#747

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November 14, 2017

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notification for the Use of Calcium Lactate in Potato and Vegetable Snacks and Sweetened Crackers
Project No. 1607280.000

Dear Sir/Madam:

In accordance with 21 CFR part 170, subpart E, PepsiCo, hereby provides a notice of a claim that the food ingredient described in the enclosed notification document is excluded from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because the notifier has concluded such use to be generally recognized as safe (GRAS), based on scientific procedures.

One paper copy of the notification is 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

1607280.000 - 0416

GRAS Conclusion for the Use of Calcium Lactate in Potato and Vegetable Snacks and Sweetened Crackers

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
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November 14, 2017

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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

Part 1: Signed Statements and Certification

PepsiCo, Inc. (PepsiCo) submits to the U.S. Food and Drug Administration (FDA) this generally recognized as safe (GRAS) notice in accordance with the 21 CFR part 170, subpart E.

Name and Address of Notifier

PepsiCo, Inc.
700 Anderson Hill Road
Purchase, NY 10577

Name of GRAS Substance

The substance that is the subject of this GRAS notice is calcium lactate (pentahydrate), CAS Registry Number 814-80-2

Intended Use and Consumer Exposure

Calcium lactate is proposed for use up to 4% in the production of potato and vegetable snacks and sweetened crackers. The intended technical effect of the proposed use of calcium lactate in the manufacturing of potato and vegetable snacks and sweetened crackers is to reduce the formation of acrylamide.

For the U.S. population age 1 year and older, the per user mean and 90th percentile intakes of calcium lactate from the proposed use in the potato and vegetable snacks and sweetened crackers were 788 and 1,575 mg/day, respectively. This corresponds to calcium intakes of 114 and 228 mg/day, respectively, and lactate intake of 674 and 1,347 mg/day, respectively. 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 1,149 and 1,902 mg/day, respectively.

Basis for Conclusion of GRAS Status

PepsiCo's conclusion of GRAS status for the intended use of calcium lactate is based on scientific procedures in accord with 21 CFR §170.30(a) and (b).

Pre-Market Approval Exclusion Claim

Use of the calcium lactate is not subject to the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because PepsiCo has concluded that such use is generally recognized as safe (GRAS) through scientific procedures.

Availability of Information

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

Exemptions from Disclosure

It is our view that none of the data and information in Parts 2 through 7 of the GRAS notice are exempt from disclosure under the Freedom of Information Act (FOIA).

Certification Statement

On behalf of PepsiCo, Inc. I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

November 14, 2017

Name: Ellen de Brabander
Title: Senior Vice-President, R&D Global Functions Governance, and Compliance
Company: PepsiCo, Inc.

Date

Part 2. Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

Identity

The substance that is the subject of this GRAS conclusion is calcium lactate (pentahydrate).

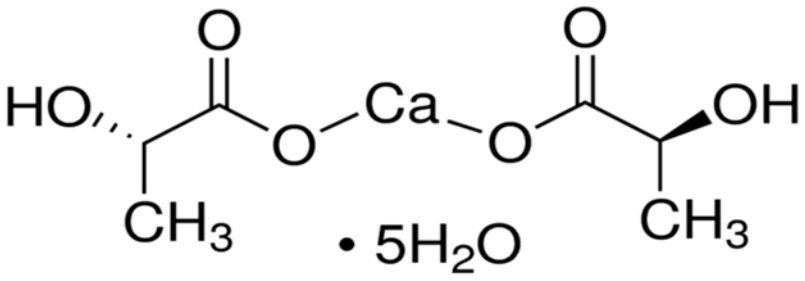
Calcium lactate is known as calcium-L-2-hydroxy-proprionate.

The general chemical abstracts service registry number (CASRN) for calcium lactate is 814-80-2

The molecular weight for calcium lactate is 218.22 (anhydrous), 308.29 (pentahydrate)

Molecular Formula: $Ca(C_3H_5O_3)_2$ (anhydrous); $Ca(C_3H_5O_3)_2 . 5\ H_2O$ (pentahydrate)

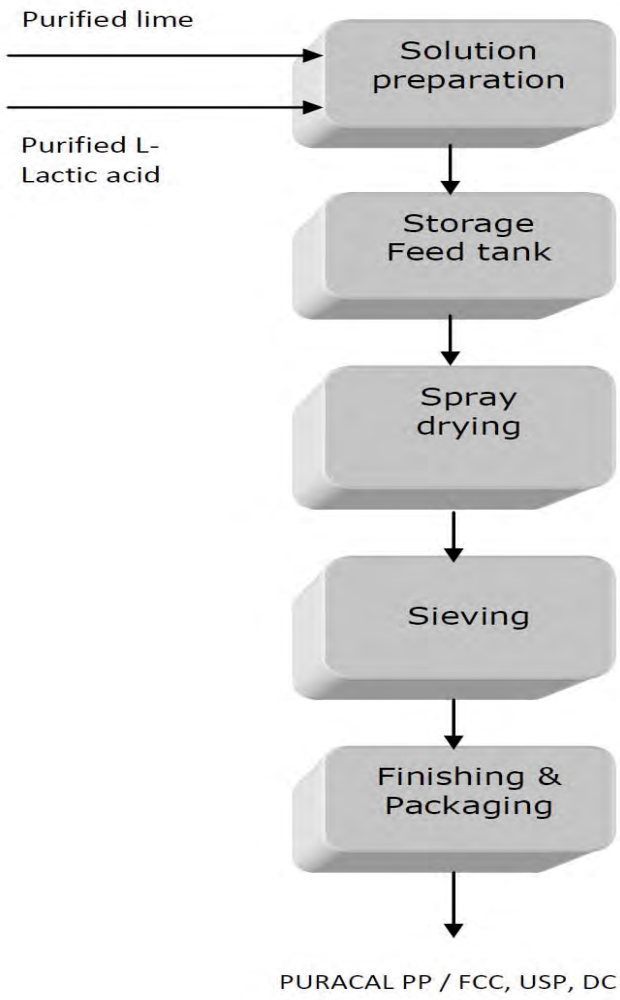
Figure 1. Structure of Calcium Lactate (Pentahydrate)



Manufacturing Information

Purified lime and purified L-Lactic acid are reacted in a controlled way to produce soluble liquid which is stored in a tank. The liquid is transformed into a powder product in a spray tower and dried. Stringent hygienic conditions are applied and the high temperature process produces a GMP product in a very short production time. Sieving eliminates dust and the final free flowing powder is stored in silos before it is finished and packaged under controlled conditions. See figure 2 for process flow diagram. All ingredients used in production of calcium lactate meet U.S. Pharmacopeia (USP) or FCC specifications. The L-lactic acid used in the production of calcium lactate is produced by fermentation and it meets the JECFA and FCC specifications.

Figure 2. Manufacturing Process Flow



Specifications

Specifications for calcium lactate have been established by Food Chemicals Codex (FCC) and the Joint Food and Agricultural Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (see Appendix A). Calcium lactate (pentahydrate) that is the subject of this GRAS conclusion, is a highly soluble calcium salt of natural L-lactic acid, which is produced by fermentation. It is a white and odorless powder with specifications, as shown in Table 1, comply with food grade specifications for physical and chemical properties and contaminants. Analytical data from representative non-consecutive batches of calcium lactate (see Appendix B for Certificate of Analysis) demonstrate that the ingredient meets product specifications appropriate for food ingredients.

