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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date: 05/13/2016.
CDER stamp date: 05/16/2016.
Product: Calcium Gluconate Injection, USP 10%.
Indication: Acute treatment of symptomatic hypocalcemia
(b) (4)
Applicant: Fresenius Kabi USA, LLC.
Review Division: Division of Metabolism and Endocrine Products.
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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	5
1.1	INTRODUCTION	5
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	5
1.3	RECOMMENDATIONS	7
2	DRUG INFORMATION	8
2.1	DRUG	8
2.4	COMMENTS ON NOVEL EXCIPIENTS	9
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	9
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	14
2.7	REGULATORY BACKGROUND	15
3	STUDIES SUBMITTED	16
3.1	STUDIES REVIEWED	16
4	PHARMACOLOGY	18
4.2	SAFETY PHARMACOLOGY	19
4.3	PHARMACOKINETICS	19
5	PHARMACODYNAMIC DRUG INTERACTIONS	24
6	GENERAL TOXICOLOGY	25
6.1	SINGLE-DOSE TOXICITY	25
6.2	REPEAT-DOSE TOXICITY	27
7	GENETIC TOXICOLOGY	30
8	CARCINOGENICITY	30
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	30
10	LOCAL TOLERANCE	32
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	32
12	APPENDIX/ATTACHMENTS	35
12.1	LITERATURE CITATIONS	35
12.2	APPLICANT SUGESSTED LABELING	40

Table of Tables

Table 1: Composition of Calcium Gluconate Injection, USP, 10% per 1000 mL	9
Table 2: Regulatory Specification for Calcium Gluconate Injection, USP 10% - Release and Stability Testing	10
Table 3: Aluminum Exposure at the Maximum Recommended Doses	12
Table 4: Key Meetings/Correspondence between the Division and the Applicant	15
Table 5: List of Nonclinical Studies from Published Literature	16
Table 6: Mean plasma total calcium, ionized calcium, inorganic phosphate, sodium and potassium values (mmol/L) and pH after IV injection of calcium gluconate	23
Table 7: Acute Toxicity in Mice	26
Table 8: Genotoxicity of Gluconic Acid and it Derivatives	30

Table of Figures

Figure 1: Calcium Distribution in Mammals20
Figure 2: Calcium Concentrations over Time in Bone, Muscle and Tissue after IV
Infusion21
Figure 3. Glomerular Filtration Rate, Urine Volume and Fractional Urinary Excretion of
Select Ions before and after IV Injection of Calcium Gluconate22
Figure 4. Percent Calcium Elimination over Time with 10, 20 and 40% Protein Diets ...24

1 Executive Summary

1.1 Introduction

Fresenius Kabi USA, LLC (the Applicant) seeks to market Calcium Gluconate Injection, USP 10% (Calcium Gluconate Injection) via the 505(b)(2) regulatory pathway. Calcium Gluconate Injection is intended for the treatment of acute, symptomatic hypocalcemia. Each milliliter of drug product contains 94 mg of calcium gluconate, 4.5 mg of calcium saccharate tetrahydrate (b) (4) hydrochloric acid and/or sodium hydroxide for pH adjustment (6.0-8.2) and water for Injection USP, q.s. Each milliliter of Calcium Gluconate contains 9.3 mg elemental calcium (i.e., 0.465 mEq/L calcium). The maximum recommended intravenous doses proposed are (b) (4)

The actual dose is dependent upon the serum calcium level of individual patients. The Applicant conducted no nonclinical studies, but instead seeks to rely on nonclinical data available in the published scientific literature.

1.2 Brief Discussion of Nonclinical Findings

Mechanism of Action, Metabolism and General Toxicity

Many vital cellular biological processes, such as blood coagulation, neuromuscular excitability, maintenance of cell membrane integrity, and cellular homeostasis are dependent on maintenance of adequate free ionized plasma calcium concentrations (Jain, 2010; Zaloga, 1992).

Calcium gluconate metabolism is limited to the gluconate component of the salt, as ionized calcium itself does not undergo direct metabolism. Gluconate is a normal product of glucose metabolism. The daily production of gluconate from endogenous sources is estimated to be about 450 mg for a 60 kg person, which amounts to 27 g, (b) (4)

(American Societies for Experimental Biology, FASEB, 1975). As an endogenous compound formed in large amounts, the general toxicological concern for gluconate is very low.

The potential for toxicity with Calcium Gluconate Injection is therefore directly related to the degree of elevated serum calcium above the normal range. The primary toxicities associated with hypercalcemia in laboratory animals are soft tissue mineralization (especially in the kidneys), hypercalciuria, nephropathy, weight loss (due to decreased food consumption), altered bone metabolism, decreased clotting time, abnormal heart rhythms and neurologic effects (altered behavior).

Mutagenic and Carcinogenic Potential

Calcium Gluconate Injection has not been evaluated in lifetime rodent carcinogenicity studies. However, calcium gluconate has been shown to be negative in mutagenicity

assays conducted in bacteria and yeast. Exposure to calcium in Calcium Gluconate Injection for short-term treatment of symptomatic hypocalcemia is considered unlikely to contribute meaningfully to any long-term carcinogenic risk. Glucono-delta-lactone (a neutral cyclic ester of gluconic acid in equilibrium with gluconic acid in solution) did not induce compound-related tumors in a 2 year feeding study in rats. Therefore, Calcium Gluconate Injection is not considered to represent a significant carcinogenic risk for its intended use.

Reproductive Toxicology

No animal reproduction studies were conducted with Calcium Gluconate Injection. Adequate calcium levels are expected to be beneficial during pregnancy. Glucono-delta-lactone administration had no effect on implantation or on maternal or fetal survival in mice, rats, hamsters and rabbits at the amounts studied (**SIDS Initial Assessment Report for SIAM 18 Paris, France, 20-23 April 2004**). Calcium Gluconate Injection use during pregnancy is therefore considered to represent a low risk for reproduction toxicity in the absence of hypercalcemia.

Excipients/Co-APIs

Calcium Gluconate Injection contains calcium saccharate (b) (4). Calcium saccharate is not listed on FDA's IIG list of excipients included in approved drugs. However, it is listed in USP Monograph for calcium gluconate therefore has been widely used clinically in the unapproved product. At least one approved and marketed injectable product produces exposures to saccharate expected to be greater than those from Calcium Gluconate Injection at the maximum recommended human dose. In addition, saccharate is a normal product of glucose metabolism formed in small amounts by mammals, including humans. No significant toxicological concern for saccharate was identified.

Impurities of Toxicological Concern

Mined calcium mineral (the source of calcium in Calcium Gluconate Injection) may naturally contain a variety of metal impurities, which may vary widely by source. Gluconate is an efficient chelator of calcium as well as metal impurities. The metal of toxicological concern identified for Calcium Gluconate Injection, based on the proposed product specifications submitted with the NDA, was aluminum. Aluminum is potentially toxic to the CNS and bone with exposure by the intravenous route, especially in patients with poor renal function, including neonates.

In response to Agency's concern, the Applicant revised the proposed aluminum specification, lowering the aluminum limit for the Calcium Gluconate Injection drug product (b) (4) to 400 mcg/L. The Applicant provided an updated finished product specification to reflect the new aluminum limit and is revising labeling to reflect the new aluminum limit. The new limit for aluminum of 400 mcg/L is acceptable.

No container leachables above the threshold of toxicologic concern were identified.

1.3 Recommendations

1.3.1 Approvability

This NDA is recommended for approval from Pharm/Tox perspective.

1.3.2 Additional Non Clinical Recommendations

The product labeling should clearly state the aluminum levels in Calcium Gluconate Injection to inform the risk of off-label use for calcium supplementation/replacement therapy (e.g., via total parenteral nutrition).

1.3.3 Labeling

Reviewer's Recommended Labeling:

INDICATIONS AND USAGE

(Established Pharmacologic Class)

Calcium Gluconate Injection is indicated for (b) (4)

8 Use in Specific Populations

8.1 Pregnancy

Risk Summary (b) (4)

8.2 Lactation

Risk Summary (b) (4)

12 Clinical Pharmacology

12.1 Mechanism of Action

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)
Calcium gluconate was not mutagenic in *S. typhimurium* strains TA-1535, TA-1537, and TA-1538, or in *S. cerevisiae*.

2 Drug Information

2.1 Drug

Name

Calcium Gluconate Injection, USP 10%.

CAS Registry Number

18016-24-5.

Generic Name

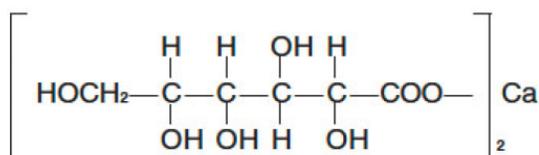
Calcium gluconate.

Chemical Name

Calcium D-gluconate (1:2) monohydrate.

Molecular Formula/Molecular Weight

$C_{12}H_{22}CaO_{14} \cdot H_2O/448.39$.

Structure or Biochemical Description**Pharmacologic Class**

Calcium

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 113171 - Calcium gluconate, Fresenius Kabi

(b) (4)

NDA 022581 - Calcium acetate, Fresenius Medical

NDA 021117 - Calcium chloride, Hospira

DMF (b) (4) - Calcium gluconate, (b) (4)

2.3 Drug Formulation

The Applicant's proposed Calcium Gluconate Injection drug product (currently marketed, but unapproved) is a super saturated solution of calcium gluconate, containing 98.0 mg/mL calcium gluconate monohydrate and 4.5 mg/mL calcium saccharate (tetrahydrate) to obtain the labeled claim of 9.317 mg/mL of calcium. Other components of the Calcium Gluconate Injection product are described in the table, below.

