dissolved in deionized water at 10% w/v and then hydrolyzed by the addition of food grade trypsin at 0.05% w/v at 50°C for 2 hours at pH 8.0 to produce a casein peptone. The pH of the casein peptone is adjusted to 9.0 by the addition of food grade NaOH and then calcium chloride ($CaCl_2xH_2O$) is added to 100 mmol/L and disodium hydrogen phosphate (Na_2HPO_4) to 60 mmol/L maintaining the pH at 9.0 by the addition of food grade NaOH. The calcium casein peptone-calcium phosphate clusters that spontaneously form are then purified by diafiltration with three to five volumes of deionized water. The calcium casein peptone-calcium phosphate represents approximately 10% w/w of the total casein peptone.

Although there is no current use of calcium casein peptone-calcium phosphate as such, the component parts (casein peptone and calcium phosphate) are approved for use as follows:

Calcium caseinate. Calcium caseinate is considered GRAS for use as an optional ingredient under the food standards of identity for ice cream and frozen custards (21 CFR 135.110) and sherbet (21 CFR 135.140).
Casein peptones. Casein peptones are GRAS affirmed for the direct addition to human foods (21 CFR 184.1553). Casein peptones have no limitations other than current good manufacturing practice and may be used as *nutrient supplements* (170.3(o)(20)), *processing aids* (170.3(o)(24)) and *surface active agents* (170.3(o)(29)).
Calcium phosphate. Calcium phosphate is listed as a multipurpose GRAS food substance (21 CFR 182.1217) with no limitation other than current good manufacturing practice.

The proposed use of calcium casein peptone-calcium phosphate is as a texturizer (21 CFR 170.3(o)(32) to be used at levels up to 5% w/w in chewing gum (21CFR 170.3(n)(6)).

CPP has been isolated from the intestinal content of rats and pigs fed a high casein diet. CPP can also be formed *in vitro* by trypsin digestion and to some extent by pepsin-digestion of casein (Hansen *et al* Pediatric Research 40(4):547-552,1996). Calcium casein and casein would

be expected to be rapidly digested to casein peptides that would be indistinguishable from those in calcium casein peptone-calcium phosphate. Based on the National Academy of Sciences (NAS) exposure estimates reported in FDA's PAFA data-base, daily consumption of casein and calcium casein as food ingredients by a 70 kg person is 1750 mg and 1204 mg. At a use level of 5% w/w in a 3 gram serving of chewing gum (21 CFR 101.12), approximately 150 mg of calcium casein peptone-calcium phosphate would be added to the diet, only a small fraction of current consumer exposure to casein peptides.

Methods for the analysis of the calcium casein peptone-calcium phosphate involve capillary electrophoresis and high performance liquid chromatography techniques as well as amino acid composition and sequence analytical methods (Adamson *et al* Journal of Chromatography 646:391-396, 1993; Reynolds *et al* Analytical Biochemistry 217:277-284, 1994).

Although there were no reported studies on the toxicity of CCP-CP, its safety can be inferred from a long history of safe use of the components, from the critical evaluations of FASEB and FDA and from nutritional/pharmacological/physiological studies.

Studies of varying lengths have been conducted in rats, pigs, chickens and humans in an attempt to determine the degree of calcium absorption and that of other ions, effects on mineral balance, bone density, shell thickness, *et cetera* (Goto *et* al Journal of the Japanese Society of Nutrition and Food Science 48(3):195-202, 1995; Hansen *et al* Pediatric Research 40(4):547-552, 1996; Nakajima *et al* Proceedings of the 7th AAAP Animal Science Congress, Bali, Indonesia pp. 153-154, 1994). Lee *et al* (Journal of the Japanese Society of Nutrition and Food Science 45(4):333-338,1992) conducted two experiments on the effects of dietary CPP on the calcium balance, breaking force of the femur and soluble calcium contents of the small intestine of young male Sprague Dawley rats. In experiment 1, weaned rats were fed on a control diet (isolated soya protein, ISP; 20%) or a CPP diet (ISP; 17.1, CPP; 3.5% CPP/Ca (w/w); 10) for 4 or 8 weeks, respectively. In experiment 2, rats were fed on a control or a CPP diet (CPP/Ca; 0.35, 2.5, 5.0, 7.5 and 10.0) for 4 weeks. All diets contained the same amounts of calcium (0.35%) and phosphorus (0.7%). A calcium balance study was performed at the 4th or 8th week. At the conclusion of the study, it was found that body weight gains were not significantly different among the groups. Calcium balance was increased to a greater extent in the CPP than in the control groups at both the 4th and 8th weeks, with decreased excretion of fecal calcium. An increase in calcium balance and plasma calcium concentration within the physiologically normal range was observed in rats fed on CPP with a weight ratio of CPP/Ca of more than 0.35. However, the dose-dependent increase in these values was not significant. The wet weight and calcium contents of the femur increased in the CPP groups, with no changes in the breaking force. The amount of calcium in the luminal contents of the small intestine 2 hours after CPP feeding was higher than those in the control rats. The authors (Lee *et al*, *ibid*, 1992) concluded that CPP supplementation may have a significant effect on calcium availability, at least under conditions of lower dietary calcium.