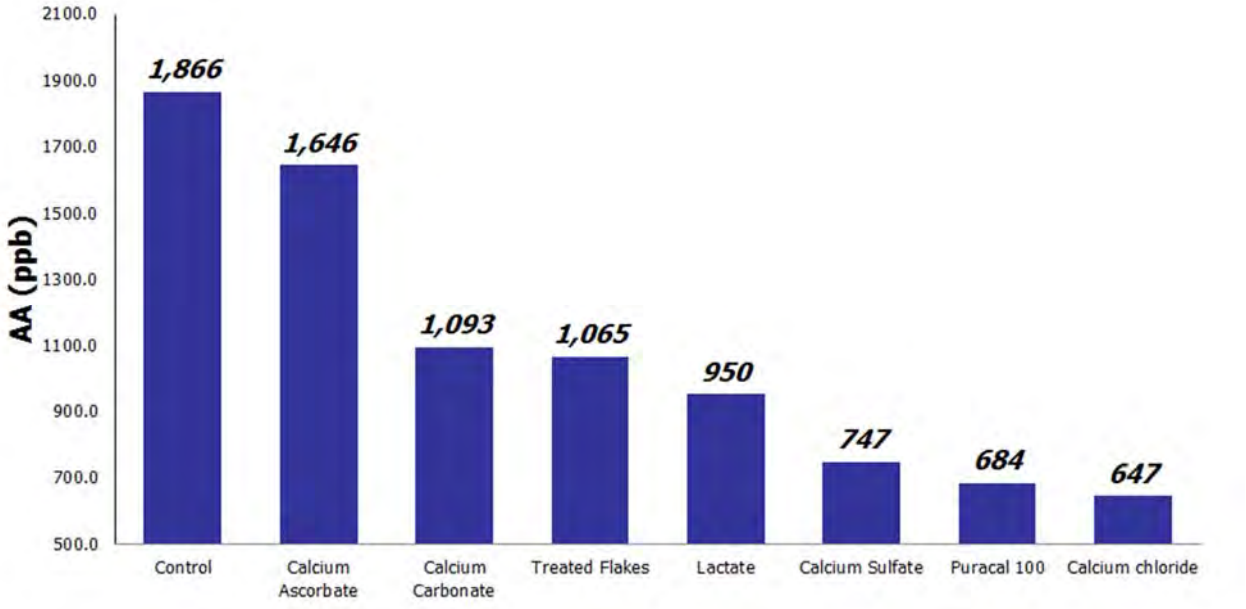
Table 1. Specifications Calcium Lactate (Pentahydrate)

Identification		**Analytical Method**
Form	Agglomerated powder	See Appendix C
Color	White	
Odor	Almost odorless	
Taste	Neutral	
Dirt (visual, reference)	Max. 6 particles	
Assay	99.0-101.0 % (w/w)	
Assay calcium	13.4-14.5 (d.s.) % (w/w)	
Stereochemical purity (L-isomer)	Min. 98 %	
Positive test for calcium	Passes test	
Positive test for lactate	Passes test	
Solubility in ethanol (95%)	Passes test	
Solubility in water (1 g/30 ml)	Passes test	
Purity		
Acidity, as lactic acid	Max. 0.19 % (w/w)	
Loss on drying	22.0-27.0 % (w/w)	
Magnesium and alkali salts	Max. 0.6 % (w/w)	
Sieve analysis max. 500 µm	Min. 98.0 %	
Sieve analysis 75 µm-425 µm	Min. 90.0 %	
Arsenic (as As)	Max. 1 mg/kg	
Chlorides	Max. 40 mg/kg	
Fluoride	Max. 15 mg/kg	
Heavy metals total	Max. 7 mg/kg	
Iron	Max. 25 mg/kg	
Lead	Max. 0.2 mg/kg	
Mercury	Max. 1 mg/kg	
Phosphates	Max. 50 mg/kg	
Sulfate	Max. 200 mg/kg	
Bromide	Max. 20 ppm	
Alkalinity	Passes test	
Barium	Passes test	
Reducing substances	Passes test	
Volatile fatty acids	Passes test	
pH (5 g product + 95 g water)	6.0-8.0	
Microbiology		
Coliform	Absent in 1g	
TAMC	Max 10 CFU/g	
TYMC	Max 10 CFU/g	

Technical Effect

Calcium lactate is proposed for use in the production of potato and vegetable snacks and sweetened crackers. The intended technical effect of the proposed use of calcium lactate in the manufacturing of these snacks is to reduce the formation of acrylamide. The effectiveness of calcium lactate as a mitigator of acrylamide levels in these snacks has been evaluated by PepsiCo. Results of an acrylamide mitigation study conducted by PepsiCo demonstrating approximately 50% or greater reduction that was observed with calcium lactate, calcium chloride, calcium sulfate and chloride/lactate blend are summarized in Figure 4 below.

Figure 3. Acrylamide mitigation study



Objective: Study effect of various calcium salts AA levels – Munchos fried pellets
Methods: 0.3% Ca^{2+} applied to Munchos dry mix, fried to 1.5% finished moisture
Results: ~50% or greater reduction was observed with calcium lactate, calcium chloride, calcium sulfate and chloride/lactate blend

Part 3. Dietary Exposure

Proposed Use and Levels

Calcium lactate is proposed for use in the production of potato and vegetable snacks and sweetened crackers. The intended technical effect of the proposed use of calcium lactate in the manufacturing of potato and vegetable snacks and sweetened crackers is to reduce the formation of acrylamide.

The proposed use level is up to 4% calcium lactate. Based on specifications, calcium assay for calcium lactate (pentahydrate) is in the range of 13.4-14.5%. For the purpose of the intake assessment, the maximum level of calcium in calcium lactate of 14.5% is assumed. Thus, the intake assessment provided herein assumed the maximum amount of calcium being added to finished product is 5.8 mg calcium/g of food.

Estimated Daily Intakes (EDI)

The estimated daily intake (EDI) of calcium lactate and calcium from the proposed use in potato and vegetable snacks and sweetened crackers 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) (2011-2012 and 2013-2014) 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 2011-2012 and 2013-2014 (2011-2014) 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 2011-2014 (NCHS 2014, 2016) 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=15,179) and was completed using Exponent's Foods Analysis 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 2013-

2014, was the main source of calcium composition data for this analysis. FNDDS 2013-2014 was based on nutrient values in the USDA National Nutrient Database for Standard Reference, Release 28 (SR 28) (USDA, 2016a), and was used by USDA to process dietary recall data reported in NHANES 2013-2014 (USDA, 2016b). When a food was unique to the 2011-2012 period (i.e., not reported in 2013-2014 by participants and thus not available in FNDDS 2013-2014), composition data was based on the earlier release of the food and nutrient database, FNDDS version 2011-2012 (USDA, 2014).

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.

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 2011-2014. 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 2011-2014 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 2011-2014 respondents reported consumption of approximately 6,400 specific foods; each food is identified by USDA by a unique 8-digit food code. The food codes representing potato and vegetable snacks and sweetened crackers that were included in the intake assessment are provided in Appendix D.

The two-day average intake of calcium lactate from consumption of potato and vegetable snacks and sweetened crackers containing calcium lactate at 4% in the finished product (i.e., food as consumed) were estimated for each individual in the NHANES 2011-2014 database. Two-day average calcium intake for the proposed use of calcium lactate in food was estimated for each individual by multiplying the calcium lactate intake by the proportion of calcium lactate that is calcium (i.e., 14.5% based on calcium lactate pentahydrate). This approach assumes that 100% of the calcium in calcium lactate is bioavailable as calcium in the human body and that all potato and vegetable snacks and sweetened crackers included in the analysis will contain calcium lactate at 4%.

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 lactate in potato and vegetable snacks and sweetened crackers.

The mean and 90th percentile of 2-day average calcium intake (from background, proposed new use, 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).

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 and vegetable snacks and sweetened crackers, (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 Analysis and Residues Evaluation Program (FARE® version 12.25) 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 lactate from the proposed use in the potato and vegetable snacks and sweetened crackers were 788 and 1,575 mg/day, respectively. This corresponds to calcium intakes of 114 and 228 mg/day, respectively, and lactate intake of 674 and 1,347 mg/day, respectively (see Table 2).

