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ORIGINAL SUBMISSION

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WRITER'S DIRECT ACCESS

August 13, 2003

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**John S. Eldred**  
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**Via Overnight Delivery**

Dr. Laura Tarantino  
Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, Maryland 20740-3835

**Re: GRAS Notification for calcium gluconate, for use as a calcium supplement in various foods**

Dear Dr. Tarantino:

Pursuant to proposed 21 C.F.R. § 170.36(c) and on behalf of our client, Purac Biochem b.v., we hereby notify the agency of our determination on the basis of scientific procedures that calcium gluconate is generally recognized as safe (GRAS) when used as a nutrient supplement, as a source of the essential mineral calcium, in various foods. As with all GRAS substances, this compound, when used in this application, is exempt from the premarket clearance requirement applicable to food additives under section 409 of the Food, Drug, and Cosmetic Act.

We trust you will find the enclosed notification acceptable. Should any questions arise during the review process, please do not hesitate to contact us, preferably by telephone, so that we may respond as quickly as possible.

Sincerely,

John S. Eldred

Enclosure (GRAS Notification in triplicate)

cc: Ton van Dongen, Purac Biochem b.v.

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Before the  
FOOD AND DRUG ADMINISTRATION  
Department of Health and Human Services  
Washington, D.C.

**GRAS NOTIFICATION**

Name of Notifier: PURAC Biochem b.v.

Post Office Address: All communications on this matter are to be sent in care of Counsel for the Notifier, John S. Eldred, Keller and Heckman LLP, 1001 G Street, N.W., Suite 500 West, Washington, D.C. 20001.

Telephone: (202) 434-4176

Name of Substance and Intended Use: Calcium gluconate, for use as a nutrient supplement in various foods.

Dated: August 13, 2003

John S. Eldred  
Counsel for PURAC Biochem b.v.

000003

**I. Claim of GRAS Status**

**A. Name and Address of Notifier:**

PURAC Biochem b.v.  
Arkelsedijk 46, P.O. Box 21  
4200 AA Gorinchem  
The Netherlands

**All communications on this matter are to be sent in care of Counsel for the Notifier:**

John S. Eldred  
Keller and Heckman LLP  
1001 G Street, N.W., Suite 500 West  
Washington, D.C. 20001.

Telephone: (202) 434-4176

**B. Common or Usual Name of the Notified Substance:**

The common or usual name of the substance that is the subject of this notification is calcium gluconate.

**C. Applicable Conditions of Use:**

Calcium gluconate is intended for use as a nutrient supplement, supplying the essential mineral calcium, in a variety of foods, including but not limited to milk, soft drinks, juices, (near) waters, dairy products, soy products, baked goods, and confectionery.

**D. Basis for GRAS Determination:**

The described use of calcium gluconate has been shown to be generally recognized as safe (GRAS) on the basis of scientific procedures, in accordance with 21 C.F.R. § 170.30, as discussed more fully in the accompanying summary of the basis for the GRAS determination.

**E. Statement of Availability of Data:**

The data and information that are the basis for the GRAS determination are available for the Food and Drug Administration's review and copying or will be sent to FDA upon request.

\* \* \* \* \*

The foregoing and attached information considered, it is respectfully submitted that the use of calcium gluconate for use in various foods as a nutrient supplement is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because it is generally recognized as safe.

Respectfully Submitted,

PURAC Biochem b.v.

By:

John S. Eldred  
Keller and Heckman LLP

COUNSEL FOR THE NOTIFIER

## II. Identity of the Notified Substance

### A. Chemical Name

The Chemical Abstract Name: D-Gluconic acid, calcium salt (2:1)

Additional Chemical Names: Calcium gluconate; calcium D-gluconate

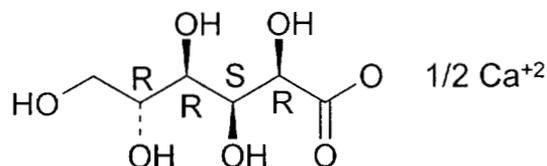
### B. Chemical Abstract Registry Number:

299-28-5 (monohydrate)

18016-24-5 (anhydrous)

C. Chemical Formula:  $C_{12}H_{22}CaO_{14}$

### D. Structure: (R & S Refer to Configuration)



E. Formula Weight 430.4 (anhydrous)

### F. Physical Properties

Calcium gluconate, anhydrous or the monohydrate, is an odorless white free flowing powder. It is soluble in water but not in alcohol or most organic solvents.

### G. Stability of Calcium Gluconate

Calcium gluconate is stable in air.