Table 1: Composition of Calcium Gluconate Injection, USP, 10% per 1000 mL

Name of ingredients	Content	Function	Quality of ingredient
Calcium Gluconate, monohydrate	98.0 mg/mL	API	USP
Calcium Saccharate, tetrahydrate	4.5 mg/mL	(b) (4)	USP
Water for injections	(b) (4)	(b) (4)	USP
Hydrochloric acid	qs	pH adjustment	NF
Sodium hydroxide	qs	pH adjustment	NF

The drug product is (b) (4) by the Fresenius Kabi USA, LLC Grand Island, NY facility.

2.4 Comments on Novel Excipients

The excipients, including amount, used in the proposed Calcium Gluconate Injection product are exactly the same as those in Fresenius Kabi's currently marketed unapproved Calcium Gluconate Injection, USP. Calcium Saccharate is listed in the USP monograph for Calcium Gluconate Injection, USP, but is not listed on the Agency's IIG (b) (4)

(b) (4) Iron sucrose (iron saccharate), a U.S. approved injectable product) is administered intravenously produces greater sucrose/saccharate exposure than from Calcium Gluconate Injection at the maximum recommended human dose. Like gluconic acid (gluconate), D-glucaric acid (saccharate) is produced naturally in small amounts by mammals, including humans and glucarate and its lactone forms (D-glucaro-1,4-lactone and D-glucaro-6,3-lactone) are end-products of the D-glucuronic acid pathway. Glucarate is transported in the blood to a number of different organs in Sprague-Dawley rats (Walaszek, 1997). Hydrochloric Acid and Sodium Hydroxide are listed in FDA's IIG list for Intravenous use. The amounts of these excipients are low as they are only used for pH adjustment.

2.5 Comments on Impurities/Degradants of Concern

No degradants of toxicological concern have been identified. The Applicant assayed six lots of Calcium Gluconate Injection USP Finished Product for 24 elemental impurities. All 24 elemental impurities are listed in ICH Q3D. The analytical results of the six (6) expired representative batches which were tested showed that all the listed elements in the ICH Q3D are (b) (4) % of the permissible daily exposure (PDE).

The aluminum content of Calcium Gluconate Injection (b) (4) was identified as a toxicological concern. The Applicant committed to lower the aluminum limit to 400 mcg/L. The Applicant committed to providing an updated method validation report and Method of Analysis (MOA) in the first week of June, 2017. The Applicant provided an updated finished product specification to reflect the new aluminum limit (see below). The Applicant is revising labeling to reflect the new aluminum limit.

The following specification for Calcium Gluconate Injection, USP 10% was submitted to FDA on April 26, 2017. This replaces the one submitted with the original NDA.

Table 2: Regulatory Specification for Calcium Gluconate Injection, USP 10% - Release and Stability Testing

Test	Acceptance Criteria	Test Method ¹
Visual Inspection		
1. Description	1. Liquid in a plastic vial	USP <1> 10-08-05-6005
2. Clarity	2. Clear	
3. Particulate Matter	3. Essentially free from visible particulate	
4. Container / Closure	4. Container is intact	
5. Visual Color	5. Colorless to slightly yellow	10-08-05-6005
Assay		
Assay (100 mg/mL) (9.317 mg Ca/mL)	(b) (4)% of Label Claim	USP
Gluconate Assay	(b) (4)% of label claim	10-08-03-6981
Saccharate Assay	(b) (4)% of label claim	10-08-03-6981
pH	6.0 - 8.2	USP <791>
Aluminum	NMT 400 µg/L	03-08-03-6037 OR PDR-ATM-APF-0009 ²
Identification ³		
1. Gluconate	(b) (4)	USP
2. Calcium		USP <191>
Test	Acceptance Criteria	Test Method ¹

Degradation Products			
Any Unspecified Impurity	NMT	(b) (4) %	10-08-03-6981
Total Degradation Products	NMT	(b) (4) %	10-08-03-6981
Container Closure Integrity ⁴			
(b) (4)			
Volume Check ³	A.	NLT (b) (4)	(Product Code 360019) USP <1>
	B.	NLT (b) (4)	(Product Code 360059)
	C.	NLT (b) (4)	(Product Code 360161)
Weight Loss ⁵	NMT	(b) (4) %	99-08-04-6001
Particulate Matter	A.	For particles (b) (4) μm :	NMT (b) (4) per container USP <788> <i>Method I</i>
	B.	For particles (b) (4) μm:	NMT (b) (4) per container
Sterility ³	Sterile		USP <71>
Bacterial Endotoxins	NMT (b) (4) EU/mg		USP <85>
Osmolality ³	(b) (4) mOsm/kg		USP <785> 10-08-03-6017 ³
Other Requirements	Meets the requirements under USP <1>		USP <1>
Statement of Compliance to USP <467> for material from Global Calcium	This finished drug product complies to the USP <467> General Chapter for Residual Solvents (b) (4)		

¹ References to compendia signify current compendia.

² Preferred method. Performed by Catalent Pharma Solutions.

³ Tested only at release.

⁴ CCIT is not a release test. Please note that the Container/Closure Integrity test is performed at 12 and 24 months in accordance with the February 2008 Guidance for Industry: Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol of Sterile Products.

⁵ Testing to be performed for the first three commercial lots only.

Test	Acceptance Criteria	Test Method ¹	Test
Microbial Bioburden	A. Total Aerobic Microbial Count:	NMT (b) (4) CFU/g	USP<61>
	B. Total Yeast and Mold:	NMT (b) (4) CFU/g	
	C. Total Combined Bioburden:	NMT (b) (4) CFU/g	
Bacterial Endotoxins	NMT (b) (4) EU/mg		USP <85>

¹ References to compendia signify current compendia.

² Testing to be performed by (b) (4)

2.5.1 Aluminum

The Applicant's noncompendial test specifications for the drug product (i.e., NMT 400 mcg/L aluminum) are based on the highest aluminum levels observed in their own historical test data. All results through 12 or 18 months have met this criterion.

The following tables provide information on the amounts of aluminum exposure at the maximum recommended doses for adults, infants and pediatric patients.

Table 3: Aluminum Exposure at the Maximum Recommended Doses

Aluminum Exposures at the MRHD for Calcium Gluconate Injection*				
Patient population	MRHD Ca Gluconate	Al dose at MRHD	Al limit**	Patient Al exposure compared to limit***
Adults (e.g., 60 kg)	(b) (4)	(b) (4)	5 mcg/kg/day	< 1-fold
Children ~1 year (e.g., 10 kg) to 17 years				
Infant (e.g., 2.5 kg)				

*Calcium Gluconate, 10% USP is prepared as a 10 mL vial; each milliliter of the product contains 100 mg calcium gluconate and up to 0.4 mcg aluminum. Therefore, one 10 mL vial of Calcium Gluconate Injection contains 1000 mg calcium gluconate and up to 4 mcg Aluminum.

**The exposure limit for aluminum is based repeated daily exposures in a sensitive population (e.g., patients with renal failure and neonates).

***Based on kg patient body weight.

CFR21 part 201 subpart 201-323 specifies that the amount of aluminum for small volume parenteral drug products shall be stated in product labeling. The proposed specification for aluminum in Calcium Gluconate Injection is 400 mcg/L. At the maximum recommended doses of Calcium Gluconate Injection, the daily aluminum exposure will be less than 5 mcg/kg/day, which is the acceptable daily intake (ADI) (**CFR21 part 201 subpart 201-323**). The ADI for intravenous exposure is 5 mcg/day, based on the observation that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 5

mcg/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration, which further supports inclusion of the aluminum levels in product labeling.

2.5.2 Leachables

On May 10, 2017, CMC informed the Pharm/Tox team that they had identified 3 potential leachables from extractable studies, (b) (4) but that none was detected at the limit of detection (LOD) of 0.20 mcg/mL. See the table, below.



While none of these potential leachables was detected in the drug product, if the amounts of (b) (4) were in fact present at the limit of detection, those levels can be compared to the maximum acceptable intake limits set for the indicated treatment duration as specified by ICH M7, titled *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk Guidance for Industry*. At the maximum indicated daily adult dose of (b) (4) patients may be exposed to a maximum of (b) (4) of each leachable. Acceptable

intake per ICH M7 for acute/short treatment durations (i.e., ≤ 1 month) for each leachable is 120 $\mu\text{g}/\text{day}$. The proposed use of the Calcium Gluconate Injection 10% USP is for acute or short-term durations and the maximum exposure of (b) (4) is below the limit of 120 mcg/day . Therefore the maximum theoretical amounts of each potential leachable is consistent with ICH M7 guidance limits and are acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Calcium Gluconate Injection is a super-saturated solution of calcium gluconate indicated for (b) (4). The dose of Calcium Gluconate Injection is dependent on the requirements of the individual patient. Calcium Gluconate Injection needs to be diluted with 5% dextrose or normal saline (b) (4) (b) (4) prior to administration in infants, pediatric and adult patients.

The proposed label recommends the following dosage for adults:



The proposed label recommends the following dosage in pediatric patients and neonates:



The proposed label indicated the following contraindications:

- [REDACTED] (b) (4)
- Concomitant use of ceftriaxone and intravenous calcium-containing products is contraindicated in neonates (28 days of age or younger).