Young adult males were estimated to have the highest intakes of calcium from the proposed uses; among males 19-30 y the estimated *per user* 90th percentile intake of calcium from potato and vegetable snacks and sweetened crackers was 307 mg/day (see Table 2). There was only one consumer of potato snacks among infants 0-5 months; not an adequate sample size to provide reliable intake estimates.

Table 2. Estimated daily intake of calcium lactate, calcium, and lactate from proposed uses by the U.S. population 1+ y and subpopulations (mg/day)

			Per User (mg/day)					
			Calcium Lactate		Calcium		Lactate	
Population	n	% Users	Mean	90th	Mean	90th	Mean	90th
U.S. 1+ y	5,142	37	788	1575	114	228	674	1347
Infants 0-5 mo	1	0.1	256	NA	37	NA	219	NA
Infants 6-11 mo	65	18	467	926.8	68	134	399	792
Males								
Children 1-3 y	267	49	612	1160	89	168	524	992
Children 4-8 y	418	50	840	1694	122	246	718	1448
Children 9-13 y	272	39	722	1280	105	186	617	1094
Adolescents 14-18 y	211	33	981	1872	142	271	838	1601
Adults 19-30 y	274	32	999	2120	145	307	854	1813
Adults 31-50 y	424	31	909	1656	132	240	777	1416
Adults 51-70 y	429	38	867	1656	126	240	741	1416
Adults 71+ y	166	32	729	1920	106	278	624	1642
Females								
Children 1-3 y	284	55	712	1663	103	241	609	1422
Children 4-8 y	349	48	721	1260	105	183	617	1077
Children 9-13y	288	41	819	1763	119	256	701	1508
Adolescents 14-18 y	229	32	731	1350	106	196	625	1154
Adults 19-30 y	313	35	824	1540	119	223	704	1317
Adults 31-50 y	511	34	724	1311	105	190	619	1121
Adults 51-70 y	508	37	671	1300	97	189	573	1112
Adults 71+ y	199	37	518	1000	75	145	443	855

n = Unweighted number of survey respondents identified as consumers of proposed foods; weighted % consumers.
NA = Not available; estimate not calculated when the unweighted number of users is 10 or less.
Estimates based on 2-day average intakes reported in NHANES 2011-2014.

Cumulative Estimated Daily Intake (CEDI) for Calcium

Cumulative intake of calcium is summarized in Table 3. 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 1,149 and 1,902 mg/day, respectively. Children 1-3 y had estimated *per user* mean and 90th percentile intakes from all sources of calcium of 1,038 and 1,594 mg/day, respectively, among males and 1,003 and 1,561 mg/day, respectively, among females. Children 4-8 y had estimated *per user* mean and 90th percentile calcium intakes of 1,167 and 1,774 mg/day, respectively, among males and 1,016 and 1,580 mg/day, respectively, among females. The estimated 90th percentile *per user* intake was highest at 2,180 mg/day among males 19-30 y.

Table 3. Estimated daily intake of calcium from background (total diet + supplements) and proposed uses of calcium lactate by the U.S. population 1+ y and subpopulations and calcium tolerable upper intake levels (mg/day)

			Estimated Daily Intakes (EDIs) of Calcium (mg/day)				
			Background Sources[a]		Cumulative[b] (background + proposed)		Tolerable Upper Intake Level (UL)[c]
Population	n	% Users	Mean	90th	Mean	90th	(mg/day)
US 1+ y	14,522	100	1107	1852	1149	1902	NA
Infants 0-5 mo	275	78	473	767	473	767	1000
Infants 6-11 mo	322	100	638	1029	651	1043	1500
Males							
Children 1-3 y	512	100	994	1541	1038	1594	2500
Children 4-8 y	818	100	1106	1681	1167	1774	2500
Children 9-13 y	750	100	1158	1783	1199	1803	3000
Adolescents 14-18 y	683	100	1192	1965	1239	2053	3000
Adults 19-30 y	912	100	1251	2129	1299	2180	2500[d]
Adults 31-50 y	1,411	100	1188	1967	1228	2007	2500[d]
Adults 51-70 y	1,378	100	1173	1922	1221	1932	2000-2500[d]
Adults 71+ y	593	100	1136	1932	1169	1947	2000-2500[d]
Females							
Children 1-3 y	529	100	946	1499	1003	1561	2500
Children 4-8 y	742	100	966	1436	1016	1580	2500
Children 9-13y	756	100	972	1551	1021	1601	3000
Adolescents 14-18 y	699	100	907	1458	941	1486	3000
Adults 19-30 y	973	100	963	1633	1005	1719	2500[d]
Adults 31-50 y	1,600	100	1021	1763	1057	1788	2500[d]
Adults 51-70 y	1,557	100	1138	1998	1175	2057	2000-2500[d]
Adults 71+ y	609	100	1220	2064	1248	2087	2000-2500[d]

n = Unweighted number of survey respondents identified as consumers of calcium; weighted % consumers. Estimates based on 2-day average intakes reported in NHANES 2011-2014.
[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 4% calcium lactate in potato and vegetable snacks and sweetened crackers.
[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 0-5 months and 6-11 months, children, adolescents and adults 19-50 y (Table 3).

Males and Females, 51-70 and 71+ 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 71+ y (2,064 mg/day), see Table 4. The per user 90th percentile cumulative estimated daily intake of calcium from background (total diet + supplements) and proposed uses of calcium lactate exceed the IOM UL among older women 51-70 y (2,057 mg/day) and 71+ y (2,087 mg/day), see Table 4. 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 4). Dietary calcium intakes among high-end consumers (i.e., per user 90th percentile) range from 1,269 mg/day among females 71+ y to 1,681 mg/day 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 of female calcium consumers and supplement consumers 71+ y of age (see Table 4). At the 90th percentile, calcium from supplement use contributes up to 50% 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 lactate in potato and vegetable snacks and sweetened crackers contributes minimally to the total cumulative calcium intake at the 90th percentiles
    - Among all calcium consumers: the proposed use contributes 5 – 9% (98 – 164 mg/day) additional calcium.

- Among non-calcium-supplement users: the proposed use contributes 7 – 9% (95 – 145 mg/day) additional calcium. The per user 90th percentile of total cumulative calcium intake from both background and proposed use of calcium lactate for the non-supplement uses ranges from 1,236 mg/day to 1,639 mg/day among females and males age 51 years and older, all well below the IOM UL.

Table 4. Estimated daily intake of calcium from food, supplements, and proposed uses of calcium lactate 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	43%	1,031	142	1,173	47	1,221	4%	982	45	1,027	4%
	90th		1,681	420	1,922	164	1,932	9%	1,615	145	1,639	9%
Males 71+ y	Mean	54%	916	219	1,136	34	1,169	3%	881	38	919	4%
	90th		1,375	710	1,932	107	1,947	6%	1,361	109	1,573	7%
Females 51-70 y	Mean	51%	838	300	1,138	36	1,174	3%	798	33	831	4%
	90th		1,330	1,002	1,998	111	2,057*	5%	1,281	95	1,292	7%
Females 71+ y	Mean	65%	818	401	1,219	28	1,248	2%	765	30	795	4%
	90th		1,269	1,000	2,064*	98	2,087*	5%	1,216	102	1,236	8%

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 4% calcium lactate in potato and vegetable snacks and sweetened crackers.
*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 lactate, 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, as measured by the USDA), calcium from dietary supplements, and calcium intake from the proposed use of calcium lactate in potato and vegetable snacks and sweetened crackers. Results of these analyses indicate that cumulative calcium intakes at the 90th percentile from all sources combined (background + proposed use in potato and vegetable snacks and sweetened crackers) are below the calcium IOM UL for the majority of the age-based subpopulations. For the two older female subpopulations (females 51-70 y and 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 50% of the total background calcium intake among supplement consumers). The calcium contribution from the proposed use of calcium lactate in potato and vegetable snacks and sweetened crackers contribute less than 10% 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 lactate ranges from 1,236 mg/day to 1,639 mg/day among females and males age 51 years and older, 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.