### H. Manufacturing Process

In the traditional process, glucose is converted to gluconic acid by a standard aerobic fermentation process. As an alternative, glucose may be converted to gluconic acid using glucose oxidase in the presence of oxygen. The hydrogen peroxide formed in this process is degraded to oxygen and water by the enzyme catalase. A third method utilizes sodium gluconate or glucono-delta-lactone as starting material. Sodium gluconate is converted to gluconic acid by cation exchange. Glucono-delta-lactone is converted to gluconic acid by hydrolysis. The gluconic acid is neutralized to calcium gluconate with calcium carbonate or calcium hydroxide. Calcium gluconate is crystallized as calcium gluconate monohydrate and then dried, or the calcium gluconate solution is directly dried to form calcium gluconate anhydrous.

Calcium gluconate is further commercially available in blends with other GRAS mineral salts, such as mineral lactates, gluconates, citrates, chlorides, phosphates, etc. These mixed salts can be produced either by dry mixing or liquid mixing of the separate salts. Liquid mixing of the separate salts is achieved by preparing a solution or slurry with the separate salts (*in situ* formulated or dissolution of the dry salts), then dried quickly to prevent crystallization and to create an amorphous mixed salt. The obtained mixed salt offers advantages in the application such as increased solubility or improved flavor.

In all manufacturing options outlined above, calcium gluconate is prepared under current Good Manufacturing Practices (cGMP) using food-grade raw materials where available or otherwise using materials of suitable purity and quality for their intended use.

### **I. Specifications for Calcium Gluconate**

Specifications for the monohydrate and the anhydrous product are provided in Appendix 1. The purity meets the requirements of the Food Chemicals Codex, 4<sup>th</sup> ed. and the U.S. Pharmacopoeia 26 (2003).

### **III. Level of Use and Intended Technical Effect**

Calcium gluconate has already been affirmed as GRAS for a number of food uses. The subject use of this Notification is as a nutrient supplement, to help ensure attainment of the Daily Reference Value (DRV) for calcium. FDA has established a DRV for calcium of 1000 mg for adults and children more than 4 years of age (21 C.F.R. § 101.9(c)(8)), but the Institute of Medicine, National Academy of Sciences, has recommended increasing this value to 1300 mg for children ages 9 to 18 and 1200 mg for those 51 years and older.<sup>1</sup> Calcium gluconate will be added to foods that are currently being fortified with other calcium compounds. Such foods may include, but are not limited to, milk, soft drinks, juices, (near) waters, dairy products, soy products, baked goods, and confectionery.

On an anhydrous basis, calcium gluconate is about 9.3% calcium. Thus, if the only source of calcium in the diet were calcium gluconate, an intake of about 10.75 g would be needed to attain the DRV for calcium. Practically, due to taste and texture considerations, the presence of endogenous calcium compounds in the diet, and the likely presence of other calcium additives, foods would rarely, if ever, be fortified with calcium gluconate at this level. A more realistic level would correspond to 10-35% of the DRV per serving.

Depending on the desired properties of the fortification, calcium gluconate may be fortified as a mixture with other GRAS calcium substances, such as calcium lactate or calcium citrate. It should be appreciated that, due to the ionic nature of these compounds, no chemical reaction will occur between calcium gluconate and calcium lactate or calcium citrate, but simply formation of the mixed salt through ionic and non-covalent chemistry, which results in improved

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<sup>1</sup> Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC. 1997.

water solubility.<sup>2</sup> HPLC, <sup>13</sup>C-NMR, and IR spectra comparing calcium gluconate lactate and a physical mixture of calcium gluconate and calcium lactate were obtained. A summary is provided in Appendix 2; full reports are available upon request. The spectra demonstrate that the products are equivalent and that the only major organic acids present are gluconic acid and lactic acid.

#### **IV. Summary of the Basis for a Conclusion that Calcium Gluconate is GRAS for its Intended Use**

Calcium gluconate has been affirmed as GRAS under 21 C.F.R. § 184.1199 for a number of uses: (1) as a firming agent, as described in 21 C.F.R. § 170.3(o)(10); (2) as a formulation aid, as described in 21 C.F.R. § 170.3(o)(14); (3) as a sequestrant, as described in 21 C.F.R. § 170.3(o)(26); (4) as a stabilizer or thickener, as described in 21 C.F.R. § 170.3(o)(28); and (5) as a texturizer, as described in 21 C.F.R. § 170.3(o)(32). According to 21 C.F.R. § 184.1199(d), calcium gluconate may be used in foods at levels not to exceed current good manufacturing practice, in accordance with 21 C.F.R. § 184.1(b)(1). Current good manufacturing practices result in a maximum level, as served, of 1.75% for baked goods, 0.4% for dairy product analogues, 4.5% for gelatins and puddings, and 0.01% for sugar substitutes.<sup>3</sup>

Prior to FDA's GRAS affirmation for calcium gluconate, the compound had been listed as GRAS in 21 C.F.R. Part 182 as a sequestrant in § 182.6199 and as a multiple purpose GRAS food substance in § 182.1199. The latter listing would have provided explicit GRAS status for use as a nutrient supplement, but there was no reported use of this calcium compound for this purpose when the affirmation proposal issued on February 16, 1979,<sup>4</sup> and only the known uses were affirmed as GRAS. However, since that time, fortification with calcium gluconate as a means of obtaining the recommended calcium intake has become desirable for certain applications.