2.7 Regulatory Background

The Applicant currently markets Calcium Gluconate Injection as an unapproved drug.

Key meetings and advice/information requests that took place during the pre-IND phase/Calcium Gluconate Injection development program are listed in the Table below.

Table 4: Key Meetings/Correspondence between the Division and the Applicant

Meeting Type	Meeting Date/Submission	Date Minutes/Correspondence Issued
Meeting Preliminary Comments	NA	November-15-2011 (PRE-IND MEETING PRELIMINARY COMMENTS)
Type B, PIND Meeting	NA	NA
Applicant Responses to FDA Preliminary Comments	NA	May-4-2012
Initial Pediatric Study Plan	Submitted on July-27-2015	October-22-2015 (Initial Pediatric Study Plan-Written Response)
Agreed Pediatric Study Plan	Submitted on March-18-2016	April-13-2016 (AGREED INITIATE PEDIATRIC STUDY PLAN)

The Applicant submitted an initial pediatric study plan (iPSP) on July 27, 2015 and received a written response from the FDA in which the FDA agreed:

- On the indication [REDACTED] (b) (4)
- No additional nonclinical studies appear necessary, and the justification for the efficacy, safety, and dosing recommendation for neonates could be supported from published literature on calcium gluconate.
- However, the Agency requested additional justification for the maximum daily dose for neonates.
- The Agency did not agree with the Applicant's original intentions [REDACTED] (b) (4). The [REDACTED].

Agency instead asked that the Applicant look for additional data to support the safety and efficacy as well as the maximum daily dose of the proposed product for pediatric patients.

The Applicant submitted an amendment to the PSP on January 18, 2016 in which additional justification on the maximum daily dose for neonates (b) (4) was provided. However, per Agency feedback, the Applicant (b) (4) submitted an agreed-iPSP on March 18, 2016 in which the Applicant committed to providing additional data to support the use of calcium gluconate in the pediatric population ages ≥ 1 month to 17 years. The available literature references were included in the NDA application. A copy of the AGREED INITIAL PEDIATRIC STUDY PLAN was also included in the NDA.

3 Studies Submitted

3.1 Studies Reviewed

The applicant submitted the following nonclinical publications from publically available literature sources to support the safety of Calcium Gluconate Injection (see Table below). While all publications were reviewed, only those relevant to the product's intended use are discussed hereafter.

Table 5: List of Nonclinical Studies from Published Literature

Source	Information Provided
Pharmacodynamics (Section 2.6.2)	
(Zaloga, 1992) (Braun, 2007) (Doze, 2008)	Mechanism of Action • Physiologic roles of calcium Efficacy in Animal Models • Treatment of hypocalcemia in lactating dairy cows
Pharmacokinetics (Section 2.6.4)	
(Cote', 1987)	Equivalence of calcium gluconate and calcium chloride with regards to ionization and cardiovascular effects in dogs
(Janle, 2000)	Distribution Distribution of Ca^{2+} after calcium gluconate infusion in sheep
(FASEB, 1978)	Metabolism • Metabolism of calcium gluconate in rats
(Maier, 1985) (Engelmann, 1975) (Wang, 1962) (Walaszek, 1997)	Excretion Calcium excretion following calcium gluconate administration in rats and pigs Gluconate and saccharate (glucarate) excretion in rats

Source	Information Provided
(Mandal, 1992) (Nola, 1970)	Pharmacokinetic Drug Interactions <ul style="list-style-type: none"> • Perturbation of tetracycline PK by induced-hypercalcemia in goats • Hypercalcemia potentiates arrhythmogenic effects of cardiac glycosides
Toxicology (Section 2.6.6)	
(Coulston, 1962) (FDA, 1974)	Single-Dose Toxicity <ul style="list-style-type: none"> • Intravenous LD₅₀ in mice Multiple-Dose Toxicity 6-month toxicity study in dogs
(Litton Bionetics Inc, 1974) (Litton Bionetics Inc, 1975)	Mutagenicity Calcium gluconate and glucono-delta-lactone are not mutagenic
(Dailey R, 1978) (van Logten, 1972) (Sigma-Aldrich, 2014)	Carcinogenicity Lack of carcinogenicity of glucono-delta-lactone in rats Calcium saccharate (calcium D-glucarate) not listed as a probable, possible, or confirmed human carcinogen
(Dailey R, 1978) (FDA, 1973)	Developmental and Reproductive Toxicology (DART) Gluconic acid had no effect on nidation or on maternal or fetal survival in mice, rats, hamsters, or rabbits Glucono-delta-lactone not teratogenic

Many of the above referenced studies were conducted prior to the implementation of GLPs, and the guidelines under which these studies were conducted are not in accordance with the current ICH guidelines and requirements. However, **OECD Screening Information Datasets (SIDS)** published a document on Gluconic Acid and its Derivatives. This document is the result of an initiative by Japanese and Belgian authorities. The industry consortium (**OECD IUCLID User Expert Panel**) collected and reviewed the existing data on nonclinical toxicology, conducted a literature search on calcium gluconate and published this document in (**The International Uniform Chemical Information Database, IUCLID**) under the OECD Cooperative Assessment Programme. The information provided in this document was used by this reviewer in addition to other information submitted to the NDA and additional literature.

The following sections focus on the review of relevant literature information provided in the above table.

Biological Role of Calcium

Calcium is the major extracellular divalent cation in mammals. More than 95% of total physiologic calcium is located within osseous tissues and the remaining amount of physiologic calcium is present in small amounts in extracellular fluids and to a minor extent within cells. In the plasma, about 45% of ionized calcium is protein bound, primarily to albumin, with about 10% being complexed with anionic buffers, such as

citrate and phosphate. The remaining fraction of “free” ionized calcium is considered to be the component that exerts physiological effects. Reduction of calcium below normal levels results in the manifestation of overt hypocalcemia symptoms. Many vital cellular biological processes, as well as cell membrane integrity and cell function, are known to be dependent upon maintenance of adequate ionized plasma calcium concentrations (**Jain, 2010; Zaloga, 1992**).

Under normal physiologic conditions, extracellular calcium levels are regulated by endocrine control; which affects calcium uptake at the level of the intestine and ultimate excretion at the level of the kidney with secondary renal associated regulation of compartmentalized calcium (within osseous tissues) enabling calcium release from sequestered stores in times of physiological need (**Cooper, 2008; Fong, 2012; Kelly, 2013; Zaloga, 1992**). Significant amounts of calcium are secreted in milk during lactation in mammals post-parturition.

The extracellular calcium concentration has three primary regulators: Calcium-sensing receptor (CaSR), parathyroid hormone (PTH), and vitamin D. The CaSR is a membrane-bound receptor found in multiple tissues, including cells of the parathyroid glands. When plasma ionized calcium concentrations are sufficient to stimulate the CaSR, the result is inhibited PTH release. When the ionized calcium concentration is low, PTH is released and is carried in blood to its target tissues: bone and kidney (**Zhou, 2009**). Intravenous administration of calcium gluconate in combination with Vitamin D has been observed to significantly increase bone mineralization in rabbits (**Lani, 2014**).

4 Pharmacology

The pharmacology of calcium gluconate is well characterized in the clinical setting and animal models, which has demonstrated a clear dose-response for increased plasma calcium and established the overall clinical efficacy of calcium gluconate in resolving hypocalcemia.

4.1 Primary Pharmacology

The pharmacodynamics of calcium gluconate in the treatment of hypocalcemic conditions have been demonstrated in studies in many animal models. Many of these studies involved the correction of parturient paresis, a condition in which animals (often observed in dairy cows) display reduced blood calcium levels which interfere with muscle function in the body. Hypocalcemia results in cramping and twitching of muscles or tetany (involuntary muscle contraction), among other symptoms. These symptoms were also treated effectively with other calcium salts (**Oetzel, 1988**).

4.2 Safety Pharmacology

Cardiovascular Safety

The risks of calcium gluconate injection for cardiovascular (CV), neurologic, and respiratory effects are essentially directly related to the plasma calcium levels. I.e., in the absence of hypercalcemia, there is no evidence of any risk of CV, neurologic, or respiratory liability.

In dogs and horses, the immediate effect of intravenous calcium administration at doses is an increase in cardiac output. Calcium gluconate has been administered safely to conscious horses at 0.1 to 0.4 mg/kg/min over a 15-minute interval and it resulted in improved cardiac function (**Grubb, et al. 1996**). In a dog model, administration of calcium gluconate was found to significantly increase coronary blood flow by 36%; coronary vascular resistance was reduced by a similar magnitude. Dogs were administered calcium chloride (4, 8, 12 mg/kg) or calcium gluconate (14, 28, 42 mg/kg) intravenously. No significant variations in mean arterial pressure (MA) following calcium gluconate administration were observed; MAP and cardiac output increased slightly for approximately 10 minutes. No significant alterations in cardiac rhythm were observed (**Cote', 1987**). Based on the results from these two animal models, it is unlikely to present a discernable cardiovascular safety risk at clinically relevant doses of intravenous calcium gluconate.)

The hemodynamic effects of calcium gluconate on conscious horses were investigated by intravenous administration by (**Grubb, et al., 1996**). All administration rates resulted in increases in both ionized and total calcium concentrations, cardiac index, stroke index, and cardiac contractility (dP/dt_{max}) relative to pre-infusion baseline. Mean arterial pressure and right atrial pressure were unchanged whereas heart rate decreased markedly during calcium administration. The authors concluded that calcium gluconate can safely be administered to conscious horses at 0.1 to 0.4 mg/kg/min and that administration resulted in improved cardiac function.