Part 4. Self-Limiting Levels of Use

Calcium lactate is proposed for use in the production of potato and vegetable snacks and sweetened crackers. The proposed use level is up to 4% calcium lactate. We are not aware of technological or palatable issues associated with the proposed use levels. Self-limiting levels of use are not applicable to this notice.

Part 5. Experience Based on Common Use in Food before 1958

The conclusion of GRAS status of the use of calcium lactate in the production of potato and vegetable snacks and sweetened crackers at level up to 4% was based upon scientific procedures. Experience based on common use in food before 1958 is not applicable to this notice.

Part 6. Narrative

Regulated Uses

Calcium lactate is approved for use as a firming agent, flavor enhancer, flavoring agent or adjuvant, leavening agent, nutrient supplement, and a stabilizer and thickener in food with no limitations other than GMPs (21CFR § 184.1207).

There are also several other 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 limitations other than GMP. Calcium glycerophosphate (§ 184.1201) and calcium pantothenate (§ 184.12 12) are both affirmed GRAS as nutrient supplements.

In addition, 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 5.

Table 5. 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
634	Calcium chloride	FDA has no questions

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

Lactic acid and other salt forms, including ferrous lactate, potassium lactate, and sodium lactate, are affirmed GRAS as direct human food ingredients (Table 6) with no limitations other than GMPs. Ferrous lactate may be used as a nutrient supplement in foods.

Table 6. Lactic Acid and Lactic Acid Salts Affirmed by FDA as GRAS for Direct Use in Food

Citation	Function[a]
§ 184.1061 – Lactic acid	Antimicrobial agent; curing and pickling agent; flavor agent and adjuvant; pH control agent; and a solvent and vehicle
§ 184.1207 – Calcium lactate	Firming agent; flavor enhancer; flavoring agent or adjuvant; leavening agent; nutrient supplement; and a stabilizer and thickener
§ 184.1311 – Ferrous lactate	Nutrient supplement and a color fixative for ripe olives[b]
§ 184.1639 – Potassium lactate	Flavor enhancer; flavoring agent or adjuvant; humectant; and a pH control agent
§ 184.1768 – Sodium lactate	Emulsifier; flavor enhancer; flavor agent or adjuvant; humectant; and a pH control agent

[a] Approved for use in food with no limitation other than cGMP
[b] May also be used in infant formula

Safety Data

Salts of lactate in aqueous environments such as the gastrointestinal (GI) tract, will dissociate into their counter-ion components. In the GI tract calcium lactate will dissociate to provide calcium cation and lactate anion, the conjugate base of lactic acid. Thus, the publicly available safety information on calcium lactate, lactic acid and calcium are summarized herein.

A. Calcium Lactate and Lactic Acid

A search for literature related to the safety of calcium lactate and lactic acid and relevant oral exposure limits established by authoritative bodies was conducted for November 2016. The CAS Registry No. (CASRN) for lactic acid is 50-21-5, 79-33-4, or 598-82-3 and the CASRN for calcium lactate is 814-80-2. Databases searched included the Toxicology Data Network (TOXNET; including ChemIDplus, Hazardous Substances Data Bank (HSDB), and Chemical Carcinogenesis Research Information System (CCRIS)), the U.S. Environmental Protection Agency (EPA; including High Production Volume Information System (HPVIS) and Integrated Risk Information System (IRIS)), the Agency for Toxicity Substances and Disease Registry (ATSDR), the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization/World Health Organization (FAO/WHO), the National Toxicology Program (NTP), European Food Safety Authority (EFSA), European Chemicals Agency (ECHA), California Office of Environmental Health Hazard Assessment (OEHHA), and the U.S. Food and Drug Administration (FDA). Search terms included lactic acid, lactate (so as to capture all salt forms in addition to the calcium salt of lactate), and the CASRNs as appropriate for each database.

Information relevant to the safety of calcium lactate or lactic acid is summarized below. Similar to evaluations conducted by authoritative bodies, safety information or determinations for salts of lactate that have biologically occurring counter-ions such as sodium are also considered, as are salts of lactate that have been evaluated for food additive use.

Biological Occurrence and Metabolism

Lactic acid is an intermediary species in normal human metabolism, and occurs naturally as L(+)-lactic acid (OECD SIDS, accessed 2016). It also exists chemically as the D(-) form, which is present in industrial formulations along with the L(+) form as a racemic mixture. Salts of lactate, in aqueous environments such as the gastrointestinal tract, will dissociate into their counter-ion components, to provide calcium cation and lactate anion, the conjugate base of lactic acid.

Lactic acid serves as fuel or energy in mammals (OECD SIDS, accessed 2016; JECFA, 2002). According to the concept of the "lactate shuttle", during hard exercise, or other situations requiring accelerated glycolysis, movement of fuel to the muscles occurs with the endogenous formation of lactic acid.

OECD reported that the estimated formation of lactic acid in a resting human of 70 kg body weight (bw) is approximately 117 g/day. Lactic acid diffuses through the muscles and is transported to the liver by the bloodstream (JECFA, 2002). There, it is converted to glucose via gluconeogenesis. It can also be catabolized further by the lactic acid cycle, or Cori cycle.

JECFA reported that following oral administration to a human volunteer, 20 to 30% of a dose of lactic acid of up to 3000 mg was excreted via the urine during a period of 14 hours (JECFA, 1974a).

Acute Toxicity

Oral LD_{50} values for lactic acid are 4875 mg/kg bw in mice, 1810 mg/kg bw in guinea pigs, >2250 mg/kg bw in quail, and 3543 mg/kg bw in rats (ChemIDplus, accessed 2016). The LDL_o for oral exposure in rabbits was 5000 mg/kg bw. Acute toxicity values were not located specifically for the calcium salt of lactate.

OECD reported additional details regarding the oral LD_{50} for lactic acid in rats that was reported by ChemIDplus via TOXNET. The LD_{50} study appears to have been conducted in male and female Charles River rats. OECD reported lethargy, ataxia, prostration, irregular breathing, piloerection, squinting, lacrimation, salivation, crusty eyes and muzzle, loose stools, damp or yellow/brown stained fur and moribund as the clinical signs observed as early as 0 – 1 hour after dosing and as late as post-dose Day 2. Four animals survived, and necropsy observations of these animals and the four animals found dead included discolored lungs, firm texture of lungs, green foci on one lung (animal not specified), several stomach lesions, discolored liver, white foci on the liver, pale capsular areas, superficial erosion, or mottled liver, discolored kidneys and red-brown exudates in the nasal and/or oral regions on the rats.

Subchronic Toxicity

In their review of lactic acid as a high production volume chemical, OECD summarized a 13 week study in F344 rats (5/sex/dose) which were administered calcium lactate via the drinking

water at 0, 0.3, 0.6, 1.25, 2.5 and 5% (reported to correspond to approximately 0, 30, 60, 125, 250 and 500 mg/kg bw/day). This study was also summarized by ECHA (2016a). Body weights and gain, food consumption, food efficiency, water consumption, hematology, clinical chemistry, and urinalysis were monitored. In addition, necropsy and gross pathological assessments were conducted. ECHA reported that histopathology data were not available from the study. No mortalities were reported to have occurred. A slight body weight gain decrease of less than 10% compared to controls was observed at all treatment concentrations. OECD reported adverse effects in a second phase of this study, but it was determined that these effects were a result of the altered calcium content that the test item in the diet provided, and were not a direct result of the lactate component. Similarly, ECHA reported that no toxicity for lactic acid was observed and that all effects reported were the result of calcium overload or imbalance. ECHA stated that the NOAEL was 5% (50,000 mg/L in drinking water), the highest dose tested, which was reported to be approximately equivalent to 500 mg/kg bw/day.

