ORIGINAL SUBMISSION

634

Exponent

Exponent
1150 Connecticut Ave., NW
Suite 1100
Washington, DC 20036
telephone 202-772-4900
facsimile 202-772-4979
www.exponent.com

GRN 000634

February 23, 2016

Paulette M. Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for the Use of Calcium Chloride in Potato Snacks

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), PepsiCo, hereby provides notice of a claim that the food ingredient described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to potato snacks to reduce the formation of acrylamide.

Three paper copies of the notification are provided as required; we also have provided a copy of the notification on the enclosed CD-ROM. If you have any questions or require additional information, please do not hesitate to contact me at 202-772-4915, or ntran@exponent.com.

Sincerely,

(b) (6)

Nga Tran, DrPH, MPH
Principal Scientist

Exponent
1150 Connecticut Ave., NW
Suite 1100
Washington, DC 20036
telephone 202-772-4900
facsimile 202-772-4979
www.exponent.com

February 23, 2016

Paulette M. Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for the Use of Calcium Chloride in Potato Snacks

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), PepsiCo, hereby provides notice of a claim that the food ingredient described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to potato snacks to reduce the formation of acrylamide.

Three paper copies of the notification are provided as required; we also have provided a copy of the notification on the enclosed CD-ROM. If you have any questions or require additional information, please do not hesitate to contact me at 202-772-4915, or ntran@exponent.com.

Sincerely,

(b) (6)

Nga Tran, DrPH, MPH
Principal Scientist

GRAS Determination for the Use of Calcium Chloride in Potato Snacks

SUBMITTED BY:

PepsiCo, Inc
700 Anderson Hill Road
Purchase, NY 10577

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
College Park, MD 20740-3835

CONTACT FOR TECHNICAL OR OTHER INFORMATION:

Nga Tran
Principal Scientist
Exponent, Inc.
1150 Connecticut Avenue, NW, Suite 1100
Washington, DC 20036

February 23, 2016

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Exhibits

List of Acronyms

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstracts Service
CDC	Center for Disease Control and Prevention
CFR	Code of Federal Regulations
CHL	Chinese Hamster Lung
CI	Confidence Interval
CVD	Cardiovascular Disease
DHHS	U.S. Department of Health and Human Services
dL	Deciliter
DRI	Dietary Reference Intake
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
ESIS	European Chemical substances Information system
EU	European Union
FAO/WHO	Joint Food and Agriculture Organization/World Health Organization
FARE	Foods and Residues Evaluation Program
FCC	Food Chemicals Codex
FCID	Food Commodity Intake Database
FDA	U.S. Food and Drug Administration
FNDDS	Food and Nutrient Database for Dietary Studies
g	Gram
GFSA	Codex General Standard for Food Additives
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
HR	Hazard Ratio
HPV	High Production Volume
IOM	Institute of Medicine
IRIS	Integrated Risk Information System
IUCLID	International Uniform Chemical Information Database
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
LD50	Lethal Dose 50
LOAEL	Low-Observed-Adverse-Effect-Level
m	Meter
mg	Milligram

mL	Milliliter
MI	Myocardial Infarction
mm	Millimeter
Mol	Moles
NCHS	National Center for Health Statistics
NCI-DHQ	National Cancer Institute Diet History Questionnaire
NHANES	National Health and Nutrition Examination Surveys
NOAEL	No-Observed-Adverse-Effect-Level
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
ppm	parts per million
PTH	Parathyroid Hormone
RCT	Randomized Control Trial
RR	Relative Risk
TOXNET	Toxicology Data Network
µg	Microgram
UL	Tolerable Upper Intake Level
U.S.	United States
USDA	U.S. Department of Agriculture
WHI	Women's Health Initiative
WWEIA	What We Eat in America
y	Years

GRAS Exemption Claim

Name and Address of Notifier

PepsiCo, Inc. (PepsiCo) hereby notifies the U.S. Food and Drug Administration (FDA) that the use of calcium chloride as described below is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because PepsiCo has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

(b) (6)

February 23, 2016

_____ _____

Name: Jan Weststrate Date
Title: Senior Vice-President, R&D Global Functions, Governance, and Compliance
Company: PepsiCo, Inc.

Name of GRAS Substance

The name of the substance that is the subject of this GRAS determination is “calcium chloride.”

Intended Use and Consumer Exposure

Calcium chloride is proposed for use in the production of potato snacks (e.g. potato chips and sticks). The intended technical effect of the proposed use of calcium chloride in the manufacturing of potato snacks is to reduce the formation of acrylamide. The effectiveness of calcium chloride as a mitigator of acrylamide levels in potato snacks has been evaluated by PepsiCo. Calcium chloride was observed to reduce acrylamide levels in three different potato snack products with percent reduction ranging from 45% to 65%. Calcium chloride is proposed to be added at a level up to a maximum of 1% in the potato flour mixtures that are extruded into pellets; the potato flour pellets are subsequently air-popped into potato snacks for consumption.

The estimated daily intake (EDI) of calcium chloride, chloride and calcium from the proposed use of calcium chloride in potato snacks and the cumulative intake of calcium (background + proposed new use) in the U.S. population was determined using food intake and supplement use data from the National Health and Nutrition Examination Survey (NHANES) (2007-2008 and 2009-2010) and nutrient composition data from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS).

For the U.S. population age 1 year and older, from the proposed use of calcium chloride in potato snacks, the *per user* mean and 90thpercentile EDI for calcium chloride were 201 and 408 mg/day, respectively. This corresponds to the per user mean and 90th percentile EDI of 128 and 261 mg/day, respectively, for chloride, and 72 and 147 mg/day, respectively, for calcium. Male adolescents were estimated to have the highest intakes of calcium from the proposed uses; among males 14-18 y the *per user* 90th percentile EDI of calcium from potato snacks was 249

mg/day, respectively. There were only 5 consumers of potato snacks among infants 6-11 months; not an adequate sample size to provide reliable intake estimates.

The *per user* mean and 90th percentile cumulative EDI (CEDI) for calcium from all sources, including background sources (diet and supplements) and the proposed uses, were 1,152 and 1,936 mg/day, respectively, for the U.S. population age 1 year and older. The *per user* mean and 90th percentile CEDI for calcium were 1,041 and 1,557 mg/day, respectively, among male children 1-3 y, and 1,029 and 1,559 mg/day, respectively, among female children 1-3 y. Children 4-8 y had *per user* mean and 90th percentile CEDI of 1,087 and 1,689 mg/day, respectively, among males and 1,008 and 1,568 mg/day, respectively, among females. Among the older subpopulations, the 90th percentile CEDI for calcium were highest among the older adults (51+ y) ranging from 1,918 mg/day among males 71+ y to 2,204 mg/day among women 51-70 y. Among infants 6-11 months the CEDI for calcium remains the same as the background estimates when the proposed use of calcium chloride in potato snacks were included (mean = 678 mg/day and 90th percentile = 1,113 mg/day).

The intake assessment was designed to conservatively estimate background intake of calcium from all food sources (i.e., all naturally-occurring and calcium-fortified food sources and approved food additive uses of calcium chloride, as measured by the USDA) and calcium from dietary supplements, as well as calcium intake from the proposed use of calcium chloride in potato snacks. No adjustment has been made to account for the potential overestimation of intakes that may result from using two days of dietary data to estimate long-term consumption nor to account for the fact that only a small percentage of PepsiCo's potato snacks will contain calcium chloride. 100% bioavailability of the calcium from the proposed use was also assumed resulting in a conservative overestimate of exposure.

The intake assessment accounting for both background sources of calcium (diet and supplements) and the proposed uses of calcium chloride in potato snacks showed that the *per user* 90th percentile CEDI were below the IOM UL for the majority of the US subpopulations. For three subgroups, the *per user* 90th percentile calcium CEDI marginally exceeded the IOM UL of 2,000 mg/day but were below the (EFSA) UL of 2,500 mg/day among the older women 51 -70 y (2,195 mg/day) and 71+ y (2,158 mg/day) as well as among men 51-70 y (2,023 mg/day). Source contribution analyses showed that background calcium intake from food sources alone are well below the IOM UL at the *per user* 90th percentile for these subpopulations, irrespective of supplement use status, with *per user* 90th percentile dietary calcium intake ranging from 1,274 mg/day among females 71+ y to 1,721 among males 51-70 y. For these older age groups, the additional calcium intake from the use of supplements drives the total background calcium intake: at the 90th percentile, calcium from supplement use contributes up to 65% of the total background calcium intake among all calcium consumers. It should also be noted that almost two-thirds (65%) of the women 71+ y reported the use of a calcium-containing supplement in the NHANES database, representing the largest supplement user group. The proposed use of calcium chloride at a level up to 1 % in potato snacks contributes minimally to the total cumulative calcium intake at the 90th percentiles among these older females and male sub-population. Among all calcium consumers, the proposed use of calcium chloride contributes from 3- 5% (31 – 59 mg/day additional calcium), among supplement consumers: 2-4% (29-59 mg/day additional calcium), and among non-calcium-supplement users: 2-7% (20 – 51 mg/day additional calcium). Among older women and men who are not taking calcium supplements, the per user 90th percentile cumulative calcium intake ranges from

1,265 mg/day to 1,639 mg/day for females and males 51-70 y, respectively, which are all well below the IOM UL of 2,000 mg/day.

Overall, the *per user* 90th percentile CEDI of calcium for the subpopulations of infants 6-11 months, children, adolescents and adults 19-50 y were below the IOM UL. For the older adults 51+ y the per user 90th percentile CEDI of calcium for males 71+ y were below the exposure limit range (2,000 – 2,500 mg/day). For women 51+ y and males 51-70 y, the per user 90th percentile background (food + dietary supplements) calcium intakes were within the exposure limit range (2,000 – 2,500 mg/day) and with the small addition of calcium (<7%) from the proposed use of calcium chloride in potato snacks the *per user* CEDI at the 90th percentile remained within the exposure limit range. Therefore, it is reasonable to conclude that the proposed use of calcium chloride in the production of potato snacks at a maximum level 1% is safe within the meaning of the FD&C Act, i.e. the proposed use meets the safety standard of reasonable certainty of no harm.

Safety of Calcium Chloride

Calcium chloride was considered to have low toxicity and an Acceptable Daily Intake (ADI) was not specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1973). Furthermore, a range of uses of calcium chloride are considered GRAS by the US FDA (SCOGS-45, 1975). An updated literature search revealed no new information that contradicts JECFA's earlier conclusion on calcium chloride or that of the US FDA.

Calcium chloride dissociates to calcium and chloride ions in the body. Chloride is the most abundant anion in all animal species. The total chloride in the adult human body is approximately 70-95 g. Chloride has historically been present in the human diet as salt (sodium chloride). The biological and toxicological effects related to both calcium deficiency and calcium excess have been extensively reviewed by both the Institute of Medicine (IOM, 2011) and the European Food Safety Authority (EFSA, 2012). Based on calcium excretion in young children and formation of kidney stones in older children and adults, the IOM established tolerable upper limits (ULs) for infants 0-6 months (1,000 mg/day), infants 6-12 months (1,500 mg/day), children 1– 8 y (2,500 mg/day), adolescents 9-18 y (3,000 mg/day), adults 19 – 50 y (2,500 mg/day), and older adults 51+ y (2,000 mg/day). The IOM concluded that there were insufficient data to determine a UL based on other effects, including increased risk of cardiovascular disease (CVD) among post-menopausal women and older men. EFSA's most recent evaluation (2012) reached similar conclusions on the lack of adverse associations between calcium intake and CVD as well as other health endpoints but did not believe the available evidence required a revision of the UL of 2,500 mg/day for adults as previously established by the Scientific Committee on Food (SCF) in 2003. Based on the currently available data and authoritative reviews by the IOM (2011) and EFSA (2012) a range of exposure limits from 2,000 to 2,500 mg/day can be reasonably relied upon to assess the safety of the proposed use of calcium chloride in potato snacks for older adults 51+ y. The literature published since the IOM review in 2011 provide no new conclusive evidence of a cause and effect that would alter the significant scientific consensus presented in the IOM (2011) or the EFSA (2012) reviews.

Basis for GRAS Determination

PepsiCo's GRAS determination for the intended use of calcium chloride is based on scientific procedures as described under 21 Code of Federal Regulations (CFR) § 170.30(b).

The intended use of calcium chloride has been determined to be safe, and has also been determined to be GRAS, by demonstrating that safety of intake under the proposed conditions of use is based on knowledge and information that is both publicly available and widely accepted by experts qualified by scientific training and experience to evaluate the safety of substances added to food.

Determination of the safety and GRAS status of calcium chloride intended to be used in the production of potato snacks (e.g. potato chips and sticks) to reduce the formation of acrylamide was made through the deliberation of an Expert Panel consisting of Gary C. Curhan, MD, ScD, FASN, Stanley M. Tarka, PhD, and Connie M. Weaver, PhD, who reviewed a dossier of information pertinent to the safety of calcium chloride as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of calcium chloride and unanimously concluded that the use of calcium chloride in the production of potato snacks at a maximum level 1%, produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications is safe. It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and information would reach the same conclusion. Therefore, the proposed use of calcium chloride in the production of potato snacks at a maximum level 1% is GRAS by scientific procedures under the conditions of use described.

Availability of Information

The data and information that serve as the basis for this GRAS determination, as well as the information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times from at the office of Nga Tran at Exponent Inc., 1150 Connecticut Ave, NW, Suite 1100, Washington, DC 20036.

Description of Substance

Identity and Chemical and Common Names

Calcium chloride (anhydrous) is the subject of this GRAS determination. Synonyms for calcium chloride include calcium dichloride and calcium (2+) chloride.

Chemical Abstracts Service (CAS) Registry Number

The CAS number for calcium chloride (anhydrous) is 10043-52-4.

Molecular Weight and Chemical and Structural Formulas

Calcium chloride (anhydrous), $CaCl_2$, has a molecular weight of 110.98 g/mol.

The structure of calcium chloride (anhydrous) is shown in Figure 1.

Figure 1. Structure of Calcium Chloride (anhydrous).

Source: www.chemicalbook.com

Product Specifications

Calcium chloride, the subject of this GRAS determination, may be dry powder or a solution (32% $CaCl_2$) and meets Food Chemicals Codex (FCC) specifications, 9th Edition. The assay of calcium chloride powder requires that the additive contains not less than 93% and not more than 100.5% by weight of calcium chloride. The assay of calcium chloride solution requires that the additive contains not less than 90% and not more than 110.0% by weight of the labeled amount of calcium chloride expressed as $CaCl_2$. The FCC 9th specifications for calcium chloride and calcium chloride solution are provided in Appendix A. Analytical data from representative non-consecutive batches of calcium chloride powder and calcium chloride solution (32% $CaCl_2$) (see Appendix B) demonstrate that the ingredient meets product specifications appropriate for food ingredients.

Manufacturing Information

The food grade calcium chloride solution (32% $CaCl_2$) intended for use in the production of potato snacks is produced from raw brine. The calcium chloride raw brine is sieved and mixed with water and hydrochloric acid. The solution is filtered and stored. Stored solution is further filtered before putting into drums, pails, totes or tank truck for transport and distribution. The manufacturing process flow for calcium chloride solution is provided in Figure 2

The food grade calcium chloride powder intended for use in the production of potato snacks is produced from raw $CaCl_2$. Raw $CaCl_2$ is evaporated and undergoes desulphation with barium chloride. Hydrochloric acid is added and liquid $CaCl_2$ is evaporated and dried for prilling. Dry $CaCl_2$ undergoes metal detection then storage and packaging for shipment to customer. The manufacturing process flow for calcium chloride powder is in figure 3.

All procedures in the production of calcium chloride solution and powder are consistent with current Good Manufacturing Practice (cGMP).

Figure 2. Manufacturing Process Flow – Calcium Chloride Solution

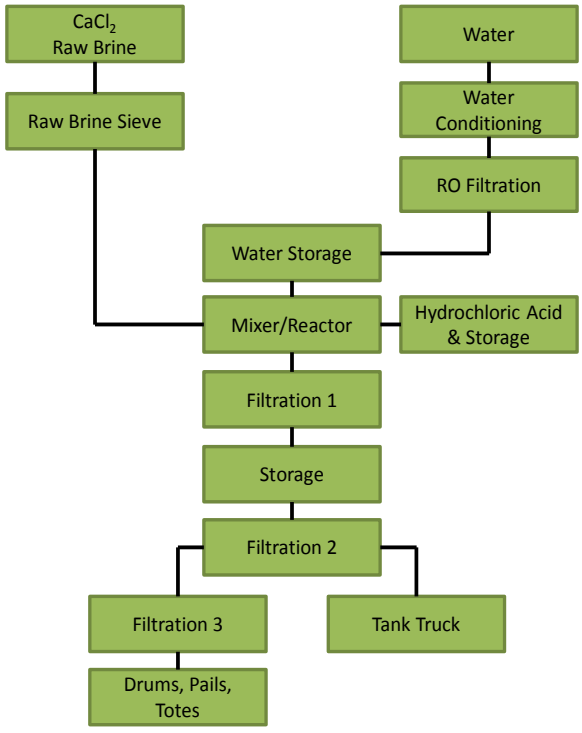
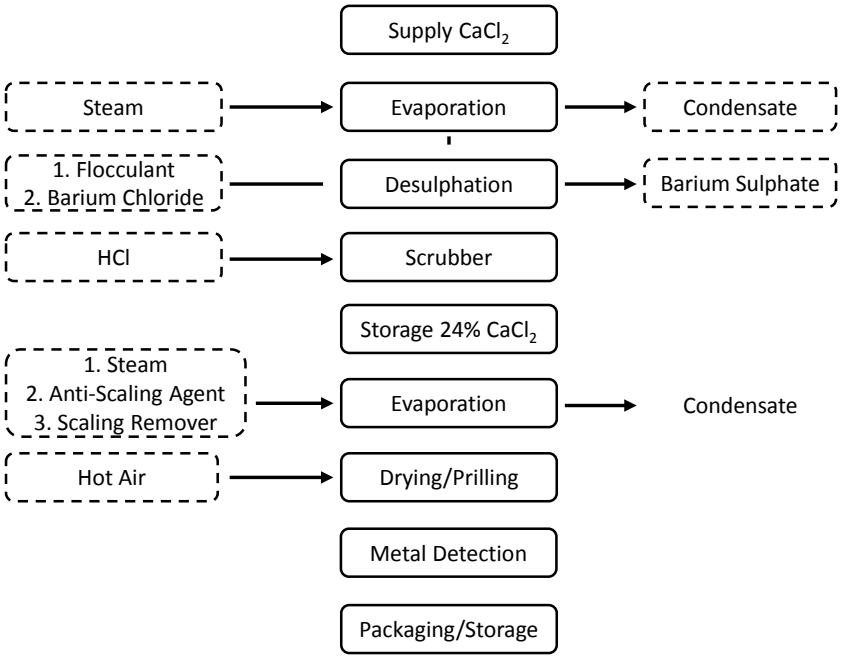


Figure 3. Manufacturing Process Flow – Calcium Chloride Powder



Current Regulated Uses

Calcium chloride has numerous food uses in the U.S. and throughout the world. Calcium chloride is listed as a food additive by Codex Alimentarius in the Codex General Standard for Food Additives (GSFA) with the functional class designation including firming agent, stabilizer, and thickener. The list of GSFA Provisions for calcium chloride is summarized at GSFA Online (FAO/WHO Food Standards Codex Alimentarius http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=197).

Calcium chloride is approved as a food additive (Group I) in the European Union (EU) for use in dehydrated milk, ripened cheese, canned or bottled fruit and vegetables, jams, jellies, marmalades and sweetened chestnut puree, and other similar fruit or vegetable spreads (E 509; https://webgate.ec.europa.eu/sanco_foods/main/index.cfm?event=substance.view&identifier=227).

In the U.S., calcium chloride is affirmed as generally recognized as safe (GRAS) (21 CFR §184.1193) for use as an anticaking, antimicrobial, curing or pickling, firming, pH control, or surface-active agent as well as a flavor enhancer, humectant, processing aid, stabilizer and thickener, synergist, and texturizer. These uses and the approved use levels for calcium chloride in select foods are summarized in Table 1.

Table 1. Permitted uses of calcium chloride in food

Category of Food	**Maximum Level (%)**
Baked goods and baking mixes, including all ready-to-eat and ready-to-bake products, flours, and mixes requiring preparation before serving.	0.3
Dairy product analogs, including nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products.	0.3
Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks.	0.22
Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip, and miscellaneous cheeses.	0.2
Processed fruits and fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, ‘‘ades’’, and drink substitutes made therefrom.	0.2
Coffee and tea, including regular, decaffeinated, and instant types.	0.32
Condiments and relishes, including plain seasoning sauces and spreads, olives, pickles, and relishes, but not spices or herbs.	0.4
Gravies and sauces, including all meat sauces and gravies, and tomato, milk, buttery, and specialty sauces.	0.2
Jams and jellies, commercial, including only commercially processed jams, jellies, fruit	0.1

Category of Food	Maximum Level (%)
Meat products, including all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.	0.25
Plant protein products, including the National Academy of Sciences/National Research Council ‘‘reconstituted vegetable protein’’ category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins.	2.0
Processed vegetables and vegetable juices, including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends.	0.4
All Other Foods	0.05

In addition to the approved uses of calcium chloride, there are several GRAS notifications involving calcium-containing compounds that have been submitted to FDA with no questions from FDA regarding the safety of the intended uses. A summary of these notices are summarized below in Table 2.

Table 2. Summary of GRAS notifications1 for calcium-containing compounds and FDA’s response

GRAS Notification No.	Substance	FDA's Response
11	Calcium casein peptone-calcium phosphate	FDA has no questions
28	Seaweed-derived calcium	FDA has no questions (additional correspondence available)
52	Whey mineral concentrate	FDA has no questions
136	Calcium gluconate	FDA has no questions
157	Calcium propionate (alternative method of manufacture)	FDA has no questions
363	Calcium disodium ethylenediaminetetraacetic acid (EDTA) and disodium EDTA	FDA has no questions
420	Calcium acid pyrophosphate	FDA has no questions
451	Calcium ascorbate with added threonate	FDA has no questions

There are also several calcium salts 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. 5 184.1 191), calcium citrate (§ 184.1 195), calcium hydroxide (§ 184.1205), calcium oxide (§ 184.121 0), and ground limestone (S 184.1409) have been affirmed as GRAS with no

[1] http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing

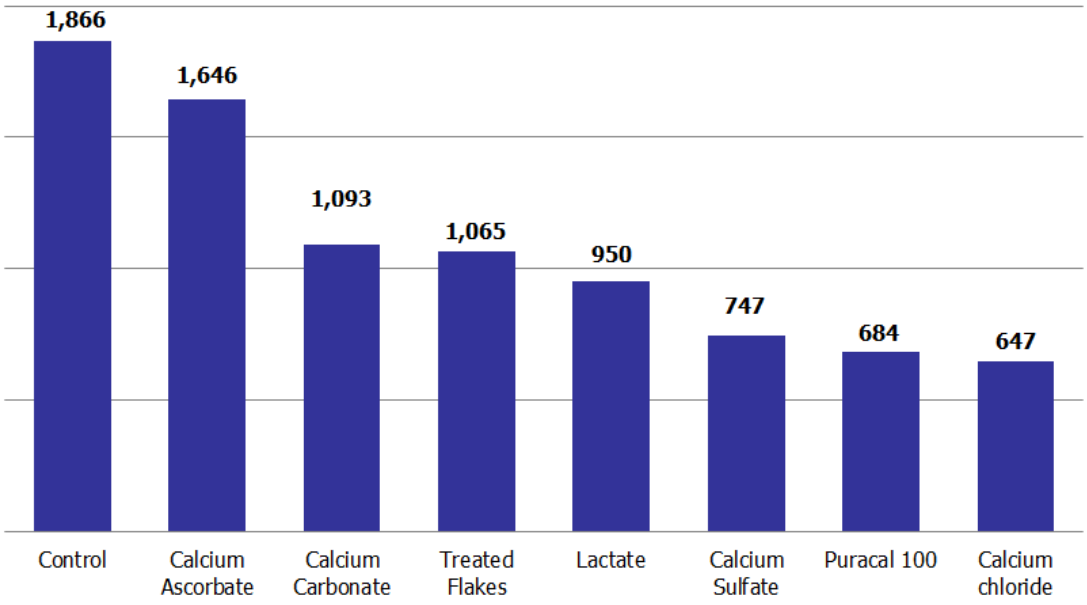
limitations other than GMP. Calcium glycerophosphate (§ 184.1201), calcium lactate (§ 184.1207), and calcium pantothenate (§ 184.12 12) have been affirmed as GRAS as nutrient supplements.

Chloride is the most abundant anion in all animal species. The total chloride in the adult human body is approximately 70-95 g (Malakooti et al., 2011). Chloride salts of alkali metals are listed as GRAS (part 182) or affirmed as GRAS (Part 184); sodium chloride (salt) is listed as GRAS (§ 21 CFR 182.1); potassium chloride (§ 184.1622) is used as a flavor enhancer, as a nutrient supplement, as a pH control agent and as a stabilizer or thickener with no limitation other than cGMP. Chloride salts of alkaline earth metals, such as magnesium chloride (§ 184.1426) is used as a flavoring agent and adjuvant and a nutrient supplement as defined at levels not to exceed cGMP; it also may be used in infant formula.