Many other calcium salts are listed as GRAS (Part 182) or affirmed as GRAS (Part 184) for uses that include use as a nutrient supplement. Calcium phosphate is both a multiple purpose GRAS food substance (21 C.F.R. § 182.1217) and GRAS as a nutrient (21 C.F.R. § 182.8217). Calcium pyrophosphate is GRAS as a nutrient (21 C.F.R. § 182.8223). Calcium carbonate (21 C.F.R. § 184.1191), calcium citrate (§ 184.1195), calcium hydroxide (§ 184.1205), calcium oxide (§ 184.1210), and ground limestone (§ 184.1409) have been affirmed as GRAS with no limitations other than GMP. Calcium glycerophosphate (§ 184.1201), calcium lactate (§ 184.1207), and calcium pantothenate (§ 184.1212) have been affirmed as GRAS as nutrient supplements.

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<sup>2</sup> Calcium gluconate lactate is also referred to as calcium gluconolactate. There are also commercial formulations of the mixed gluconate, lactate, and carbonate salts. We note that a combination of calcium gluconate, calcium lactate, calcium citrate, and other salts is approved as optional ingredients in the manufacture of artificially sweetened fruit jelly, preserves, and jams. See 21 C.F.R. §§ 150.141; 150.161.

<sup>3</sup> § 184.1199(d).

<sup>4</sup> 44 Fed. Reg. 10078.

Other calcium salts are listed as GRAS or affirmed as GRAS for various uses other than as nutritional supplements. Such uses include anticaking agents, chemical preservatives, sequestrants, and thickeners.

Certain gluconate salts, other than calcium gluconate, are also listed as GRAS in Part 182 or affirmed as GRAS in Part 184. Sodium gluconate is GRAS as a sequestrant in § 182.6757. Zinc gluconate is GRAS as a nutrient in § 182.8988. Copper gluconate (§ 184.1260), ferrous gluconate (§ 184.1380), and manganese gluconate (§ 184.1452) are affirmed as GRAS as nutrient supplements. Glucono delta lactone, which in solution is in equilibrium with gluconic acid, has been affirmed as GRAS (§ 184.1318) for a number of uses, but not as a nutrient supplement.

Because calcium gluconate is ionic, calcium and gluconate ions may be considered separately.

#### **A. Calcium**

As noted above, calcium gluconate is intended to provide a portion of the dietary reference value for calcium. Typical fortification levels would correspond to 25% of the DRV of 1000 mg Ca. Assuming that 250 mg of Ca is provided by calcium gluconate, the amount of calcium gluconate ingested would be about 2.7 g. The gluconic acid salt of calcium is an efficient means for introducing a significant fraction of the DRV for calcium, currently 1,000 mg, due to the increased solubility of this salt relative to calcium carbonate. In this regard, there is no question that ingestion of calcium is safe at the levels considered for supplementation. In fact, it is thought that the average calcium intake in this country is below the adequate intake (AI) of 1000-1300 mg, as recommended by the NAS.

In its 1997 report, the NAS identified three possible adverse effects of excessive calcium intake: kidney stone formation (nephrolithiasis), hypercalcemia and renal insufficiency, and possible reduced absorption of other essential minerals. Considering these effects, the NAS determined an upper limit (UL) of 2,500 mg for adults 19 through 70 years and recommended the same UL for toddlers, children and adolescents ages 1 through 18 years and for older adults >70 years. A UL was not established for infants up to 12 months of age. As stated above, calcium gluconate fortification is expected to supply about 250 mg calcium in the daily diet. It is most unlikely that the UL will be exceeded due to this level of fortification.

#### **B. Gluconate**

Gluconic acid and gluconate salts, including calcium gluconate, were evaluated in reports of the Federation of American Societies for Experimental Biology, Life Sciences Research Office (FASEB). More recently the toxicity profile has been discussed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

## 1. FASEB Evaluations

As reported in FDA's GRAS Affirmation proposal for calcium acetate, chloride and gluconate,<sup>5</sup> FASEB concluded that "no evidence in the available information on calcium acetate, calcium chloride, calcium gluconate, and calcium phytate demonstrates or suggests reasonable grounds to suspect a hazard to the public when those substances are used at levels that are current or that might reasonably be expected in the future."<sup>6</sup> In addition, the 1978 FASEB report on gluconate salts discussed gluconate metabolism, and concluded that gluconate was a normal product of glucose oxidation in mammals.<sup>7</sup> The daily production of gluconate from endogenous sources was estimated to be about 450 mg/kg for a 60 kg person, which amounts to 27 g or more than ten times the estimated daily intake of gluconate from the proposed use of calcium gluconate as a calcium supplement. "The body can easily cope with this load since only a small amount of 6-phosphogluconate is found in the liver (approximately 10 mg/kg). This reflects the efficiency of the liver in the conversion of gluconate."<sup>8</sup> FASEB concluded that "Evidence suggests that any possible toxicity [of gluconate salts] is a function of the cation rather than of the gluconate portion of these substances."<sup>9</sup>