Chronic Toxicity

Maekawa et al. (1991), conducted a two year drinking water study in F344 rats which was supported by the subchronic range-finding study described above. This study was also reviewed and summarized in the ECHA database (2016b) and by OECD (SIDS accessed 2016). In this study calcium lactate was administered to F344 rats (50/sex/dose, randomized) for two years in the drinking water. Rats were administered 0, 2.5, or 5% levels in water, which was offered ad libitum, starting around 6 weeks of age. The study reported the group mean total intake of calcium lactate per rat, but did not translate this to a mg/kg bw/day dose level, and likewise ECHA and OECD did not report estimates of dose. The test substance was 97-101% pure. Following the two-year exposure period, rats were maintained in a recovery phase for nine weeks prior to terminal sacrifice. The study was published in 1991, and although a specific guideline was not followed, the study contains major features of modern guideline studies including body weight and clinical parameters, hematology, clinical chemistry, necropsy for gross findings, and histopathological assessment of representative tissues and all lesions. All rats that died during the study, and those surviving until termination, were subjected to a full necropsy. Body weights were measured once per week for the first 13 weeks, then every four weeks thereafter. Sample collections for hematology and clinical chemistry were obtained at terminal sacrifice following the recovery period. Animals were examined macroscopically and microscopically for gross lesions and neoplastic and non-neoplastic changes. ECHA summarized that there were no statistically significant treatment-related differences in terms of clinical signs or mortality between treated animals and controls, and there were no remarkable effects on hematology or clinical chemistry. The study authors, as well as ECHA and OECD, concluded that the study did not demonstrate carcinogenic potential for calcium lactate when administered via the drinking water for two years at the doses tested. OECD stated that the highest dose resulted in a significant reduction in body weight, which was approximately 13% as reported in the study by the authors. However, despite this finding of an approximately 13% decrease in body weight at the high dose, the study authors concluded that calcium lactate was neither toxic nor carcinogenic in F344 rats when given continuously through the drinking water for two years. Body weight changes are typically considered adverse if they are greater than approximately 10%, which could have led the study authors and others to conclude that the finding was non-adverse. The authors reported that there were no changes in clinical chemistry or hematology, and any organ weight

changes observed were not found to be toxicologically significant and did not correlate with any histopathological alterations in those organs.

Genotoxicity

Lactic acid (L(+)) was negative using multiple strains of *Salmonella typhimurium* (TA 97, 98, 100, 104) in Ames assays with and without metabolic activation (OECD, accessed 2016; EFSA, 2009) ECHA reported that lactic acid was negative in Ames assays with strains TA92, 1535, 100, 1537, 94, 98, and 2637) (ECHA, 2016c). EFSA (2009) reported that positive results had been obtained in some assays, but when experiments were corrected for pH, these effects were mitigated (EFSA, 2009). OECD (accessed 2016) further reported that *in vitro* chromosomal aberration tests using Chinese hamster ovary (CHO) cells did not demonstrate clastogenic activity for lactic acid both with and without metabolic activation, and following neutralization of culture medium to a physiologically relevant pH of 6.4. OECD (accessed 2016) concluded that overall, L(+) lactic acid was not mutagenic, and ECHA (2016c, d) reported this same interpretation of the weight of the evidence demonstrating a lack of genotoxic potential for lactic acid.

Reproductive and Developmental Toxicity

Reproductive toxicity studies were not located for lactic acid or salts of lactate in the publically available scientific literature. Likewise, ECHA, OECD and other reviewing authoritative bodies did not summarize such information, nor was such information considered by these authorities to be critical to the safety review for lactic acid or common salts of lactate, and ECHA specifically stated in their summary files that developmental and reproductive toxicity studies were not scientifically justified under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (ECHA, 2016e,f).

A developmental toxicity study was summarized by OECD (SIDS accessed 2016) in which lactic acid was administered by gavage to pregnant CD-1 mice from gestation days (GD) six through 15. OECD concluded that lactic acid was neither toxic to dams or offspring at doses of 0 or 570 mg/kg bw/day, and that 570 mg/kg bw/day was considered the NOAEL. This study did not appear summarized in the ECHA database, nor was it cited by JECFA or EFSA in their reviews, and the OECD document did not contain information from which the original study could be located.

Established Oral Exposure Limits

The safe use of lactic acid and salts of lactate have been reviewed by authoritative bodies including JECFA, EFSA, ECHA, and the Cosmetic Ingredient Review (CIR). In particular, JECFA and EFSA have reviewed lactic acid or common salts of lactate as they pertain to use in food and food contact applications.

In 1974, JECFA established an ADI of "not limited" for calcium lactate, stating that D(-) forms and racemic mixtures shall not be utilized in infant formula (JECFA, 1974c). Previous to this, in 1973, JECFA established an acceptable daily intake (ADI) of "not limited" for lactic acid, which was upheld in 2001 for use as a flavoring agent and acidifier (JECFA 1973, 2001). Likewise, an ADI of "not limited" was established for sodium lactate (JECFA, 1974b) as an acidifying agent,

humectant, and antioxidant synergist with the exception of uses of D(-) or racemic mixtures of D(-) and L(+) in infant formula.

EFSA has also reviewed lactic acid or salts of lactate within various EFSA Panels. It was evaluated as a flavoring in 2009 (EFSA, 2009), based on no safety concern at the current levels of intake and the role of lactic acid in health, normal mammalian metabolism. EFSA (2011) also had no concerns in a more recent review by their Panel on Food Contact Materials, Enzymes, Flavourings, Processing Aids (CEF). Most recently, lactic acid and calcium lactate have been reviewed for use in animal food by EFSA's Panel on Additives and Products or Substances used in animal feed (FEEDAP) (EFSA, 2015). In this application, EFSA considered that the proposed uses of lactic acid (lactate) were safe considering the endogenous nature of lactic acid, and that the proposed uses reviewed by the FEEDAP panel would not greatly alter the total intake from other food sources.

Safety Data Summary

Lactic acid is a naturally occurring compound in foods, and is a ubiquitous intermediate in the course of normal, healthy mammalian metabolism. Some positive results have been obtained in genotoxicity assays for lactic acid. However, when experiments are adjusted for acidic pH, a genotoxic effect is not observed, and OECD (SIDS accessed 2016) and EFSA (2009) concluded that overall lactic acid does not demonstrate genotoxic potential. Reproductive and developmental studies for lactic acid or common salts of lactate were not located in the publically available literature, but based on the endogenous, ubiquitous nature of lactic acid, ECHA (accessed 2016) and JECFA did not cite this as a deficiency in the database and ECHA specifically stated that such studies were not warranted. A single subchronic and a single chronic repeated dose study exist for the calcium lactate salt, and both studies tested doses of up to 5% calcium lactate in the drinking water. In the 13-week subchronic study, toxicologically relevant adverse effects were not observed even at the highest dose tested. In the two-year study with 9 week recovery phase, although there was a decrease in body weight at the highest dose, the authors of the study found no other adverse or non-adverse treatment-related effects in the study at the highest test dose and reported that calcium lactate was neither toxic nor carcinogenic under the conditions of the two year study.

The safe use of lactic acid and common salts of lactate has been reviewed by EFSA and JECFA for food applications. The ADI for lactic acid and all common salts of lactate including calcium, sodium and ammonium, was "not limited" (JECFA, 1973, 2001, 1974a,b,c) and there were no concerns at current levels of use for such applications as flavoring, buffering agent or acidifier, and humectant. Likewise, in EFSA reviews from several different Panels, there was no concern expressed regarding the use of lactic acid or common salts of lactate at current use levels based largely on the endogenous and ubiquitous nature of lactic acid and lactate (EFSA 2009, 2011, 2015).