Proposed Use and Levels

Calcium chloride is proposed for use in the production of potato snacks (e.g. potato chips and sticks). The intended technical effect of the proposed use of calcium chloride in the manufacturing of potato snacks is to reduce the formation of acrylamide. The effectiveness of calcium chloride as a mitigator of acrylamide levels in potato snacks has been evaluated by PepsiCo. Calcium chloride was observed to reduce acrylamide levels in three different potato snack products with percent reduction ranging from 45% to 65%. Results of an acrylamide mitigation study conducted by PepsiCo demonstrating greater than 50% reduction of acrylamide levels are summarized in Figure 4 below.

Figure 4. Acrylamide mitigation study



Source: PepsiCo; Study Objective: Study effect of various calcium salts on acrylamide (AA) levels in Munchos fried pellets; Methods: 0.3% Ca^{2+} applied to Munchos dry mix, fried to 1.5% finished moisture; Results: Greater than 50% reduction was observed with calcium chloride, Puracal, and calcium sulfate.

Calcium chloride is proposed to be added at a level up to a maximum of 1% in the potato flour mixtures that are extruded into pellets; the potato flour pellets are subsequently air-popped into potato snacks for consumption. For the purpose of the intake assessment, the maximum concentration of calcium chloride at 1% in the potato snacks (e.g. potato chips and sticks) was assumed.

Estimated Daily Intake (EDI)

The estimated daily intake (EDI) of calcium chloride, chloride and calcium from the proposed use of calcium chloride in potato snacks at a maximum concentration of 1%, and the cumulative intake of calcium (background + proposed new use) in the U.S. population was determined using two main sources of data: (1) food intake and supplement use data from the National Health and Nutrition Examination Survey (NHANES) (2007-2008 and 2009-2010) and (2) nutrient composition data from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS). The following sections describe the data and method used in this analysis in more detail.

NHANES Data

Data from the combined 2007-2008 and 2009-2010 (2007-2010) What We Eat in America (WWEIA), the dietary recall component of the National Health and Nutrition Examination Survey (NHANES) was used to conduct the intake assessment. The WWEIA/NHANES 2007-2010 (NCHS 2010, 2012) is a complex multistage probability sample designed to be representative of the civilian U.S. population. The WWEIA survey collects two days of food intake data, in addition to nutrition, demographic, and health information. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population and the selected age-gender subgroups. The analysis was limited to respondents with complete and reliable two-day dietary records as determined by the NCHS (N=16,244) and was completed using Exponent's Foods and Residue Evaluation Program (FARE®) software.

Food and Nutrient Database for Dietary Studies (FNDDS)

For each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) database provides information on the amount of energy and on approximately 60 nutrients or food constituents per 100 g of each food. The most recent version of FNDDS, version 5.0 (FNDDS 5), was the main source of calcium composition data for this analysis. FNDDS 5 was based on nutrient values in the USDA National Nutrient Database for Standard Reference, Release 24 (SR 24) (USDA, 2012a), and was used by USDA to process dietary recall data reported in NHANES 2009-2010 (USDA, 2012b). When a food was unique to the 2007-2008 period (i.e., not reported in 2009-2010 by participants and thus not available in FNDDS 5), composition data was based on the earlier release of the food and nutrient database, FNDDS version 4.1 (USDA, 2010).

The FNDDS database represents the nutrient content of foods currently on the market and consumed by the US population. The FNDDS database is used in numerous research projects to calculate the amounts of nutrients in foods consumed by the U.S. population. Applications of the FNDDS database include the What We Eat in America (WWEIA – NHANES), MyPyramid Tracker, the Food Commodity Intake Database (FCID) developed by the US EPA and USDA-ARS, and the National Cancer Institute Diet History Questionnaire (NCI-DHQ). In fact, the IOM 2011 report on Calcium and Vitamin D used an earlier version of this database to estimate usual

intake of calcium from dietary sources in the US population and select subpopulations. Therefore, it is reasonable to assume that the FNDDS database is comprehensive and provides a complete estimate of the total amount of calcium in foods from all sources, including naturally occurring (e.g., milk), all calcium fortification uses, and regulated uses of calcium chloride.

24-hour Dietary Supplement Use

Starting in 2007-2008, NHANES collected supplement use data along with food consumption data as part of the 24-hour dietary recall data collection. The data collection for the 24-hour dietary supplement use is administered by trained dietary interviewers. During the 24-hour recall, NHANES participants who reported taking supplements in the past 30 days in the household questionnaire were asked if they took these supplements in the previous 24 hours, and if so how much they took. All participants in the 24-hour recall were also asked if they took any other supplements not reported during the 30-day supplement use household interview, and if so, they were asked to report how much they took. The use of non-prescription antacids containing calcium and/or magnesium is included in this database. NHANES has preprocessed the supplement recall data and derived nutrient intakes from supplements for NHANES 2007-2010. Therefore, estimated calcium intake from supplements as provided by NHANES was integrated into the EDI.

Analysis

Background Sources of Calcium

Estimates of calcium intake from background sources included reported intakes of calcium from all dietary sources and supplements. Estimates of calcium from background food sources were derived from food consumption data reported in the NHANES 2007-2010 in combination with calcium level in foods as provided in the USDA FNDDS database. As described above, the dietary recall portion of the NHANES survey consists of two non-consecutive 24-hr recalls. For each subject with a complete 2-day dietary recall, intake of calcium was derived by summing an individual's intake of calcium on day 1 and day 2 of the survey and dividing that sum by 2. If a survey participant consumed food that contained calcium on only one of the survey days, their calcium intake from that day was divided by two, to obtain their 2-day average intake. Intake of supplemental calcium by each respondent was added to the intake of calcium from food sources to estimate the total potential intake of calcium per person from both dietary and supplemental sources.

Proposed Use

NHANES 2007-2010 respondents reported consumption of approximately 5,600 specific foods; each food is identified by USDA by a unique 8-digit food code. The following food codes representing potato snacks were included in the intake assessment:

NHANES Food Code	NHANES Food Description
71201010	White potato, chips
71201015	White potato chips, regular cut
71201020	White potato chips, ruffled, rippled, or crinkle cut
71201050	White potato, chips, reduced fat
71201080	White potato, chips, fat free
71201090	White potato chips, fat free, made with Olean
71201100	White potato, chips, restructured*
71201200	White potato, chips, restructured*, reduced fat and reduced sodium
71201210	White potato, chips, restructured*, fat free, made with Olean
71201250	White potato, chips, restructured*, baked
71202000	White potato, chips, unsalted
71202100	White potato, chips, unsalted, reduced fat
71205000	White potato, sticks
71211000	White potato skins, chips

*represented extruded

The two-day average intake of calcium chloride from consumption of potato snacks containing calcium chloride at 1% in the finished product (i.e., food as consumed) were estimated for each individual in the NHANES 2007-2010 database. Two-day average calcium intake for the proposed use of calcium chloride in potato snacks was estimated for each individual by multiplying the calcium chloride intake by the proportion of calcium chloride that is calcium (i.e., 36.1%). This approach assumes that 100% of the calcium in calcium chloride is bioavailable as calcium in the human body and that all potato snacks included in the analysis will contain calcium chloride at 1%.

Cumulative EDI – Calcium

To estimate the cumulative EDI for calcium from all potential sources, each individual's current background calcium intake (food and supplement) was added to his/her potential calcium intake from the proposed use of calcium chloride in potato snacks.

The mean and 90th percentile of 2-day average calcium intake (from background, proposed new use in potato snacks , and cumulative total from background and proposed new use) were calculated for the total US population 1+ y and several subpopulations as defined by the IOM-Dietary Reference Intake (DRI). Infants 0 to 5 months were excluded from the analysis due to the fact that potato snacks are not infant foods and in the NHANES 2007-2010, there was no reported consumption of potato snacks among infants 0 to 5 months.

The estimates based on 2-day average intakes do not necessarily represent long-term intakes, since they (1) may not capture infrequent consumers of occasionally eaten food such as potato snacks, (2) assume that subjects who consumed such a food on both survey days actually consume it every day of the year, and (3) do not adjust for potential day-to-day variation in intake. A 2-day average typically overestimates long-term (chronic) daily intake.

All estimates of intake per person were generated using Exponent's Foods and Residues Evaluation Program (FARE® version 10.05) software. Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

Results

EDI from Proposed Uses

For the U.S. population age 1 year and older, the *per user* mean and 90th percentile intakes of calcium chloride from the proposed use in the potato snacks were 201 and 408 mg/day, respectively. This corresponds to calcium intakes of 72 and 147 mg/day, respectively, and chloride intake of 128 and 261 mg/day, respectively (see Table 3).

The major form of dietary sodium is sodium chloride, i.e. 90% (IOM, 2004). Thus, based on the usual mean daily intake for sodium among the US population 1 year and older (i.e. 3.44g/day, USDA 2013) and on a molar equivalent basis, a usual mean daily intake of 4.77 g of chloride from existing dietary sources can be estimated. The EDI for chloride from the proposed use of calcium chloride is a small fraction (3-8%) of this existing background dietary exposure to chloride from sodium chloride sources.

Male adolescents were estimated to have the highest intakes of calcium from the proposed uses; among males 14-18 y the estimated *per user* 90th percentile intake of calcium from potato snacks was 249 mg/day, respectively (see Table 3). There were only 5 consumers of potato snacks among infants 6-11 months; not an adequate sample size to provide reliable intake estimates.

Table 3. Estimated daily intake of calcium chloride and calcium from proposed uses by the U.S. population 1+ y and subpopulations (mg/day)

			Per User (mg/day)					
			Calcium Chloride		**Calcium**		**Chloride**	
Population	**Unwtd-N**	**% Users**	**Mean**	**90th**	**Mean**	**90th**	**Mean**	**90th**
U.S. 1+ y	3,177	21	201	408	72	147	128	261
Infants 0-5 months	0	0	--	--	--	--	--	--
Infants 6-11 months	5	0.7	27	NA	9.8	NA	17.2	NA
Males								
Children 1-3 y	125	19	111	200	40	72	71	127
Children 4-8 y	213	28	177	290	64	105	113	185
Children 9-13 y	181	28	202	376	73	136	129	240
Adolescents 14-18 y	134	19	289	691	104	249	185	441
Adults 19-30 y	181	20	253	419	91	151	161	267
Adults 31-50 y	314	22	264	569	95	205	169	363
Adults 51-70 y	317	22	189	331	68	120	121	212
Adults 71+ y	115	15	162	326	58	118	103	209
Females								

Population	Unwtd-N	% Users	Per User (mg/day)					
			Calcium Chloride		Calcium		Chloride	
			Mean	90th	Mean	90th	Mean	90th
Children 1-3 y	116	20	102	164	37	59	65	105
Children 4-8 y	186	26	161	306	58	110	103	194
Children 9-13y	199	28	180	295	65	106	115	188
Adolescents 14-18 y	129	21	169	306	61	111	108	196
Adults 19-30 y	195	20	168	280	61	101	107	179
Adults 31-50 y	367	22	199	354	72	128	127	226
Adults 51-70 y	269	19	205	490	74	177	131	313
Adults 71+ y	136	17	122	212	44	76	78	135

NA = Not available; sample size is not adequate to provide a reliable estimate.

Cumulative Estimated Daily Intake (CEDI) for Calcium

Cumulative intake of calcium is summarized in Table 4. For the U.S. population age 1 year and older, the *per user* mean and 90th percentile levels of intake of calcium from all sources, including background sources and the proposed uses, were estimated at 1,152 and 1,936 mg/day, respectively. Children 1-3 y had estimated *per user* mean and 90th percentile intakes from all sources of calcium of 1,041 and 1,557 mg/day, respectively, among males and 1,029 and 1,559 mg/day, respectively, among females. Children 4-8 y had estimated *per user* mean and 90th percentile calcium intakes of 1,087 and 1,689 mg/day, respectively, among males and 1,008 and 1,568 mg/day, respectively, among females. Among the older subpopulations, estimated 90th percentile intakes were highest among the older adults (51+ y) ranging from 1,918 mg/day among males 71+ y to 2,204 mg/day among women 51-70 y. Infants 6-11 months cumulative calcium intake remains the same as the background estimates when the proposed use of calcium chloride in potato snacks were included (mean = 678 mg/day and 90th percentile = 1,113 mg/day).

Table 4. Estimated daily intake of calcium from background (total diet + supplements) and proposed uses of calcium chloride by the U.S. population 1+ y and subpopulations and calcium tolerable upper intake levels (mg/day)

Population	n	% Users	Estimated Daily Intakes (EDIs) of Calcium (mg/day)				Tolerable Upper Intake Level (UL)[c] (mg/day)
			Background Sources[a]		Cumulative[b] (background + proposed)		
			Mean	90th	Mean	90th	
U.S. 1+ y	15,498	100	1,138	1,926	1,152	1,936	NA
Infants 0-5 months	382	100%	406	724	NA	NA	1,000
Infants 6-11 months	364	100%	678	1,113	678	1,113	1,500
Males							
Children 1-3 y	641	100	1,033	1,557	1,041	1,557	2,500
Children 4-8 y	806	100	1,069	1,678	1,087	1,689	2,500
Children 9-13 y	718	100	1,127	1,769	1,147	1,813	3,000
Adolescents 14-18 y	680	100	1,305	2,027	1,325	2,138	3,000

Population	n	% Users	Estimated Daily Intakes (EDIs) of Calcium (mg/day)				Tolerable Upper Intake Level (UL)[c] (mg/day)
			Background Sources[a]		Cumulative[b] (background + proposed)		
			Mean	90th	Mean	90th	
Adults 19-30 y	884	100	1,233	2,157	1,251	2,186	2,500[d]
Adults 31-50 y	1,529	100	1,205	1,966	1,226	1,982	2,500[d]
Adults 51-70 y	1,552	100	1,195	2,023*	1,210	2,084*	2,000 – 2,500[d]
Adults 71+ y	804	100	1,110	1,901	1,119	1,918	2,000-2,500[d]
Females							
Children 1-3 y	601	100	1,022	1,557	1,029	1,559	2,500
Children 4-8 y	713	100	993	1,573	1,008	1,568	2,500
Children 9-13 y	741	100	1026	1,625	1,044	1,621	3,000
Adolescents 14-18 y	635	100	930	1,497	943	1,507	3,000
Adults 19-30 y	995	100	982	1,557	993	1,574	2,500[d]
Adults 31-50 y	1,759	100	1,055	1,816	1,071	1,821	2,500[d]
Adults 51-70 y	1,565	100	1,280	2,195*	1,294	2,204*	2,000-2,500 [d]
Adults 71+ y	875	100	1,230	2,158*	1,238	2,162*	2,000-2,500 [d]

n = Unweighted number of survey respondents identified as consumers of calcium; weighted % consumers.
Estimates based on 2-day average intakes reported in NHANES 2007-2010.
NA= Not applicable; there was no reported consumption of potato snacks in this age group.
[a] EDIs include naturally occurring calcium and calcium that may be added to foods as noted in 21 CFR and reported use of calcium-containing dietary supplements.
[b] EDIs include naturally occurring calcium and calcium that may be added to foods as noted in 21 CFR, reported use of calcium-containing dietary supplements, and the calcium from the proposed maximum use of 1% calcium chloride in potato snacks.
[c] Calcium ULs as reported in IOM 2011.
[d] Calcium ULs as reported in EFSA 2012.
*EDI within the range of exposure limits for calcium (IOM UL 2000 mg/day – EFSA UL 2500 mg/day).

Overall, the cumulative (background + proposed use) *per user* 90th percentile intakes of calcium were below the IOM UL for the subpopulations of infants 6-11 months, children, adolescents and adults 19-50 y and males 71+ y (Table 4).

Women 51-70 and 71+ years and Males 51-70 years

The background (food sources + supplement) per user 90th percentile calcium intakes based on 2-day averages exceeded the IOM UL of 2,000 mg/day (but below the EFSA UL of 2,500 mg/day) among older women 51 -70 y (2,195 mg/day) and 71+ y (2,158 mg/day) as well as among men 51-70 y (2,023 mg/day), see Table 5. These findings are consistent with the 2011 IOM report of usual calcium intakes exceeding the UL at the 95th and 99th percentiles (as analyzed by Bailey et al. 2010 with further data provided by staff at the National Cancer Institute – National Institutes of Health).

Further source contribution analyses stratified based on 1) all calcium consumers (i.e. supplement and non-supplement consumers combined); 2) supplement consumers and 3) non-supplement consumers showed the following:

- Background calcium intake from food sources alone are below the IOM UL at the per user 90th percentile for these subpopulations, irrespective of supplement use status (see Table 5). Dietary calcium intakes among high-end consumers (i.e., per user 90th percentile) range from 1,274 mg/day among females 71+ y to 1,721 among males 51-70 y.
- The additional calcium intake from the use of supplements drives total background calcium intake to exceed the IOM UL at the 90th percentile for all calcium consumers and supplement consumers (see Table 5). At the 90th percentile, calcium from supplement use contributes up to 65% of the total background calcium intake among all calcium consumers. It should also be noted that almost two-thirds (65%) of the women 71+ y reporting use of a calcium-containing supplement in the NHANES database, representing the largest supplement user group.
- The proposed use of calcium chloride in potato snacks contributes minimally to the total cumulative calcium intake at the 90th percentiles
    - Among all calcium consumers: the proposed use contributes 3- 5% (31 – 59 mg/day) additional calcium.
    - Among non-calcium-supplement users: the proposed use contributes 2-7% (20 – 51 mg/day) additional calcium. The per user 90th percentile of total cumulative calcium intake from both background and proposed use of calcium chloride for the non-supplement uses ranges from 1,265 mg/day to 1,639 mg/day among females and males 51-70 y, respectively, all well below the IOM UL.

Table 5. Estimated daily intake of calcium from food, supplements, and proposed uses of calcium chloride by older adults (51+ y) (mg/day) and contribution to total calcium intake (%)

Population		% reporting calcium supplement use	Estimated Daily Intakes (EDIs) of Calcium (mg/day)									
			All Users						Non-supplement users			
			Food[1]	Supplement[2]	Total background	Proposed use[3]	Cumulative	% from proposed use	Food[1]	Proposed use[3]	Cumulative	% from proposed use
Males 51-70 y	Mean	42%	1,036	160	1,195	15	1,210	1%	974	13	987	1%
	90th		1,721	500	2,023*	59	2,084*	5%	1,625	51	1,639	5%
Males 71+ y	Mean	52%	904	207	1,110	9	1,119	1%	851	7	858	1%
	90th		1,471	600	1,901	34	1,918	3%	1,343	20	1,343	2%
Females 51-70 y	Mean	58%	878	402	1,280	14	1,294	1%	780	17	797	2%
	90th		1,438	1,200	2,195*	48	2,204*	5%	1,249	51	1,265	7%
Females 71+ y	Mean	65%	788	442	1,230	7	1,238	1%	735	7	742	1%
	90th		1,274	1,200	2,158*	31	2,162*	3%	1,266	32	1,266	4%

1.Calcium intake from food; include naturally occurring calcium and calcium that may be added to foods as noted in 21 CFR
2.Calcium intake from reported use of calcium-containing dietary supplements.
3.Calcium intake from proposed maximum use of 1% calcium chloride in potato snacks.
*EDI exceeds the IOM UL for calcium.

Summary

The EDIs presented in this analysis are based on 2-day average estimates. No adjustment has been made to account for the potential overestimation of intakes that may result from using two days of dietary data to estimate long-term consumption and that not all of the calcium consumed will be bioavailable. Given this conservative approach and that not all of the foods included in the proposed food category in this assessment will contain the calcium chloride, the estimated exposures to calcium for each population group are likely overestimates of actual calcium intake.

In summary, these analyses were designed to estimate background intake of calcium from all food sources (i.e., all naturally-occurring and calcium fortified food sources and approved food additive uses of calcium chloride, as measured by the USDA), calcium from dietary supplements, and calcium intake from the proposed use of calcium chloride in potato snacks. Results of these analyses indicate that cumulative calcium intakes at the 90th percentile from all sources combined (background + proposed use in potato snacks) are below the calcium IOM UL for the majority of the age-based subpopulations. For the three older subpopulations (males and females 51-70 y and females 71+ y), the 90th percentile background calcium intake falls within the range of exposure limits (the IOM UL of 2,000 mg/day and the EFSA UL of 2,500 mg/day). For these older populations, calcium from dietary supplements was the main contributing source of exposure (supplement use contributes up to 69% of the total background calcium intake among supplement consumers). The calcium contribution from the proposed use of calcium chloride in potato snacks contribute less than 7% of the cumulative total calcium intake among these older age groups. Among the non-supplement consumers, the per user 90th percentile of total cumulative calcium intake from both background and proposed use of calcium chloride ranges from 1,265 mg/day to 1,639 mg/day among females and males 51-70 y, respectively, which are well below the IOM UL of 2,000 mg/day. All EDIs for all population groups were below the EFSA ULs established in 2012.

Safety Evaluation

Introduction

Calcium chloride readily dissociates into calcium and chloride ions in water. Calcium is a nutrient for which dietary recommendations have been established. The current dietary recommendations for calcium intake for the U.S. population, which were initially released on November 30, 2010, were developed by an ad hoc consensus committee of 14 scientists established by the IOM. These recommendations supersede the recommendations released by the IOM in 1997 (IOM 1997). As part of the recent IOM review and establishment of the current recommendations, the toxicology, metabolism, and overall safety of calcium was analyzed in detail by the IOM's Food and Nutrition Board through the work of its Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. This analysis was published in 2011 as part of the report, *Dietary Reference Intakes for Calcium and Vitamin D* (hereinafter referred to as the "IOM report") (IOM 2011).

A toxicological assessment of calcium chloride (IUPAC name, calcium dichloride; chemical formula, $CaCl_2$; molecular weight, 110.98; CAS No. 10043-52-4) based on preclinical and clinical literature regarding the safety of calcium chloride, as well as on other calcium salts, and its component ions, Ca^{2+} and chloride (Cl^-), was conducted and is summarized below. The pharmacokinetics data for calcium in humans was also evaluated and is also summarized.

Several searches were conducted between October 2013 and October 2015. The following resources were searched for safety information in October 2013 using the CAS number or chemical name: TOXNET, National Toxicology Program (NTP), European Food Safety Authority (EFSA), European Chemical substances Information system (ESIS) including IUCLID, Chemical Safety Information from Intergovernmental Organizations (Inchem), MedlinePlus (health information related to medicine), Organization for Economic Cooperation and Development (OECD), Joint FAO/WHO Expert Committee on Food Additives (JECFA), FDA GRAS inventory, EPA's IRIS and High Production Volume (HPV) databases, and Food and Chemical Toxicology journal. Searches of PubMed, Google Scholar and Google were also conducted in October 2013 using the following terms:

> "calcium chloride OR calcium dichloride OR calcium salts AND (mutagenicity OR acute toxicity OR subchronic toxicity OR reproductive OR developmental OR carcinogenicity OR absorption OR metabolism OR digestion OR excretion OR toxicity OR safety)"

The October 2013 PubMed search captured 1,114 citations, of which 15 were relevant to the safety of calcium chloride. This search was limited to animal studies and the "title/abstract" filter was applied to the search terms. This search was updated in October 2015 and captured 426 citations, but no additional relevant studies were identified. The TOXNET search yielded 3,100 records. On further inspection of these records, most of them were not relevant to the safety of calcium chloride and those that were relevant had been previously captured in the PubMed search. Most of the publications captured following Google Scholar or Google searches were previously retrieved through the PubMed search and TOXNET search. Several authoritative bodies have evaluated the safety of calcium or calcium chloride. A safety assessment report

(OECD, 2002) for calcium chloride was identified from the Screening Information Data Set (SIDS) of the Organization for Economic Cooperation and Development (OECD). This report titled "SIDS Initial assessment report for SIAM 15" and dated October 2002 consists of a thorough evaluation of the safety of calcium chloride. A review by the IOM-DRI report of 2011 for calcium and vitamin D (IOM, 2011) was captured along with EFSA's Panel on Dietetic Products, Nutrition and Allergies scientific opinion on the UL of calcium (EFSA, 2012).

Another PubMed search for human health information on calcium published after the IOM report (2011) was conducted in October 2013 to capture any relevant studies published between June 1, 2010 and June 20, 2014. Subsequent literature searches were also performed in June 2014 and October 2015 for human health information published between June 2014 and January 2016 (to capture publications ahead of print). Details of the results of these searches are provided in Appendix C. An overview of the metabolism and toxicity of calcium and calcium chloride; a discussion of the safety of chloride, and a summary of the current DRIs for calcium, including the UL established by the IOM and EFSA in their separate re-evaluations of calcium in 2011 and 2012, respectively, and a review of safety data published since release of the IOM and EFSA reports are summarized herein.

Absorption, Distribution, Metabolism, and Excretion

Calcium Chloride

Calcium chloride is a salt that readily dissociates into calcium ion and chloride counter-ion in water. The absorption, distribution and excretion of calcium and chloride in animals is regulated separately (OECD, 2002) and as such is described separately below. In addition, calcium may be encountered in several forms of salt not limited to calcium chloride, including calcium carbonate and calcium citrate.

Chloride

Chloride is the most abundant anion in all animal species. The total chloride in the adult human body is approximately 70-95 g. Eighty percent of the chloride is located extracellularly. The intracellular concentration of chloride is ~ 100-140 ug/mL. Chloride is absorbed via co-transporters in the intestine, which transport sodium and chloride ions (Malakooti et al., 2011), as well as by co-transport re-uptake systems in the kidney (Richardson and Alessi, 2009). Although chloride is absorbed efficiently from the intestine, the chloride concentration in plasma is maintained around 3.55-3.90 mg/mL (OECD, 2002). Control of chloride levels is maintained by the balance of excretion and uptake, with participation of active re-uptake and excretion in the kidney (Richardson and Alessi, 2009; Malakooti et al., 2011). Because chloride is a monoatomic ion, it is not metabolized to another species. Chloride is secreted from the renal tubular lumen by active transport systems, and also by passive diffusion (OECD, 2002).