## 2. JECFA Evaluation

The safety of glucono-delta-lactone and the calcium, magnesium, potassium, and sodium salts of gluconic acid was evaluated in the Fifty-first meeting of JECFA.<sup>10</sup> In aqueous media, glucono-delta-lactone is in equilibrium with D-gluconic acid.

Two new 28-day ingestion studies in rats were evaluated in the JECFA report. In the first study, groups of 12 male and 12 female Sprague-Dawley rats were given sodium gluconate by gavage at doses of 0, 500, 1000, or 2000 mg/kg bw/day. No gluconate-related effects were observed in urinalysis or hematological parameters. Statistically significant increases were noted in the relative weights of the kidneys of males at 1000 and 2000 mg/kg bw/day and in the absolute weights of the adrenal glands of males at 1000 mg/kg bw/day, but these differences were not dose related. The only treatment-related histopathological effect reported was an increased incidence of thickening of the limiting ridge of the stomach in 5/12 males receiving 2000 mg/kg bw/day, but, as the limiting ridge is specific to rodents, this effect is not considered toxicologically significant in humans. This effect, apparently, was not observed in the subsequent dietary study.

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<sup>5</sup> 44 Fed. Reg. 10078 (Feb. 16, 1979). FDA's decision to affirm calcium gluconate as GRAS was based on the 1975 FASEB Report PB-254 539, Evaluation of the Health Aspects of Certain Calcium Salts as Food Ingredients, as well as the later 1978 FASEB report on gluconate salts, discussed below.

<sup>6</sup> Id., at page 10079.

<sup>7</sup> FASEB (1978). Evaluation of the Health Aspects of Sodium, Potassium, Magnesium and Zinc Gluconates as Food Ingredients. National Technical Information Service Report PB-288 675.

<sup>8</sup> Id., at page 7.

<sup>9</sup> Id., at page 9.

<sup>10</sup> JECFA. Safety Evaluation of Certain Food Additives. WHO Food Additive Series: 42. World Health Organization, Geneva, 1999.

In the second study, conducted in the same laboratory, groups of 10 male and 10 female rats were fed a diet containing sodium gluconate at doses equivalent to 0, 1200, 2000, or 4100 mg/kg bw/day for males and 0, 1000, 2000, or 4400 mg/kg bw/day for females. Occasional significant differences in urinalysis or hematological parameters were not considered treatment-related. Histopathologic examination did not reveal any treatment related-changes. JECFA concluded that neither of these two studies was suitable for establishing a no-observed-effect level, primarily because of the small group sizes.

On the basis of re-evaluation of data previously considered as well as the new studies, JECFA extended the previous ADI "not specified" for glucono-delta-lactone to a group ADI for glucono-delta-lactone and the calcium, magnesium, potassium, and sodium salts of gluconic acid.

### 3. Other Considerations

As reported in JECFA, manganese gluconate tested negative in the Ames assay. In a study by Litton Bionetics, reported in TOXLINE, sodium gluconate also was negative in the Ames assay.

Calcium gluconate is the preferred intravenous preparation for the treatment of hypocalcemia.<sup>11</sup>

In water, glucono-delta-lactone (GDL) is in equilibrium with D-gluconic acid, and under acidic conditions is completely converted to D-gluconic acid. Hence, the safety of glucono-delta-lactone is directly relevant to the safety of D-gluconic acid. The substance has been affirmed as GRAS under 21 C.F.R. § 184.1318. The safety of GDL was evaluated in a 1981 FASEB report.<sup>12</sup> This report summarizes a 29 month feeding study designed to study the effects of nitrites in cured meats. In that study, groups of 30 male and 30 female Wistar rats received a diet of 40% meat, , 40% meat treated with 0.5% sodium nitrite and 1% GDL, 40% meat treated with 0.02% sodium nitrite and 1% GDL and 40% meat treated with 1% GDL. Rats receiving GDL added to meat in the diet did not differ from controls with respect to growth, feed intake, mortality and histopathology. GDL was not mutagenic in the Ames assay (*S. typhimurium* strains TA 1535, TA 1537 and TA 1538) or in *Saccharomyces cerevisiae* with or without metabolic activation.