4.3 Pharmacokinetics

Absorption

The applicant product, Calcium Gluconate Injection is intended for intravenous administration and therefore the bioavailability of the Sponsor's product is presumed to be 100%.

Distribution

Calcium in the body is located within 3 primary compartments: i) osseous tissues), ii) soft tissues, and iii) extracellular fluids. Approximately 99% of total body calcium is located within the skeleton (as hydroxyapatite crystals), with the remainder being located within cellular membranes, mitochondria, the endoplasmic reticula (0.9%), as

well as within extracellular fluids (0.1%). In general, cytosolic free calcium concentrations are low (**Toribio et al., 2010**).

In equine blood, calcium is found in free or ionized form (50%–58%); bound to proteins (40%–45%); and complexed to anions, such as citrate, bicarbonate, phosphate, and lactate (5%–10%). Free ionized calcium (Ca^{2+}) is the biologically active form of calcium. Albumin is the main calcium-binding protein in plasma and its affinity for Ca^{2+} is pH-dependent. In acidosis there is decreased Ca^{2+} binding to albumin (higher free Ca^{2+}); whereas, in alkalosis the free Ca^{2+} concentrations are lower (**Toribio et al., 2010**).

Figure 1: Calcium Distribution in Mammals

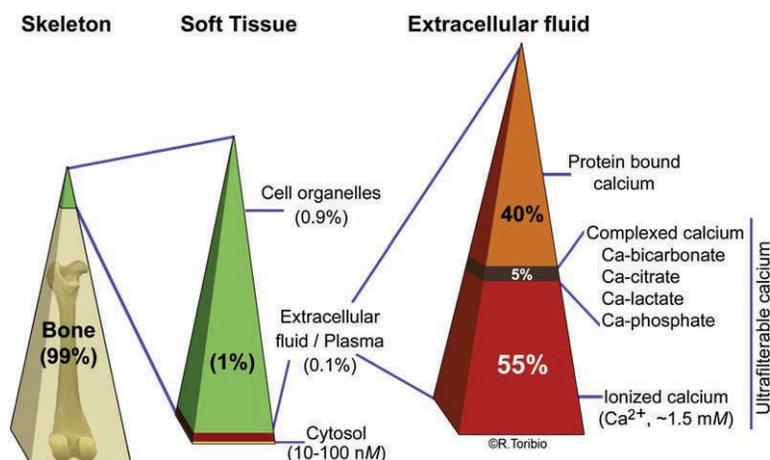
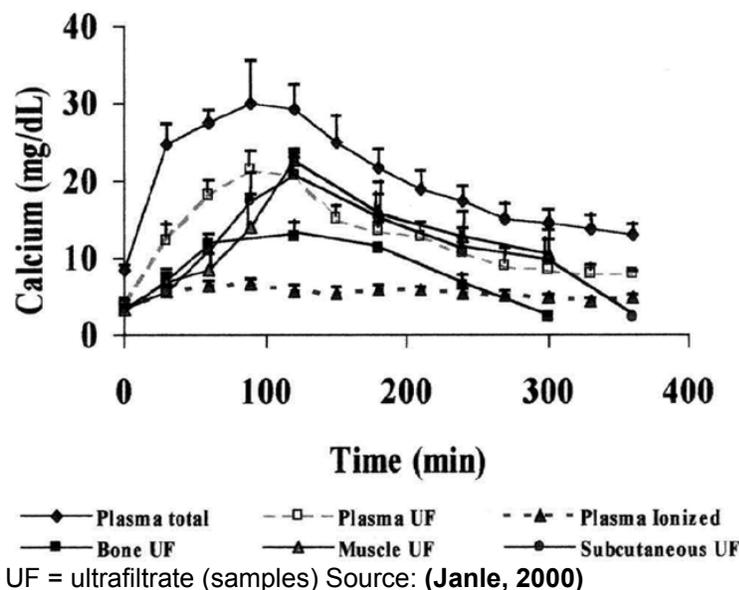


Fig. 1. Calcium distribution in the body. Around 99% of the total calcium in the body is in bone with the remaining 1% present in the extracellular fluid (0.1%) and cellular organelles (0.9%). In the extracellular compartment, calcium exists as a free, ionized or active form (Ca^{2+}), bound to proteins, and complexed to anions, such as bicarbonate, phosphate, citrate, and lactate. In horses, serum ionized calcium represents 50% to 58% of the total extracellular calcium. Ionized and complex calcium are filtered through the glomerulus (ultrafilterable) but are rapidly reabsorbed by the nephron. **Toribio, et al. (2010)**

The amounts of calcium and magnesium are usually measured in blood, urine, and fecal samples, which provides the only information on whole-body averages, but gives no indication of differences between tissues. (**Janle et al., 2000**) investigated the distribution of tissue calcium (gluconate) using ultrafiltration probes in sheep. This study showed that a uniform rise in tissue interstitial ultrafilterable calcium concentrations did not follow the rise in plasma calcium levels. Tissue interstitial ultrafilterable calcium rose more slowly than did blood ultrafilterable calcium. From 30 to 90 min, ultrafilterable calcium in the blood was significantly higher ($p < 0.05$) than that in bone, muscle, or subcutaneous tissue. Maximum blood levels occurred at 120 min. Peak tissue concentrations occurred 30 min later. Muscle and subcutaneous tissue showed similar peak ultrafilterable calcium concentrations, whereas that in bone was less. At 120 min, the calcium concentration in bone was significantly less ($p < 0.05$) than that in the subcutaneous tissue and muscle. Since the increase in the calcium level is sooner and the calcium level is significantly higher in the blood than in the tissues, the measurement of blood glucose to titrate the dose is reasonable.

Figure 2: Calcium Concentrations over Time in Bone, Muscle and Tissue after IV Infusion



Metabolism

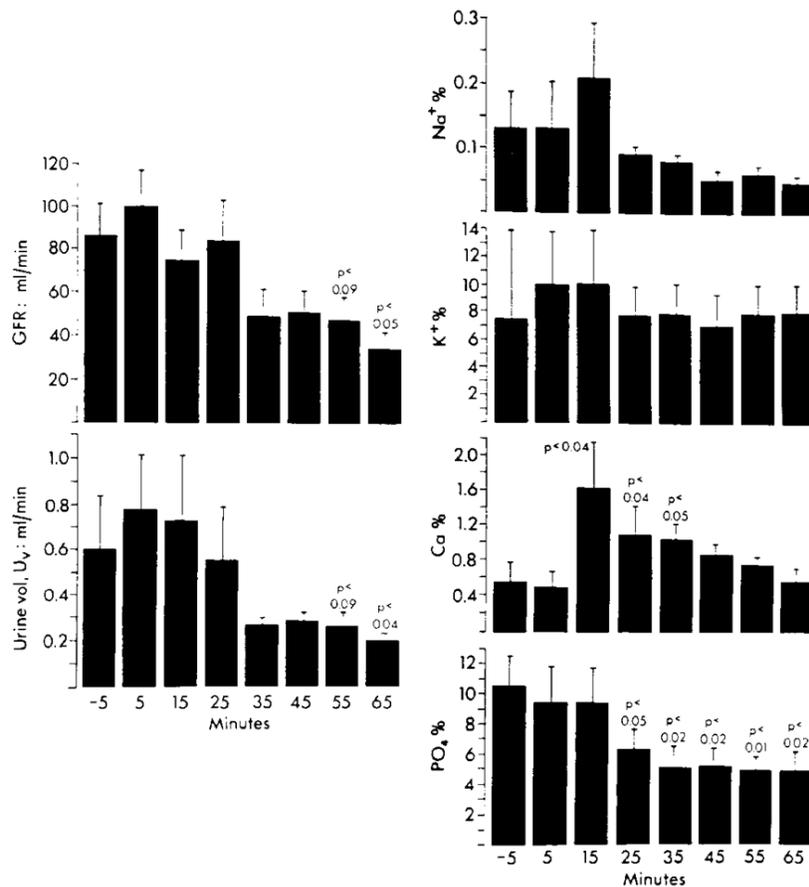
It has been shown that the release of ionized calcium following intravenous administration of calcium gluconate is direct and as clinically effective as that of a calcium chloride injection; liver metabolism is considered to be minimal with respect to the calcium gluconate product (Cote', 1987; Jaros, 1982). Both ionized calcium and gluconate are normal physiologic components of body fluids. Calcium gluconate metabolism, to the extent that such metabolism may occur, is limited to the gluconate component of the salt - as ionized calcium itself does not undergo direct metabolism. Further, gluconate is a normal product of glucose oxidation (Stetten, Jr., 1955; White, 1973); the greatest levels are in the liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis and the lactating mammary gland. The daily production of gluconate from endogenous sources corresponds to approximately 450 mg/kg for a 60 kg person (Life Science Research Office, 1978).

In studies in which sodium gluconate radio-labeled with ^{14}C and deuterium was prepared, mixed and administered to normal and phlorizin (competitive inhibitor of SGLT1 and SGLT2; sodium/glucose transporters types 1 and 2) treated adult rats, extensive biological oxidation of gluconate was confirmed by the presence of 14% ^{14}C as expired CO_2 , with 57% of ^{14}C gluconate being excreted in the urine. The urinary glucose from the phlorizinized rat and the liver glycogen from the normal rat were found to be approximately uniform with respect to ^{14}C . These findings support gluconate conversion into glucose in a direct manner (Stetten, 1950). As a natural component of the body that is readily metabolized, gluconate is unlikely to represent a toxicological concern.