Calcium

Absorption

The efficiency of calcium absorption is affected by the presence of dietary components including phosphorus (Gueguen and Pointillart, 2000), by the vitamin D and calcium status of the body, and also by the physiological state of the individual such as growth, age, pregnancy, disease, and lactation (Allen, 1982). For calcium to be absorbed through the wall of the intestine, it must be in a soluble form, generally ionized (Ca^{2+}) in the upper small intestine or bound to a soluble organic molecule (Gueguen and Pointillart, 2000; EFSA, 2012; IOM, 2011; OECD, 2002). The solubility of calcium complexes appears to increase when gastric acid is present (Allen, 1982). The pH of the intestine after food consumption is reported to be about 6.0. Calcium tends to precipitate from solutions with pH > 6.1, such that dietary calcium is present in a more absorbable form in the duodenum and proximal jejunum. In addition, the calcium binding protein is found mainly in the duodenum and proximal jejunum. Hence, most absorption of calcium takes place in the duodenum and proximal jejunum because of the combination of acid pH and calcium binding protein in these areas of the small intestine (Allen, 1982). Absorption is a result of active transport across cells, mainly in the duodenum and upper jejunum, and by passive diffusion which occurs throughout the small intestine, but mainly in the ileum and partially in the colon (Allen, 1982; Gueguen and Pointillart, 2000).

The mean calcium absorption (also referred to as “fractional calcium absorption”), which is the percentage of a given dose of calcium that is absorbed, has been determined by a number of investigators, and while the absorption values may vary between calcium salts, they do not vary dramatically between chloride and the other commonly consumed salts, except for the oxalate salt (Guegen and Pointillart, 2013; Sheikh et al., 1987). EFSA reports that the mean absorption for calcium in general ranges from approximately 10 to 40% with approximately 25% as the average for adults (EFSA, 2012). In a series of tightly controlled metabolic in-house feeding studies conducted by the USDA in men and non-pregnant women (n =155) across a wide age range, the mean calcium absorption was demonstrated to be approximately 25 percent of calcium intake (Hunt and Johnson, 2007). The mean calcium absorption from calcium chloride specifically was reported to be 30.6 %, with an average of 23 to 37% for all calcium salts (Gueguen and Pointillart 2000). In the same publication, based on reviews of several references, the mean absorption of calcium salts was reported to vary from 13.2% in oxalate salt and oxalate–rich products to 26.4 (fasting) and 29 (fed) for carbonate salt, to 23.5 (fasting citrate) and 37 (fed) for the citromalate salt. In this compilation of absorption values, those for the oxalate salt are by far the lowest; however absorption values for the other salts do no vary greatly. In a study reporting primary data on calcium absorption, the mean (± standard error of the mean (SEM)) net calcium absorption was calculated in eight healthy fasting subjects after oral administration of 500 mg dose of calcium from five different calcium salts with various degrees of water solubility. Absorption from milk was reported to be 32 ± 4 % from calcium acetate, 32 ± 4 % from calcium lactate, 27 ± 3 % from calcium gluconate, 30 ± 3 % from calcium citrate, and 39 ± 3% from calcium carbonate, in decreasing order of the solubility of the salts. The differences in absorption were not statistically significant. Calcium absorption from whole milk (31 ± 3 %) was similar to absorption from calcium salts (Sheikh et al., 1987).

Mean calcium absorption (fractional calcium absorption) varies during critical periods of life. During pregnancy calcium absorption doubles (Kovacs and Kronenberg ,1997; Kovacs, 2001, as cited in IOM, 2011), probably due to increased maternal and fetal calcitriol levels, rise in maternal parathyroid hormone level, and increased active transport in the jejunum (Allen, 1982). Calcium absorption in newborns is reported to be largely passive and facilitated by the lactose content of breast milk (Kocian et al., Kobayashi et al., 1975, as cited in IOM, 2011). With age, passive absorption declines in the newborn and calcitriol-mediated active intestinal calcium uptake becomes more important (Grishan et al., 1980; Halloran and DeLuca, 1980; Ghrishan et al., 1984, as cited in IOM 2011). In infancy, it is high at approximately 60 percent, although the range is large. With aging and after menopause, fractional calcium absorption has been reported to decline on average by 0.21 % per year after 40 y (Heaney et al., 1989, as cited in IOM, 2011). Calcium absorption is also influenced by metabolic status, such that severe obesity is associated with higher calcium absorption and dieting reduces the fractional calcium absorption by 5 percent (IOM, 2011).

There is some indication that different forms of calcium (ionic or complexed) and different forms of calcium salts (citrate compared to carbonate, etc.) are absorbed differently, presumably based on the relative solubilities of the different species. In Sprague-Dawley rats, ionic calcium (Ca^{2+}) was demonstrated to be more effectively absorbed from the gut than calcium complexed with lactate, malate, and fumarate (Favus and Pak, 2001). Shiga et al. (1998) demonstrated that dietary calcium is dissolved in the stomach and absorption occurs predominantly in the small intestine. In 5-week old male Wistar/ST rats (n = 24) fed 0.2% calcium diets containing soluble calcium salts, calcium was mostly absorbed in the small intestine; in contrast, in rats fed a 0.2% calcium diet containing an insoluble calcium salt (calcium carbonate), calcium was not sufficiently absorbed in the small intestine. However, the large intestine compensates for the small intestinal calcium absorption (Shiga et al., 1998).

Distribution

The majority of calcium absorbed (99%) is stored in the skeleton and teeth (EFSA, 2012) and total calcium concentration in serum is tightly regulated to remain between 8.5 and 10.5 mg/dL (2.12 and 2.62 mmol/L) (IOM, 2011). Regulation of serum calcium levels is maintained through an endocrine system, that includes a major role for vitamin D metabolites, principally calcitriol, and parathyroid hormone (PTH). If serum calcium level drops slightly, PTH secretion increases as the calcium sensing receptor in the parathyroid gland senses changes in circulating ionic calcium. Increased PTH levels induce enzyme activity (1α-hydroxylase) in the kidney, which converts vitamin D to its active hormonal form, calcitriol. In turn, calcitriol stimulates enhanced calcium absorption from the gut, thereby raising serum calcium levels. As the serum calcium level rises, the feedback mechanism causes the calcium sensing receptor to be turned off and PTH secretion to drop. If there is a sudden rise in serum calcium levels, the parafollicular cells of the thyroid gland secrete calcitonin, which can block bone calcium resorption, helping to keep serum calcium levels in the normal range.

ADME Summary

Calcium chloride readily dissociates into its component ions, calcium and chloride, under aqueous conditions and in the gut. Absorption of calcium occurs in the small intestine, primarily in the duodenum and proximal jejunum by active transport and also by passive diffusion. The mean calcium absorption is about 25% of calcium intake (10 – 40%) (EFSA, 2012). Chloride absorption occurs via co-transporters in the gastrointestinal tract as well as by active re-uptake systems in the kidney. Absorbed calcium is distributed mainly in the skeleton and teeth and excess calcium is excreted in urine, feces and sweat. Chloride, which is the most abundant anion in living species, is distributed extracellularly throughout the body, and plasma concentrations are maintained between 100 – 110 mmol/L. Chloride excretion occurs mainly via the kidneys.

Safety Data

Calcium Chloride

Acute Toxicity

The lethal dose (LD_{50}) value of calcium chloride in mice was determined using OECD guideline 401, with the exception that mortality was determined by the up and down method after a 3-day observation period. Calcium chloride was administered orally to groups of 15 ICR male and female mice (n=3/dose/sex) and the LD_{50} values for each sex were determined by the up and down method after a 3-day observation period. The oral LD_{50} was determined to be 2.045 g/kg bodyweight (bw) in male mice and 1.94 g/kg bw in female mice (Akatsuka et al., 1977, as cited in OECD 2002).

The LD_{50} values of calcium chloride for male and female rats were determined by administering the substance orally to a total of 15 Wistar rats (n=3/dose/sex). The LD_{50} values were determined by the up and down method after a 3-day observation period. The oral LD_{50} was 3.798 g/kg in male rats and 4.179 g/kg bw in female rats (Akatsuka et al., 1977, as cited in OECD, 2002). In another study, the oral LD_{50} of calcium chloride in the rat was determined to be approximately 5g/kg bw (Barnes and Eltherington, 1964, as cited in SCOGS-45, 1975).

Four studies on acute oral toxicity of calcium chloride in rabbits were carried out using a method similar to OECD Test Guideline 401 under GLP guidelines except for a few modifications in number of doses or animals/dose. Several forms of calcium chloride were administered orally by gavage to New Zealand white rabbits (males) at doses of 250 to 2,000 mg/kg bw to determine the respective LD_{50} values. Weight loss in the surviving animals was observed in the first two days after dosing, which was then recovered. Gross post-mortem examination revealed perforation and severe ulceration of the stomach in the dead animals. Old ulcers were also detected in the stomach of some of the surviving animals (Koopman and Pot, 1986, as cited in OECD, 2002). The oral LD_{50} values determined for calcium chloride in the rabbit ranged from 500 – 1,000 mg/kg bw/day in this group of studies. The LD_{50} has also been reported elsewhere as 1.38 g/kg bw in the rabbit (SCOGS-45, 1975) although a specific primary reference for the study was not clearly cited.

The LD_{50} was found to be above 2 g/kg bw for the dog (Mahorner, 1937, as cited in SCOGS-45, 1975).

Subchronic Toxicity

Calcium chloride was administered at a level of 1% in the drinking water (10,000 ppm or 1 g/kg bw; n=24) or 2% in a goitrogenic basal diet (20,000 ppm or 2 g/kg bw; n = 71) over a period of 12 weeks to male and female rats that were 4 to 5 weeks old. Growth and survival of the animals were unaffected. Calcium chloride caused no thyroid enlargement when compared to that produced by the basal diet except for a slight increase in thyroid weight when vitamin D was present. No microscopic alterations were observed (Sharpless et al., 1943, as cited in OECD, 2002).

Although the cow is not one of the animal models recommended in either the OECD guidelines or FDA guidance, a short-term toxicity study of calcium chloride was conducted in cows where food and water consumption, body weight, milk production, and clinical chemistry and hematology parameters were evaluated. A 0.3 % solution of calcium chloride was given *ad libitum* to dairy cows as the sole source of water for a period of 75 days. No significant changes in feed consumption, body weight or milk production were observed. Average daily water intake was increased by approximately 20%, and signs of slight gastro-intestinal irritation were observed (softer than usual feces with mucus present). Major alterations in blood hemoglobin levels, hematocrit, total and differential white blood cell counts or thrombocyte numbers were not observed or reported to be attributable to treatment. No significant effect on the serum calcium, chloride, magnesium, potassium, or sodium content was observed. The level of inorganic phosphate in the serum rose to higher, but still normal values. Throughout the experiment, urine pH was abnormally acidic in these dairy cows. Electrocardiograms taken after 45 days of calcium chloride administration were reported to be normal. When 0.1 and 0.2% solutions were given as the sole source of water for a period of 81 days, the cows remained in good condition, and no alterations in appetite, body weight or milk production were observed. In summary, the administration of $\leq$ 0.3% calcium chloride for periods of 75 and 81 days to cows did not cause any clinical sign of toxicity (Mathieu and Pelletier, 1966).

Chronic Toxicity

A group of twenty 40-day old rats were administered 20 mg calcium chloride/g diet for 12 months (OECD, 2002). Based on the food consumption (22 g diet/day), the daily intake of calcium chloride was estimated to be 440 mg. Given that 1 mg/g diet is equivalent to 100 and 50 mg/kg bw/day for young and old rats, respectively, the dose used in this study (20 mg/g diet) corresponded to 1,000 to 2,000 mg/kg bw/day. No difference in mortality, weight gain, or daily food consumption was observed between the test and the control groups. In addition, no neoplastic lesions were observed in gastrointestinal tract, urinary tract, liver, heart, brain or spleen of the animals. These results indicate that oral chronic administration of calcium chloride to rats at 1000 – 2000 mg/kg bw/day does not induce any adverse effects to rats.

Genotoxicity

Two studies were conducted by the method similar to OECD Test Guideline 471. In a Salmonella mutation test, doses of calcium chloride up to 5 mg/plate were examined using *S. typhimurium* TA92, TA94, TA98, TA100, TA1535 and TA1537 with metabolic activation (Ishidate et al., 1984). In another Salmonella mutation test, using *S. typhimurium* TA97 and TA102, doses up to 10 mg/plate were examined with or without metabolic activation (Fujita et al., 1987 as cited in OECD, 2002). There were no significant increases in mutation frequencies in either study.

Two additional genetic toxicity studies with bacteria have also been reported. In a recombination mutagenicity assay in *Bacillus subtilis* H17 (rec+) and H45 (rec-), the potential of calcium chloride to damage cellular DNA was examined at concentrations of 0.005 - 0.5 M (Kanematsu et al. 1980, as cited in the OECD, 2002). The result of the test was negative. In an *Escherichia coli* test, the potential of calcium chloride to induce cellular DNA damage was tested at doses up to 1 mM (0.1 – 111 mg/L). This test was also negative (Olivier and Marzin, 1987, as cited in the OECD, 2002).

The ability of calcium chloride to induce chromosomal aberrations in Chinese hamster lung (CHL) cells was studied by Ishidate et al. (1984). This test was carried out in accordance with OECD guideline 473. The CHL cells were exposed to calcium chloride doses up to 4 mg/mL for 24 and 48 h respectively, without metabolic activation. The maximum dose was selected by a preliminary test, in which the dose needed for 50% cell-growth inhibition was estimated using a cell densitometer. Colcemid (final concentration 0.2 mg/mL) was added to the culture 2 h before cell harvesting and chromosome preparations were made. A hundred well-spread metaphases were observed and the incidence of polyploid cells as well as of cells with structural chromosomal aberrations was reported. No significant increase in polyploid formation or structural chromosome aberration was observed (Ishidate et al., 1984).

In summary, the body of genotoxicity studies for calcium chloride indicates that it is not genotoxic.

Reproductive and Developmental Toxicity

The reproductive toxicity of calcium chloride has not been evaluated. A developmental toxicity study examined the effect of calcium chloride on embryo lethality and teratogenicity in mice, rats and rabbits (Food and Drug Research laboratories 1974, as cited in OECD, 2002 and the GRAS Substances Database (SCOGS-45, 1975); Table 6). The method used in this study was equivalent to OECD guideline 414, although this study was conducted before the establishment of this testing guideline.

Table 6. Summary of developmental toxicity studies in mice, rats and rabbits

Species	**Strain**	**No. of animals/ group**	**Doses (mg/kg/day)**	**Administration (days of gestation)**	**Caesarian section (days of gestation)**
Mouse	CD-1	25	1.89, 8.78, 40.8, 189	6-15	17
Rat	Wistar	25	1.76, 8.18, 38.0, 176	6-15	20
Rabbit	Dutch	16-22	1.69, 7.85, 35.6, 169	6-15	29

All animals were observed daily for appearance, behavior and signs of maternal toxicity (reductions in body weight and food consumption). The numbers of implantation sites, resorption sites, and live and dead fetuses were recorded when all dams were subjected to Caesarean section. All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations. The remaining two-thirds were examined for skeletal defects. No clearly noticeable effect on implantation or on maternal or fetal survival as a result of calcium chloride was observed in mouse, rat or rabbit. In addition, 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 control group. Oral administration of calcium chloride of up to 189 mg/kg bw/day in mice (day 6 through 15 of gestation), up to 176 mg/kg bw/day in rats (day 6 through 15), and up to 169 mg/kg bw/day in rabbits (day 6 through 18), had no untoward effects on maternal or fetal survival.

In another study by Varnai et al. (2003), suckling Wistar rats (n=12) were administered by artificial feeding calcium chloride in water at a total calcium dose of 340 mg for 6 to 14 days after birth. Pups were removed from their mothers daily and given calcium chloride by pipet drip for 7 hours per day, then returned to their home cages. Pups supplemented with calcium chloride had a lower body weight gain and carcass wet weights compared to controls. The authors concluded that calcium chloride had an adverse effect on pup growth, however, it is of critical importance to note that all other test groups and control pups received cow's milk as a vehicle for dosing, while the calcium chloride group, for reasons of solubility, received calcium chloride in water. It is therefore not possible to make a scientifically sound comparison, in particular on metrics of growth, between the calcium chloride treated animals and controls in this study.

In summary, calcium chloride at doses up to 189 mg/kg bw/day in mouse, 176 mg/kg bw/day in rat and 169 mg/kg bw/day in rabbit did not cause any toxic effects on dams or fetuses in a reliable developmental toxicity study.

Summary for Calcium Chloride

The body of genotoxicity studies for calcium chloride indicates that it is not genotoxic. Calcium chloride at doses up to 189 mg/kg bw/day in mouse, 176 mg/kg bw/day in rat and 169 mg/kg bw/day in rabbit did not cause any toxic effects on dams or fetuses in a reliable developmental toxicity study. Oral chronic administration of calcium chloride to rats at 1000 – 2000 mg/kg bw/day does not induce any adverse effects to rats. There is no established ADI for calcium chloride. JECFA evaluated calcium chloride in 1973 and put no limit on the ADI for calcium chloride. Many calcium salts, including calcium chloride, are used in dietary and supplemental sources of calcium in humans.

Chloride

Calcium chloride readily dissociates to bioavailable calcium and chloride ions in the body, and chloride is the most abundant anion in living species. There are no established upper safety levels for chloride and excess chloride is readily secreted into the renal tubular lumen by active transport systems, and also by passive diffusion (OECD 2002).

Chloride ion is not only safe at concentrations relevant to the subject of this dossier, but also an essential and ubiquitous electrolyte. It is the major extracellular and intracellular counter anion

to sodium and potassium; 70% of chloride is found in the extracellular fluid in the body and the remainder is in the intracellular space, connective tissue and bone (Pallas, 2013). The intracellular concentration of chloride is ~ 100-140 ug/mL (Malakooti et al. 2011). Chloride, in association with sodium (i.e., sodium chloride), is the principal osmotically active anion in the extracellular fluid and is also important in maintaining fluid and electrolyte balance. It also serves as an important component of gastric juice in the form hydrochloric acid (IOM, 2004). Chloride is the most abundant anion in all animal species. The total chloride content in the average adult human body is approximately 70-95 g (Malakooti et al. 2011).

Absorption of chloride occurs primarily in the small intestine and is approximately 98 percent across a wide intake range (IOM, 2004). Chloride is absorbed via co-transporters in the intestine, which transport sodium and chloride ions (Malakooti et al. 2011), as well as by co-transport re-uptake systems in the kidney (Richardson and Alessi 2009). Although chloride is absorbed efficiently from the intestine, the chloride concentration in plasma is maintained around 3.55-3.90 mg/mL (OECD 2002). Because chloride is a monoatomic ion, it is not metabolized to another species. Chloride is secreted from the renal tubular lumen by active transport systems, and also by passive diffusion (OECD 2002). Control of chloride levels is maintained by the balance of excretion and uptake, with participation of active re-uptake and excretion in the kidney (Richardson and Alessi 2009; Malakooti et al. 2011).

The Institute of Medicine (IOM) reviewed potential adverse effects of chloride (in association with sodium) intake in humans. The tolerable upper intake level (UL) for chloride for children 1 – 3 years is 2.3 g/day; 4-8 years is 2.9 g/day; 9-13 years is 3.4 g/day and for ≥ 14 years is 3.6 g/ day (IOM, 2004). This is based on the lowest-observed adverse-effect level (LOAEL) for dietary sodium, set at 2.3 g/day (100 mmol/day) based on the direct and progressive relationship between sodium intake and blood pressure and an uncertainty factor (UF) of 1.

Based on the usual mean daily intake for sodium among the US population 1 year and older (i.e. 3.44g/day, USDA 2013), the major form of dietary sodium is sodium chloride, i.e. 90% (IOM, 2004), and on a molar equivalent basis, a usual mean daily intake of 4.77 g of chloride from existing dietary sources can be estimated. In addition, the mean daily chloride intakes in Europe range from 5-7 g (8 -11g salt) (Pallas, 2013). These background intake estimates for chloride from sodium chloride salt are all well in excess of the EDI for chloride from proposed uses of $CaCl_2$.

In summary, the chloride present in calcium chloride is not of any safety concern based on the following reasons: i) chloride is endogenously present as the principal anion in extracellular fluid; ii) the total chloride in the adult human body is approximately 70 - 95 g; and iv) historical exposures to chloride.

Calcium

Calcium is the fifth most abundant element in the human body and provides the structural strength of bones (Heaney et al., 2012). The majority of the calcium in the body (>99%) resides in the skeleton as a calcium phosphate mineral crystal ($Ca_{10}[PO_4]_6[OH]_2$). Calcium is constantly diffusing in and out of the bone, and the kidneys are responsible for filtering as much as 10,000 mg of calcium per day, the majority of which is reabsorbed by the kidney. Inadequate calcium intake results in loss of calcium from the bone and in an increased risk for fractures. The skeletal

benefit of calcium intake is well established, however, recent controversy has arisen about the concept of "more is better", particularly since calcium is being increasingly added to food and calcium supplement use, especially among older adults, is widespread. To address these concerns, the IOM, among others, recently reviewed and assessed the current data with the charge to update the current DRIs for calcium (and vitamin D). There was a targeted focus on skeletal as well as non-skeletal benefits (e.g., reduction in cancer or diabetes risk) to determine if either could be used to specify adequate or excess intake of calcium. The results of their review are summarized in the 2011 IOM Report. The review of the hazards associated with calcium consumption in humans was initially excerpted from the chapter on calcium from the IOM Report (IOM, 2011). As mentioned previously, EFSA's expert panel also re-evaluated the tolerable upper limit for calcium in 2012 following the IOM review. A further review of the scientific literature published subsequent to these two reviews was also conducted. The IOM review and conclusions, the EFSA review, and newly published and relevant data on any potential adverse effect of calcium intake in humans are summarized below.

IOM Report on Calcium and Safety in Humans

As defined by the IOM, UL represents "the highest average daily intake of a nutrient that is likely to pose no risk of adverse health effects for nearly all persons in the general population." The IOM also notes that "as intake increases above the UL, the potential risk for adverse effects increases" and the UL therefore provides a reference value to guide policymakers and scientists involved in ensuring a safe food supply and protecting public health.

Excess intake of calcium may result in hypercalcemia, hypercalciuria, gastrointestinal issues (i.e., constipation), nephrolithiasis (kidney stones), interference with iron and zinc absorption, possible vascular and soft tissue calcification, and renal and cardiovascular damage

The determination of the UL was evaluated separately for selected life stages. Among the younger age groups, the ULs were based on a no-observed-adverse-effect level (NOAEL) established using calcium excretion as an indicator of excess calcium. Among the older age groups, a LOAEL with kidney stone formation was used as the basis for the UL.

The Committee determined that in the case of calcium, little new information had become available since the last DRI determination in the IOM report from 1997 (IOM, 1997) with the exception of a calcium excretion database among infants. The basis for the UL among infants is a NOAEL of 1,750 mg calcium/day determined from a report by Sargent et al. (1999) on calcium excretion measures in infants 3 to 9 months. This NOAEL was reduced by a factor of 2 and rounded to a UL of 1,000 mg/day among infants 0-6 months to adjust for the weight difference in the younger infants. Among the older infants (7-12 months), the NOAEL of 1,750 mg/day was reduced to a UL of 1,500 mg/day due to a lack of data.

The Committee determined that no new data on adverse outcomes based on excess calcium intake among children and adolescents since the 1997 report (IOM, 1997) has emerged and therefore, the 1997 UL of 2,500 mg/day is not too low to provide protection for this group. However, the Committee determined that the UL should be increased among the older children and adolescents 9 to 18 y due to increased tolerance as result of metabolic increases and growth spurts associated with bone accretion. According to the 2011 IOM report, "…based on a biologically reasonable adjustment intended to take into account increased need and therefore increased capacity to tolerate a slight increase in a UL value…" the Committee opted to increase the UL established for

younger children by 500 mg/day. The UL for children 1 to 8 y was set at 2,500 mg/day, while the UL for older children and adolescents (9 to 18 y) was increased to 3,000 mg/day.

Among the adult age groups, kidney stone formation was selected as the indicator for excess intake and the UL, most notably among post-menopausal women. Other indicators such as prostate cancer had confounded evidence, while vascular calcification, had conflicting evidence with no thresholds available for establishing a UL. Data on constipation and nutrient interaction did not support these outcomes serving as an indicator for the UL. Data from the Women's Health Initiative (WHI) on women 50-79 y and the study by Jackson et al. (2006) served as the basis for the selection of kidney stones as an adverse outcome and established a LOAEL of 2,000 mg/day for adults 50+ y. The WHI was a double-blind, placebo controlled clinical trial designed to test whether calcium plus vitamin D supplementation would reduce fractures (hip and total) as well as colorectal cancer. No uncertainty factors were applied to the LOAEL because the LOAEL is very close to recommended and adequate intakes. Therefore, the UL for adults 51+ y was established to be 2,000 mg/day. This is 500 mg/day lower than the UL established in the 1997 IOM report. The Committee notes that it is very difficult to achieve excess calcium intakes from diet alone and therefore the adverse outcomes seen in the WHI are most likely due to supplementation added to dietary intake.