### C. Clinical Studies

A number of clinical studies involving calcium gluconate or mixed salts of calcium, such as calcium lactate gluconate (calcium gluconolactate), and calcium lactate gluconate carbonate (calcium gluconolactate-carbonate) have recently been carried out. Although these studies are

<sup>11</sup> Levenson, D.I. and Bockman, R.S. (1994). A review of calcium preparations. *Nutr Rev.* **52(7)**, 221-232.

<sup>12</sup> FASEB (1981) Evaluation of the Health Aspects of Glucono Delta-Lactone as a Food Ingredient. Report No. PB82-108663.

not classical toxicology studies, they do provide support that the use of calcium gluconate or mixed calcium salts are safe.

### 1. Single Dose Studies

Acute clinical studies on calcium gluconate or mixed calcium salts containing gluconate are generally designed to determine net calcium absorption. In the context of this Notification, they are useful in demonstrating that effects of ingestion of calcium gluconate do not differ qualitatively from effects of other calcium salts. The following studies have been published:

a) **Sheikh *et al.* (1987).**<sup>13</sup>

Eight healthy fasting men each received 500 mg calcium from 5 calcium salts and from milk. The 5 salts were calcium acetate, calcium lactate, calcium gluconate, calcium citrate, and calcium carbonate. The order of administration of the agents given was randomly determined. The differences in calcium absorption from all sources did not differ significantly. The authors suggest that acid dissolution in the gastrointestinal tract was responsible for the similar absorption.

b) **Marchandise *et al.* (1987).**<sup>14</sup>

48 subjects of various age and health received 12.4 mg/kg calcium pyrrolidone carboxylate (GPC) and 18.8 mg/kg calcium gluconate. Fractional absorption of calcium from GPC was higher by a factor of about 1.4 than corresponding absorption of the gluconate.

c) **Reginister *et al.* (1993).**<sup>15</sup>

In this study, 10 male volunteers received different preparations of calcium gluconolactate and carbonate (mixed calcium salt of lactate, gluconate, and carbonate), tricalcium phosphate, and calcium citrate. A 1000 mg dose of each salt, as calcium, was ingested at weekly intervals. All calcium supplements induced significant increases in serum calcium and suppression of parathyroid hormone (PTH). In this study, calcium citrate was found to induce a larger increase in serum calcium and decrease in serum PTH.

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<sup>13</sup> Sheikh, M.S., Santa Ana, C.A., Nícar, M.J., *et al.* (1987). Gastrointestinal absorption of calcium from milk and calcium salts. *N. Engl J. Med* **317(9)**, 532-536.

<sup>14</sup> Marchandise, X., Pagniez, D., Ythier, H., Gilquin, B., Duquesnoy, B., and Wemeau, J-L (1987). Influence of accompanying anion on intestinal radiocalcium absorption. *Calcif. Tissue Int* **40**, 8-11.

<sup>15</sup> Reginister, J.Y., Denis, D., Bartsch, V., Deroisy, R., Zegels, B and Franchimont, P. (1993). Acute biochemical variations induced by four different calcium salts. *Osteoporosis Int* **3**, 271-275

**d) Gonnelli et al. (1995).<sup>16</sup>**

In this study, 10 women received 1000 mg calcium in the form of calcium citrate, and 10 women received the same dose of calcium in the form of calcium gluconolactate and carbonate. Both calcium salts resulted in an increase of serum calcium and a decrease of PTH. In this study also, calcium citrate induced the larger increase in serum calcium and a smaller suppression of PTH, but the effects were statistically significant in both cases.

**e) Gonnelli et al. (1995).<sup>17</sup>**

This study was similar to the previous one, except that there were two groups of 8 women. Results were similar.

**f) Deroisy et al. (1997).<sup>18</sup>**

Eighteen male volunteers received one day per week a control tablet containing no calcium, calcium carbonate (500 mg Ca), calcium gluconolactate and carbonate (500 mg Ca), calcium citrate (500 mg Ca), calcium pidolate and carbonate (500 mg Ca), and an ossein-hydroxyapatite complex (516 mg Ca). Each of the 5 calcium salts induced significant increases in serum calcium and decreases in serum PTH compared to the controls. There were no statistically significant differences in serum calcium elevation from the 5 calcium salts, but the carbonate and the citrate salts induced a greater decrease in PTH.

**g) Praet, et al. (1998).<sup>19</sup>**

This study compared calcium absorption of calcium citrate in soluble and solid form and calcium gluconolactate-carbonate in 15 young and 20 elderly women. All three salts of calcium resulted in increased serum calcium. Soluble calcium citrate produced the highest serum levels, followed by calcium gluconolactate-carbonate, and solid calcium citrate.

**h) Other**

In their summary article, Levenson and Bockman (1994)<sup>20</sup> compared the absorptive fractions of calcium for 9 different salts and milk in normal subjects. Although many different

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<sup>16</sup> Gonnelli, S., Cepollaro, C., Camporeale, A., Nardi, P., Rossi, S. and Gennari, C. (1995). Acute biochemical variations induced by two different calcium salts in healthy perimenopausal women. *Calci. Tissue Int* **57**, 175-177.