Excretion

Effect of intravenous bolus administration of calcium gluconate (2.2 mM calcium gluconate) on glomerular filtration rate and electrolyte excretion in the kidney of conscious pigs was investigated. In the plasma, both ionic and total calcium concentrations increased but returned to normal within 35 minutes, while sodium, potassium and inorganic phosphate did not change significantly. The fraction of calcium excretion via urine was measured over each 10-minute interval and comparison was made to baseline values. The response of the kidney to the bolus calcium injection was an immediate increase in urine volume, an increase in glomerular filtration rate (GFR) and increased fractional excretion of sodium. Fractional excretion of potassium and inorganic phosphate remained constant for the calcium only increased during the second 10-minute period after the injection (Figure 3).

Figure 3. Glomerular Filtration Rate, Urine Volume and Fractional Urinary Excretion of Select Ions before and after IV Injection of Calcium Gluconate



p < 0.05 indicated significance to pre-injection values GFR = Glomerular filtration rate; U_v = urinary volume Source: (Maier, 1985)

Table 6: Mean plasma total calcium, ionized calcium, inorganic phosphate, sodium and potassium values (mmol/L) and pH after IV injection of calcium gluconate

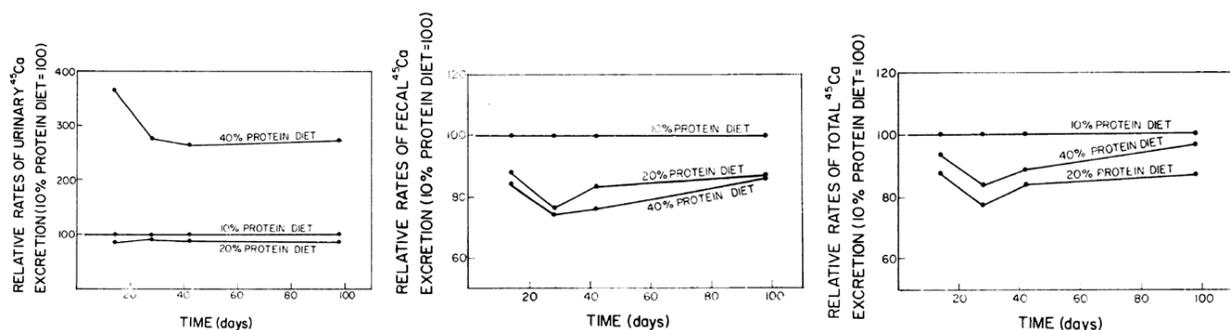
Time (minutes)	-5	+5	+15	+25	+35	+45	+55	+65
Total Ca²⁺	2.31 (0.04)	2.67 (0.06)*	2.52 (0.06)*	2.47 (0.07)*	2.31 (0.05)	2.27 (0.04)	2.27 (0.05)	2.29 (0.06)
Ca²⁺	1.37 (0.016)	1.7 (0.032)*	1.54 (0.021)*	1.47 (0.021)*	1.40 (0.02)	1.35 (0.015)	1.34 (0.015)	1.3 (0.013)
P (inorganic)	2.23 (0.11)	2.16 (0.07)	2.08 (0.09)	2.10 (0.09)	2.15 (0.012)	2.10 (0.11)	2.05 (0.10)	2.08 (0.11)
Na⁺	134 (24)	135 (1.5)	132 (2.1)	132 (2.0)	132 (2.8)	132 (2.7)	132 (2.4)	133 (2.4)
K⁺	3.8 (0.1)	3.8 (0.11)	4.1 (0.09)	4.1 (0.1)	4.0 (0.1)	4.0 (0.09)	4.1 (0.11)	4.15 (0.18)
pH	7.5 (0.02)	7.5 (0.02)	7.48 (0.05)	7.53 (0.05)	7.5 (0.03)	7.5 (0.03)	7.49 (0.03)	7.49 (0.02)

Data are expressed as mean (\pm SE); *significant change from baseline values
Source: (Maier, 1985)

The exact mechanism for the effect of calcium on the GFR is still unclear, but two possibilities exist. Calcium could have an effect on the glomerular ultrafiltration coefficient or on the factors influencing glomerular filtration pressure. The latter include arterial pressure, total renal resistance and osmotic pressure. Calcium ions seem to affect most of these factors. It has been reported that prolonged intravenous infusions of calcium solutions were associated with significant increases in cardiac output, systemic arterial pressure and calculated total peripheral vascular resistance. Further it was shown that a reduction of the calcium ion concentration caused by ethylene diamine tetra-acetic acid led to hypotension and subsequently to renal failure (**Maier, 1985**). The maintenance of physiological calcium level in the blood to effect on the glomerular ultrafiltration coefficient or on the factors influencing glomerular filtration pressure is essential for kidney function in hypocalcemia patients.

(Engelmann, 1975) conducted a study to determine the influence of dietary protein intake on calcium metabolism in adult rats. The results are presented in Figure 4.

Figure 4. Percent Calcium Elimination over Time with 10, 20 and 40% Protein Diets (Left, Urinary Excretion; Center, Fecal Excretion; Right, Total Excretion).



Source: (Engelmann, 1975)

Engelmann, et al., concluded that there was a pronounced shift in the route of endogenous Ca^{2+} excretion from the fecal route to that of urinary elimination in association with increasing protein intake in rats. Other factors such as plasma calcium concentration, glomerular filtration rate, and parathyroid hormone levels have been found to affect urinary calcium excretion (Engelmann, 1975). It is, however, noted that high dietary protein intake had no effect on bone composition or bone resorption (Creedon, 2000). In view of this observation, it is reasonable to postulate that dietary intake by patients receiving Calcium Gluconate Injection for hypocalcemia would be unlikely to alter relative calcium distribution.

Other studies are consistent with the preceding findings in that data suggest that a significant portion (60-85%) of parenterally administered gluconate is excreted unchanged in the urine in rats (Wang, 1962), with the primary route renal excretion of gluconate being via tubular secretion (Herken, 1975).

5 Pharmacodynamic Drug Interactions

Aminoglycoside antibiotics can produce a complete neuromuscular blockade at the isolated phrenic nerve-hemidiaphragm preparation of the rat. Verapamil, also produce this same blockade. However, blockade caused by either of these agents, alone or in combination, can be reversed by the administration of ionized calcium (Paradelis, 1988). The interactions between calcium, aminoglycosides and/or verapamil form the basis for the use of calcium salt infusion in the treatment of respiratory depression or prolonged apnea caused by aminoglycoside antibiotics and/or verapamil. Independent of mechanisms associated with aminoglycoside or tetracycline reversal, calcium administration is also known to abrogate tetracycline antibacterial activity by calcium binding to the antibiotic itself (Mandal, 1992). IV administration of calcium gluconate increases the $t_{1/2}$, AUC and volume of distribution (Vd) of oxytetracycline in the goat (Mandal, 1992). Intravenous administration of calcium gluconate in combination with

Vitamin D significantly increased bone mineralization in rabbits (whereas as vitamin D alone was not effective) (**Lani, 2014**).

6 General Toxicology

The Applicant performed literature searches using PubMed, the Select Committee on GRAS Substances (SCOGS) and the World Health Organization (WHO)/Joint Expert Committee on Food Additives (JECFA) Toxnet specifically, TOXLINE, DART, HSDB, and CCRIS databases. The search terms used include “calcium gluconate”, “gluconic acid”, “glucono-delta-lactone”, “calcium saccharate”, “calcium gluconate”, and “glucaric acid”. In addition, the National Toxicology Program (NTP) databases was searched using the “calcium gluconate”, “gluconate”, “gluconic acid”, “calcium saccharate”, “glucarate”, and “glucaric acid”.

The Applicant provided nonclinical toxicology information for calcium gluconate, calcium saccharate, as well as ionized calcium, gluconic acid, gluconolactone, and glucaric acid as they relate to the Sponsor’s formulation for Calcium Gluconate Injection. Since calcium gluconate and calcium saccharate (glucarate) are ionic compounds, calcium, gluconate and glucarate ions may also be considered separately with respect the nonclinical toxicology of the proposed drug product. Since in aqueous solution, gluconic acid is in equilibrium with its lactone form, glucono-delta-lactone studies of the lactone form are relevant to gluconic acid. The literature references submitted by the applicant for assessing the toxicity of reproductive and developmental studies were performed with glucono-delta-lactone and not with calcium gluconate.

The toxicological studies referenced in support of this NDA by the applicant were, for the most part, conducted before the implementation of the Good Laboratories Practices and the Guidelines for conducting toxicological studies.

6.1 Single-Dose Toxicity

Mice- Intravenous Toxicity

Mice in groups of ten were injected intravenously with calcium kinate gluconate (a mixture of calcium kinate and calcium gluconate in an approximate molar ratio of 2:1), calcium gluconate, or calcium chloride. Calcium kinate gluconate and calcium gluconate had approximately the same acute intravenous toxicity in terms of 24-hour LD₅₀ and on the basis of total compound administered, but they were less toxic than calcium chloride. On basis of calcium content, no significant difference in the acute intravenous toxicity was observed among the three compounds. The 7-day LD₅₀ was the same as that at 24 hours (**Coulston, 1962**). These data are presented in Table below.