The UL for younger adults (19-50 y) uses the established LOAEL among the older adults as a starting point. Kidney stone formation in young adults, while notable and with a higher incident rate compared to older adults, does not appear to be driven by supplement use; younger adults are less likely to use supplements. Given the UL of 3,000 mg/day for adolescents up to 18 y and the knowledge that younger adults are able to tolerate higher levels of calcium than older adults with declining kidney function, the UL for adults 19 to 50 y was based on an extrapolation between 2,000 and 3,000 mg/day resulting in a UL of 2,500 mg/day.

The ULs for pregnant and/or lactating women are the same as the ULs for non-pregnant and non-lactating women of the same age as there is no evidence showing that the calcium requirements are different between these two groups.

The UL for calcium established by the IOM in 2011 for the youngest infants, namely infants in the first year of life, was newly established. For young children 1-8 y, the UL established in 2011 is the same as the UL established in the previous review. The UL among older children and adolescents (9-18 y) is 500 mg/day higher than the previous UL. Alternatively, the ULs among the older adults (51 y and older) is 500 mg/day lower due to new evidence on the association of excess calcium intake with kidney stone formation.

EFSA's Scientific Opinion on the UL of Calcium

The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) re-evaluated the safety of calcium in 2012 at the request of the European Commission (EFSA, 2012). The main objective was to determine if the UL of 2,500 mg/day established in 2003 for adults including pregnant and lactating women needed to be revised in light of new scientific evidence. The panel reviewed the same studies the IOM reviewed but concluded that among older adults, the UL established in 2003 was sufficient and no new evidence supports its revision. In contrast to the IOM which based the UL of 2,000 mg/day in older adults on risk of kidney stones in women participating in the WHI by Jackson et al. (2006), the NDA reported that the risk of kidney stones in the WHI population was not significantly different between the treatment and placebo groups when the

analysis was restricted to subjects that complied with the study protocol (HR=1.21; 95%CI: 0.98-1.34). Further, NDA concluded that the Jackson et al. (2006) study did not provide evidence on the risk of kidney stones in association with total calcium intakes from diet and supplement use but rather on the risk of stone formation from an additional amount of calcium "…over widely variable baseline calcium intakes from food and personal supplements" (EFSA, 2012, page 13). The panel noted that calcium intakes up to 2,400 mg/day have not been associated with hypercalciuria or impaired kidney function. This is a broad statement and clinically, it is observed that patients with high calcium intake and urine calcium often show decreases in their urine calcium with decreasing calcium intakes. However, based on these findings and further evaluation of all newly available data, the NDA concluded the UL for adults remain at 2,500 mg/day.

The EFSA panel concluded that there was no new evidence to allow for the establishment of a UL for infants, children or adolescents but also that no risk has been associated with the highest current intakes of calcium in these population groups.

Safety Data Published Subsequent to the IOM Review of Calcium

A review of the recent literature on risk of adverse effects from excessive calcium intake was conducted to identify relevant studies that may not have been included in the 2011 IOM report or 2012 EFSA opinion. PubMed searches were conducted to identify studies indexed since June 1, 2010 to identify reports of any new clinical trials or epidemiology studies related to adverse effects of excessive calcium intake. The searches were conducted using "calcium" and key words including toxicity, tolerable, adverse, safety, hypercalciuria, hypercalcemia, prostate cancer, cardiovascular, and nephrolithiasis. The initial search was conducted on October 31, 2013 with updated searches conducted in June 2014, October 2015, and February 2016. The search strategies are outlined in Appendix C.

A total of 3317 citations were generated in the initial search covering the period of June 1, 2010 through October 31, 2103, an additional 246 citations were identified in the updated search conducted in June 2014 covering the period from October 32, 2013 through June 20, 2014; an additional 948 citations were identified in the updated search conducted in October 2015 covering the period from June 2014 through October 2015 and the most recent search conducted in February 2016, spanning publication dates from November 2015 through February 2016, produced 56 additional citations. Given the large volume of published data on calcium, for each search period, when available, meta-analyses and systematic reviews were first selected for review. If there were no published meta-analyses or systematic reviews, then all identified individual clinical trials and epidemiologic studies within the specified time-frame were reviewed, with emphasis on higher quality studies (i.e., those with a prospective design). Supplementary literature searches by examining the reference lists of all relevant articles not identified in the initial PubMed search were also conducted. In addition, full articles identified in the earlier reviews on chronic disease outcomes were examined for relevance to human safety data.

*Inclusion and Exclusion Criteria***:** Clinical trials and epidemiological studies that examined dietary and/or supplemental calcium intakes or serum calcium as a biomarker of calcium intake or as a measure of calcium status in normal, healthy individuals were considered eligible for

review. Studies that examined associations between calcium deficiency and disease were excluded.

The title and abstracts of the references identified in the literature searches were reviewed to identify potentially relevant papers. Abstracts contained one or more of the following terms: tolerable, safety, toxic, toxicity, adverse, hypercalciuria, hypercalcemia, kidney stones, mortality, cancer, cardiovascular, myocardial infarction, and stroke, were closely examined. A major focus of the search was to identify and evaluate the potential for an increased cardiovascular risk from excess calcium intake. This risk has been highly debated since the publication of a meta-analysis investigating the effect of calcium supplements on the risk of myocardial infarction (MI) and cardiovascular events in post-menopausal women by Bolland and colleagues in 2010 (Bolland et al. 2010). This analysis included 11 randomized control trials of calcium supplementation (≥500 mg/day) without vitamin D in 12,000 older patients and showed a 31% increased risk of MI (Hazard ratio (HR) = 1.31 (95%CI: 1.02-1.67); p-value = 0.035) using patient level data from five of the studies. This report was reviewed by the IOM and determined to be lacking sufficient evidence to change their UL determination among older adults due to several important limitations including the size of the studies, low event frequency, cardiovascular events were not the primary outcome, and many important covariates, including renal function, were not evaluated. Further, the total calcium intake (including diet) was unknown in many of the studies supplementing individuals with 1,000 to 1,200 mg/day. The IOM concluded that since dietary intake was unknown, the adverse events could be occurring from calcium intakes higher than 2,000 mg/day and that it is difficult to apply causality to calcium intakes of 1,000 to 1,200 mg/day. Overall, the scientific community's general consensus on this topic is that additional research is needed where these adverse outcomes of concern are the primary measured outcomes, and all potential confounders are appropriately measured and included in analyses. To date, there is currently insufficient scientific evidence to change the IOM's conclusions regarding the safety of calcium intake.

From the published literature, it appears there are no new or ongoing calcium trials being conducted, and thus, the most recent scientific literature on calcium and cardiovascular risk and any other adverse event is mainly secondary analyses of existing trials and observational studies where cardiovascular events were not the primary outcome. Information relevant to the safety of calcium from these meta-analyses, analyses/re-analyses of individual clinical studies, and observational studies are summarized herein.

Calcium and CVD - Clinical Trials

Meta-analyses

As described above, Bolland et al. (2010) published a meta-analysis of 15 clinical trials showing a 31% increased risk of MI among calcium supplement users from five studies with patient-level data and a 27% increased risk of MI among calcium supplement users from 11 studies with trial-level data. This analysis was a follow-up to earlier studies by many of the same researchers that first suggested serious adverse effects from calcium supplementation (Bolland et al., 2008; Reid et al., 2008). This same group of researchers followed up their 2010 meta-analysis by publishing a study (Bolland et al., 2011a) that included a re-analysis of the study conducted by Jackson et al. (2006) using the Women's Health Initiative (WHI) dataset (see discussion below) and updated their 2010 meta-analysis with the restricted analysis results from the WHI study among women with no personal supplement use. Pooling trial-level data from three placebo-controlled trials of

calcium with vitamin D (CaD) supplementation (including the WHI restricted analysis) showed a significant increased risk of MI, stroke, and the composite of MI or stroke (HR=1.21, 1.20, and 1.16, respectively). However, all HRs had a lower 95% CI ranging from 1.00 to 1.03 indicating the borderline statistical significance of these results. When the analysis was expanded to include nine placebo-controlled trials examining calcium supplementation with or without vitamin D, there was again a significant increased risk of MI and the composite of MI and stroke (HR = 1.24; 95%CI: 1.07-1.45 and HR=1.15; 95%CI: 1.03-1.27, respectively). It is important to note that the WHI restricted analysis results were heavily weighted in these meta-analysis ranging from 75-81% in the CaD trials and 47-56% in the Ca ± vitamin D trials.

Most recently, Lewis et al. (2015) performed a meta-analysis of randomized controlled trials to investigate the effect of calcium supplementation on CHD events in post-menopausal women. The analysis, which covered literature from 1966 through May 24, 2013, included data from 18 randomized controlled trials enrolling 63,564 participants, including 5 trials (48,460 participants, 3,390 CHD events) of calcium supplementation and CHD events, and 17 trials (62,383 participants, 4,157 deaths) of calcium supplementation and all cause-mortality. There was no statistical significant association between calcium supplementation and CHD risks; across five trials the risk ratio was 1.02 (95%CI: 0.96 – 1.09). Similarly, there were no statistical significant association between calcium and other health endpoints. For all-cause mortality, the analysis included 17 trials and found a risk ratio of 0.96 (95%CI: 0.91 – 1.02). For MI, the risk ratio was 1.08 (95%CI: 0.93 – 1.25), and for angina pectoris with acute coronary syndrome the risk ratio was 1.09 (95%CI: 0.95 – 1.24). For chronic CHD, the risk ratio was 0.92 (95%CI: 0.73 – 1.15). No significant heterogeneity was observed across studies for any outcome. The results of this meta-analysis are in contrast to the results of the Bolland et al (2011a) meta-analysis where a significant association with MI and stroke were reported. Several key differences between the studies may contribute to this difference. All outcomes included in the Lewis et al (2015) analysis were verified by clinical review, hospital record, or death certificates. This is in contrast to the Bolland et al meta-analysis (2011a) that included outcomes that were a mix of verified as well as self-reported outcomes. In addition, the Lewis et al (2015) meta-analysis is based on five trials of CHD with a greater number of events compared to the three trials with a smaller number of events included in the Bolland et al (2011a) analysis.

Analyses of WHI

As noted earlier, Boland et al. (2011a) re-analyzed the study conducted by Jackson et al. (2006) using the WHI dataset, a large, seven year, randomized, placebo-controlled trial, that originally found no adverse effects of calcium on any CVD outcomes. The re-analysis by Bolland et al. (2011a) involved limiting the study population to only include women with no reported personal use of calcium supplementation at baseline. The WHI dataset included 36,282 women 51-82 y supplemented with oral calcium carbonate at 1,000 mg/day or a placebo. Bolland et al. (2011a) reported that 54% of the women were taking personal calcium supplements at baseline and hypothesized that the previous analysis by Jackson et al. (2006) was attenuated due to the frequent personal use of supplements among the study population. The re-analysis by Bolland et al. (2011a) resulted in a borderline significant risk of MI (HR = 1.22; 95%CI: 1.00-1.50).

Following the Boland et al (2011a) re-analysis of the WHI, Jackson and other researchers, including Rossouw from the National Health, Lung, and Blood Institute at NIH (Prentice et al., 2013) examined the question further using the same dataset in combination with an observational study that included a study population of women drawn from the same study areas as the

participants in the WHI to help improve measurements of long-term supplement use. This analysis adjusted for usual calcium intake and including years from supplement initiation as a time-varying covariate. Contrary to the conclusions of Bolland et al. (2008, 2011a), there were no significant associations between calcium supplement intake, either among the total study population or a subset of women who were non-supplement users at baseline, and any cardiovascular endpoint. Hazard ratios ranged from 0.81 (95%CI: 0.60-1.09) for stroke among non-supplement users to 0.97 (95%CI: 0.86-1.10) for total CVD among all study participants (Prentice et al., 2013).

It is important to not over-interpret subgroup analyses in clinical trials. These findings should be used for hypothesis generation and subsequent research recommendations must be verified and validated by repeated experiments and consistently strong associations. A major criticism of the Bolland et al. analyses are that the CVD outcomes were not primary outcomes in any of the trials and they were based on self-reporting without adjudication (IOM, 2011; Heaney et al., 2012). The potential for ascertainment bias was investigated by Lewis et al. (2012), and their assessment showed an attenuation of the HR for MIs when the self-reported cardiovascular events were adjudicated. In an analysis of two randomized control trials that used self-reported MI as an outcome (Bolland et al., 2008; Prince et al., 2006), the HR based on self-report was 1.69 (95%CI: 1.09-2.61) compared to an HR = 1.45 (95%CI: 0.88-2.45) when based on adjudicated outcomes (Lewis et al., 2012). Further, many of these meta-analyses including the WHI trial where women were supplemented with CaD were weighted heavily and therefore, it is difficult to separate out any potential adverse effects of calcium versus vitamin D.

The analyses of the WHI trial described above are based on the seven years of follow-up during active intervention. In a post-intervention analysis, Cauley et al. (2013) reported effects of CaD supplementation on health outcomes of women in the WHI trial including 4.9 years following the intervention for a total of 11.1 years of follow-up. The post-intervention period showed similar effects as the intervention period and overall HRs for CVD events among women who received CaD supplements were not significantly increased for overall CHD (HR=1.03; 95%CI: 0.94-1.13), CHD deaths (HR=0.99; 95%CI: 0.84-1.18), clinical MI (HR=1.03; 95%CI: 0.92-1.15), stroke (HR=1.04; 95%CI: 0.93-1.16) and CVD deaths (HR=1.03; 95%CI: 0.92-1.17). These findings were similar among both women who reported taking supplements at baseline and those who did not.

More recently, Donneyong et al. (2015) investigated the risk of heart failure (HF) among 35,983 post-menopausal women in the WHI and whether the risk differed among those at high or low risk of HF. The authors concluded that 1000 mg/day calcium plus 400 IU D_3 (CaD) did not significantly reduce HF incidence in the overall cohort (HR = 0.95;95% CI:0.82-1.09), was beneficial in women lacking major HF risk factors (HR = 0.63; 95%CI:0.46-0.87) and had no effect among women at high risk for HF (HR = 1.06;95%CI:0.90-1.24).

Other Randomized Control Trials

Two recent RCTs both showed null results for any association of calcium supplementation and CVD outcomes, however, in these trials CVD events were not the primary outcomes. Lewis et al. (2011) analyzed data from a 5-year RCT (Calcium Intake Fracture Outcome Study) with 4.5 y of follow-up in 1,460 women 70+ y randomized to receive either 1,200 mg calcium carbonate per day or placebo. There was no association between supplementation and atherosclerotic vascular mortality or first hospitalization from atherosclerotic disease during the 5 year RCT (HR=0.938;

95%CI: 0.690-1.275). Wang et al. (2010a) also showed that there was no association between dietary and supplemental calcium intake and two established risk factors for cardiovascular disease, abdominal aortic calcification (AAC) and coronary artery calcification (CAC), among 1471 healthy post-menopausal women receiving 1,000 mg calcium/day and in 323 healthy older men receiving 600 or 1,200 mg calcium/day. Most recently, Bristow et al (2016) reported results from an RCT in 100 healthy post-menopausal women in New Zealand to compare the acute and 3–month effect of 1,000 mg calcium/day on blood pressure and acute effects on blood coagulation. These outcomes were secondary outcomes with the primary outcomes described as serum Ca and bone turnover markers. Both systolic and diastolic blood pressure were reduced at 2 hour intervals between 2 and 8 hours post supplementation; however the changes were smaller in the Ca supplement group compared to the placebo at 2 hours. The systolic changes were also observed to be significantly smaller compared to the placebo group at 4 and 6 hours. At the 3-month follow-up, blood pressure was not significantly different from baseline nor were there any difference between the treatment and placebo groups (Bristow et al., 2016).

Observational Studies

Meta-Analysis

Wang et al. (2014) conducted a meta-analysis of prospective studies to investigate the association between dietary calcium intake and mortality risk from CVD and all causes (use of calcium supplements was a covariate in the fully adjusted model for the dietary calcium assessment). The analysis, which covered literature from 1950 through December 30, 2013, utilized 11 prospective studies which drew from 12 independent cohorts (757,304 participants). There was not a statistically significant association between dietary calcium intake and CVD mortality; the relative risk when comparing the highest to lowest level of intake (9 studies, 709,499 subjects, >21,457 deaths[2]) was 0.97 (95%CI: 0.89 – 1.07), with no significant heterogeneity across studies (I^2 = 18.8%; p = 0.276). The authors also reported a non-statistically significant association between dietary calcium intake and all-cause mortality, the relative risk when comparing the highest to lowest level of intake (6 studies with 225,189 subjects, >21,055 deaths[1]) was 0.83 (95%CI: 0.70 – 1.00, P = 0.05). There was significant heterogeneity among the studies (I^2 = 74.9%; P = 0.003).

In a random-effects dose response meta-analysis, Wang et al (2014) observed a non-linear association between dietary calcium intake and CVD and all-cause mortality (i.e., U-shaped dose response). Based on the mathematical models (cubic splines), the study authors used 800 mg calcium/day as the reference intake upon which to base the estimated relative risks for CVD mortality. At intakes below 800 mg/day, there was a non-significant higher risk of CVD mortality, whereas there was higher risk of CVD mortality associated with calcium intake above this reference point. At 1,200 mg/day, there was a statistically significant association with a relative risk of CVD mortality of 1.05 (95%CI: 1.01 – 1.09) when compared to individuals with dietary calcium intakes at 800 mg/day and at 1,400 mg/day, the relative risk was 1.10 (95%CI: 1.02 – 1.18). Similarly, for all-cause mortality, the study authors reported an inflection point

[2] Wang et al (2014) reports that the exact number of deaths is unknown because one study did not report the number of deaths.

(reference intake) of approximately 900 mg calcium/day. Specifically, when compared with the reference intake of 900 mg/day, lower intake was associated with increased risk for all-cause mortality while there was no reduction in risk at intakes above 900 mg/day.

The mathematically derived non-linear dose response model relied upon in this study, however, needs further explanation regarding the selection of the number of knots to determine the inflection point (reference intake). The authors used a cubic spline with knots at the 10th, 50th, and 90th percentiles of the pooled exposure data, and selected 800 mg calcium/day as a reference to estimate all relative risks for CVD mortality. Although the authors do not clearly state how they selected the reference intake of 800 mg calcium/day, they state that "Intakes around 800 mg/day conferred the lowest risk of cardiovascular mortality" (Wang et al., 2014). An inspection of the spline curves shown in Figure 3 of the manuscript indicates that the spline curve is essentially flat between 800 mg/day and 1,000 mg/day, which would imply that the reference intake could have been selected to be any point between 800 and 1,000 mg/day. Further, and more importantly, the authors do not explain the reason why they selected to use three knots for the cubic spline. Had they used more knots, say four, it is likely that they would have seen different spline curves and therefore potentially different inflection points and a different "lowest risk dose". Given this uncertainty, coupling with the limitation of exposure information inherent with observational studies, as acknowledged by study authors, the dose response data from this analysis would need to be subject to further assessment and validation.

Wang et al. (2014) also reviewed six studies that investigated the relationship between calcium supplementation, rather than dietary calcium, and CVD mortality and concluded that none of these studies found a significant association between calcium supplementation and risk of CVD mortality (RR = 0.96, 95%CI: 0.82 – 1.13).

Cohort Studies

Several recent cohort analyses showed mixed findings regarding the risk of CVD and calcium intake.

Two prospective studies reported null findings of calcium intake and CAC among men and women in the Framingham Offspring Study with a mean age of 60 y (Samelson et al., 2012) and serum calcium and CVD events or mortality among 1,040 and 1,298 Scottish men and women, respectively between the ages of 45 and 64 y (Welsh et al., 2012). Prentice et al (2013) found no association between calcium supplementation and CVD events in a prospective study of 46,892 postmenopausal women in the same catchment area as the WHI clinical trial. Similarly, in a prospective cohort analysis of 74,245 women in the Nurses' Health Study (1984-2008) free of CVD and cancer at baseline, supplemental calcium intake was not associated with increased incidence of CVD ($RR_{>1000 \text{ vs } 0 \text{ mg/day}}$=0.82; 95%CI: 0.74-0.92), CHD (fatal or non-fatal MI; ($RR_{>1000 \text{ vs } 0 \text{ mg/day}}$=0.71; 95%CI: 0.61-0.83) or stroke ($HR_{>1000 \text{ vs } 0 \text{ mg/day}}$=1.03; 95%CI: 0.87-1.21) in multivariate models adjusted for dietary factors and known health behaviors that may confound this relationship (Paik et al., 2014).

On the contrary, in 2012, Li et al. published a study examining the association of dietary calcium intake and calcium supplementation with MI and stroke risk as well as CVD mortality among a cohort of 23,980 participants 35-64 y in the European Prospective Investigation in Cancer and Nutrition (EPIC-Heidelberg) study (Li et al., 2012). This observational study showed an increased risk of MI among calcium supplement users (HR=2.39; 95%CI: 1.12-5.12). There was no association with stroke or CVD mortality (HR=0.34; 95%CI: 0.05=2.47 for stroke and

HR=1.20 (95%CI: 0.38-3.78 for CVD mortality). However, there was a decreased risk of MI among the third quartile of total *dietary* calcium intake compared to the lowest quartile (HR=0.69; 95%CI: 0.50-0.94). This analysis failed to ascertain the dose of calcium supplement consumed.

Michaelsson et al. (2013) measured the association between long-term intake of calcium (dietary and supplements) and mortality from all causes and CVD among a Swedish cohort of 61,433 women who were followed-up for a median of 19 y. Many of the cardiovascular associations were null but they did find a significant association among calcium tablet users (500 mg calcium/tablet) with dietary calcium intakes >1,400 mg/day and all-cause mortality (HR = 2.57; 95%CI: 1.19-5.55).

Xiao et al. (2013) conducted a prospective study of 388,229 men and women ages 50-71 y in the National Institutes of Health (NIH) – American Association of Retired Persons (AARP) Diet and Health Study to assess the association between dietary and supplemental calcium intake and CVD mortality. After an average 12 y of follow-up, increased mortality from CVD was associated with supplemental calcium intake in men ($RR_{>1000\ v\ 0\ mg/day}$=1.20; 95%CI: 1.05-1.36), but not women (RR=1.06; 95%CI: 0.96-1.18). CVD mortality was not associated with dietary calcium in men ($RR_{Q5\ vs\ Q1}$=1.04; 95%CI: 0.97-1.12) or women ($RR_{Q5\ vs\ Q1}$=1.04; 95%CI: 0.94-1.15).

Van Hemelrijk et al., (2013) published an analysis using NHANES data. This study showed an increased risk of death from ischemic heart disease in the NHANES –III Mortality Follow-up Study among women with serum calcium in the top 5% compared to those in the mid 90% (HR=1.72; 95%CI: 1.13-2.61), but no association between any CVD death and dietary ($HR_{>1300\ vs\ <500mg/day}$=0.90; 95%CI: 0.59-1.35) or supplemental calcium intake ($HR_{\geq 2000\ vs\ 0\ mg/day}$=1.62; 95%CI: 0.27-9.75).

Cross-Sectional Studies

Cross-sectional studies published since the IOM 2011 report also yielded mixed results regarding the risk of CVD and calcium intake.

In a cross-sectional study, Kwak et al. (2014) investigated the relationship between dietary calcium intake and serum calcium levels and the risk of coronary artery calcification (CAC). Participants (23,652 Korean men (83.5%) and women) who did not have kidney disease or clinically overt CVD were included in the analysis. When comparing the highest and lowest intake categories, there was no association between dietary calcium intake and risk of CAC.

Similarly, Raffield et al. (2014) found no significant association between dietary calcium intake or calcium supplementation and measures of vascular calcification in type 2 diabetic patients in a cross-sectional study. When comparing categories of intake, this study also found no association between dietary calcium intake and all-cause and CVD mortality, but a modest reduction in risk of all-cause mortality (HR: 0.62, 95%CI: 0.42 – 0.92) with supplemental calcium intake in women.

In contrast, Huang et al. (2014) in another cross-sectional study in type 2 diabetic patients, reported that high (> 600 mg/day) or low (< 402 mg/day) dietary calcium intakes increased levels of C-reactive protein (CRP), a biomarker associated with CVD risk. Patients with high calcium intakes (>600 mg/day) had significantly higher CRP levels ($P < 0.05$) compared with patients with moderate calcium intakes (402 – 600 mg/day).

Uemura et al. (2014) conducted a cross-sectional analysis of 574 men (35-69 years of age) enrolled in the baseline survey of a prospective cohort study in Japan. They report an inverse association between dietary calcium intake and arterial stiffness, an emerging biomarker for CVD risk, among Japanese men (p-trend = 0.020).

Summary on Calcium Intake and CVD Risk

The recent studies that re-analyzed the WHI database suggest an association between calcium supplementation and cardiovascular risk and these have received significant attention considering the wide-spread use of supplements. This potential causal pathway has not yet been very carefully studied. However, as pointed out by many researchers in the field, the methods used and results of these studies do not stand up to the standards of assigning causality on their own or in combination (Heaney et al., 2012; Nordin et al., 2011). The most recent meta-analysis of experimental RCTs that investigated the use of calcium supplements on health outcomes by Lewis et al (2015) show no significant association between calcium supplement use and CHD events, all-cause mortality, MI, angina pectoris and acute coronary syndrome, and chronic CHD. This is supported by an earlier systematic review of the literature in 2010 by Wang et al. (2010b) which concluded that calcium supplements have minimal cardiovascular effects with four randomized trials (pooled RR=1.14; 95%CI: 0.92-1.41) showing no difference in incidence of CVD between calcium supplement users and non-users. Similar conclusions have been made based on prospective cohort studies. In a more recent review, Heaney et al. (2012) concluded that "Among 16 studies reviewed in this article, involving >358,000 individuals, there was no indication of a connection between calcium intake and atherosclerotic heart disease or stroke." They further note the inconsistencies in the direction of the effect as well as the strength of any association between calcium intake and/or supplementation and CVD risk varies greatly among the studies. In the most recent systematic evidence review of vitamin and mineral supplements conducted by the U.S. Preventive Services Task Force, they concluded there was no evidence of an effect of calcium supplements on CVD (Fortmann et al., 2013). Heaney et al. (2012) outline the lack of evidence for causality which is also repeated by many researchers and echoes the statements made by the IOM in 2011 (Bockman et al., 2011; Nordin et al., 2011; Biggs, 2008; Heiss, 2010; Prince et al., 2011; IOM, 2011).