<sup>17</sup> Gonnelli, S., Nardi, P., Cepollaro, C., Monotomoli, M., Palumbo, F. and Gennari, C. (1995). Acute metabolic variations induced by two different calcium salts in healthy perimenopausal women. *Challenges of Modern Medicine* **7**, 279-284.

<sup>18</sup> Deroisy, R., Zartarian, M. et al (1997). Acute changes in serum calcium and parathyroid hormone circulating levels induced by the oral intake of five currently available calcium salts in health male volunteers. *Clinical rheumatology* **16**, 249-253.

<sup>19</sup> Praet, J P , Peretz, A., Mets, T. and Rozenberg, S. (1998). Comparative study of the intestinal absorption of three salts of calcium in young and elderly women. *J Endocrinol Invest* **21**, 263-267.

studies were referenced, the percent of calcium absorption was very similar, with the exception of calcium oxalate, which was much lower. Percent absorption varied from about 22% to 36%. Average absorption of calcium gluconate was reported to be 34%, close to the percentage for milk (33%). The results summarized by Levenson and Bockman are supported by Heaney *et al.* (1990), who demonstrated that the absorbability of calcium from different salts is only weakly related to the solubility of the salt at neutral pH.<sup>21</sup> Heaney studied 7 salts of calcium, but not the gluconate.

## 2. Longer Term Studies

### a) Patton *et al.* (1952).<sup>22</sup>

In an eight week study, nine women received 400 mg calcium in the form of the lactate, gluconate, sulfate, and carbonate salts. No significant differences in the utilization of calcium from these salts were found.

### b) Spencer *et al.* (1966).<sup>23</sup>

Eight subjects received 1,600 mg/day calcium as calcium gluconate or calcium lactate. The average period of administration was 45 days for the gluconate and 28 days for the lactate. In this study, calcium was better absorbed as the lactate than as the gluconate.

### c) Cappuccio *et al.* (1987).<sup>24</sup>

Eleven men and 7 women with untreated mild to moderate essential hypertension received calcium gluconolactate (400 mg Ca) for one month and a placebo for one month in a double blind, randomized crossover study. A significant increase in urinary and plasma calcium and a decrease in urinary phosphate were observed in the treatment phase, but there was no effect on blood pressure.

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<sup>20</sup> Levenson, D.I. and Bockman, R.S. (1994). A review of calcium preparations. *Nutr. Rev* **52(7)**, 221-232.

<sup>21</sup> Heaney, R.P., Recker, R.R., and Weaver, C.M. (1990). Absorbability of calcium sources: the limited role of solubility. *Calcif. Tissue Int* **46**, 300-304.

<sup>22</sup> Patton, M.B. and Sutton, T S (1952). The utilization of calcium from lactate, gluconate, sulfate and carbonate salts by young college women. *J Nutrition* **48**, 443-52

<sup>23</sup> Spencer, H., Scheck, J., Lewin, I And Samachson, J. (1966). Comparative absorption of calcium gluconate and calcium lactate in man. *J Nutrition* **89**, 283-292.

<sup>24</sup> Cappuccio, F P., Markandu, N.D., Singer D R.J. *et al* (1987). Does oral calcium supplementation lower high blood pressure? A double blind study *J. Hypertension* **5**, 67-71.

**d) Lau et al. (1992).<sup>25</sup>**

Fifty Chinese women, ages 62-92, were randomly selected to enter one of 4 groups. Two of the 4 groups received 800 mg calcium (as calcium gluconolactate), one group with exercise and one group without exercise, for 10 months. Significant increases in bone mineral density (BMD) were observed in subjects on the calcium supplement, and PTH levels declined. The albumin-adjusted calcium levels increased significantly in the subjects on the calcium supplements, but no significant changes in urinary calcium/creatinine and hydroxyproline/creatinine ratios were observed.

**e) Kohls and Kies (1992).<sup>26</sup>**

Two groups of 10 subjects received about 500 mg calcium for 28 days from various calcium compounds: oyster shell, calcium gluconate, calcium carbonate, dolomite (calcium magnesium carbonate), calcium lactate, a mixed calcium-vitamin D preparation, and skim milk. Each substance was administered for 10 days. Mouth-to-anus transit times were lengthened with oyster shell and calcium carbonate, but did not significantly differ among the other supplements. There were no major differences in absorption among the various calcium supplements. Gastrointestinal complaints were registered with milk, calcium carbonate and calcium lactate, but not the gluconate.