Table 7: Acute Toxicity in Mice

ACUTE TOXICITY IN MICE INJECTED INTRAVENOUSLY WITH CALCIUM SALTS		
Medication	I.v. 24-hr ^a LD ₅₀ (mg/kg ± S.E.)	
	As Compound	As Ca
Calcium kinate gluconate	1050 ± 57	83 ± 4
Calcium gluconate	950 ± 83	86 ± 8
Calcium chloride	215 ± 14	78 ± 5

^a Same LD₅₀ at 7 days.

From Coulston, 1962

Dog - Intravenous Tolerance

Two groups of six unanesthetized dogs were injected intravenously with calcium kinate gluconate or calcium gluconate. One week later a crossover experiment was done in which each dog was injected with the alternate solution. The preparations were injected into the saphenous vein at a rate of 3 mg Ca per kilogram per minute. This rate of injection was approximately twice that which is recommended for therapeutic purposes. Maximum calcium tolerance was determined at the moment vomiting occurred and the total milligrams of calcium of the injected solution per kilogram body weight was noted.

Calcium kinate gluconate was better tolerated than calcium gluconate in terms of total calcium administered when injected intravenously into dogs. The mean maximum tolerated dose of calcium kinate gluconate as determined by occurrence of vomiting was 19.7 ± 2.6 mg calcium per kg and that of calcium gluconate was 13.2 ± 1.7 mg Ca per kg (**Coulston, 1962**).

Dog - Intravenous Cardiovascular Effects

Four dogs were anesthetized intravenously with approximately 15 mg/kg of sodium thiopental followed by 250 g/kg of sodium barbital. Femoral blood pressure was recorded. Calcium kinate gluconate or calcium gluconate were injected intravenously in doses ranging from 1.35 mg to 7.5 mg calcium per kilogram at a rate of 7.5 mg calcium per kilogram per minute. The electrocardiograms and blood pressures were recorded prior to the injections and at 10, 20, 30, 60, 90, 120, 180, and 240 minutes after the injections. A crossover experiment was done on the same day so that each dog received both solutions.

Calcium kinate gluconate or calcium gluconate produced no significant changes in the electrocardiograms, heart rates, or blood pressures in dogs when injected intravenously at doses up to 7.5 mg calcium per kilogram (**Coulston, 1962**).

6.2 Repeat-dose Toxicity

Dog – Chronic Intravenous Tolerance

Fifteen dogs were divided into five groups of three dogs each. Calcium kinate gluconate was administered intravenously to two groups at 2 or 10 mg calcium per kilogram, calcium gluconate to one group at 10 mg calcium per kilogram, and sodium kinate to two groups at 75 or 375 mg/kg, 3 times per week for six months. A sixth group of two dogs received physiological saline as a control of the experimental procedure. At the end of the experiment, the control and treated dogs were sacrificed and the thoracic and abdominal viscera were examined grossly. Sections of tissues were fixed, stained and examined microscopically.

The dogs injected intravenously with calcium kinate gluconate or calcium gluconate three times weekly for 6 months were normal in appearance and behavior throughout the experiment. The body weight gain of the medicated and control dogs was normal. No symptoms of hypercalcemia occurred and no evidence of irritation was observed in any of the sites of repeated intravenous injections at any time. The rectal body temperature of the medicated dogs was within normal limits throughout the experiment.

No significant change attributable to medication occurred at any time in color, odor, turbidity, or the chemical or microscopic properties of urine of the dogs. No significant macroscopic or microscopic lesions attributable to medication were observed in any of the dogs autopsied at the termination of the experiment (**Coulston, 1962**).

Dog and Monkey – Chronic Intravenous Hematological Studies

Hematologic studies were done on the same dogs used for the 6-month intravenous tolerance study. The dosage regimen of these dogs is described in the previous section.

Another experiment with monkeys. Six rhesus monkeys (*Macaca mulatta*) were divided into three equal groups. Calcium kinate gluconate (CKG) or calcium gluconate (CG) at 7.5 mg calcium/kg or saline in an equivalent volume to the CG dosage were injected three times intravenously on alternate days to one group each. Total red and white blood cell counts, differential counts, hematocrits, and hemoglobin concentrations were determined before medication and approximately once a month thereafter on dogs, and before medication and at 1 and 4 days after the last injection on monkeys. Platelet counts were made, from the blood of dogs twice during the fourth and fifth months, and of monkeys following each injection. Clotting time of blood from dogs receiving the high doses of calcium and from the control dogs was determined by the capillary tube method three times during the fourth and fifth months; clotting time of blood from monkeys was determined after each injection. Serum calcium levels were determined at monthly intervals on dogs and following each injection on monkeys (**Coulston, 1962**).

Total red and white blood cell counts, differential counts, hematocrits, and the hemoglobin concentrations of dogs or monkeys injected with calcium kinate gluconate or calcium kinate were normal as compared to control dogs or monkeys.

A significant increase in the serum calcium level was observed in the dogs injected with CKG or CG when compared to the controls. The maximum increase was reached within 15 minutes following the injection and was still slightly elevated 3 hours later. Similarly, a significant increase was observed in the monkeys injected with CKG or CG when compared to the controls. This increase reached a maximum within 5 minutes following the injections and returned to an approximately normal level within 90 minutes. Serum calcium levels are presented in Table below.

SERUM CALCIUM VALUES AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication ^c	Species	Serum calcium (mg/100 ml) at minutes after injection							
		Pre-med.	5'	15'	30'	60'	90'	120'	180'
Saline control	Dog ^a	10.3	—	10.4	—	10.5	—	10.7	10.7
CKG 10 mg Ca/kg	Dog	10.3	—	14.4	—	13.2	—	12.7	12.0
CG 10 mg Ca/kg	Dog	10.7	—	16.1	—	14.0	—	13.4	12.2
Na kinate 375 mg/kg	Dog	10.7	—	10.9	—	10.5	—	10.7	10.5
Saline control	Monkey ^b	11.8	11.3	11.5	11.6	—	11.8	—	—
CKG 7.5 mg Ca/kg	Monkey	10.8	14.2	13.5	13.0	—	11.7	—	—
CG 7.5 mg Ca/kg	Monkey	12.1	15.0	14.3	13.4	—	12.5	—	—

^a Averages of 3 dogs in each medicated group and 2 in the controls from 6 determinations at monthly intervals following chronic administration.

^b Averages of 2 monkeys in each group following three injections on alternate days.

^c CKG = calcium kinate gluconate; CG = calcium gluconate.

Clotting time in dogs injected with the calcium solutions at 10 mg calcium per kilogram or with sodium kinate at 375 mg/kg for 3-4 months was faster than in the controls. Usually, this was observed within 15 minutes after each injection, and the clotting time returned to the premedication time within 45 minutes. Determinations made at 60 minutes and at 1 and 2 days following the last injection were within normal limits. A decrease in clotting time was noted also in both groups of monkeys medicated with CKG or CG. The decrease was observed within 15 minutes after each injection and reached a maximum by 30 minutes. The clotting time returned to normal at 90 minutes following injections of CG, whereas after CKG the clotting time returned to normal at 150 minutes or more. Four days after the last injection the clotting time of all monkeys was normal. The clotting time values are presented in Table below.

CLOTTING TIME IN DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS										
Medication ^c	Species	Clotting time								
		Pre-med.	Time after medication							
			5'	15'	30'	60'	90'	150'	24 Hr	48 Hr
Saline control	Dog ^a	2'15"	—	2'57"	2'30"	2'57"	—	—	2'30"	2'50"
CKG 10 mg Ca/kg	Dog	2'55"	—	2'45"	2'01"	2'53"	—	—	2'48"	2'57"
CK 10 mg Ca/kg	Dog	2'20"	—	1'51"	1'40"	2'18"	—	—	2'27"	2'27"
Na kinate 375 mg/kg	Dog	2'30"	—	2'21"	1'53"	2'41"	—	—	2'43"	2'48"
Saline control	Monkey ^b	1'23"	1'18"	1'18"	1'18"	—	1'18"	—	—	—
CKG 7.5 mg Ca/kg	Monkey	1'27"	1'18"	0'51"	0'41"	—	0'53"	1'08"	—	—
CG 7.5 mg Ca/kg	Monkey	1'31"	1'15"	0'53"	0'41"	—	1'23"	—	—	—

^a Averages of 3 dogs in each medicated group and 2 in the controls at 3-4 months of medication.

^b Averages of 2 monkeys in each group following 3 injections on alternate days.

^c CKG = calcium kinate gluconate; CG = calcium gluconate.

A slight increase in platelet counts was observed in dogs and monkeys injected with CKG and in dogs injected with sodium kinate within 15 or 30 minutes after the injections. No definite increase was noted in dogs or monkeys injected with CG. The platelet counts are presented in Table below.

PLATELET COUNTS OF DOGS AND MONKEYS AFTER INTRAVENOUS INJECTIONS OF CALCIUM SALTS

Medication ^c	Species	Platelet count (1000/mm ³)						
		Pre-med.	Time after medication					
			5'	15'	30'	60'	90'	24 Hr
Saline control	Dog ^a	400	—	—	354	454	—	346
CKG 10 mg Ca/kg	Dog	389	—	—	475	476	—	429
CK 10 mg Ca/kg	Dog	422	—	—	435	380	—	366
Na kinate 375 mg/kg	Dog	379	—	—	435	448	—	388
Saline control	Monkey ^b	296	287	301	300	—	305	—
CKG 7.5 mg Ca/kg	Monkey	292	361	407	429	—	375	—
CG 7.5 mg Ca/kg	Monkey	412	407	445	405	—	387	—

^a Averages of 3 dogs in each medicated group and 2 in the controls at 3-4 months of medication.