Calcium and Other Adverse Outcomes

There are limited data available on other adverse outcomes related to calcium supplementation and intake published after the 2011 IOM report. One re-analysis of the WHI dataset showed that among women not taking calcium supplements at randomization, calcium and vitamin D supplementation significantly decreased the risk of total, breast, and invasive breast cancer by 14-20% and showed a non-significant decrease in colorectal cancers by 17% (Bolland et al., 2011b). The post-intervention analysis conducted by Cauley et al. (2013) using 11.1 years of follow-up reported no significant difference between the CaD supplement and placebo group in incidence of colorectal cancer (HR=0.95; 95%CI: 0.80-1.13), invasive breast cancer (HR=1.04; 95%CI: 0.94-1.14), and all-cause mortality (HR=0.96; 95%CI: 0.90-1.03). Another group of researchers used the same WHI dataset to investigate the occurrence of kidney stones and found that neither total calcium intake ($HR_{\geq 1490.12 \text{ vs } <674.58 \text{ mg/day}}$=1.12; 95%CI: 0.83-1.50) nor the use of calcium supplements at baseline (HR=1.10; 95%CI: 0.79-1.53) was associated with an increased risk of

stone formation (Wallace et al., 2011). Incidence of self-reported kidney stones was significantly higher in the supplementation group (1,000 mg calcium/day) compared to the placebo group (HR=1.17; 95%CI: 1.02-1.34). Total calcium intake from supplementation and dietary sources was not measured, and therefore, one cannot conclude that the increased risk of stones is due to calcium intakes in the 1,000 mg/day range.

Payne et al. (2014) reported that users of calcium supplements had significantly greater brain lesion volumes, an indicator of ischemic events, than non-use of calcium supplements (p=0.0011). A dose response relationship was not observed, however. Furthermore, the study was controlled for dietary calcium intake making the role of total calcium unclear. Calcium has also been reported to be associated with age-related macular degeneration in older adults with an OR of 1.85 (95%CI: 1.25 – 2.75) when comparing the highest (> 800 mg/day) and lowest (≤ 100 mg/day) intake quintiles (Kakigi et al. 2015). However, similar to the study by Payne et al. (2014), no dose response was established.

One meta-analysis and an update to a cohort study, both assessing calcium intake and prostate cancer risk, were also identified as being published after the IOM report. In the meta-analysis, dietary calcium intake was associated with a prostate cancer relative risk of 1.05 per 400 mg calcium/day (95%CI: 1.02 – 1.09, n = 15 cohorts) (Aune et al. 2015). In this analysis, nine cohort studies were selected which measured both dietary and supplemental (total) calcium intake. For total calcium, there were 33,127 cases of prostate cancer among 750,275 study participants. Comparing the highest and lowest total calcium intake levels, the relative risk was1.10 (95%CI:1.01-1.21) for prostate cancer. The relative risk per 400 mg calcium/day was 1.02 (95%CI:1.01-1.04) However, in the Health Professionals Follow-up Study cohort of 47,885 men, there was no association between calcium intake and prostate cancer when adjustment for phosphorous intake was performed (Wilson et al. 2015).

In summary, the ongoing controversy relative to the potential association between calcium supplementation and cardiovascular disease requires further investigation in controlled trials with a specific focus on those outcomes, with controls in place for known and potential cardiovascular risk factors, and accurate measurements of total calcium intake (diet and supplements) included. In light of the inconsistent and inconclusive findings from recent meta-analyses, systematic reviews, and recent clinical and observational studies for cardiovascular outcomes as well as any other potential adverse outcomes, at the preparation of this report, there is no new conclusive evidence of a cause and effect that would alter the significant scientific consensus presented in the IOM (2011) or the EFSA (2012) reviews.

Safety Data Summary

Calcium chloride is considered to have low toxicity and an ADI was not established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1973). Furthermore, a range of uses of calcium chloride are considered GRAS by the US FDA (SCOGS-45, 1975). An updated literature search revealed no new information that contradicts the JECFA's earlier conclusion on calcium chloride.

Calcium chloride dissociates to calcium and chloride ions in the body. Calcium and chloride are both essential nutrients required by all forms of life. Calcium and chloride have well-established mechanisms of action in the human body. Chloride is the most abundant anion in all animal

species. The total chloride in the adult human body is approximately 70-95 g. The toxicity of calcium compounds depends upon their bioavailability and the resultant release of calcium. The amount of calcium absorbed depends upon many factors including dietary components, the source of the dietary calcium, total calcium content of the diet and the body's need for calcium.

The biological and toxicological effects related to both calcium deficiency and calcium excess have been extensively reviewed by the IOM (2011) and EFSA (2012). The IOM-established ULs for calcium are lowest among infants (1,000 mg/day and 1,500 mg/day for infants 0-6 months and 6-12 months, respectively). Among older adults (51+ y the UL ranges from 2,000 mg/day based on the IOM evaluation to 2500 mg/day based on EFSA's evaluation. The ULs for the remaining life stages are 2,500 mg/day for children 1– 8 y (IOM, 2011) and adults 19 – 50 y (IOM, 2011; EFSA, 2012) and 3,000 mg/day for adolescents 9-18 y (IOM 2011). As summarized above, the ULs for calcium established by the IOM was based on calcium excretion in young children and formation of kidney stones among older children and adults. The IOM concluded that there were insufficient data to determine a UL based on other effects, including increased risk of CVD among post-menopausal women and older men. EFSA's most recent evaluation reached similar conclusions on the lack of increase of CVD and other health endpoints but did not believe the available evidence required a revision of the 2003 UL established among adults of 2,500 mg/day. Both the IOM and EFSA expert panels had also noted that it is difficult to measure the precise amount of daily calcium intake from both diet and supplements among the study subjects in the WHI and can result in considerable uncertainty in the upper intake level associated with any adverse effects. Reviews of the recent published literature on the same endpoints considered by the IOM in 2011 and a complete search for other potential health outcomes not considered by the IOM, while adding to the body of literature, do not offer any conclusive evidence of cause and effects and do not appear to impact the IOM and EFSA conclusions on the safety of dietary calcium and the UL.

Table 7. Calcium and CVD -- Summary of clinical trials and meta-analyses published subsequent to the IOM 2011 report

Citation	Study Design	Population	Findings	Considerations
Meta-analyses				
Bolland et al., 2010 (Included in IOM review but summarized here)	Randomized, double blind, placebo controlled trials (1966-March 2010) with a study duration > 1 y	Studies with 100 or more male and female participants of mean age > 40 y	• 15 trials included in analysis o 5 with patient level data o 11 with trial-level data • Patient-level analysis: increased risk of MI in those allocated to calcium (HR=1.31; 95% CI 1.02- 1.67); no increased risk of stroke, MI/stroke/sudden death, or death • Trial-level analysis: increased incidence of MI in those allocated to calcium (pooled relative risk=1.27; 95% CI: 1.01-1.59)	• CVD outcomes in the RCTs included in meta-analysis were not the primary outcomes • CVD outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured • WHI study heavily weighted in analyses • Low CVD event frequency • Lack of ability to control for important confounding factors including renal failure and several known CVD risk factors • Findings from this study provide no new information as this analysis was reviewed by the IOM as part of setting the current ULs.
Bolland et al., 2011a	Randomized, double blind, placebo controlled trials (1966-March 2010) with a study duration > 1 y	Studies with 100 or more male and female participants of mean age > 40 y Updated Bolland et al 2010 meta-analysis with the restricted analysis of the WHI CaD study among women not reporting use of calcium supplements at randomization 28,072 participants from 8 trials of calcium supplements	• Increased risk of MI among calcium/vit D supplementation group (RR= 1.24; 95%CI:1.07-1.45) and MI/stroke (RR =1.15; 95%CI: 1.03-1.27)	• CVD outcomes in the RCTs included in meta-analysis were not the primary outcomes • CVD outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured • WHI study heavily weighted in analyses • Low CVD event frequency • Lack of ability to control for important confounding factors including renal failure and several known CVD risk factors • This analysis is an update of the 2010 meta-analysis (Bolland et al 2010) and has the same limitations described in the IOM 2011 report.

Citation	Study Design	Population	Findings	Considerations
Lewis et al., 2015	18 randomized placebo-controlled trials (1966 - May 24, 2013, with study duration >1y and calcium dose >0.5g	63,564 post-menopausal women, mean cohort age >50 y. CHD: 48,460 participants (5 trials), 3390 CHD events All-cause mortality: 62,383 participants (17 trials), 4157 deaths MI: 51,111 participants (7 trials), 1,123 events Angina pectoris with acute coronary syndrome: 48,033 participants (4 trials), 876 events Chronic CHD: 48,033 participants (4 trials), 1,506 events	• No statistically significant increase in risk of CHD events (pooled RR = 1.02; 95%CI:0.96-1.09) from 5 trials • No statistically significant increase in risk of all-cause mortality (pooled RR = 0.96; 95%CI:0.91-1.02) from 17 trials • No statistically significant increase in risk of MI (RR=1.08;95%CI:0.93, 1.25) from 7 trials • No statistically significant increase in risk of angina pectoris with acute coronary syndrome (RR=1.09;95%CI:0.95-1.24) from 4 trials • No statistically significant increase in risk of chronic CHD (RR=0.92;95%CI:0.73-1.15) from 4 trials	• Heterogeneity among trials was low for CHD events and all-cause mortality (I^2 = 0%) • Total dietary intake of calcium was not measured • WHI study heavily weighted in analyses • Outcomes in the RCTs included in meta-analysis were not the primary outcomes • Only outcomes verified by clinical review, discharge record, or death certificate included
Analysis of WHI				
Bolland et al., 2011a	Randomized, blinded, placebo-controlled trial (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y	• Interaction observed between personal supplement use at enrollment and allocated calcium and vitamin D for CVD events • Among the 16,718 women (46%) not taking personal calcium supplements at randomization, borderline significant increase in risk for MI (HR=1.22; 95%CI:1.00-1.50) • Among women taking personal calcium supplements, no increased risk observed	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • CVD outcomes were not the primary outcomes • CVD outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured • Low CVD event frequency

Citation	Study Design	Population	Findings	Considerations
				• Lack of ability to control for important confounding factors including renal failure and several known CVD risk factors • This analysis is a subgroup restricted analysis of a previous analysis (Bolland et al 2008) and has the same limitations described in the IOM 2011 report.
Prentice et al., 2013	Randomized, blinded, placebo-controlled trial (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y	• No association between calcium supplementation and CVD events among all study subjects or among study subjects with no reported personal use of supplements at randomization • Hazard ratios ranged from 1.00 (95%CI: 0.86-1.18) for all heart disease to 1.18 (95%CI: 0.88-1.59) for MIs among women with no report use of supplements at randomization.	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • CVD outcomes were not the primary outcomes • Analysis accounts for duration of supplement use • Low CVD event frequency • Lack of ability to control for important confounding factors including renal failure and several known CVD risk factors
Cauley et al., 2013	Randomized, blinded, placebo-controlled trial – Post intervention analysis (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y; 29,868 (86%) women included in post-intervention follow-up (4.9 years).	• The post-intervention period showed similar effects as the intervention period • Overall HRs among women who received CaD supplements were not significantly increased for overall CHD (HR=1.03; 95%CI: 0.94-1.13), CHD deaths (HR=0.99; 95%CI: 0.84-1.18), clinical MI (HR=1.03; 95%CI: 0.92-1.15), stroke (HR=1.04; 95%CI: 0.93-1.16) and CVD deaths (HR=1.03; 95%CI: 0.92-1.17). • Findings similar among both women who reported taking supplements at baseline and those who did not.	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • CVD outcomes were not the primary outcomes • CVD outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured • Low CVD event frequency

Citation	Study Design	Population	Findings	Considerations
Donneyong et al., 2015	Randomized, double-blind, placebo-controlled trial (secondary analysis of WHI randomized trial)	35,983 women from WHI, age 50-79 y, with 744 adjudicated incident heart failure (HF) cases Supplemented with 1,000 mg/day calcium with 400 IU/day vitamin D	• No increase in risk of HF with calcium supplementation (HR=0.95;95%CI:0.82-1.09) • Baseline risk factors affected HR risk; low risk (HR=0.63;95%CI:0.46-0.87), high risk (HR=1.06;95%CI:0.90-1.24)	
Other Clinical Trials				
Wang et al., 2010	Randomized, placebo controlled trial	1,471 postmenopausal women supplemented with 1 g calcium/day (5 y) 323 men >40 y supplemented with calcium at 600 or 1,200 mg/day (2 y)	• Dietary or supplemental calcium intake was not associated with abdominal aortic calcification (AAC) changes; calcium supplementation also was not related to coronary artery calcification (CAC) scores in men.	
Lewis et al., 2011	Randomized, double-blind, placebo controlled trial (Calcium Intake Fracture Outcome Study (CAIFOS)) 5-y trial; 4.5 y follow-up	1,460 Australian women aged 75.1±2.7 y at baseline (1998) Supplemented with 1,200 mg/day of calcium carbonate daily or placebo	• No increased risk of death or first-time hospitalization from atherosclerotic vascular disease (HR=0.938; 95% CI: 0.690–1.275) during RCT • Similar null findings during 9.5 y of observational study (HR=0.919, 95% CI 0.737–1.146).	• Adjustment for many CVD risk factors included in analysis • Outcomes were based on verified hospitalization and death registries • Potential lower bioavailability of calcium from calcium carbonate however this is not well-established

Citation	Study Design	Population	Findings	Considerations
Bristow et al., 2016	Randomized, placebo-controlled trial	100 postmenopausal New Zealand women with 1 g calcium/day (3 months);	• Systolic blood pressure (BP) significantly lower at 2, 4, 6, and 8 hours post initial treatment in all groups • Smaller reduction in BP for Ca group compared to placebo (Systolic BP at 2, 4, and 6 hours; diastolic BP at 2 h). • No significant difference in change in BP from baseline to 3 months • No difference in BP between groups at 3 months	• BP was not the primary outcome • Total dietary intake of calcium is not measured • Type of calcium supplement varied among the treatment groups (citrate, carbonate, two preparations of microcrystalline hydroxyapatite • Population of older women; findings may not be generalizable to the total population • Small control group (N=20)

Table 8. Calcium and CVD - summary of observation studies and meta-analysis published subsequent to the IOM 2011 report

Citation	Study Design	Population	Findings	Considerations
Meta-analysis				
Wang et al., 2014	11 prospective studies (12 independent cohorts)(1950 - Dec 30, 2013)	757,304 male and female participants, 4+y CVD mortality: 704,499 participants (9 studies), >21,457 deaths All-cause mortality: 225,189 participants (6 studies),>21,055 deaths	• For risk of CVD mortality, pooled RR=0.97 (95%CI:0.89-1.07) when comparing "highest" and "lowest" dietary calcium intake • For all-cause mortality, RR = 0.83 (95%CI: 0.70-1.00) when comparing "highest" with "lowest" dietary calcium intake. • In a dose-response analysis, non-linear association between dietary calcium intake and risk of CVD mortality observed (p<0.01 for non-linearity); when compared to individuals with calcium intakes of 800 mg/d, significantly increased risk of CVD mortality with 1200 mg/day calcium intakes (RR=1.05; 95%CI:1.01-1.09) and calcium intakes of 1400 mg/day (RR=1.10; 95%CI:1.02-1.18) • In a dose response analysis, nonlinear association between dietary calcium intake and risk of mortality from all causes observed (p<0.01 for non-linearity; when compared with the reference intake of 900 mg/day, lower intake was associated with increased risk for all-cause mortality while there was no reduction in risk at intakes above 900 mg/day. • No statistically significant association between supplemental calcium and CVD mortality (6 studies; RR=0.96; 95%CI:0.82-1.13)	Study did not find statistically significant association between calcium intake and CVD mortality based on pooled RR across nine studies. The mathematically derived non-linear dose response model needs further explanation regarding the selection of the number of knots to determine the reference point. This coupling with the limitation of exposure information inherent with observational studies, as acknowledged by study authors, the dose response data from this analysis would need to be subject to further assessment and validation.
Cohort studies with findings of no or inverse association				

Citation	Study Design	Population	Findings	Considerations
Samelson et al., 2012	Prospective (Framingham Offspring Study)	669 women; 532 men (age= 60 y; range: 36–83 y); baseline clinic visit in 1998-2001; CT exam in 2002-2005	• Total (diet + supplements) calcium intake: o 1185 ± 565 mg/day (women) o 891 ± 461 mg/day (men) • Inverse association between mean age-adjusted coronary artery–calcification Agatston score and total calcium intake. • Results were similar for dietary calcium and calcium supplement use.	• Measured total calcium intake • Adjusted for several known CVD risk factors
Welsh et al., 2012	Prospective (MIDSPAN Family Study); 14.4 y median follow-up	1,040 men and 1,298 women from the West of Scotland recruited in 1996; age 45-64 y	• No association between albumin-corrected serum calcium levels and CVD events (ICD-10 I00-I99 coded on death certificate or discharge record)	
Prentice et al., 2013	Prospective cohort	46,892 postmenopausal women in the same catchment area as the WHI clinical trial	• No association between calcium supplementation and CVD events	• CVD outcomes were not the primary outcomes • Analysis accounts for duration of supplement use
Paik et al., 2014	Prospective cohort; 24 y follow-up	74,245 female registered nurses (30-55 y) free of CVD and cancer at baseline	• Dietary and supplemental calcium intake measured through a semi-quantitative food frequency questionnaire • Calcium intake not associated with increased incidence of fatal or non-fatal MI ($RR_{>1000 \text{ vs } 0 \text{ mg/day}}$=0.71; 95%CI: 0.61-0.83) or stroke ($HR_{>1000 \text{ vs } 0 \text{ mg/day}}$=1.03; 95%CI: 0.87-1.21)	• Multivariate models adjusted for dietary factors and known health behaviors that may confound this relationship • Large number of events and long follow-up with repeated measure of Ca intake • Study population is female and predominantly white – not generalizable to men and/or other races/ethnicities. Conclusion: This study provides no new adverse associations to call the current calcium UL into question.
Cohort Studies with some findings of an association				

Citation	Study Design	Population	Findings	Considerations
Li et al., 2012	Prospective cohort (European Prospective Investigation into Cancer and Nutrition study (EPIC)-Heidelberg); 11 y follow-up	23,980 German participants in the EPIC study, aged 35-64 y CVD-free at recruitment	• 354 MIs; 260 stroke cases; 267 CVD deaths • Significant reduction in MI risk among the third quartile of total dietary calcium intake compared to the lowest (HR=0.69; 95% CI: 0.50 - 0.94) • No association with stroke and CVD mortality • Increased risk of MI in users of calcium supplements compared to non-users (HR=1.86; 95% CI: 1.17-2.96); • Larger risk observed among calcium supplement only users (HR=2.39; 95% CI: 1.12- 5.12).	• Dose of calcium supplement unknown • Close to half of supplement users (44.5%) did not report name of supplement; prevalence of calcium supplement use is lower than observed in a German elderly population or that observed in the US.
Michaelsson et al., 2013	Prospective longitudinal cohort; 19 y median follow-up	Swedish mammography cohort, population-based (1987-90); 61,433 women (born 1914-1948)	• No association between calcium tablet use (500 mg calcium per tablet) and all cause or cause specific mortality • Dietary calcium intake >1400 mg/day among calcium tablet users was associated with increased risk of mortality (HR=2.57; 95% CI: 1.19 to 5.55)	• Dietary calcium intake based on food frequency questionnaires with standard portion sizes, not measured portion sizes, which tend to overestimate intake • Results from this observational study are not consistent with other cohort analyses nor are they confirmed by clinical trials.
Van Hemelrijk et al., 2013	Prospective analysis using NHANES III mortality linkage follow-up	US population 17+ y eligible for mortality follow-up and free from history of heart disease (n=18,714)	• ~10% of population died of cardiovascular disease (N=1870); majority were ischemic heart disease (IHD; 5.4%) • Increased risk of IHD death among women with serum calcium levels in top 5% compared to those in the mid 90% (HR: 1.72; 95%CI: 1.13-2.61), • Among men, low serum calcium was related to increased IHD mortality (HR= 2.32; 95%CI: 1.14-3.01) • No associations observed with dietary or supplemental calcium intake	• Adjusted for many dietary and known risk factors for CVD • Dietary calcium assessed using a 24-hour diary which may not reflect long-term intake • Supplement intake is self-reported but NHANES records the supplement name directly from the label
Xiao et al., 2013	Prospective cohort (National	388,229 men and women in the US aged 50–71 y	• 7904 CVD deaths in men; 3874 CVD deaths in women	• Adjusted for dietary variables • No data on duration of supplement use

Citation	Study Design	Population	Findings	Considerations
	Institutes of Health (NIH)–AARP Diet and Health Study); 12 y follow-up		• Calcium-containing supplement use was 51% and 70% in men and women, respectively. • In men, supplemental calcium intake was associated with CVD mortality ($RR_{>1000 \text{ vs. } 0 \text{ mg/day}}$ =1.20; 95% CI: 1.05–1.36), heart disease mortality (RR=1.19; 95% CI: 1.03–1.37), but not cerebrovascular disease mortality (RR=1.14; 95% CI: 0.81–1.61). • No association between calcium supplements and CVD events in women	• Incomplete adjustment for other CVD risk factors including nutrients
Cross-sectional studies with findings of no association				
Kwak et al., 2014	Cross-sectional	23,652 Korean men and women, asymptomatic for CVD, without kidney disease, with mean age 40.8 y	• Comparing the highest (≥478.2 mg/day) and lowest (<221.8 mg/day) quartiles of dietary calcium intake, tomographic score ratios of coronary artery calcification (CAC), a risk factor for CVD, were not associated with dietary calcium intake (0.84;95%CI:0.58-1.20). • Comparing the highest (≥ 9.7 mg/dL) and lowest (< 9.3 mg/dL) quartiles of serum, serum calcium levels were positively associated with CAC score ratios; no association was described as to the relationship of serum calcium to estimated calcium intake.	• Details of supplement use were not described. • Relationship of serum calcium to calcium intake not described. • Concurrent assessment of intake and risk.
Raffield et al., 2014	Cross-sectional (Diabetes Heart Study)	720 male and female type 2 diabetics (T2D) enrolled in Diabetes Heart Study	• No significant association of dietary calcium or supplements with measures of vascular calcified plaques in men or women. • No significant association of dietary calcium intake with all-cause or CVD mortality risk in men and women; no significant association of supplemental calcium with CVD mortality risk in men and women or all-cause mortality in men	• Quintiles of energy-adjusted total calcium intake (dietary and supplement) utilized for analysis. • Concurrent assessment of calcium intake and risk.

Citation	Study Design	Population	Findings	Considerations
			• For women, HR=0.62 (95%CI:0.42-0.92) for all-cause mortality associated with supplemental calcium use when comparing highest and lowest intakes (>500 mg/day compared to 0 mg/day)	
Cross-sectional studies with findings of an association				
Huang et al., 2014	Cross-sectional	197 male and female type 2 diabetics (T2D), age >65 y	• Patients whose dietary calcium intake was high (>600 mg/day) or low (<402 mg/day) had higher C-reactive protein (CRP) levels, an emerging biomarker for CVD risk, than those with moderate (402 – 600 mg/day) calcium intake (moderate vs high, $p<0.05$).	• CVD risk, CVD mortality, or all-cause mortality were not primary outcomes. • Publication does not mention supplement use or inclusion/exclusion of patients utilizing calcium supplements. • Concurrent assessment of intake and risk.
Uemura et al., 2014	Cross-sectional analysis of a prospective cohort (J-MICC cohort)	535 men with dietary calcium intake data, 35-69 y, in cohort from Tokushima Prefecture, Japan	• When comparing the highest (>497.3 mg/day) and lowest (≤ 351.8 mg/day) quartiles of dietary calcium intake, measurements of brachial-ankle pulse wave velocity, a measure of arterial stiffness, were significantly inversely associated with dietary calcium intake (p for trend=0.02).	• Exclusion or inclusion of subjects based on supplement use was not described.