**f) Antoniazzi et al. (1999).<sup>27</sup>**

In this study, 40 girls, affected by central precocious puberty and treated with gonadotropin-releasing hormone agonists (GnRHa), were assigned to 1 of 3 groups. The first group received solely GnRHa for 24 months, the second group received GnRHa for 12 months and then supplementation with calcium gluconolactate and carbonate (1 g as calcium) for another 12 months, and the third group received the combined treatment for 24 months. BMD declined in those in the first group, declined for one year in the second group and then increased, and increased in the third group. The increase in the third group was statistically significant relative to the first group, which received no calcium supplement.

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<sup>25</sup> Lau, E.M.C., Woo, J., Leung, P.C., Swaminathan, R. and Leung, D. (1992). The effects of calcium supplementation and exercise on bone density in elderly Chinese women. *Osteoporosis Intl.* **2(4)**, 168-173.

<sup>26</sup> Kohls, K.J. and Kies, C. (1992). Calcium bioavailability: a comparison of several different commercially available calcium supplements. *J Appl Nutr* **44**, 50-61.

<sup>27</sup> Antoniazzi, F., Bertoldo, F., Lauriola, S. et al. (1999). Prevention of bone demineralization by calcium supplementation in precocious puberty during gonadotropin-releasing hormone agonist treatment. *Journal of Clinical Endocrinology & Metabolism* **84(6)**, 1992-1996.

g) **Deroisy et al. (2002).**<sup>28</sup>

One hundred post-menopausal women, having serum 25(OH)D levels below 18 ng/mL were randomly assigned for a duration of 90 days to daily supplementation of either one tablet of calcium gluconolactate and carbonate (500 mg Ca) or a mixture of calcium carbonate (500 mg Ca), citric acid, and cholecalciferol (200 IU) (CaD group). In this study, PTH levels were not significantly reduced in the calcium-only group but were reduced in the CaD group. During the 90 days of the study, 2 patients in the CaD group and 3 patients in the Ca group withdrew from the study; these early discontinuations were not drug-related. Compliance with the two treatments was not significantly different.

**3. Conclusions from Clinical Studies**

The results of single and longer term treatment with calcium gluconate or mixed salts of calcium with gluconate demonstrate that there is nothing unique about calcium gluconate that would somehow preclude its use as a nutrient supplement.

**D. Consumer Exposure**

As noted above, calcium gluconate is intended to provide a portion of the dietary reference value for calcium. Typical fortification levels would correspond to 25% of the DRV of 1000 mg Ca. Assuming that 250 mg of Ca is provided by calcium gluconate, the amount of calcium gluconate ingested would be about 2.7 g.

**V. Conclusion**

Due to the demonstrated safe history of use of calcium gluconate and other similar calcium salts, the desirability of increased calcium in the diets of most people, and the lack of any safety concerns over gluconic acid, PURAC Biochem b.v. respectfully concludes that use of calcium gluconate as a nutrient supplement is Generally Recognized as Safe, as demonstrated through scientific procedures.

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<sup>28</sup> Deroisy, R., Collette, J., Jupsin, A.I. and Reginster, J-Y (2002) Administration of a supplement containing both calcium and vitamin D is more effective than calcium alone to reduce secondary hyperparathyroidism in postmenopausal women with low 25(OH)vitamin D circulating levels. *Aging Clin Exp Res* 14, 13-17

APPENDIX 1

Specifications

# Gluconates

## Product Data Leaflet



GLUCONA

## Gluconal® CAA-P-IN

### Chemical name

Calcium gluconate anhydrous; (CAS-No.: 18016-24-5).  
Intended for use in the preparation of injectable dosages.

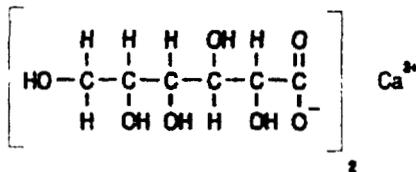
### Molecular formula

$C_{12}H_{22}O_{14}Ca$

### Molecular mass

430.4

### Structure



### Specifications

Appearance	: white, free flowing powder	
Assay	: 98.0 - 102.0%	(1)
Calcium content	: 8.8 - 9.5%	(2)
Loss on drying	: 3.0% max.	(1)
Chloride content	: 50 ppm max.	(1)
Sulphate content	: 50 ppm max.	(1)
Phosphate	: 100 ppm max.	(1)
Oxalate	: 100 ppm max.	(1)
Magnesium and alkali metals	: 0.4% max.	(1)
Iron	: 5 ppm max.	(1)
Heavy metals content	: 10 ppm max.	(1)
Lead	: 1 ppm max.	(1)
Arsenic content	: 3 ppm max.	(1)
pH (10% solution)	: 6.0 - 8.2	(1)
Reducing substances	: 1.0% max.	(1)
Colour of 10% solution	: colourless	(3)
Total aerobic viable count	: 1,000 cfu/g max.	(3)
Organic volatile impurities	: passes test	(1)
Yeasts	: 10 cfu/g max.	(3)
Moulds	: 50 cfu/g max.	(3)
Enterobacteriaceae	: absent in 1 g	(3)
Salmonella	: absent in 50 g	(3)
Endotoxins	: 170 EU/g max.	(1)

Purity meets the requirements of the United States Pharmacopoeia 24. Gluconal CAA-P-IN complies with the requirements of the California Proposition 65.