B. Calcium

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). EFSA had also extensively reviewed calcium and issued its report in 2012 -- the EFSA's Panel on Dietetic Products, Nutrition and Allergies scientific opinion on the UL of calcium (EFSA, 2012). To capture human health information on calcium that have been published since the IOM and EFSA reviews and publications, a PubMed search 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, October 2015, February 2016, and October 2016 for human health information published between June 2014 and January 2017 (to capture publications ahead of print). Details of the results of these searches are provided in Appendix D. 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

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 the absorption values may vary between calcium salts. 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 average calcium absorption from all calcium salts

was reported to be in the range of 23 to 37% (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 not 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

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).

Safety Data

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, February 2016, October 2016, and October 2017. The search strategies are outlined in Appendix D.

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 938 citations were identified in the updated search conducted in October 2015 covering the period from June 2014 through October 2015, an additional 56 publications spanning publication dates from November 2015 through February 2016, an additional 421 citations covering publications from February 2016 through October 2016, and the most recent search conducted in October 2017, produced 705 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. In total, this safety review relies upon evidence from three meta-analyses of randomized control trials (RCTs) investigating the association between calcium intake and CVD outcomes as well as 4 published analyses of the Women's Health Initiative (WHI) trial and two additional RCTs. Observational data from three published meta-analyses of calcium intake and CVD outcomes as well as eleven prospective cohort studies and 4 cross-sectional studies provided additional evidence. For the safety review of calcium intake with other health outcomes including all-cause mortality and cancer outcomes, there were four RCTs, five observational studies and one meta-analysis of observational studies that provided evidence.

*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. Until recently, 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.

In late October 2016, the National Osteoporosis Foundation (NOF) and the American Society for Preventive Cardiology (ASPC) published a clinical guideline that stated their position that there was "moderate-quality evidence (B level) that calcium with or without vitamin D intake from food or supplements has no relationship (beneficial or harmful) to the risk for cardiovascular and cerebrovascular disease, mortality, or all-cause mortality in generally healthy adults at this time." (Kopecky et al 2016) This clinical guideline was informed by a review commissioned by the NOF and ASPC on the effects of calcium intake (both dietary and supplement sources) with or without vitamin D on CVD risk in healthy adults (Chung et al 2016). This review included RCTs as well as prospective cohort or nested case-control studies published between 2009 and July 2016 that evaluated the association between calcium intake and incident CVD risk. No meta-analysis of the RCTs identified was conducted due to heterogeneous definitions of outcomes reported. A dose-response meta-regression of fifteen prospective cohort studies found no statistically significant associations (linear or non-linear) between dietary and/or total calcium intake and CVD outcomes. This report concluded that based on assessments of "internal validity, precision of risk estimates, and consistency of results from randomized trials and prospective cohort studies" there is no association between calcium intake at levels within the recommended tolerable intake range of 2000 to 2500 mg/day and CVD risks in generally healthy adults (Chung et al 2016). The findings of the Chung et al (2016) review were consistent with recent meta-analyses included in the current review of both RCTs (Lewis et al 2015) and observational data (Asemi et al 2015).

From the published literature, it appears there are no new or ongoing calcium trials being conducted where CVD outcomes are the primary outcome, 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.

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

In the most recent review by Chung et al (2016), the authors conducted a meta-regression of prospective cohort studies to evaluate the association between calcium intake and CVD among generally healthy adults. Included studies reported associations between total calcium intake (from food and/or supplements) among adults in the U.S., Europe, Asia, and Australia with cohort sizes ranging from 755 to 388,229 and follow-up ranging from 8 to 30 years. The majority of the calcium intake was estimated using food frequency questionnaires and the outcomes were largely mortality outcomes. Total calcium intakes were reported to range from 400 to 2400 mg/day but with the majority of the intakes less than 1600 mg/day. The authors report the risk of bias in the studies included in the meta-regression as moderate due to potential residual bias, reporting bias as to in adequate justification of statistical methods and lack of detail on dietary assessment methods used. Overall, there were no consistent associations between calcium intake and CVD outcomes with linear and non-linear dose-response models showing no statistically significant association between any type of calcium intake and CVD. Total calcium intake and CVD/IHD mortality as well as total stroke events were both reported as HR=0.99 (95%CI: 0.97-1.01). The authors note that these studies are still limited in that a precise measure of total calcium intake can not be assessed and many of the reported intakes are below the range of tolerable upper intakes of 2000-2500 mg/day.

In a recent meta-analysis of observational studies evaluating the evidence on calcium intake and mortality from all causes as well as CVD, Asemi et al. (2015) included 22 studies that measured total calcium intake as well as dietary and/or supplemental calcium intake from a systematic

search of the literature through May 2014 with duration of follow-up ranging from 4.6 to 28 years in both males and females throughout the US, Europe, Japan, Australia, Canada, and China. Among six studies contributing eight data points, there was no significant association between total calcium intake (diet and supplements) and CVD mortality (RR= 1.05; 95%CI: 0.83-1.34) with significant heterogeneity across studies (I^2 = 60.8%; p = 0.01). When this analysis was limited the four studies that were among the US population, the heterogeneity was reduced and the summary risk estimate was in the opposite direction (RR= 0.88; 95%CI: 0.66-1.215; I^2 = 27.6%; p = 0.25). Both male and female sub-group analyses showed similar non-significant associations between total calcium intake and CVD mortality (males: RR=1.04; 95%CI: 0.81-1.34; females: RR=1.02; 95%CI: 0.66-1.58). The only sub-group analysis that showed a potential adverse association was when the analysis was limited to three studies where the duration of follow-up was ≥10 years (RR=1.35; 95%CI: 1.09-1.68; I^2 = 21.8%; p = 0.28). However, it cannot be determined what amount of total calcium this association is based on since these are summary measures from three observational studies where dietary calcium intake is measured from food frequency questionnaires and intake may vary largely among the populations studied. When the analysis focused on calcium intake from supplements only, a similar lack of association was observed based on eight studies contributing nine data points with significant heterogeneity among studies (RR= 0.95; 95%CI: 0.82-1.10; I^2 = 73.9%; p <0.001). Sub-group analyses limited to US only as well as stratified by gender showed similar non-significant associations (US only: RR=0.96; 95%CI: 0.83-1.12; males: RR=0.98; 95%CI: 0.59-1.64; females: RR=0.93; 95%CI: 0.80-1.08).

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

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

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 (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.

Most recently, 2158 men and 2153 women in the Korean Ansung-Ansan cohort followed up for a mean duration of 8.9 y and 9.2 y, respectively, for self-reported CVD outcomes showed no association between dietary calcium intake and CVD outcomes with the exception of a reduction in incident CVD in women (Kong et al 2017). Dietary calcium intake was estimated based on baseline responses to a 103 question semi-quantitative FFQ and Food Composition Tables of the Korean Nutrition Society. Intakes ranged from a median of approximately 200 mg/day at the 1st quartile to approximately 650 mg/day at the 4th quartile. CVD was not associated with increasing energy-adjusted dietary calcium intake in men ($HR_{4th\ quartile\ vs\ 1st\ quartile}$ = 1.47, 95%CI: 0.86 – 2.51) but was associated with a statistically significant reduction among women ($HR_{4th\ quartile\ vs\ 1st\ quartile}$ = 0.49, 95%CI: 0.28 – 0.83). There was no association between dietary calcium intake and incident stroke among men or women (men: $HR_{4th\ quartile\ vs\ 1st\ quartile}$ = 1.41, 95%CI: 0.62 – 3.21; women: $HR_{4th\ quartile\ vs\ 1st\ quartile}$ = 0.56, 95%CI: 0.27 – 1.14).

In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, men and women ages 45-84 years from four major race/ethnicity groups and free from CVD at baseline were recruited from six U.S. locations (Raffield et al, 2016). An FFQ was used to ascertain intake of dietary and supplemental calcium at baseline and individuals were followed-up for an average of 10.3 years.