Table 9. Calcium and Other Outcomes - Summary of published subsequent to the IOM review of calcium

Citation	Study Design	Population	Findings	Considerations
Clinical Trials				
Bolland et al., 2011b	Randomized, blinded, placebo-controlled trial (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y	• Among the 16,718 women (46%) not taking personal calcium supplements at randomization, significant reductions observed for total cancer, total breast cancer, and invasive breast cancer (HRs ranging from 0.80 – 0.86). • In women taking personal calcium or vitamin D supplements, trial supplementation did not alter cancer risk (HR: 1.06–1.26)	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • Cancer outcomes were not the primary outcomes • Cancer outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured
Wallace et al., 2011	Randomized, blinded, placebo-controlled trial (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y	• 449 women in the CaD group and 381 women in the placebo group reported a stone during the trial. • Increased incidence of self-reported clinically diagnosed urinary tract stones in women supplemented with CaD compared to placebo (HR= 1.17; 95% CI: 1.02- 1.34). • The rates of self-reported stones did not differ between various demographic, anthropomorphic, dietary, and other hypothesized risk factors. • Increased incidence (17%) of self-reported clinically diagnosed urinary tract stones in the vitamin D/calcium group relative to the placebo group. • Neither the total calcium intake nor the use of calcium supplements at baseline was associated with the risk of stones.	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • Small absolute difference in occurrence of urinary tract stones between groups: 0.35 vs. 0.30% • Findings from this study provide no new information relevant to determination of the UL, as the increased risk for kidney stones had been published (Jackson et al. 2006, as cited by IOM 2011) before the IOM set the present UL.

Citation	Study Design	Population	Findings	Considerations
Cauley et al., 2013	Randomized, blinded, placebo-controlled trial – Post intervention analysis (WHI: Women's Health Initiative)	36,282 post-menopausal women ages 50-79 y; subjects consumed placebo or 400 IU vitamin D_3 with 1,000 mg calcium carbonate daily for an average of 7 y; 29,868 (86%) women included in post-intervention follow-up (4.9 years).	• The post-intervention period showed similar effects as the intervention period • No significant difference between the CaD supplement and placebo group in incidence of colorectal cancer (HR=0.95; 95%CI: 0.80-1.13), invasive breast cancer (HR=1.04; 95%CI: 0.94-1.14), and all-cause mortality (HR=0.96; 95%CI: 0.90-1.03).	• No control group as all subjects took calcium supplement with vitamin D; can't isolate effects of supplemental calcium • Population of older women; findings may not be generalizable to the total population • Cancer/mortality outcomes were not the primary outcomes • Outcomes are based on self-report events and were not adjudicated • Total dietary intake of calcium is not measured
Observational Studies and Meta-Analyses				
Aune et al., 2015	Meta-analysis of prospective studies of dietary, supplemental, and total calcium	Total calcium: 750.275 participants (9 cohorts), 33,127 cases Dietary calcium: 800,879 participants (15 cohorts), 35,493 cases	• Total calcium intake associated with increased prostate cancer risk per 400 mg calcium/day (RR=1.02;95%CI:1.01-1.04) and when comparing the highest and lowest intake levels (RR=1.10;95%CI:1.01-1.21) for prostate cancer risk. • Total dietary calcium associated with increased prostate cancer risk per 400 mg calcium/day (RR=1.05;95%CI: 1.02–1.09), and when comparing highest and lowest intake levels (RR=1.18; 95%CI:1.08-1.30)	• Studies included both the NIH-AARP cohort and the HPFS cohort • Exclusion of the NIH-AARP reduced the RR for total calcium and prostate cancer risk to 1.03 (95%CI:1.02-1.05) and exclusion of the HPFS cohort reduced the RR to 1.02 (95%CI:1.01-1.03) • Published concurrently with Wilson et al. (2015) • Study by Wilson et al. (2015) suggests that correction for phosphorous intake may attenuate association between prostate cancer and total calcium found in this analysis.
Wilson et al., 2015	Prospective study based on Health Professionals Follow-up Study (HPFS). Study collected data from 1986 -	47,885 men from HPFS cohort aged 40-75 y; 5,861 cases of prostate cancer including 789 lethal cancers (defined as fatal or metastatic)	• Comparing intake categories, calcium intake of ≥2000 mg/day (compared to 500-749 mg/day) associated with greater risk of total (RR=1.24;95%CI:1.02-1.51), lethal (RR=1.66;95%CI:1.09-2.53), and high-grade (RR=1.88;95%CI:1.13-3.12) prostate cancer. All significance attenuated after adjustment for phosphorous intake.	• Cancer diagnosis initially self-reported followed by confirmation by review of medical records and pathology reports • No increased risk was found when correction for phosphorous intake was conducted.

Citation	Study Design	Population	Findings	Considerations
	2010, every 4 years			
Kakigi et al., 2015	Cross-sectional study of calcium supplementation and age-related macular degeneration (AMD)	3,191 male and female participants aged 40+ y, from NHANES; 248 (7.8%) diagnosed with AMD	• Comparing the highest and lowest quintiles of self-reported calcium supplementation, supplementation with greater than 800 mg/day calcium had higher odds of AMD diagnosis compared to those reporting no (≤ 100 mg/day) supplementation (OR=1.85; 95%CI:1.25-2.75); no association was observed when comparing the other quintiles of supplementation • For older participants (>67y) the odds of AMD diagnosis was higher (OR=2.63;95%CI:1.52-4.54)	• Supplement intake was self-reported • Comorbidities (confounders) were not adjudicated, but self-reported • No accounting for dietary or total calcium intake • A clear dose-response was not established. Lack of dose response limits strength of findings.
Payne et al., 2014	Cross-sectional study of calcium supplementation and brain lesion volume	227 male and female participants age >60 y;149 supplement users, and 78 non-users	• Users of calcium supplements (yes/no), after controlling for dietary calcium intake, had significantly greater lesion volumes than non-use of calcium supplements (p=0.0011) • Among supplement users, the amount of supplement consumed was not associated with lesion volume (p=0.81), therefore no dose response. • For users with duration information (n = 106), there was no association between lesion volume and supplement use duration (p=0.35)	• No dose response established; daily supplement intake ranged from 37-1130 mg/day (mean 744.2 mg/day) • Duration of supplementation only available for 106 or 149 participants • Exposure assessment could not distinguish between calcium-only and calcium/vitamin D containing supplements • Concurrent assessment of intake and risk. • Lack of dose response and duration response limit strength of findings.

HR: hazard ratio; For RCTs, RR refers to risk ratio, while for observational studies in this table, RR refers to relative risk.

Acceptable Daily Intake

Calcium chloride was considered to have low toxicity and an ADI was not specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1973). Furthermore, a range of uses of calcium chloride are considered GRAS by the US FDA (SCOGS-45, 1975). An updated literature search revealed no new information that contradicts the JECFA's earlier conclusion on calcium chloride or that of the US FDA.

Calcium chloride dissociates to calcium and chloride ions in the body. Chloride is the most abundant anion in all animal species. The total chloride in the adult human body is approximately 70-95 g. Although chloride is absorbed efficiently from the intestine, the chloride concentration in plasma is maintained around 3.55-3.90 mg/mL (OECD, 2002). Control of chloride levels is maintained by the balance of excretion and uptake, with participation of active re-uptake and excretion in the kidney (Richardson and Alessi, 2009; Malakooti et al., 2011). Because chloride is a monoatomic ion, it is not metabolized to another species. Chloride is excreted from the renal tubular lumen by active transport systems, and also by passive diffusion (OECD, 2002).

The biological and toxicological effects related to both calcium deficiency and calcium excess have been extensively reviewed by both the IOM (2011) and EFSA (2012). Based on calcium excretion in young children and formation of kidney stones in older children and adults, the IOM established ULs for infants 0-6 months (1,000 mg/day), infants 6-12 months (1,500 mg/day), children 1– 8 y (2,500 mg/day), adolescents 9-18 y (3.000 mg/day), adults 19 – 50 y (2500 mg/day), and older adults 51+ y (2,000 mg/day). The IOM concluded that there were insufficient data to determine a UL based on other effects, including increased risk of CVD among post-menopausal women and older men. EFSA's most recent evaluation (2012) reached similar conclusions on the lack of adverse associations between calcium intake and CVD as well as other health endpoints but did not believe the available evidence required a revision of the UL of 2,500 mg/day for adults as previously established by the Scientific Committee on Food (SCF) in 2003. Based on the currently available data and authoritative reviews by the IOM (2011) and EFSA (2012) a range of exposure limits from 2,000 to 2,500 mg/day can be reasonably relied upon to assess the safety of the proposed use of calcium chloride in potato snacks for older adults 51+ y. The literature published since the IOM review in 2011, provide no new conclusive evidence of a cause and effect that would alter the significant scientific consensus presented in the IOM (2011) or the EFSA (2012) reviews.

Safety Conclusion

The intake assessment was designed to conservatively estimate background intake of calcium from all food sources (i.e., all naturally-occurring and calcium-fortified food sources and approved food additive uses of calcium chloride, as measured by the USDA) and calcium from dietary supplements, as well as calcium intake from the proposed use of calcium chloride in potato snacks. No adjustment has been made to account for the potential overestimation of intakes that may result from using two days of dietary data to estimate long-term consumption nor to account for the fact that only select potato snacks will contain calcium chloride. 100% bioavailability of the calcium from the proposed use was also assumed resulting in a conservative overestimate of exposure. Results of these analyses indicate that the per user 90^{th} percentile

cumulative calcium intakes (background + proposed use) were below the IOM UL for the majority of the US subpopulations. For three subgroups, the per user 90th percentile calcium intakes from background sources (food sources + dietary supplement) marginally exceeded the IOM UL of 2,000 mg/day but were below the EFSA UL of 2,500 mg/day among the older women 51 -70 y (2,195 mg/day) and 71+ y (2,158 mg/day) as well as among men 51-70 y (2,023 mg/day). These findings are consistent with the 2011 IOM report of usual calcium intakes exceeding the IOM UL at the 95th and 99th percentiles (as analyzed by Bailey et al., 2010 with further data provided by staff at the National Cancer Institute – National Institutes of Health). Source contribution analyses showed that background calcium intake from food sources alone are well below the IOM UL at the per user 90th percentile for these subpopulations, irrespective of supplement use status, with per user 90th percentile dietary calcium intake ranging from 1,274 mg/day among females 71+ y to 1,721 among males 51-70 y. For these older age groups, the additional calcium intake from the use of supplements drives the total background calcium intake: at the 90th percentile, calcium from supplement use contributes up to 65% of the total background calcium intake among all calcium consumers. It should also be noted that almost two-thirds (65%) of the women 71+ y reported the use of a calcium-containing supplement in the NHANES database, representing the largest supplement user group.

The proposed use of calcium chloride at a level up to 1 % in potato snacks contributes minimally to the total cumulative calcium intake at the 90th percentiles among these older females and male sub-population. Among all calcium consumers, the proposed use of calcium chloride contributes from 3- 5% (31 – 59 mg/day additional calcium), among supplement consumers: 2-4% (29-59 mg/day additional calcium), and among non-calcium-supplement users: 2-7% (20 – 51 mg/day additional calcium). Among older women and men who are not taking calcium supplements, the per user 90th percentile cumulative calcium intake ranges from 1,265 mg/day to 1,639 mg/day for females and males 51-70 y, respectively, which are all well below the IOM UL of 2000 mg/day.

Overall, the *per user* 90th percentile cumulative calcium intakes for the subpopulations of infants 6-11 months, children, adolescents and adults 19-50 y were below the IOM UL. For the older adults 51+ y the per user 90th percentile cumulative calcium intake for males 71+ y were below the exposure limit range (2,000 – 2,500 mg/day). For women 51+ y and males 51-70 y, the per user 90th percentile background (food + dietary supplements) calcium intakes were within the exposure limit range (2,000 – 2,500 mg/day) and with the small addition of calcium (<7%) from the proposed use of calcium chloride in potato snacks the per user cumulative intake at the 90th percentile remained within the exposure limit range. Therefore, it is reasonable to conclude that the proposed use of calcium chloride at a maximum level 1% in potato snacks is safe within the meaning of the FD&C Act, i.e. the proposed use meets the safety standard of reasonable certainty of no harm.

Discussion of Information Inconsistent with GRAS Determination

PepsiCo, is not aware of information that would be inconsistent with a finding that the proposed use of calcium chloride in potato snacks meeting appropriate specifications and used according to GMP, is GRAS.

Basis for Conclusion that there is Consensus Regarding Safety

The intended use of calcium chloride has been determined to be safe through scientific procedures as set forth in 21 CFR§170.30(b), thus satisfying the so-called "technical" element of the GRAS determination. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination. Determination of the safety and GRAS status of calcium chloride for addition to foods under its intended conditions of use has been made through the deliberations of an Expert Panel of individuals qualified by scientific training and experience to evaluate the safety of substances intended to be added to food. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that calcium chloride produced consistent with Good Manufacturing Practice and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of calcium chloride are GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food ingredients would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit 1 to this document.

References

Allen LH. Calcium bioavailability and absorption: a review. Am J Clin Nutr. 1982;35: 783-808

Akatsuka K, Hashimoto T, Takeuchi K. Pharmacological studies on Ca meso- tartrate I. Acute and subacute toxicity tests. Ouyou-Yakuri. 1977;14: 963-75. (in Japanese) (as cited in OECD Screening Information Database Set, 2002)

Aune D, Navarro Rosenblatt DA, Chan DSM, Vieira AR, Greenwood DC, Vatten LJ, Norat T. Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies. Am J Clin Nutr. 2015; 101:87-117.

Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, Sempos CT, Picciano MF. Estimation of total usual calcium and vitamin D intakes in the United States. J. Nutr. 2010;140:817-22.

Biggs, WS. Calcium supplementation: data were misrepresented. BMJ. 2008;336:404.

Bockman RS, Zapalowski C, Kiel DP, Adler RA. The challenges of the single micronutrient study: Commentary on calcium supplements and cardiovascular events. Position paper of the ASMBR Professional Practice committee. Washington DC: American Society for Bone and Mineral Research; 2011.

Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ. 2010;341:1-9.

Bolland MJ, Grey A, Avenell A, Gamble G, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ. 2011a;342:1-9.

Bolland MJ, Grey A, Gamble GD, Reid IR. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. Am J Clin Nutr. 2011b;94(4):1144-9.

Bonny O, Rubin A, Huang CL, Frawley WH, Pak CY, and Moe OW. Mechanism of urinary calcium regulation by urinary magnesium and pH. J Am Soc Nephrol. 2008;*19*(8), 1530-1537.

Bristow SM, Gamble, GD, Stewart A, Horne AM, Reid IR. Acute effects of calcium supplements on blood pressure and blood coagulation: secondary analysis of a randomised controlled trial in post-menopausal women. Brit J Nutr. 2015;114:1868-1874.

Bronner F. Intestinal calcium absorption: mechanisms and applications. J Nutr. 1987;117(8):1347-52.

Cauley J, Rowan T, Chlebowski MD, Wactawski-Wende J, Robbins JA, Rodabough RJ, Chen Z, Johnson KC, O'Sullivan MJ, Jackson RD, and Manson JE. Calcium plus vitamin D supplementation and health outcomes five years after active intervention ended: The Women's Health Initiative. J Women's Health. 2013;22(11): 915-29.

Donneyong MM, Hornung CA, Taylor KC, Baumgartner RN, Myers JA, Eaton CB, Gorodeski EZ, Klein L, Martin LW, Shikany JM, Song Y, Li W, Manson JE. Risk of heart failure among post-menopausal women: A secondary analysis of the randomized trial of vitamin D plus calcium of the Women's Health Initiative. Circulation:Heart Failure. 2015; 8(1):49-56.

European Food Safety Authority (EFSA). Scientific opinion on the tolerable upper intake level of calcium. EFSA Journal. 2102;10(7):2814.

U.S. Food and Drug Administration Generally Recongized as Safe (GRAS) Database, SCOGS-45. 1975. Evaluation of the health aspects of certain calcium salts as food ingredients.

Food and Drug Research Laboratories, Inc. Teratologic evaluation of FDA 71-87 (calcium chloride) in mice, rats and rabbits. 1974. Report No. FDABF-GRAS-251. NTIS PB-234 879 (as cited in OECD Screening Information Data Set, 2002).

Food Chemicals Codex (FCC). 2014. Calcium Chloride pp. 188-189. 9th edition, U.S. Pharmacopeial (USP) Convention, Rockville, MD

Food Chemicals Codex (FCC). 2014. Calcium Chloride Solution pp. 189-190. 9th edition, U.S. Pharmacopeial (USP) Convention, Rockville, MD

Fortmann S, Burda BU, SEnger CA, Lin JS and Whitlock EP. Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: An updated systematic evidence review for the U.S. Preventive Services Task Force. Ann Int Med. 2013;159(12): 824-834.

Fujita H, Sasaki M. Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102 (II). Ann Rep Tokyo Metr Res Lab. P.H. 1987;38, 423-430. (in Japanese) (as cited in OECD Screening Information Data Set, 2002)

Gueguen L, Pointillart A. The bioavailability of dietary calcium. J Am Coll Nutr. 2000;19(2 Suppl), 119S-136S.

Hanes DA, Weaver CM, Wastney ME. Calcium and oxalic acid kinetics differ in rats. J Nutr. 1999;129(1), 165-169.

Heaney RP, Kopecky S, Maki KC, Hathcock, J, MacKay D, Wallace TC. A review of calcium supplements and cardiovascular disease risk. Adv Nutr. 2012;3(6):763-77.

Heiss, G, Hsia J, Pettinger M, Howard BV, Anderson G. Editorial was confusing. BMJ. 2010;341:570.

Hunt CD, Johnson LK. Calcium requirements: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. Am J Clin Nutr. 2007;86(4):1054-63.

Institute of Medicine (IOM). Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. 1997. Washington, DC: National Academies Press.

Institute of Medicine (IOM). Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate 2004. Washington, DC: National Academies Press.

Institute of Medicine (IOM). Dietary Reference Intakes for Calcium and Vitamin D. 2011. Washington, DC: The National Academies Press.

Ishidate M, Sofumi T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A. Primary mutagenicity screening of food additives currently used in Japan. Fd Chem Toxic. 1984;22:623-36.

Joint FAO/WHO Expert Committee on Food Additives (2001) Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956- 2001) (First through fifty-seventh meetings) Available at: http://jecfa.ilsi.org/

Kakigi CLM, Singh K, Wang SY, Enanoria WT, Lin SC. Self-reported calcium supplementation and age-related macular degeneration. JAMA Ophthamol. 2015; 133(7):746-754.

Kanematsu N, Hara M, Kada T. Rec assay and mutagenicity studies on metal compounds. Mutation Res. 1980;77: 109-16.

Koopman TSM, Pot TE. Acute oral toxicity study with anhydrous calcium chloride in male rabbits. 1986. Duphar Report No. 56645/52/86 (as cited in OECD Screening Information Data Set, 2002)

Koopman TSM, Pot TE. Acute oral toxicity study with calcium chloride 33% in male rabbits. 1986. Duphar Report No. 56645/50/86 (as cited in OECD Screening Information Data Set, 2002)

Koopman TSM, Pot TE. Acute oral toxicity study with calcium chloride dihydrate in male rabbits. 1986. Duphar Report No. 56645/51/86 (as cited in OECD Screening Information Data Set, 2002)

Koopman TSM, Pot TE. Acute oral toxicity study with calcium chloride hexahydrate in male rabbits. 1986. Duphar Report No. 56645/49/86 (as cited in OECD Screening Information Data Set, 2002)

Kuangron L, Kaks R, Linseisen J, Rohrmann S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg). Heart. 2012;98(12):920-5.

Kwak SM, Kim JS, Choi Y, Chang Y, Kwon M, Jung J, Jeong C, Kim HS, Shin H, Ryu S. Dietary intake of calcium and phosphorus and serum concentration in relation to the risk of coronary artery calcification in asymptomatic adults. Arterioscler Thromb Vasc Biol. 2014;34:1763-1769.

Lewis JR, Calver J, Zhu K, Flicker L, Prince RL. Calcium supplementation and the risks of atherosclerotic vascular disease in older women: results of a 5-year RCT and a 4.5-year follow-up. J Bone Miner Res. 2011;26(1):35-41.

Lewis JR, Radavelli-Bagatini S, Rejnmark L, Chen JS, Simpson JM, Lappe JM, Mosekilde L, Prentice RL, Prince RL. The effects of calcium supplementation on verified coronary heart disease hospitalization and death in postmenopausal women: A collaborative meta-analysis of randomized controlled trials. Journal of Bone and Mineral Research. 2015; 30(1):165-175.

Lewis JR, Zhu K, Prince RL. Adverse events from calcium supplementation: relationship to errors in myocardial infarction self-reporting in randomized controlled trials of calcium supplementation. J Bone Miner Res. 2012;27(3):719-22.

Michaelsson K, Melhus H, Lemming EW, Wolk A. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. BMJ. 2013;346:1-13.

Miller DD. Calcium in the diet: food sources, reecommended intakes, and Nutritional availability. Adv Food Nutr Res. 1989;3;103-56.

National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data 2009-2010. 2012. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available via: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes09_10.aspx.

National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data 2007-2008. 2010. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available via: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/nhanes07_08.htm.

Nordin BE, Lewis JR, Daly RM, Horowitz J, Metcalfe A, Lange K, Prince RL. The calcium scare – what would Austin Bradford Hill have thought? Osteoporos Int. 2011;22:3073-7.

Olivier, Ph. and Marzin, D. Study of the genotoxic potential of 48 inorganic derivatives with the SOS chromotest. Mutation Res. 1987;189, 263-9.

Organization for Economic Corporation (OECD) Screeining Information Data Set (SIDS) intial assessment Report for calcium chloride, UNEP Publications 2002.

Paik JM, Curhan GC, Sun Q, Rexrode KM, Manson JE, Rimm EB, Taylor EN. (2014). Available Online-May 2014. Calcium supplement intake and risk of cardiovascular disease in women. Osteoporos Int. 2014, 25:2047-2056.

Payne ME, McQuoid DR, Steffens DC, Anderson JJB. Elevated brain lesion volumes in older adults who use calcium supplements: a cross-sectional observational study. British Journal of Nutrition. 2014; 112, 220-227.

Prentice RL, Pettinger MB, Jackson RD, Wactawski-Wende J, LaCroix AZ, Anderson GL, Chlebowski RT, Manson JE, Van Horn L, Vitolins MZ, Datta M, LeBlanc ES, Cauley JA, Rossouw JE. Osteoporos Int. Health risks and benefits from calcium and vitamin D supplementation: Women's Health Initiative clinical trial and cohort study. 2013;24(2):567-580.

Prince RL, Zhu K, Lewis JR. Evidence of harm is unconvincing. BMJ. 2011;342:1274.

Raffield LM, Agarwal S, Cox AJ, Hsu F, Carr JJ, Freedman BI, Xu J, Bowden DW, Vitolins MZ. Cross-sectional analysis of calcium intake for associations with vascular calcification and mortality in individuals with type 2 diabetes from the Diabetes Heart Study. Am J Clin Nutr. 2014; 100:1029-35.

Samelson EJ, Booth SL, Fox CS, Tucker KL, Wang TJ, Hoffmann U, Cupples LA, O'Donnell CJ, Kiel DP. Calcium intake is not associated with increased coronary artery calcification: the Framingham Study. Am J Clin Nutr. 2012;96(6):1274-80.

Sheikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS. Gastrointestinal absorption of calcium from milk and calcium salts. N Engl J Med. 1987;317(9):532-56.

Shiga K, Hara H, Kasai T. The large intestine compensates for insufficient calcium absorption in the small intestine in rats. J Nutr Sci Vitaminol (Tokyo). 1998;44(6):737-44.

Uemura H, Katsuura-Kamano S, Yamaguchi M, Nakamoto M, Hiyoshi M, Arisawa K. Association between dietary calcium intake and arterial stiffness according to dietary vitamin D intake in men. British Journal of Nutrition. 2014; 112,1333-1340.

U.S. Department of Agriculture, Agricultural Research Service (USDA). Usual Intake from Food and Beverages 2007-2010 Compared to Dietary Reference Intakes. July 2013.

U.S. Department of Agriculture, Agricultural Research Service (USDA). USDA National Nutrient Database for Standard Reference, Release 24. 2012a. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

U.S. Department of Agriculture (USDA). USDA Food and Nutrient Database for Dietary Studies, 5.0. 2012b. Beltsville, MD: Agricultural Research Service, Food Surveys Research Group.

U.S. Department of Agriculture (USDA). USDA Food and Nutrient Database for Dietary Studies, 4.1. 2010. Beltsville, MD: Agricultural Research Service, Food Surveys Research Group.

Van Hemelrijck M, Michaelsson K, Linseisen J, Rohrmann S. 2013. Calcium intake and serum concentration in relation to risk of cardiovascular death in NHANES III. PLoS ONE. 8(4):1-9.

Varnia VM, Saric M, Mokrovic G, Piasek M, Blanusa M, Buljan Culej J, Matek Saric M, Kostial K. The effect of dietary supplementation with calcium salts on skeletal calcium in suckling rats. Arh Hig Rada Toksikol. 2003;54:119-125.

Walker RM, Linkswiler HM. Calcium retention in the adult human male as affected by protein intake. J Nutr. 1972;102(10), 1297-1302.

Wallace RB, Wactawski-Wende J, O'Sullivan MJ, Larson JC, Cochrane B, Gass M, Masaki K. Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements. Am J Clin Nutr. 2011;94(1):270-7.

Wang TK, Bolland MJ, Van Pelt NC, Horne AM, Mason BH, Ames RW, Grey AB, Ruygrok PN, Gamble GD, Reid IR. Relationships between vascular calcification, calcium metabolism, bone density, and fractures. J Bone Miner Res. 2010a;25(12):2777-2785.

Wang L, Manson JE, Song Y, Sesso HD. Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. Ann Intern Med. 2010b;152(5):315-323.

Wang X, Chen H, Ouyang Y, Liu J, Zhao G, Bao W, Yan M. Dietary calcium intake and mortality risk from cardiovascular disease and all causes: a meta-analysis of prospective cohort studies. BMC Medicine. 2014; 12:158.