^b Averages of 2 monkeys in each group following three injections on alternate days.

^c CKG = calcium kinate gluconate; CG = calcium gluconate.

Intravascular clotting is considered the greatest danger associated with intravenous calcium therapy, in that it occurs without warning; the effects of which can be fatal (Lieberman, 1930).

7 Genetic Toxicology

Calcium gluconate did not exhibit genotoxicity at 2.5% in the *S. typhimurium* strains TA-1535, TA-1537, and TA-1538 or *S. cerevisiae* strain D4 (**Litton Bionetics Inc., 1975**).

Table 8: Genotoxicity of Gluconic Acid and its Derivatives

Substance and End-Point	Test System	Test Article Concentration	Result	Source
Calcium Gluconate	<i>S. cerevisiae</i> D4 <i>S. typhimurium</i> TA 1535, TA 1537, and TA 1538	2.5%	Negative ^a	(Litton Bionetics Inc, 1975)
Manganese Gluconate Reverse Mutation	<i>S. typhimurium</i> TA 98, TA 100, TA 1535, TA 1537, and TA 1538	0.033, 0.1, 0.33, 1, 3.3, 10 mg/plate	Negative ^a	(Prival, 1991)
Manganese Gluconate Tryptophan reversion	<i>E. coli</i> WP2	0.033, 0.1, 0.33, 1, 3.3, 10 mg/plate	Negative	(Prival, 1991)

With and without metabolic activation

8 Carcinogenicity

In a 2-year toxicity study, 6 groups of 30 male and 30 female SPF-derived Wistar rats were fed a diet containing 40% canned meat treated with either nitrite and 1% glucono-delta-lactone or glucono-delta-lactone (neutral cyclic ester of gluconic acid) alone. In aqueous solution, glucono-delta-lactone is in equilibrium with D-gluconic acid. There was no evidence of changes in hematology, clinical chemistry, gross, or microscopic histopathology that suggested pre-neoplastic changes or tumor formation attributable to the diet (**van-Logten, 1972**).

Calcium D-gluconate (calcium saccharate) is not identified as a probable, possible, or confirmed human carcinogen by the **International Agency for Research on Cancer (IARC)**, as a carcinogen or potential carcinogen by the **American Conference of Governmental Industrial Hygienists (ACGIH)**, as a known or anticipated carcinogen by the **National Toxicology Program (NTP)**, or as a carcinogen or potential carcinogen by the Occupational Safety and Health Administration (OSHA) (**Sigma-Aldrich, 2014**).

9 Reproductive and Developmental Toxicology

No reproductive studies with Calcium Gluconate Injection were provided in the NDA. The reproductive and developmental toxicity studies in referenced literature were conducted with repeated dosing of gluco-delta-lactone by routes of administration other than the intended clinical route. The intended route of administration of Calcium Gluconate Injection is acute IV treatment for symptomatic hypocalcemia (b) (4). Therefore, the information provided below on the reproductive and developmental toxicities by repeat dosing of the drug may not be directly relevant to the safety evaluation of the gluconate component of Calcium Gluconate Injection.

Since the reproductive and developmental toxicity studies were conducted by administering gluco-delta-lactone instead of calcium gluconate and the doses were administered not by the intended intravenous dosing, it is not possible to establish a safety factor for the proposed clinical doses. This issue is further complicated by the uncertainty in quantifying the bioavailability of orally administered calcium gluconate. Approximately one-fifth to one-third of orally administered calcium is absorbed in the small intestine, depending on presence of vitamin D metabolites, pH in lumen, and on dietary factors, such as calcium binding to fiber or phytates (**Thomson/Micromedex, 2006**).

A teratologic evaluation of glucono-delta-lactone was conducted by (**Food and Drug Research Laboratories Inc., 1973**). Virgin adult females of several species, including mice, rats, hamsters and rabbits, were mated with males of their respective species. After pregnancy had been determined, the females were given doses of glucono-delta-lactone via oral intubation. CD-1 mice, on Days 6-15 of gestation were dosed with 6.95 mg/kg, 32.5 mg/kg, 150 mg/kg, or 695 mg/kg. A negative control was sham treated and a positive control group received 150 mg/kg aspirin. Likewise Wistar rats were dosed with 5.94 mg/kg, 27.6 mg/kg, 128 mg/kg, or 594 mg/kg on gestational day 6-15. Hamsters received doses of 5.60 mg/kg, 26.0 mg/kg, 121.0 mg/kg and 560.0 mg/kg for 5 consecutive days, while rabbits were given doses of 7.80, 36.2, 168.5 and 780 mg/kg for 13 consecutive days following pregnancy. Test parameters included appearance, weight gain, and behavior, the number of implantation sites, resorption sites, and live and dead fetuses. Results with the experimental animals did not differ significantly from the controls with respect to the evaluations mentioned above. Additionally, the urogenital tract of each female was examined for anatomical normality. All fetuses were examined grossly for external congenital abnormalities. One third of the fetuses of each species underwent detailed visceral examinations employing the Wilson technique. The remaining two-thirds were cleared in potassium hydroxide and examined for skeletal abnormalities.

The results of the study are discussed below.

Mice (Albino CD1 outbred mice)

The administration of up to 695 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Rat (Albino rats - Wistar derived stock)

The administration of up to 594 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of

the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Hamster (Golden hamsters from an outbred strain)

The administration of up to 560 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Rabbits (Dutch-belted female rabbits)

The administration of up to 780 mg/kg (Body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Based on results from 4 different species, it was concluded that the administration of glucono-delta-lactone at the various test dosages in mice, rats, hamsters and rabbits of species had no effect on implantation or on maternal or fetal survival.

10 Local Tolerance

Injection of 10% calcium gluconate into the skin of rabbits produced ulcerated lesions consistent with iatrogenic calcinosis cutis caused by extravasation into the surrounding tissue. Injection of 1% or 10% calcium gluconate into the proximal artery of the ileum results in dose-dependent necrosis, hemorrhage and villous atrophy.

11 Integrated Summary and Safety Evaluation

The Applicant filed this NDA under 505(b)(2) pathway for marketing Calcium Gluconate Injection intended for the acute treatment of symptomatic hypocalcemia (b) (4). The Applicant currently markets Calcium Gluconate Injection, as an *unapproved drug* and did not provide human exposure information of their *unapproved drug*. Safety findings noted in literature on the use of calcium gluconate 10% administered intravenously include increased BP, hypercalcemia, nausea or faintness unless given slowly over 5 to 8 minutes, increased urine excretion, increased parathyroid gland sensitivity, increased serum calcitonin levels, transient flush sensation that lasted for one minute, severe cardiac arrhythmias when administered as a bolus dose, calcinosis cutis, calcifications in vessel walls and eccrine sweat glands, localized skin necrosis was observed in 4 cases, local tissue necrosis, calcification of kidney. Most of these are from case reports, and the source of calcium gluconate used is unknown.

Per the drug proposed labeling, (b) (4)
[REDACTED]. Doses are

ultimately dependent upon the serum calcium level of patients. The Applicant seeks to rely on the clinical and nonclinical data published in the scientific literature.

Oral calcium acetate, indicated for reduction of serum phosphorus in patients with end-stage renal disease (NDA 022581) and 10% Calcium Chloride Injection for the treatment of hypocalcemia (NDA 021117) are U.S. approved calcium containing products.

Many vital cellular biological processes, including cell membrane integrity and cell function are known to be dependent upon maintenance of adequate ionized plasma calcium concentrations. Calcium gluconate metabolism is limited to the gluconate component of the salt as ionized calcium itself does not undergo direct metabolism. As in humans, gluconate is a normal product of glucose metabolism in the rat and 60-85% of parenterally administered gluconate is excreted unchanged in the urine via tubular secretion.

The Applicant provided literature references to demonstrate that intravenous calcium gluconate has been used successfully in dogs and cats with hypocalcemia, secondary to hypoparathyroidism, parturient paresis in cows (hypocalcemia associated with post-parturition lactation leading to myasthenia and coma in dairy cows) and cats have been treated successfully with IV and/or subcutaneous administration of calcium salt solutions. Administration of calcium gluconate to horses resulted in increases in both ionized and total calcium concentrations, cardiac index, stroke index, and cardiac contractility (dP/dtmax) relative to pre-infusion baseline and in dogs increased coronary blood flow and reduced coronary vascular resistance. In Rhesus monkeys calcium gluconate injections produced transient decrease in blood clotting time on Day 1 after the dose which returned to normal by Day 4 after the last dose.

Pharmacodynamic drug interactions are possible between calcium (calcium gluconate) and various other drug products, including but not limited to: digoxin, verapamil, aminoglycosides, and tetracycline antibiotics.