Among this cohort, there were 208 incidence MIs and 641 CVD events including strokes, deaths, definite angina, and resuscitated cardiac arrests. Outcomes were ascertained using self-report with confirmation from death certificates and medical records and adjudication from two physicians. There was no statistically significant associations between calcium supplement use and MI ($HR_{\geq 1000 \text{ mg v } 0 \text{ mg}}$=0.87, 95%CI: 0.52 – 1.44; p-trend=0.315) or total CVD events ($HR_{\geq 1000 \text{ mg v } 0 \text{ mg}}$=1.16, 95%CI: 0.88 – 1.53; p-trend=0.501). Similarly, there was no statistically significant association between total energy-adjusted dietary calcium intake and MI ($HR_{Q4 \text{ v } Q1}$=0.91, 95%CI: 0.60 – 1.37; p-trend=0.617) or total CVD events ($HR_{Q1 \text{ v } Q4}$=0.94, 95%CI: 0.74 – 1.21; p=trend=0.695). Total dietary calcium intake ranged from approximately 48 mg/day to 3328.2 mg/day (Raffield et al 2016). In sub-group analyses, these findings held true among postmenopausal women (Raffield et al 2016).

In a study of a prospective cohort of 132,823 men and women in the Cancer Prevention Study II Nutrition Cohort were followed from baseline (1992/3) through 2012 for mortality outcomes, including CVD, estimated supplemental, dietary and total calcium intake from an FFQ collected at baseline and updated twice in 1999 and 2003 (Yang et al, 2016). All analyses were stratified by gender due to previously reported heterogeneity in associations and dietary calcium was adjusted for in the supplement analyses and vice versa. The mean age of the participants was 62.6 years (SD=6.3 years). Among the 43,186 deaths observed throughout the follow-up, 13,916 were from CVD. There was no association between supplemental calcium intake and CVD mortality among men ($RR_{\geq 1000 \text{mg/d vs } 0 \text{ mg/d}}$= 1.22; 95%CI: 0.99-1.51; p-trend=0.39) while there was a significant inverse (protective) association between supplemental calcium intake and CVD mortality among women ($RR_{\geq 1000 \text{mg/d vs } 0 \text{ mg/d}}$= 0.84; 95%CI: 0.74-0.94; p-trend<0.01). Similarly, there was no association between total calcium intake and CVD mortality among men ($RR_{\geq 1000 \text{mg/d vs } 0 \text{ mg/d}}$= 0.97; 95%CI: 0.91-1.05; p-trend=0.77) while there was a significant inverse (protective) association between total calcium intake and CVD mortality among women ($RR_{\geq 1000 \text{mg/d vs } 0 \text{ mg/d}}$= 0.84; 95%CI: 0.74-0.89; p-trend<0.01). A similar pattern of no association among men and a significant protective association among women was further observed with supplemental and total calcium intake and both CHD and stroke.

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 most recent comprehensive review of both RCTs and observational data concluded that there is no association between calcium intake and CVD risk (Chung et al 2016). While studies that re-analyzed the WHI database suggest an association between calcium supplementation and cardiovascular risk, receiving significant attention considering the wide-spread use of supplements, the potential causal pathway has not yet been very carefully studied. In fact, clinical guideline published by NOF and ASPC cited a recent animal study that found effect of diets high in calcium on coronary artery calcium phosphate deposition in swine as support to the fact that there is no currently established biological mechanism to explain any potential causal pathway between calcium and CVD (Kopecky et al 2016). Further, as pointed out by many researchers in the field, the methods used and results of several of the WHI re-analyses 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 the NOF/ASPC commissioned review (Chung et al 2016) as well as 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. The most recent meta-analyses of calcium intake and CVD mortality outcomes reported no significant associations between both total and supplemental calcium intake with CVD outcomes with the exception of one analysis limited to studies with ≥10 years of follow-up where an adverse association between total calcium intake and CVD mortality was observed. However, this conclusion is limited to three studies and the level of total calcium intake associated with this observed risk was not reported (Asemi et al, 2105). In a 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 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. Researchers involved in a recent meta-analysis and an individual prospective cohort study both presented findings from an evaluation of the association between dietary, supplemental, and total calcium intake with mortality outcomes including all-cause and cancer mortality (Asemi et al, 2016; Yang et al, 2016). In a meta-analysis of total calcium intake and all-cause mortality, data from three studies with significant heterogeneity (I^2=87.1%; p<0.001) showed a non-significant association (RR=1.16; 95%CI: 0.83-1.64). Similarly, no significant association between total calcium intake and cancer mortality was observed, though this was based on only two studies with moderate heterogeneity (I^2=53.4%; p=0.143). When restricted to supplemental calcium intake only, a significant inverse association was observed with all-cause mortality based on five data points from four studies with no heterogeneity ($RR_{all\text{-}cause}$=0.91; 95%CI: 0.88-0.94; I^2=0.0%; p=0.954). No association was observed with cancer mortality from three studies with significant heterogeneity (RR_{cancer}=1.22; 95%CI: 0.81-1.84; I^2=87.5%; p=<0.001).

In the recent study of a prospective cohort of 132,823 men and women in the Cancer Prevention Study II Nutrition Cohort followed from baseline (1992/3) through 2012 for mortality outcomes (Yang et al, 2016), supplemental calcium intake was associated with a significant increased risk of all-cause mortality in men only when comparing intakes of ≥1000mg/day versus none ($RR_{\geq 1000mg/d \text{ vs } 0 mg/d}$ =1.17; 95%CI: 1.03-1.33; p-trend = 0.18). However, when including dietary calcium plus supplement intake, the association was attenuated to null and no longer significant ($RR_{\geq 1000mg/d \text{ vs } 0 mg/d}$ =1.00; 95%CI: 0.95-1.04). No other significant associations were observed among men between supplemental or total calcium intake and cancer mortality as well as cancer-specific mortality endpoints including colorectal, prostrate, and lung cancer when comparing high to low intakes. Among women, significant inverse (protective) associations between total calcium and all-cause, cancer, and colorectal cancer was observed with reductions ranging from 44% (95%CI: 25%-59%) for colorectal cancer when comparing the extreme quintiles to 15% (95%CI: 10%-19%) reduction in all-cause mortality. When restricted to supplemental calcium intake, the only significant association was a reduction in all-cause mortality (RR=0.93; 95%CI: 0.87-0.99; p-trend<0.01).

In a recent double-blind, placebo-controlled RCT, postmenopausal women 55+ y living in rural Nebraska were given 2000 IU/day vitamin D3 and 1500 mg/day calcium carbonate for four years and followed-up every six months for incident cancer including breast, lung, colon, lymphoma, leukemia, and myeloma (Lappe et al, 2017). Study participants in the treatment and placebo groups were not significantly different with regards to dietary calcium intake or calcium supplement intake at baseline and annual visits. In the intention to treat analysis, there was no significant difference in total cancer incidence (HR = 0.70; 95%CI: 0.47 – 1.02) nor breast cancer incidence (HR=0.79, 95%CI: 0.43 – 1.43). 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, an increasing number of researchers are providing evidence that supports the conclusion that there is no association between calcium intake and CVD (Chung et al 2016; Lewis et al 2015; Asemi et al 2015). The recent review by Chung et al (2016) suggests that the relationship between total, dietary and supplemental calcium intake and cardiovascular disease be investigated in future prospective cohorts studies with a specific focus on using validated dietary assessment methodology, standardized outcome ascertainment, and prospectively published study protocols, power calculations, and analysis plans. In light of the 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

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.

Acceptable Daily Intake

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 lactate in potato and vegetable snacks and sweetened crackers 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.