Welsh P, Doolin O, McConnachie A, Boulton E, McNeil G, Macdonald H, Hardcastle A, Hart C, Upton M, Watt G, Sattar N. Circulating 25OHD, dietary vitamin D, PTH, and calcium associations with incident cardiovascular disease and mortality: the MIDSPAN family study. J Clin Endocrinol Metab. 2012;97(12):4578-87.

Wilson KM, Shui IM, Mucci LA, Giovannucci E. Calcium and phosphorous intake and prostate cancer risk: 2 24-y follow-up study. Am J Clin Nutr. 2015; 101:173-183.

Xiao Q, Murphy RA, Houston DK, Harris TB, Chow W, Park Y. Dietary and supplemental calcium intakes in relation to mortality from cardiovascular diseases in the NIH-AARP Diet and Health Study. JAMA Intern Med. 2013;173(8):639-46.

Appendices

the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it.
Acceptance criteria: The weight of the residue does not exceed 10 mg. (NMT 0.2%)

- **ARSENIC,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 10 mL of 2.7 N hydrochloric acid
  Acceptance criteria: NMT 3 mg/kg
- **FLUORIDE,** *Fluoride Limit Test, Method III,* Appendix IIIB
  Acceptance criteria: NMT 0.005%
- **LEAD,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Cautiously dissolve 5 g of sample in 25 mL of 1:2 hydrochloric acid and evaporate the solution to dryness on a steam bath. Dissolve the residue in about 15 mL of water and dilute to 25 mL (200 mg/mL).
  Control: 12 µg Pb (12 mL of *Diluted Standard Lead Solution*)
  Analysis: Use 20 mL of *Sample solution.*
  [NOTE—As an alternative to the above test, determine as directed in the *Lead Limit Test, APDC Extraction Method,* Appendix IIIB.]
  Acceptance criteria: NMT 3 mg/kg
- **MAGNESIUM AND ALKALI SALTS**
  Sample: 1 g
  Analysis: Mix the *Sample* with 40 mL of water, carefully add 5 mL of hydrochloric acid, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS. Then, add 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL graduated cylinder, dilute to 100 mL with water, and let stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper and place 50 mL of the clear filtrate in a platinum dish. Add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight.
  Acceptance criteria: The weight of the residue does not exceed 5 mg. (NMT 1%)

SPECIFIC TESTS

- **LOSS ON DRYING,** Appendix IIC: 200° for 4 h
  Acceptance criteria: NMT 2%

Calcium Chloride

First Published: Prior to FCC 6
Last Revision: FCC 9

$CaCl_2$ — Formula wt, anhydrous 110.98
$CaCl_2 \cdot 2H_2O$ — Formula wt, dihydrate 147.01
CAS: anhydrous [10043-52-4]
CAS: dihydrate [10035-04-8]
INS: 509
UNII: M4I0D6VV5M [calcium chloride]

DESCRIPTION

Calcium Chloride occurs as white, hard fragments, granules, or powder. It is anhydrous or contains two molecules of water of hydration. It is deliquescent. It is soluble in water; slightly soluble in alcohol. The pH of a 1:20 aqueous solution is between 4.5 and 11.0.
Function: Firming agent
Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **CALCIUM,** Appendix IIIA
  Sample solution: 100 mg/mL
  Acceptance criteria: Passes test
- **CHLORIDE,** Appendix IIIA
  Sample solution: 100 mg/mL
  Acceptance criteria: Passes test

ASSAY

- **PROCEDURE**
  Sample: 1.5 g
  Analysis: Transfer the *Sample* into a 250-mL volumetric flask, dissolve it in a mixture of 100 mL of water and 5 mL of 2.7 N hydrochloric acid, dilute with water to volume, and mix. Transfer 50 mL of this solution into a suitable container and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Then, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator. Continue the titration to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 5.55 mg of calcium chloride ($CaCl_2$) or 7.35 mg of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$).
  Acceptance criteria
  Anhydrous: NLT 93.0% and NMT 100.5% of $CaCl_2$
  Dihydrate: NLT 99.0% and NMT 107.0% of $CaCl_2 \cdot 2H_2O$

IMPURITIES

Change to read:

Inorganic Impurities

- **ACID-INSOLUBLE MATTER (ANHYDROUS SALT)**
  Filter assembly: Place a 32-mm (od) ▲▲ FCC9 disk filter[1] in a suitable filter assembly comprised of a 2.5-L screw-cap bottle cut in half horizontally and fitted with a rubber washer (35-mm od and 25-mm id), followed by the ▲ disk filter,▲ FCC9 a 20-mesh stainless steel screen (35-mm od), and a bottle cap with a 25-mm hole in the top.
  Sample solution: Dissolve 1 kg sample in 3 L of water containing 10 mL of glacial acetic acid. Allow the solution to cool.
  Analysis: Wash the *Filter assembly,* with the filter at the bottom, with 100 mL of 1:300 acetic acid, followed by 100 mL of water. Remove the disk from the assembly, place it on a watch glass, dry the combination at 105° for 2 h, let cool, and weigh. Filter the *Sample solution* through the lintine disk. Rinse the walls of the *Filter assembly* so that all insoluble matter is transferred to

[1] Visible Sediment Test Card Company (www.visible-sediment.com) part # 1.25 filter disc, or equivalent.

the disk, and wash with 100 mL of water. Place the disk on the same watch glass mentioned above, dry at 105° for 2 h, let cool and weigh the combination, being careful at all times not to lose any particles that may be on the disk. The difference in the two weights is the weight of the acid-insoluble matter. Place the disk under a low-power magnifier (4× to 10× magnification). Using a millimeter rule, measure the largest dimension of each particle (or as many as may be necessary) on the disk.

Acceptance criteria

Anhydrous: NMT 0.02%; no particles of sample greater than 2 mm in any dimension are present

- **Arsenic,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 10 mL
  Acceptance criteria: NMT 3 mg/kg
- **Fluoride,** *Fluoride Limit Test, Method III,* Appendix IIIB
  Acceptance criteria: NMT 0.004%
- **Lead,** *Lead Limit Test,* Appendix IIIB
  Sample solution: 1 g in 20 mL
  Control: 5 µg Pb (5 mL of *Diluted Standard Lead Solution*)
  Acceptance criteria: NMT 5 mg/kg
- **Magnesium and Alkali Salts**
  Sample: 1 g
  Analysis: Dissolve the *Sample* in 50 mL of water, add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS. Then add 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute with water to 100 mL, and let it stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, and transfer 50 mL of the clear filtrate to a platinum dish. Add 0.5 mL of sulfuric acid to the dish, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight.
  Acceptance criteria
  Anhydrous: NMT 25 mg of residue (NMT 5.0%)
  Dihydrate: NMT 20 mg of residue (NMT 4.0%)

OTHER REQUIREMENTS

- **Labeling:** Indicate whether the salt is anhydrous or dihydrate.

Calcium Chloride Solution

First Published: Prior to FCC 6

UNII: OFM21057LP [calcium chloride anhydrous]

DESCRIPTION

Calcium Chloride Solution occurs as a clear to slightly turbid, colorless or slightly colored liquid at room temperature. It is nominally available in a concentration range of about 35% to 45% of $CaCl_2$.

Function: Sequestrant; firming agent

Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Calcium,** Appendix IIIA
  Sample solution: 100 mg/mL ($CaCl_2$ basis)
  Acceptance criteria: Passes tests
- **Chloride,** Appendix IIIA
  Sample solution: 100 mg/mL ($CaCl_2$ basis)
  Acceptance criteria: Passes test

ASSAY

- **Procedure**
  Sample: Quantity equivalent to 1 g of $CaCl_2$
  Analysis: Transfer the *Sample* into a 250-mL volumetric flask, add 5 mL of 2.7 N hydrochloric acid and 100 mL of water to dissolve; dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Then add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator. Continue the titration to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 5.55 mg of $CaCl_2$.
  Acceptance criteria: NLT 90.0% and NMT 110.0%, by weight, of the labeled amount of calcium chloride, expressed as $CaCl_2$

IMPURITIES

Inorganic Impurities

- **Fluoride,** *Fluoride Limit Test, Method III,* Appendix IIIB
  Sample: Quantity equivalent to 1 g of $CaCl_2$
  Acceptance criteria: NMT 0.004%, calculated on the amount of $CaCl_2$ as determined in the *Assay*
- **Lead,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Quantity of sample equivalent to 1 g of $CaCl_2$, diluted to 10 mL
  Control: 4 µg Pb (4 mL of *Diluted Standard Lead Solution*)
  Acceptance criteria: NMT 4 mg/kg, calculated on the amount of $CaCl_2$ as determined in the *Assay*
- **Magnesium and Alkali Salts**
  Sample solution: Quantity of sample equivalent to 1 g of $CaCl_2$, diluted to 50 mL
  Analysis: To the *Sample solution,* add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute to 100 mL with water, and let it stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper and transfer 50 mL of the clear filtrate to a platinum dish. Add 0.5 mL of sulfuric acid to the dish and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely

decomposed and volatilized. Finally, ignite the residue to constant weight.
Acceptance criteria: The weight of the residue does not exceed 25 mg, calculated on the amount of $CaCl_2$ as determined in the *Assay* (NMT 5.0%).

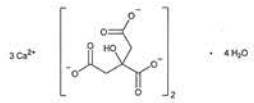
SPECIFIC TESTS

- **ALKALINITY (AS $Ca(OH)_2$)**
  Sample solution: Quantity of sample equivalent to 5 g of $CaCl_2$ diluted to 50 mL
  Analysis: Add phenolphthalein TS to the *Sample solution* and titrate with 0.1 N hydrochloric acid. Each mL of 0.1 N hydrochloric acid is equivalent to 3.71 mg of $Ca(OH)_2$.
  Acceptance criteria: NMT 0.3%

Calcium Citrate

First Published: Prior to FCC 6

Tricalcium Citrate



$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$ Formula wt 570.50
INS: 333 CAS: [5785-44-4]
UNII: MLM29U2X85 [calcium citrate]

DESCRIPTION

Calcium Citrate occurs as a fine, white powder. It is very slightly soluble in water, but it is insoluble in alcohol.
Function: Sequestrant; buffer; firming agent
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **A. PROCEDURE**
  Sample: 500 mg
  Analysis: Dissolve the *Sample* in 10 mL of water and 2.5 mL of 1.7 N nitric acid. Add 1 mL of mercuric sulfate TS, heat to boiling, and then add potassium permanganate TS.
  Acceptance criteria: A white precipitate forms.
- **B. PROCEDURE**
  Sample: 500 mg
  Analysis: Completely ignite the *Sample* at as low a temperature as possible. Cool the residue and dissolve it in a mixture of 10 mL of water and 1 mL of glacial acetic acid. Filter and add 10 mL of ammonium oxalate TS to the filtrate.
  Acceptance criteria: A voluminous, white precipitate forms that is soluble in hydrochloric acid.

ASSAY

- **PROCEDURE**
  Sample: 350 mg, previously dried
  Analysis: Dissolve the *Sample*, in a mixture of 10 mL of water and 2 mL of 2.7 N hydrochloric acid, and dilute to about 100 mL with water. While stirring, preferabl with a magnetic stirrer, add about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Add 15 mL of 1 sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 8.300 mg of $Ca_3(C_6H_5O_7)_2$.
  Acceptance criteria: NLT 97.5% and NMT 100.5% o $Ca_3(C_6H_5O_7)_2$, on the dried basis

IMPURITIES

Inorganic Impurities

- **FLUORIDE,** *Fluoride Limit Test, Method III,* Appendix IIIB
  Sample solution: Prepare as directed using 10 mL of hydrochloric acid instead of 20 mL.
  Analysis: Prepare a calibration curve as directed usinc 1.0, 5.0, and 10.0 mL of the *Sodium Fluoride Solutior* (equivalent to 5.0, 25.0, and 50.0 mg/kg of fluoride respectively).
  Acceptance criteria: NMT 0.003%
- **LEAD,** *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 2 mg/kg

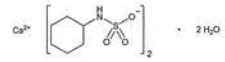
SPECIFIC TESTS

- **LOSS ON DRYING,** Appendix IIC: 150° for 4 h
  Acceptance criteria: Between 10.0% and 14.0%

Calcium Cyclamate

First Published: Third Supplement, FCC 7

Calcium Cyclohexanesulfamate
Calcium Cyclohexylsulfamate



$C_{12}H_{24}CaN_2O_6S_2 \cdot 2H_2O$ Formula wt, anhydrous 396
Formula wt, dihydrate 432
INS: 952(ii) CAS: anhydrous [139-0(
dihydrate [5897-1(

DESCRIPTION

Calcium Cyclamate occurs as colorless to white crystals o crystalline powder. It is soluble in water and sparingly soluble in ethanol.
Function: Sweetener
Packaging and Storage: Store in tight containers in a cool, dry place.

IDENTIFICATION

- **CALCIUM,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes test
- **INFRARED ABSORPTION,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Calcium Cyclamate RS
  Sample and standard preparation: *K*

Appendix B. Certificates of Analysis

LINCOLN FINE INGREDIENTS

NEDMAG

CERTIFICATE OF ANALYSIS

CALCIUM CHLORIDE PRILLS 94 - 97%

FOOD GRADE

Nedmag Industries

Rilitonweg 1
9641 KZ Veendam
P.O. Box 241
9640 AE Veendam
The Netherlands

T +31 598 651 911
F +31 598 651 206
E info@nedmag.nl
I www.nedmag.com

COMPANY : [redacted]
YOUR ORDERNR. : F201600023
VOLUME : 12 ML
DELIVERY DATE : 11-1-2016
NEDMAG ORDERNR. : 790000829
NEDMAG BATCHNR. : 0873
BATCH PRODUCTION : 15-12-2015 TO 16-12-2015
EXPIRY DATE : 15-12-2017 TO 16-12-2017

PARAMETER	UNIT	MEASURED VALUE	MINIMUM	MAXIMUM	ANALYTICAL METHOD
$CaCl_2$	%	98,4	94		Titrimetric
KCl**	%	1,3			ICP-AES
NaCl**	%	1,1			ICP-AES
$MgCl_2$**	%	0,01			ICP-AES
Sum Mg+alkali salts	%	2,5		5	
SO_4	%	0,02		0,1	ICP-AES
Ba	%	0,03		0,08	ICP-AES
Fe	mg/kg	< 0,25		5	ICP-AES
F *	mg/kg	< 10		10	Titrimetric
Cu *	mg/kg	0,36		2	ICP-AES
Zn *	mg/kg	0,11		2	ICP-AES
As *	mg/kg	0,005		0,03	ICP-Hydride
Pb *	mg/kg	< 1,5		2	ICP-AES
Hg *	mg/kg	< 0,004		0,03	ICP-Hydride

* on anhydrous basis
** sum **<5%** on anhydrous basis
*** sum Cu and Zn max ***50*** mg/kg

LABORATORY APPROVED (b) (6)

LINCOLN FINE INGREDIENTS

NEDMAG

CERTIFICATE OF ANALYSIS

CALCIUM CHLORIDE PRILLS 94 - 97%

FOOD GRADE

Nedmag Industries
Mining & Manufacturing B.V.
Billitonweg 1
9641 KZ Veendam
P.O. Box 241
9640 AE Veendam
The Netherlands
T +31 598 651 911
F +31 598 651 226
E info@nedmag.nl
I www.nedmag.com

COMPANY : [redacted]
YOUR ORDERNR. : Mail 15/12
VOLUME : 22 Mt
DELIVERY DATE : 16-12-2015
NEDMAG ORDERNR. : 790000818
NEDMAG BATCHNR. : 0866
BATCH PRODUCTION : 21-11-2015 TO 22-11-2015
EXPIRY DATE : 21-11-2017 TO 22-11-2017

PARAMETER	UNIT	MEASURED VALUE	MINIMUM	MAXIMUM	ANALYTICAL METHOD
CaCl2	%	96,2	94		Titrimetric
KCl**	%	1,5			ICP-AES
NaCl**	%	1,2			ICP-AES
MgCl2**	%	0,02			ICP-AES
Sum Mg+alkali salts	%	2,7		5	
SO4	%	0,02		0,1	ICP-AES
Ba	%	0,04		0,08	ICP-AES
Fe	mg/kg	0,44		5	ICP-AES
F *	mg/kg	< 10		10	Titrimetric
Cu *	mg/kg	0,53		2	ICP-AES
Zn *	mg/kg	0,21		2	ICP-AES
As *	mg/kg	0,005		0,03	ICP-Hydride
Pb *	mg/kg	< 1,5		2	ICP-AES
Hg *	mg/kg	< 0,004		0,03	ICP-Hydride

* on anhydrous basis
** sum **<5%** on anhydrous basis
*** sum Cu and Zn max ***50*** mg/kg

LABORATORY APPROVED b) (6)

Trade register Groningen 02331543
VAT no. NL 004327084B01
NedMag is ISO 9001 ISO 14001

LINCOLN FINE INGREDIENTS

NEDMAG

Nedmag Industries
Mining & Manufacturing B.V.

Billitonweg 1
9641 KZ Veendam
P.O. Box 241
9640 AE Veendam
The Netherlands

T +31 598 651 911
F +31 598 651 226
E info@nedmag.nl
I www.nedmag.com

CERTIFICATE OF ANALYSIS

CALCIUM CHLORIDE PRILLS 94 - 97%

FOOD GRADE

COMPANY	[redacted]
YOUR ORDERNR.	AV2304/2613
VOLUME	4 Mt
DELIVERY DATE	20-1-2016
NEDMAG ORDERNR.	790000834
NEDMAG BATCHNR.	0881
BATCH PRODUCTION	23-12-2015 TO 24-12-2015
EXPIRY DATE	23-12-2017 TO 24-12-2017

PARAMETER	UNIT	MEASURED VALUE	MINIMUM	MAXIMUM	ANALYTICAL METHOD
CaCl2	%	96,2	94		Titrimetric
KCl**	%	1,2			ICP-AES
NaCl**	%	1,1			ICP-AES
MgCl2**	%	0,01			ICP-AES
Sum Mg+alkali salts	%	2,4		5	
SO4	%	0,02		0,1	ICP-AES
Ba	%	0,03		0,08	ICP-AES
Fe	mg/kg	0,30		5	ICP-AES
F *	mg/kg	< 10		10	Titrimetric
Cu *	mg/kg	< 0,28		2	ICP-AES
Zn *	mg/kg	0,08		2	ICP-AES
As *	mg/kg	0,005		0,03	ICP-Hydride
Pb *	mg/kg	1,5		2	ICP-AES
Hg *	mg/kg	< 0,004		0,03	ICP-Hydride

* on anhydrous basis
** sum **<5%** on anhydrous basis
*** sum Cu and Zn max ***50*** mg/kg

LABORATORY APPROVED (b) (6)

Trade register Groningen 0733545
VAT no. NL 004270840B01

NedMag is ISO 9001, ISO 14001
[illegible] and feed

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

Certificate of Analysis

32% Calcium Chloride
FCC Grade Material

Consignee Name:	[redacted]	Bill of Lading #:	37396
Batch#:	(b) (6)	Customer PO#:	587750
Carrier:	Roy's Transfer		
pH(as is):	6.50	Specific Gravity:	1.325 @ 70 F
Date of Manufacture:	September 28, 2015		

Certification	Results
Color:	< 40 platinum-cobalt units
Concentration:	32.12%
Fluoride:	0.00050%
Arsenic:	NMT 1 mg/kg
Lead*:	NMT 1 mg/kg
Alkalinity:	NMT 0.3%
Magnesium and Alkali Salts*:	NMT 5%

*Guaranteed analysis checked at intervals according to plant schedule.

Meets or exceeds Food Chemicals Codex 7th Edition Specifications.

(b) (6)

By: (b) (6)

Kathy Kelley
Quality Control

Friday, October 02, 2015

110 East Avenue H
Rochelle IL 61068
www.fbcindustries.com
Phone 847.839.0880

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

Certificate of Analysis

32% Calcium Chloride
FCC Grade Material

Consignee Name: [redacted]
Batch#: (b) (6)
Carrier: Customer Pick-up
pH(as is): 6.27
Date of Manufacture: September 22, 2015

Bill of Lading #: 37351
Customer PO#: 1090116
Specific Gravity: 1.325 @ 70 F

Certification	Results
Color:	< 40 platinum-cobalt units
Concentration:	32.21%
Fluoride:	0.00043%
Arsenic:	NMT 1 mg/kg
Lead*:	NMT 1 mg/kg
Alkalinity:	NMT 0.3%
Magnesium and Alkali Salts*:	NMT 5%

*Guaranteed analysis checked at intervals according to plant schedule.

Meets or exceeds Food Chemicals Codex 7th Edition Specifications.

By: (b) (6)
Kathy Kelley (b) (6)
Quality Control
Friday, October 02, 2015

110 East Avenue H
Rochelle IL 61068
www.fbcindustries.com
Phone 847.839.0880

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

Certificate of Analysis

32% Calcium Chloride
FCC Grade Material

Consignee Name:	[redacted]	Bill of Lading #:	37398
Batch#:		Customer PO#:	3560896185
Carrier:	Roy's Transfer		
pH(as is):	6.34	Specific Gravity:	1.325 @ 70 F
Date of Manufacture:	September 28, 2015		

Certification	Results
Color:	< 40 platinum-cobalt units
Concentration:	32.26%
Fluoride:	0.00047%
Arsenic:	NMT 1 mg/kg
Lead*:	NMT 1 mg/kg
Alkalinity:	NMT 0.3%
Magnesium and Alkali Salts*:	NMT 5%

*Guaranteed analysis checked at intervals according to plant schedule.

Meets or exceeds Food Chemicals Codex 7th Edition Specifications.

By: [redacted]

Lisa Hayes
Quality Control

Friday, October 02, 2015

110 East Avenue H
Rochelle IL 61068
www.fbcindustries.com
Phone 847.839.0880

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

Certificate of Analysis

32% Calcium Chloride
FCC Grade Material

Consignee Name: [redacted]

Batch#: (b) (6)

Carrier: Roy's Transfer

pH(as is): 6.27

Date of Manufacture: September 25, 2015

Bill of Lading #: 37397

Customer PO#: 3560896184

Specific Gravity: 1.325 @ 70 F

Certification	Results
Color:	< 40 platinum-cobalt units
Concentration:	32.24%
Fluoride:	0.00050%
Arsenic:	NMT 1 mg/kg
Lead*:	NMT 1 mg/kg
Alkalinity:	NMT 0.3%
Magnesium and Alkali Salts*:	NMT 5%

*Guaranteed analysis checked at intervals according to plant schedule.

Meets or exceeds Food Chemicals Codex 7th Edition Specifications.

By: (b) (6)

Kathy Kelley (b) (6)
Quality Control

Friday, October 02, 2015

110 East Avenue H
Rochelle IL 61068
www.fbcindustries.com
Phone 847.839.0880

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

Certificate of Analysis

32% Calcium Chloride
FCC Grade Material

Consignee Name: [redacted]

Batch#:

Carrier: Roy's Transfer

pH(as is): 6.25

Date of Manufacture: September 29, 2015

Bill of Lading #: 37466

Customer PO#: 58828

Specific Gravity: 1.325 @ 70 F

Certification	Results
Color:	< 40 platinum-cobalt units
Concentration:	32.22%
Fluoride:	0.00045%
Arsenic:	NMT 1 mg/kg
Lead*:	NMT 1 mg/kg
Alkalinity:	NMT 0.3%
Magnesium and Alkali Salts*:	NMT 5%

*Guaranteed analysis checked at intervals according to plant schedule.

Meets or exceeds Food Chemicals Codex 7th Edition Specifications.

By:

Lisa Hayes
Quality Control

Friday, October 02, 2015

110 East Avenue H
Rochelle IL 61068
www.fbcindustries.com
Phone 847.839.0880

LINCOLN FINE INGREDIENTS

NEDMAG

Nedmag Industries
Mining & Manufacturing B.V.
Billitonweg 1
9641 KZ Veendam
The Netherlands

T +31 598 651 911
F +31 598 651 205
E sales@nedmag.nl
I www.nedmag.com

$CaCl_2$ food

Calcium chloride prills 94-97%

Food grade E 509

Product description

$CaCl_2$ **food** prills are small white prills of anhydrous calcium chloride.

$CaCl_2$ **food** is mainly used in cheese production as a source for calcium; and in beer bottling where it serves to regulate the hardness of water.

$CaCl_2$ **food** is also used for canned food, to increase the firmness of the fruits and vegetables.

Product quality

$CaCl_2$ **food** meets or exceeds the requirements of:

- Current EU Food Additive Regulation (E509)
- Current Edition Food Chemical Codex
- 19th JECFA (1975) and 63rd JECFA (2004) ($CaCl_2$ **food** passes Chloride and Calcium test)

Packaging

$CaCl_2$ **food** prills are available in 25 kg bags.