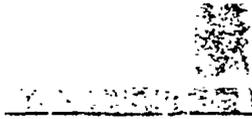
- (1) Method of analysis according to USP 24.  
(2) Calculated from assay on as is basis.  
(3) Method of analysis available upon request.

® Registered trade mark.

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# Gluconates



GLUCONA

## Product Data Leaflet

### Gluconal® CAM-P-OR

#### Chemical name

Calcium gluconate monohydrate; (CAS-No: 299-28-5).  
Not intended for use in the preparation of injectable dosages.

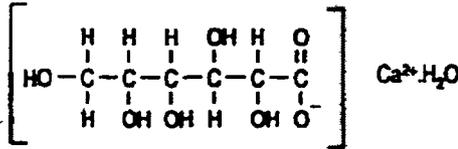
#### Molecular formula

$C_{12}H_{22}O_{14}Ca \cdot H_2O$

#### Molecular mass

448.4

#### Structure



#### Specifications

Appearance	: white free flowing powder	
Assay	: 98.5-102.0%	(1A, 1B)
Calcium content	: 8.8-9.1%	(2)
Loss on drying	: 2.0% max.	(1A, 1B)
Chloride content	: 200 ppm max.	(1C-EP)
Sulphate content	: 100 ppm max.	(1C-EP)
Magnesium and alkali metals	: 0.4% max.	(1A)
Heavy metals content	: 10 ppm max.	(1A, 1B)
Lead	: 1 ppm max.	(1A, 1B)
Arsenic content	: 3 ppm max.	(1A, 1B)
Reducing substances	: 1.0% max.	(1A, 1B)
Organic volatile impurities	: passes test	(1A)
Total aerobic count	: 1,000 cfu/g max.	(3)
Yeasts	: 10 cfu/g max.	(3)
Moulds	: 50 cfu/g max.	(3)
Enterobacteriaceae	: absent in 1 g	(3)
Salmonella	: absent in 50 g	(3)

#### United States Pharmacopoeia and European Pharmacopoeia (monograph 172)

Purity meets the requirements of the United States Pharmacopoeia 24 and the European Pharmacopoeia 3<sup>rd</sup> edition. Gluconal CAM-P-OR complies with the requirements of the California Proposition 65.

- (1A) Method of analysis according to USP 24.
- (1B) Method of analysis according to EP 3<sup>rd</sup> edition
- (1C) Meets the narrowest specifications
- (2) Calculated from assay on as is basis.
- (3) Method of analysis available upon request.

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APPENDIX 2

**Summary of Analyses**



Chemicals Division  
Research Centre Deventer

MEMORANDUM

Reference: MUL/KOI  
Doc code : RCD 922-1004  
Date : 15-07-1992

Department : Analytical department  
From : <sup>O</sup>  
Name : R.J. Mulder  
Signature:

To : See distributionlist

File RCM

Page 1 of 1

Subject

CALCIUM LACTOGLUCONATE  
ANALYSIS

Herewith I send you three (3) reports, giving the evidence that calcium lactogluconate, a spraydried solution of calciumgluconate and calcium-lactate in a molar ratio of 2:1 merely consists of calciumgluconate and calciumlactate.

Below the essentials of the reports are briefly described.

- **E.P. Geluk, Spraydried calcium lactogluconate vs a physical mixture under acidic conditions. Doc. code: RCD 924-0645; date: 09-07-1992.**

In this report is shown that the spraydried product and the physical mixture exhibit the same free calcium ion concentration under acid condition (stomach).

So complex constants are not different, indicating that no new products have been formed during spraydrying.

- **J.C. Speelman, Calcium lactogluconate, physical mixture versus spray-dried product. Doc. code: RCD 914-727; date: 17-06-1991.**

No spectroscopic differences are observed in solution (<sup>13</sup>C-NMR) and in solids (Diffuse Reflectance IR spectroscopy and solid state C<sup>13</sup>-NMR). Thin Layer chromatography according to the USP method for calcium-gluconate shows no differences either.

These findings strongly support the identicalness of both products.

- **M. de Lange and F.A. Buytenhuis, Calcium lactogluconate analysis. Doc. code: CR4 F92081; date: 24-06-1992.**

Unambiguous evidence for the identicalness of both products was obtained from HPLC under acidic conditions, using UV and RI as detection. The chromatograms were identical in all aspects. No reaction product(s) could be observed with this sensitive technique in the calcium lactogluconate.

SUBMISSION END

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