The Applicant has not conducted any toxicology study with Calcium Gluconate Injection. The referenced literature information indicated that intravenous calcium gluconate tolerance in dogs, when measured as the moment of emesis, was found to be 13.2 ± 1.7 mg of calcium/kg. When dogs were injected intravenously with 10 mg/kg of calcium gluconate 3 times/ week for 6 months, they were normal in appearance, behavior, and body weight gain throughout the study. No symptoms of hypercalcemia occurred and no evidence of irritation was observed in any of the sites of repeated intravenous injections time. No changes in the rectal temperature and in the chemical and microscopic properties of urine were observed in dogs. No significant macroscopic or microscopic lesions attributable to medication were observed in any of the dogs autopsied at the termination of the experiment. Clotting time in dogs injected with the calcium solutions at 10 mg calcium per kilogram or with sodium kinate at 375 mg/kg for 3-4 months was faster than in the controls. No definite increase in platelet counts was noted in dogs or monkeys injected with calcium gluconate. Injection of 10% calcium

gluconate into the skin of rabbits produces ulcerated lesions consistent with iatrogenic calcinosis cutis caused by extravasation into the surrounding tissue.

The Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office and FAO/WHO Expert Committee on Food Additives (JECFA) discussed the toxicity profile of Calcium Gluconate. These reports are summarized in a GRAS Notification for (oral) Calcium Gluconate as a Food Supplement and the subsequent FDA Response to the GRAS notice. However, it is noted that the intended route of the Calcium Gluconate Injection, USP 10% is intravenous, not oral. 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.

Each mL of Calcium Gluconate Injection contains 94 mg of calcium gluconate, 4.5 mg of calcium saccharate tetrahydrate, hydrochloric acid and/or sodium hydroxide for pH adjustment (6.0-8.2) and water for Injection, USP. (b) (4)

Calcium saccharate is not listed on FDA's IIG list, but like gluconic acid, D-glucaric acid (saccharate) is produced naturally in small amounts by mammals, including humans and glucarate and its lactone forms (D-glucono-1,4-lactone and D-glucono-6,3-lactone) are end-products of the D-glucuronic acid pathway. Glucarate is transported in the blood to a number of different organs in Sprague-Dawley rats.

Calcium gluconate was not mutagenic in either bacteria or yeast. Glucono-delta-lactone was not carcinogenic in a 2 year feeding study in rats. Calcium D-glucarate (calcium saccharate) is not identified as a human carcinogen by the International Agency for Research on Cancer (IARC).

No references were provided by the Applicant to directly address the reproductive and development effects of calcium gluconate. However, the Applicant provided reproductive and developmental studies using glucono-delta-lactone. In aqueous solution, glucono-delta-lactone is in equilibrium with D-gluconic acid, and under acidic conditions is completely converted to D-gluconic acid. The glucono-delta-lactone was used as a surrogate to assess the reproductive and developmental toxicity of gluconic ions. Glucono-delta-lactone had no effect on implantation or on maternal or fetal survival in mice, rats, hamsters and rabbits at the highest doses tested. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from those of the controls. The administration of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from that of the controls (**SIDS Initial Assessment Report For SIAM, 2004**). Since reproductive and developmental toxicity studies were conducted by administering gluco-delta-lactone instead of calcium gluconate and the doses were administered not by the intended intravenous dosing, it is not possible to establish a safety factor for the proposed clinical doses based on these data. There is additional uncertainty in quantifying the bioavailability of orally administered calcium gluconate. Approximately one-fifth to one-third of orally administered calcium is absorbed in the

small intestine, depending on presence of vitamin D metabolites, pH in lumen, and on dietary factors, such as calcium binding to fiber or phytates (**Thomson/Micromedex. 2006**).

CFR21 part 201 subpart 201-323 specifies that the amount of aluminum for small volume parenteral drug products shall be stated in product labeling. The proposed specification for aluminum in Calcium Gluconate Injection is 400 mcg/L. At the maximum recommended doses of Calcium Gluconate Injection, the daily aluminum exposure will be less than 5 mcg/day, which is less than the acceptable daily intake (ADI). The ADI for intravenous exposure is 5 mcg/day, based on the observation that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 5 mcg/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration.

The Applicant assayed six lots of Calcium Gluconate Injection USP Finished Product for 24 elemental impurities. All 24 elemental impurities listed in ICH Q3D are treated as potential elemental impurities in all inorganic reagents. The analytical results of the six (6) expired representative batches which were tested showed that all the listed elements in the ICH Q3D are (b) (4) % of the PDE. The Specification for Calcium Gluconate, USP 10% states the limit for (b) (4) is NMT (b) (4) ppb which is less than the ICH Q3D threshold of 1.5 ppm (for parenteral products).

The high aluminum content of Calcium Gluconate Injection USP, were identified as an impurity of toxicological concern. In support of the revised proposed aluminum limit, the Applicant committed to lower aluminum limit (400 mcg/L versus (b) (4)). The Applicant provided an updated finished product specification to reflect the new aluminum limit. Applicant is revising labels to reflect the new aluminum limit.

Calcium Gluconate Injection, USP 10% (Calcium Gluconate Injection) is intended for the treatment of acute, symptomatic hypocalcemia (b) (4). This NDA is recommended for approval for the proposed indication.

12 Appendix/Attachments

12.1 LITERATURE CITATIONS

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12.2 APPLICANT SUGESSTED LABELING

INDICATIONS AND USAGE

Calcium Gluconate Injection is a super saturated solution of calcium gluconate indicated

(b) (4)

8.1 Pregnancy

Risk summary

(b) (4)

8.2 Lactation

Risk summary

Calcium is present in human milk as a natural component of human milk. It is not known whether intravenous administration of Calcium Gluconate Injection can alter calcium concentration in human milk.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

(b) (4) intravenous administration of calcium gluconate increases serum ionized calcium level (b) (4). Calcium gluconate dissociates (b) (4) ionized calcium in plasma. (b) (4) ionized calcium and gluconate are normal constituents of the body fluids.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ARULASANAM K THILAGAR
05/11/2017

CALVIN L ELMORE
05/11/2017
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: NDA 208418

Applicant: Fresenius Kabi USA, LLC (FK USA)

Stamp Date: 5/16/2016

PIND Number: 113171

Drug Name: Calcium Gluconate Injection, USP 10%

NDA/BLA Type: 505(b)(2)

On **initial** overview of the NDA/BLA application for filing: The NDA is fileable from Pharm/Tox point of view.

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		<p>The Sponsor did not conduct any nonclinical studies to define the pharmacodynamics, pharmacokinetics (PK), safety pharmacology, or toxicology of intravenous (IV) calcium gluconate administration. Sponsor stated that due to the long history of clinical use of the product, both the safety and efficacy of calcium gluconate in humans are reasonably considered to be well understood.</p> <p>Sponsor performed up-to-date (January 25, 2016) searches of non-clinical data from scientific literature and plans to rely on them to support the approval of this 505(b)(2) NDA. These pivotal nonclinical studies published in the scientific literature are listed in the NDA.</p> <p>Sponsor markets Calcium Gluconate Injection, USP 10% which is currently an unapproved drug. Sponsor intends to rely on the clinical and nonclinical data contained in the scientific published literature medical guidelines and text books of medicine to support the approval of its proposed product.</p>

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
				This NDA also contains a request for the waiver of <i>in vivo</i> bioavailability / bioequivalence studies.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			<p>Sponsor presented the nonclinical toxicology information from published literature for calcium gluconate, calcium saccharate, as well as ionized calcium, gluconic acid, gluconolactone, and glucaric acid as they relate to the Sponsor's formulation for Calcium Gluconate Injection, USP 10%.</p> <p>Nonclinical intravenous injection studies referenced in this NDA were performed using calcium gluconate USP formulations and it is not clear whether they contained calcium saccharate. Calcium Saccharate is not listed on FDA's IIG list. However Calcium Saccharate is listed in the USP monograph for Calcium Gluconate Injection, USP. (b) (4)</p> <p>(b) (4)</p> <p>Each mL of drug product contains 94 mg of calcium gluconate, 4.5 mg of calcium saccharate tetrahydrate, hydrochloric acid and/or sodium hydroxide for pH adjustment (6.0 -8.2) and Water for Injection USP, q.s. Each mL provides 9.3 mg elemental calcium (0.465 mEq/L). This is the same formulation that is currently being marketed as an unapproved drug.</p>
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	Yes		In many nonclinical studies referenced from the literature in the NDA, the same (IV) route of administration was used as the intended human exposure route.
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?			N/A There is no reference to GLP compliance for the nonclinical studies referenced from the literature in the NDA. Some of the referenced studies were conducted prior to the implementation of GLPs and the

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
				guidelines under which these studies were conducted are not in accordance with current ICH guidelines and requirements.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		N/A	Prior to the submission of this NDA, Division did not request any special studies.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?		N/A	<p>No human dose multiples is expressed in either mg/m² or comparative serum/plasma levels). Dose is dependent upon the serum calcium level of patients.</p> <p>There are no adequate or well-controlled studies of Calcium Gluconate Injection in pregnant women. It is not known whether Calcium Gluconate Injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. No information on the animal reproductive effects of calcium gluconate is provided in this NDA.</p> <p>Nonclinical data to assess the potential adverse effects on the breastfed child from Calcium Gluconate Injection are not provided in this NDA.</p>
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		N/A	This is a new drug application for a marketed unapproved drug
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	Yes		<p>Sponsor intends to rely on the clinical and nonclinical data contained in the scientific published literature medical guidelines and text books of medicine to support the approval of its proposed product.</p> <p>No nonclinical bridging study was performed by the sponsor.</p>

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ARULASANAM K THILAGAR
06/29/2016

CALVIN L ELMORE
06/29/2016