Kitts *et al* (1992) designed a study to determine possible influences of CPP and calcium on blood pressure of hypertensive rats. The first goal was to determine if CPP-induced enhancement of paracellular Ca absorption would have an effect on blood pressure and the second goal to determine if CPP contained any peptide sequences associated with hypertension. ^{45}Ca absorption and temporal systolic blood pressure measurements were recorded in spontaneously hypertensive and normotensive rats fed on casein and soybean protein isolate (soyprotein) diets, containing 0.5, 5 and 20 g Ca/kg. Animals were on diet for two weeks at the start of data gathering, which

continued for about 6 weeks total. Calcium absorption from the small intestine was measured by an *in situ* ligated intestinal loop technique on the final day of the experiment, at 14 weeks of age. Similar measurements were also taken in spontaneously hypertensive rats fed only casein and 5 g Ca/kg, or soyprotein and 5 g Ca/kg diets supplemented with 30 g casein phosphopeptides (CPP)/kg. The investigators found that absorption of ^{45}Ca from the ileal loop was equivalent in both types of rats and was largely affected by the level of dietary calcium. In addition, animals fed on casein diets exhibited significantly ($P<0.05$) greater ileal absorption of ^{45}Ca compared with the soyprotein-fed animals. This result was attributed to the presence of CPP in the casein and a greater ($P<0.05$) proportion of soluble ^{45}Ca in the contents of the ileum. Animals fed on soyprotein diets supplemented with CPP confirmed this finding. As expected, the systolic blood pressure of spontaneously hypertensive rats was higher ($P<0.01$) than normotensive controls after 9-10 weeks of age, confirming that the development of hypertension in spontaneously hypertensive rats was independent of dietary influence. The severity of hypertension in the spontaneously hypertensive rats was affected only by dietary calcium deficiency, and not by calcium supplementation or CPP enhancement of calcium bioavailability. These findings suggest that tryptic digestion products of casein in milk can enhance calcium bioavailability by increasing its solubility; however, this action had no effect in reducing hypertension in spontaneously hypertensive rats. (Kitts *et al* Br. J. Nutr. 68(3):765-781, 1992). Nagasawa *et al* (Nutrition Research 11:819-830, 1991) reported similar enhancement of paracellular calcium uptake in casein and milk protein concentrate fed (normotensive) rats, and no differences were noted in either systolic or mean blood pressure compared to soy protein-fed controls.

Unlike other peptides in casein, CPP are not associated with opioid angonistic or antagonistic activity, inhibition of angiotensin converting enzyme or immunomodulatory effects (Meisel, Biopolymers 43(2):119-128, 1997).

In an *in vitro* experiment with pig oocytes and boar sperm and CPP in the incubation medium at the time of fertilization, there was an enhanced uptake of Ca^{2+} by the spermatozoa resulting in higher fertilization rates, enhanced penetration rates of oocytes by spermatozoa and, in the case of pre-incubation with CPP, the spermatocytes retained the ability to penetrate oocytes for a longer time than spermatozoa preincubated without CPP (Mori *et al* Theriogenology 39(1):272,1993; Mori *et al* Biology of Reproduction 55(2):364-369,1996).

The Select Committee on GRAS Substances (SCOGS) reviewed the scientific literature on protein hydrolysates published from 1920 to 1977 and supplemental material on enzymatic casein hydrolysates. The SCOGS concluded that "no evidence in the available information on enzymatically hydrolyzed casein demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used as a nutrient in special dietary foods at levels that are now current or that might reasonably be expected in the future" (SCOGS-37-b-suppl., LSRO, FASEB, 1980). FDA has undertaken its own evaluation of all available information on protein hydrolysates and enzymatically hydrolyzed casein and "concurs with the conclusions of the Select Committee that these substances are GRAS". (Federal Register 48(237):54992, December 8, 1993).

Conclusion

Based upon a critical evaluation and analysis of the information summarized above, the Expert Panel determined that calcium casein peptone-calcium phosphate, meeting the specifications given above and produced by current good manufacturing practice (21 CFR 182.1(b)), to be generally recognized as safe (GRAS) by scientific procedures as a texturizer in chewing gum at a level up to 5% w/w.

Signatures:

Joseph F. Borzelleca, Ph.D., F.A.T.S.	19 Nov 1998 Date
George A. Burdock, Ph.D., D.A.B.T.	18 Nov 98 Date
W.Gary Flamm, Ph.D., F.A.C.T.	11/18/98 Date

SUBMISSION END

000013

[redacted]