Storage and handling

- $CaCl_2$ **food** prills are very hygroscopic
- Store it in a dry place to avoid uptake of moisture
- Quickly reseal open bags

Shelf life

- 2 years, if stored as indicated before

Chemical composition

		Typical	Specification
$CaCl_2$	%	96	> 94
Mg and alkali salts *	%	3	< 5
SO_4	%	< 0,05	< 0,1
Ba	%	< 0,05	< 0,08
Fe	mg/kg	< 2	< 5
Cu*	mg/kg	< 0,28	< 2
Zn*	mg/kg	0,15	< 2
F*	mg/kg	< 10	< 10
As*	mg/kg	< 0,005	< 0,03
Pb*	mg/kg	< 1,5	< 2
Hg*	mg/kg	< 0,004	< 0,03

* on anhydrous basis

Physical properties

	Typical
pH (aqueous solution 10%)	10

Nedmag is ISO 9001, ISO 14001, ISO 22000 and GMP+ certified

Date of issue: July 2015

FBC INDUSTRIES, INC.
Creating Solutions for the Food and Beverage Industries

MATERIAL SAFETY DATA SHEET

24 Hour Emergency Phone, CHEMTREC 800- 424-9300
EFFECTIVE: March 2, 2010, Supersedes February 23, 2005

MANUFACTURER'S NAME AND ADDRESS:

FBC INDUSTRIES
1933 N. MEACHAM, SUITE 550
SCHAUMBURG, IL 60173
(847) 839-0880 PHONE, (847) 839-0884 FAX

FBC INDUSTRIES (PLANT LOCATION)
P.O. BOX 173/110 EAST AVENUE H
ROCHELLE, IL 61068
(815) 562-8169 PHONE, (815) 562-3018 FAX

SECTION I: PRODUCT IDENTITY

CHEMICAL NAME	30-42% CALCIUM CHLORIDE SOLUTION
TRADE NAME	CALCIUM CHLORIDE
CHEMICAL FAMILY SYNONYMS	$CaCl_2$
MOLECULAR WEIGHT	110.99 (ACTIVE WEIGHT)
CAS REG. #/NAME	10043-52-4 CALCIUM CHLORIDE
FORMULA	$CaCl_2$

SECTION II: HAZARDOUS INGREDIENTS

WARNING! CAUSES IRRITATION TO SKIN AND EYES. HARMFUL IF SWALLOWED OR INHALED.

SECTION III: PHYSICAL DATA

FLASH POINT: NA; FLAMMABLE LIMITS: NA; BOILING PT: 230°F - 251°F; VAPOR PRESSURE: NOT FOUND; VAPOR DENSITY: NOT FOUND, COMPLETELY MISCIBLE; SPECIFIC GRAVITY: 1.297-1.437 AT 25°C; APPEARANCE: CLEAR LIQUID SOLUTION; ODOR: NONE

SECTION IV: FIRE AND EXPLOSION HAZARDS

CALCIUM CHLORIDE WILL NOT BURN.
UNUSUAL FIRE AND EXPLOSION HAZARDS: NONE

MATERIAL SAFETY DATA SHEET

SECTION V: REACTIVITY DATA

CALCIUM CHLORIDE IS STABLE AND HAZARDOUS POLYMERIZATION WILL NOT OCCUR.
CONDITIONS TO AVOID: ELEVATED TEMPERATURES.
MATERIALS TO AVOID: STRONG OXIDIZING AGENTS
HAZARDOUS DECOMPOSITION PRODUCTS INCLUDE: NONE KNOWN

SECTION VI: HEALTH HAZARDS

EYES: MODERATE TO SEVERE IRRITATION, ENCOUNTERED AT ELEVATED TEMPERATURES. SKIN: PROLONGED EXPOSURE MAY CAUSE IRRITATION, BURN AT ELEVATED TEMPERATURES. INHALATION: NOT APPLICABLE. INGESTION: LOW TOXICITY. SYSTEMIC AND OTHER EFFECTS: COMPONENTS OF THIS PRODUCT ARE NOT LISTED BY IARC OR OSHA AS A CARCINOGEN FOR HAZARD PURPOSES. RESULTS OF TESTS HAVE BEEN NEGATIVE.

SECTION VII: TOXICOLOGICAL INFORMATION

ORL-RAT LD50 13,431 mg/kg
CANCER LISTS

	NTP CARCINOGEN		
INGREDIENT	Known	Anticipated	IARC Category
Calcium Chloride (10043-52-4)	No	No	None

SECTION VIII: SPILL, LEAK AND WASTE PROCEDURES

FLUSH AREA WITH PLENTY OF WATER. WALKING SURFACE MAY REMAIN WET LONGER DUE TO MOISTURE BEING HELD BY SPILLED MATERIAL. DISPOSAL METHOD: WASH AWAY WITH LARGE EXCESS OF WATER. KEEP OUT OF DRINKING WATER SOURCES. COMPLY WITH LOCAL, STATE, AND FEDERAL REGULATIONS.

SECTION IX: SPECIAL PROTECTION

EYE PROTECTION: NORMAL INDUSTRIAL EYE PROTECTION PRACTICES SHOULD BE EMPLOYED. SKIN PROTECTION: NO SPECIAL EQUIPMENT IS REQUIRED. HOWEVER, GOOD PERSONAL HYGIENE PRACTICES SHOULD ALWAYS BE FOLLOWED. RESPIRATORY PROTECTION: NO SPECIAL REQUIREMENTS UNDER ORDINARY CONDITIONS OF USE AND WITH ADEQUATE VENTILATION. VENTILATION: NO SPECIAL REQUIREMENTS UNDER ORDINARY CONDITIONS OF USE.

MATERIAL SAFETY DATA SHEET

SECTION X: SPECIAL PRECAUTIONS

SPECIAL PRECAUTIONS SHOULD BE TAKEN IN HANDLING AND STORAGE: PRODUCT MAY BE SHIPPED HOT, AVOID EYE AND SKIN CONTACT. LEATHER CLOTHING AND SHOES WILL BE DAMAGED BY $CaCl_2$.

SECTION XI: DOT REQUIREMENTS

DOT PROPER SHIPPING NAME:	NOT REGULATED
DOT HAZARD CLASS/I.D. NO.:	NOT REGULATED
DOT LABEL:	N/A

SECTION XII REGULATORY INFORMATION

Chemical Inventory Status- Part 1

Ingredient	TSCA	EC	Japan	Australia
Calcium Chloride (10043-52-4)	Yes	Yes	Yes	Yes

Chemical Inventory Status- Part 2

		-----Canada-------		
Ingredient	Korea	DSL	NDSL	Phil.
Calcium Chloride (10043-52-4)	Yes	Yes	No	Yes

Federal, State & International Regulations- Part 1

	--SARA 302--		----------SARA 313--------	
Ingredient	RQ	TPQ	List	Chemical Categories
Calcium Chloride (10043-52-4)	No	No	No	No

Federal, State & International Regulations- Part 2

		--RCRA--	--TSCA--
	CERCAL	261.33	8 (d)
Ingredient			
Calcium Chloride (10043-52-4)	No	No	No

SARA 311/312: Acute: Yes (As Solid) Chronic: No Fire: No Pressure: No

MANUFACTURES DISCLAIMER

FBC INDUSTRIES, INC. INFORMATION GIVEN HEREIN IS OFFERED IN GOOD FAITH AS ACCURATE, BUT WITH OUT GUARANTEE AND WILL NOT BE RESPONSIBLE FOR DAMAGES RESULTING FROM USE OR RELIANCE UPON THIS INFORMATION. CONDITIONS OF USE AND SUITABILITY OF THE PRODUCT ARE THEREFORE ASSUMED BY THE USER AND WE EXPRESSLY DISCLAIM ALL WARRANTIES OF EVERY KIND AND NATURE, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE IN RESPECT TO THE USE AND SUITABILITY OF THE PRODUCT. NOTHING IS INTENDED AS A RECOMMENDATION FOR USES, WHICH INFRINGE VALID PATENTS, OR AS EXTENDING LICENSE UNDER VALID PATENTS. APPROPRIATE WARNINGS AND SAFE HANDLING PROCEDURES SHOULD BE PROVIDED TO THE HANDLERS AND USERS.

Appendix D. PubMed Literature Search Strategy

Summary of PubMed Literature Searches for Safety Data on Calcium Published Subsequent to the IOM's Review

		Hits (n)			
Search Terms	**Limits**	**Initial search (10/31/13)**	**Updated search (6/20/2014)**	**Updated Search (10/2015)**	**Updated Search (2/2016)**
calcium AND (hypercalcemia OR hypercalciuria OR nephrolithiasis OR prostate cancer OR cardiovascular OR toxicity OR UL OR tolerable OR safety OR adverse)	Published since 6/1/2010, Humans, Dietary supplements, English language, with abstracts	857	65	202	13
calcium AND (hypercalcemia OR hypercalciuria OR nephrolithiasis OR prostate cancer OR cardiovascular OR toxicity OR UL OR tolerable OR safety OR adverse)	Published since 6/1/2010, Humans, Clinical trials, English language, with abstracts	845	70	264	14
calcium AND (hypercalcemia OR hypercalciuria OR nephrolithiasis OR prostate cancer OR cardiovascular OR toxicity OR UL OR tolerable OR safety OR adverse) AND (cross-sectional or cross-sectional or case-control or cohort or NHANES or epidemiology)	Published since 6/1/2010, Humans, English language, with abstracts	1615	111	472	43
Total citations reviewed	-	3317	246	938	56

Exhibits

Exhibit 1. Report of the Expert Panel

EXPERT PANEL OPINION
THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE PROPOSED USE OF CALCIUM CHLORIDE IN POTATO SNACKS

Introduction

The undersigned, an independent panel of experts, qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "Expert Panel"), was specially convened on behalf of Frito-Lay, Inc., and asked to evaluate the safety and "generally recognized as safe" ("GRAS") status of the proposed use of calcium chloride in the production of potato snacks at a level up to 1% to reduce the acrylamide concentration formed during the production of these foods.

Calcium chloride is a salt that is produced through the extraction of aqueous brine from natural underground deposits. Lime is added to the brine to precipitate magnesium as magnesium hydroxide which is removed by filtration and clarification. The remaining calcium chloride rich brine is concentrated by water evaporation and dried to produce white, free-flowing pellets. Calcium chloride is most often used in food to enhance the flavor and/or texture of food products. Calcium chloride, the subject of this GRAS determination, meets the specifications requirement as noted under 21 CFR §184.1193 and §582.1193 (i. e. Food Chemicals Codex (FCC) specifications, 9th Edition (FCC, 2014)).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a review in 1973 and determined that calcium chloride was of low toxicity and therefore established an acceptable daily intake (ADI) as "not specified". Calcium chloride dissociates to calcium and chloride ions in the body. Calcium and chloride are both essential nutrients required by all forms of life. Many calcium salts, including calcium chloride, are dietary and supplemental sources of calcium. Therefore, to evaluate the safety of calcium chloride proposed for use in potato snacks, the safety of calcium was also evaluated and the estimated daily intake (EDI) is based on intake of calcium from background sources in the diet including supplements in addition to the proposed use in potato snacks.

Exponent Inc. ("Exponent") performed a comprehensive search of the scientific literature in October 2013 and June 2014 relating to the safety of calcium chloride and calcium for human consumption. Exponent summarized the results of the literature search and prepared a safety dossier, "GRAS Determination for the Use of Calcium Chloride in Potato Snacks," for consideration by the Expert Panel.

The Expert Panel critically evaluated Exponent's safety documentation (the dossier), and other available data and information that the members of the Expert Panel believed to be pertinent to the safety of calcium chloride under the conditions of intended use. In addition, the Expert Panel critically evaluated the method of production and specifications for calcium chloride, analytical

data confirming compliance with appropriate food-grade specifications and consistency of production, the conditions of its intended use as a component of the food production process, and the estimated dietary exposure to calcium and calcium chloride. After independent review, the Expert Panel convened via telephone conference call on February 5, 2014, and subsequently reviewed additional documentation incorporated into the dossier on July 1, 2014. The Expert Panel independently, jointly, and unanimously concluded that the intended use of calcium chloride in the production process of potato snacks, produced consistent with current good manufacturing practice (cGMP) and meeting appropriate food-grade specifications, is safe and suitable. The Expert Panel further concluded that such intended use is safe and GRAS based on scientific procedures. It is also the opinion of this Expert Panel that other qualified experts would concur with these conclusions.

Summarized below is the Expert Panel's scientific analysis supporting our conclusions.

Description

The calcium chloride solution proposed for use contains 32% calcium chloride by weight and has limits on lead, fluoride, and magnesium and alkali salt contaminants. The calcium chloride solution complies with requirements of 21 CFR §184.1193 and 21 CFR §582.1193, which in turn indicates that the calcium chloride meets the specifications of the Food Chemicals Codex (FCC), 9th Edition.

Manufacturing Process

The food grade anhydrous calcium chloride that is intended for use in the production of potato snacks is a salt produced through the extraction of aqueous brine from natural underground deposits. The production process is consistent with the production of calcium chloride for various technical functions as specified in 21 CFR §184.1193. The calcium chloride raw brine is filtered by compressed air and mixed with water and hydrochloric acid. The solution is sieved to control for particle size distribution and then added to drums, pails, and totes or a tank truck for transport to a third party company for packaging and distribution.

Intended Use and Estimated Intake

Calcium chloride meeting the specifications as described in 21 CFR §184.1193 and §582.1193 (i. e. Food Chemicals Codex (FCC) specifications, 9th Edition (FCC, 2014)) is proposed for use in the production of potato snacks to reduce the acrylamide concentration that is formed during the production of these foods. Acrylamide has been suggested to be a human carcinogen and the use of technological advances such as calcium chloride addition in reducing its formation during production of this food is desirable in reducing overall exposure to dietary sources of acrylamide. Calcium chloride will be added at a level up to a maximum of 1% in the potato flour mixtures that are extruded into pellets; the potato flour pellets are subsequently air-popped into potato snacks for consumption. For the purpose of the intake assessment, the maximum level of calcium chloride present in the finished product (i.e., food as consumed) is assumed to be 1%. Examples of potato snacks include potato chips and all potato-containing snack foods (e.g., potato sticks).

The *per user* mean and 90th percentile intakes of calcium from the proposed use of calcium chloride in potato snacks were estimated at 72 and 147 mg/day, respectively, for the population age 1+ yrs. Male adolescents were estimated to have the highest intakes of calcium from the proposed uses (249 mg/day at the 90th percentile of intake). For the U.S. population age 1+ yrs, the *per user* mean and 90th percentile cumulative intakes of calcium from all sources (background food and dietary supplement sources and the proposed use of calcium chloride in potato snacks), were estimated at 1152 and 1936 mg/day, respectively. Children 1-3 yrs had estimated *per user* mean and 90th percentile cumulative calcium intakes of 1041 and 1557 mg/day, respectively, among males, and 1029 and 1559 mg/day, respectively, among females. Children 4-8 yrs had estimated *per user* mean and 90th percentile cumulative calcium intakes of 1087 and 1689 mg/day, respectively, among males, and 1008 and 1568 mg/day, respectively, among females. The cumulative intake estimates for infants 6-11 months remain the same as their background calcium intake when the proposed use of calcium chloride in potato snacks was added (mean = 678 mg/day and 90th = 1113 mg/day). Among the older subpopulations, estimated 90th percentile cumulative calcium intakes were highest among the older adults (51+ yrs) ranging from 1918 mg/day among males 71+ yrs to 2204 mg/day among women 51-70 yrs.

Safety

The safety of calcium chloride was evaluated by a critical analysis of the safety of calcium and calcium chloride. The safety evaluation for calcium chloride included an evaluation of the adsorption, distribution, metabolism, and excretion of calcium chloride in the human body; a review of the Institute of Medicine's (IOM's) 2011 evaluation of calcium (IOM 2011); a review of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) revaluation of the safety of calcium in 2012 (EFSA, 2012); a review of the recent literature on risk of adverse effects from excessive calcium intake that may not have been included in the 2011 IOM report or 2012 EFSA opinion; and a toxicological assessment of calcium chloride based on preclinical and clinical literature. All the information critically evaluated that formed the basis for this GRAS determination is available in the public literature.

Calcium chloride readily dissociates into its component ions, calcium and chloride, under aqueous conditions in the gut. Absorption of calcium occurs in the small intestine, primarily in the duodenum and proximal jejunum by active transport and also by passive diffusion. The mean calcium absorption is about 25% of calcium intake (10 – 40%) (EFSA, 2012). Chloride absorption occurs via co-transporters in the gastrointestinal tract as well as by active re-uptake systems in the kidney. Absorbed calcium is distributed mainly in the skeleton and teeth and excess calcium is excreted in urine, feces and sweat. Chloride, which is the most abundant anion in living species, is distributed extracellularly throughout the body, and plasma concentrations are maintained between 100-110 mmol/L. Chloride excretion occurs mainly via the kidneys.

The toxicity of calcium chloride was evaluated through pre-clinical and clinical studies. Calcium chloride is considered to have low toxicity and an ADI was not established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1973). Furthermore, calcium chloride is considered GRAS by the US FDA (SCOGS-45, 1975). An updated literature search revealed no new information that contradicts the JECFA or FDA's earlier conclusion on calcium

chloride. Several acute toxicity studies in mice, rats, and rabbits resulted in LD_{50} values ranging from 500 mg/kg bw in rabbits to 5 g/kg bw in rats. While reproductive toxicity has not been evaluated, a reliable developmental toxicity study showed that calcium chloride at doses up to 189 mg/kg bw/day in mouse, 176 mg/kg bw/day in rat and 169 mg/kg bw/day in rabbit did not cause any toxic effects on dams or fetuses. Results from a series of in vitro and in vivo genotoxicity studies demonstrate that calcium chloride is not mutagenic. Results of a 1 year study in rats indicate that oral chronic administration of calcium chloride at 1000 – 2000 mg/kg bw/day did not induce any adverse effects. The EDI for calcium chloride from the proposed use in potato snacks is several orders of magnitude lower than the lowest dose tested in rats with no observed adverse effects following chronic oral administration (i.e., 1000 mg/kg bw/day).

The biological and toxicological effects related to both calcium deficiency and calcium excess have been extensively reviewed by the IOM and EFSA. The IOM-established ULs for calcium are lowest among infants (1,000 mg/day and 1,500 mg/day for infants 0-6 months and 6-12 months, respectively). Among older adults (51+ yrs the UL ranges from 2,000 mg/day based on the IOM evaluation to 2500 mg/day based on EFSA's evaluation. The ULs for the remaining life stages are 2500 mg/day for children 1– 8 yrs (IOM, 2011) and adults 19 – 50 yrs and 3000 mg/day for adolescents 9-18 yrs. The ULs for calcium established by the IOM were based on calcium excretion in young children and formation of kidney stones among older children and adults. The IOM concluded that there were insufficient data to determine an UL based on other effects, including increased risk of CVD among post-menopausal women and older men. EFSA's most recent evaluation reached similar conclusions on the lack of increase of CVD and other health endpoints and concluded that the available evidence did not support a revision of the 2003 UL established among adults of 2500 mg/day. Both the IOM and EFSA expert panels had also noted that it is difficult to measure the precise amount of daily calcium intake from both diet and supplements among the study subjects in the Women's Health Initiative Trial, a randomized placebo-controlled clinical trial in post-menopausal women which investigated the effect of calcium and vitamin D supplementation on risk of hip and other fractures and was the source of data to evaluate the effect of calcium supplementation on secondary outcomes such as cardiovascular events. This measurement imprecision can result in considerable uncertainty in the upper intake level associated with any adverse effects. Reviews of the recent published literature on the same endpoints considered by the IOM in 2011 and a complete search for other potential health outcomes not considered by the IOM, while adding to the body of literature, do not offer any conclusive evidence of cause and effects contrary to the IOM and EFSA conclusions on the safety of dietary calcium and the UL.

Summary and Conclusion

The intake assessment was designed to conservatively estimate background intake of calcium from all food sources (i.e. all naturally-occurring and calcium fortified food sources and approved food additive uses of calcium chloride, as measured by the USDA), and also included intake of calcium from dietary supplements, and projected calcium intake from the proposed use of calcium chloride in all potato snacks. No adjustment has been made to account for the potential overestimation of intakes that may result from using two days of dietary data to estimate long-term consumption nor to account for the fact that only select Frito-Lay potato snacks will contain calcium chloride. Additionally, 100% bioavailability of the calcium from the

proposed use was also assumed resulting in a conservative overestimate of exposure. Results of these analyses indicate that the *per user* 90th percentile cumulative calcium intakes (background + proposed use) were below the IOM UL for the majority of the US subpopulations. For three subgroups, the *per user* 90th percentile calcium intakes from background sources (food sources + dietary supplement) marginally exceeded the IOM UL of 2000 mg/day but were below the EFSA UL of 2500 mg/day among the older women 51 -70 yrs (2195 mg/day) and 71+ yrs (2158 mg/day) as well as among men 51-70 yrs (2023 mg/day). These findings are consistent with the 2011 IOM report of usual calcium intakes exceeding the IOM UL at the 95th and 99th percentiles (as analyzed by Bailey et al. 2010 with further data provided by staff at the National Cancer Institute – National Institutes of Health). Source contribution analyses showed that background calcium intake from food sources alone are well below the IOM UL at the *per user* 90th percentile for these subpopulations, irrespective of supplement use status, with *per user* 90th percentile dietary calcium intake ranging from 1274 mg/day among females 71+ yrs to 1721 among males 51-70 yrs. For these older age groups, the additional calcium intake from the use of supplements drives the total background calcium intake: at the 90th percentile, calcium from supplement use contributes up to 65% of the total background calcium intake among all calcium consumers. It should also be noted that almost two-thirds (65%) of the women 71+ yrs reported the use of a calcium-containing supplement in the NHANES 2007-2010 database, representing the largest supplement user group.

The proposed use of calcium chloride at a level up to 1 % in potato snacks contributes minimally to the total cumulative calcium intake at the 90th percentiles among these older female and male sub-populations. Among all calcium consumers, the proposed use of calcium chloride contributes from 3- 5% (31 – 59 mg/day additional calcium), among supplement consumers: 2-4% (29-59 mg/day additional calcium), and among non-calcium-supplement users: 2-7% (20 – 51 mg/day additional calcium). Among older women and men who are not taking calcium supplements, the per user 90th percentile cumulative calcium intake ranges from 1265 mg/day to 1639 mg/day for females and males 51-70 yrs, respectively, which are all well below the IOM UL of 2000 mg/day.

Overall, the *per user* 90th percentile cumulative calcium intakes for the subpopulations of infants 6-11 months, children, adolescents and adults 19-50 yrs were below the IOM UL. For the older adults 51+ yrs the *per user* 90th percentile cumulative calcium intake for males 71+ yrs were below the exposure limit range (2000 - 2500 mg/day). For women 51+ yrs and males 51-70 yrs, the *per user* 90th percentile background (food + dietary supplements) calcium intakes were within the exposure limit range (2000 – 2500 mg/day) and with the small addition of calcium <7%) from the proposed use of calcium chloride in potato snacks the *per user* cumulative intake at the 90th percentile remained within the exposure limit range. Therefore, it is reasonable to conclude that the proposed use of calcium chloride at a maximum level 1% in potato snacks is safe within the meaning of the FD&C Act, i.e. meets the standard of reasonable certainty of no harm.

Expert Panel Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the proposed use in potato snacks at a maximum level of 1% calcium chloride in the finished product, produced consistent with cGMP and meeting appropriate food-grade specifications, and unanimously conclude that it is safe and "generally recognized as safe" (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

02 July 2014

Stanley M. Tarka, Jr., Ph.D.
Chair of the Expert Panel
President, The Tarka Group, Inc.
United States

Date

Gary C. Curhan, MD, ScD, FASN
Professor of Medicine, Harvard School of Medicine
Professor of Epidemiology, Harvard School of Public Health
United States

Date

Connie M. Weaver, Ph.D.
Distinguished Professor and Department Head
Director, Women's Global Health Institute
Department of Nutrition Science
Purdue University, United States

Date

Expert Panel Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the proposed use in potato snacks at a maximum level of 1% calcium chloride in the finished product, produced consistent with cGMP and meeting appropriate food-grade specifications, and unanimously conclude that it is safe and "generally recognized as safe" (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

Stanley M. Tarka, Jr., Ph.D.
Chair of the Expert Panel
President, The Tarka Group, Inc.
United States

Date

(b

Gary C. Curhan, MD, ScD, FASN
Professor of Medicine, Harvard School of Medicine
Professor of Epidemiology, Harvard School of Public Health
United States

7/9/14
Date

Connie M. Weaver, Ph.D.
Distinguished Professor and Department Head
Director, Women's Global Health Institute
Department of Nutrition Science
Purdue University, United States

Date

Expert Panel Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the proposed use in potato snacks at a maximum level of 1% calcium chloride in the finished product, produced consistent with cGMP and meeting appropriate food-grade specifications, and unanimously conclude that it is safe and "generally recognized as safe" (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

By:

Stanley M. Tarka, Jr., Ph.D.
Chair of the Expert Panel
President, The Tarka Group, Inc.
United States

Date

Gary C. Curhan, MD, ScD, FASN
Professor of Medicine, Harvard School of Medicine
Professor of Epidemiology, Harvard School of Public Health
United States

Date

[redacted]

Connie M. Weaver, Ph.D.
Distinguished Professor and Department Head
Director, Women's Global Health Institute
Department of Nutrition Science
Purdue University, United States

7/7/14

Date

REFERENCES:

Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, Sempos CT, Picciano MF. Estimation of total usual calcium and vitamin D intakes in the United States. J. Nutr. 140: 817-822, 2010.

European Food Safety Authority (2012). Scientific opinion on the tolerable upper intake level of calcium. EFSA Journal;10(7):2814.

Food and Drug Administrattion Generally Recongized as Safe (GRAS) Database, SCOGS-45 (1975). Evaluation of the health aspects of certain calcium salts as food ingredients.

Food Chemicals Codex (FCC). (2014). Calcium Chloride Solution pp. 188-190. 9th edition, U.S. Pharmacopeial (USP) Convention, Rockville, MD

Institute of Medicine (IOM). 2011. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press.

SUBMISSION END