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				
Chung et al, 2016	15 prospective cohort studies included in meta-regression (N=11 dietary calcium; N=6 total calcium intake)	Male and female populations in U.S., China, Australia, Europe, Japan.	• Total calcium and CVD mortality: linear RR=0.99 (95%CI: 0.97-1.01); quadratic RR=0.89 (95%CI: 0.80-0.98) • Total/dietary calcium and stroke mortality: linear RR=1.00 (95%CI: 0.82-1.01); quadratic RR=0.97 (95%CI: 0.90-1.05) • Total/dietary calcium and total stroke: linear RR=0.99 (95%CI: 0.97-1.01); quadratic RR=0.93 (95%CI: 0.84-1.04)	• No statistically significant association between calcium intake (total or dietary) and CVD outcomes based on pooled RR using both a linear and quadratic (non-linear) dose-response model. • Overall risk of bias in included studies was determined to be moderate. • Meta-regression analysis • Total calcium intake ranged from 400 to 2400 mg/day with few >1600 mg/day
Asemi et al, 2015	21 prospective studies; 1 nested case-control study (1992-2013)	2,346,368 male and female participants, 8 to >65 y 81,298 total deaths Total calcium and mortality: 559,666 participants 25-79 y Dietary calcium and mortality: 832,549 participants 8->65 y Supplemental calcium and mortality: 954,153 participants 35 - >65 y	• Total calcium and CVD mortality: RR=1.05 (95%CI: 0.83-1.34) • Total calcium and CVD mortality limited to studies with >10 years follow-up: RR=1.35 (95%CI: 1.09-1.68) • Supplemental calcium and CVD mortality: RR=0.95 (95%CI: 0.82-1.10)	• Study did not find statistically significant association between calcium intake (total or supplemental) and CVD based on pooled RR. • Analysis limited to three studies with >10 years of follow-up reported significant increased risk between total calcium intake and CVD mortality; however results inconsistent with other findings within the same study as well as other published meta-analyses
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	• 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.	• 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

Citation	Study Design	Population	Findings	Considerations
		All-cause mortality: 225,189 participants (6 studies), >21,055 deaths	• 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)	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				

Kong et al, 2017	Prospective (Ansung-Asan Cohort Study)	2153 Korean women (mean age = 60.5 ± 5.3 y); 2,158 Korean men (mean age = 59.2 ± 5.8 y)
		
		Women:
		- 100 deaths
		- 150 CVD events
		- 82 stroke events
		- 292 incident fractures
		
		Men:
			• Dietary calcium intake
			  • $Q4_{men} \geq 694$ mg/d
			  • $Q4_{women} \geq 645$ mg/d
			• Dietary calcium intake and mortality
			  • Men ($HR_{\geq 694 \text{ mg/d vs. } \leq 249 \text{ mg/d}} = 0.77$; 95% CI: 0.51, 1.15; p-trend = 0.21)
			  • Women ($HR_{\geq 654 \text{ mg/d vs. } \leq 209 \text{ mg/d}} =$ 1.08; 95% CI: 0.59,1.99; p-trend = 0.26)
			• Dietary calcium intake and CVD
			  • Men ($HR_{\geq 694 \text{ mg/d vs. } \leq 249 \text{ mg/d}} = 1.47$; 95% CI: 0.86, 2.51; p-trend = 0.09)
				• Self-reported CVD, stroke and incident fracture outcomes; deaths reported by family
				• Adjusted for several known CVD risk factors
				• Excluded participants taking calcium supplements
				• Not generalizable to other races/ethnicities

Citation	Study Design	Population	Findings	Considerations
		- 242 deaths - 149 CVD events - 58 stroke events - 211 incident fractures	• Women ($HR_{\geq 654\ mg/d\ vs.\ \leq 209\ mg/d}$ = 0.49; 95% CI: 0.28, 0.83; p-trend = 0.01) • Dietary calcium intake and stroke • Men ($HR_{\geq 694\ mg/d\ vs.\ \leq 249\ mg/d}$=1.41; 95% CI: 0.62, 3.21; p-trend = 0.64) • Women ($HR_{\geq 654\ mg/d\ vs.\ \leq 209\ mg/d}$ = 0.56; 95% CI: 0.27, 1.14; p-trend = 0.33) • Dietary calcium intake and incident fracture • Men ($HR_{\geq 694\ mg/d\ vs.\ \leq 249\ mg/d}$ = 0.90; 95% CI: 0.58, 1.41; p-trend = 0.80) • Women ($HR_{\geq 654\ mg/d\ vs.\ \leq 209\ mg/d}$ = 1.13; 95% CI: 0.78, 1.64; p-trend= 0.36) • Among women, higher energy-adjusted dietary calcium intake was associated with reduced CVD risk after adjustment; no such association found among men. • No association between dietary calcium intake and mortality, stroke, and incident fracture in both men and women.	
Raffield et al, 2016	Prospective (Multi-Ethnic Study of Atherosclerosis Cohort)	Men and women ages 45-84 y from 6 different locations in the U.S; free of clinical CVD at baseline (N=6,236) 208 MIs 641 CVD events	• Supplemental calcium intake: • Q4 ≥ 1000 mg/d • Supplemental calcium and MI: • $HR_{\geq 1000mg/d\ vs\ 0\ mg/d}$ = 0.87; 95% CI: 0.52, 1.44; p-trend = 0.315) • Supplemental calcium and CVD events: • $HR_{\geq 1000mg/d\ vs\ 0\ mg/d}$ = 1.16; 95% CI: 0.88, 1.53; p-trend = 0.501) • Total energy-adjusted dietary calcium intake: • Q4 ≥ 960 mg/d • Total energy-adjusted dietary calcium intake and MI:	• Measured total calcium intake • Adjusted for several known CVD risk factors • Models for supplemental calcium were adjusted for dietary calcium intake and vice versa

Citation	Study Design	Population	Findings	Considerations
			• HR $_{Q4 \text{ vs. } Q1}$ = 0.91; 95% CI: 0.60, 1.37; p-trend = 0.617 • Total energy-adjusted dietary calcium intake and CVD events: • HR $_{Q4 \text{ vs. } Q1}$ = 0.94; 95% CI: 0.74, 1.21; p-trend = 0.695 • No association was found among postmenopausal women between total dietary calcium intake or supplemental calcium intake and MI or CVD events	
Yang et al, 2016	Prospective (Cancer Prevention Study II Nutrition Cohort)	73,079 women; 59,744 men (mean age =62.6 ±6.3 y) 43,186 deaths 13,157 cancer deaths 13,916 CVD deaths	• Total (diet + supplements) calcium intake: o $Q5_{men}$ >1100 mg/day o $Q5_{women}$ >1300 mg/day • Supplemental calcium intake and CVD mortality: • Men ($RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$= 1.22; 95%CI: 0.99-1.51; p-trend=0.39) • women ($RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$= 0.84; 95%CI: 0.74-0.94; p-trend<0.01). • Total calcium intake and CVD mortality • Men ($RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$= 0.97; 95%CI: 0.91-1.05; p-trend=0.77) • Women ($RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$= 0.84; 95%CI: 0.74-0.89; p-trend<0.01). • Supplemental calcium intake and all-cause mortality • Men: $RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$ =1.17; 95%CI: 1.03-1.33; p-trend = 0.18. • Women: RR=0.93; 95%CI: 0.87-0.99; p-trend<0.01 • Total calcium intake and all-cause mortality • Men: $RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$ =1.00; 95%CI: 0.95-1.04 • No other significant associations were observed among men between	• Measured total calcium intake • Adjusted for several known CVD risk factors • Models for supplemental calcium were adjusted for dietary calcium intake and vice versa

Citation	Study Design	Population	Findings	Considerations
			supplemental or total calcium intake and cancer mortality as well as cancer-specific mortality endpoints including colorectal, prostrate, and lung cancer when comparing high to low intakes. • Among women, significant inverse (protective) associations between total calcium and all-cause, cancer, and colorectal cancer was observed with reductions ranging from 44% (95%CI: 25%-59%) for colorectal cancer when comparing the extreme	
Samelson et al, 2012	Prospective (Framingham Offspring Study)	669 women; 532 men (mean 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

Citation	Study Design	Population	Findings	Considerations
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				
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	US population 17+ y eligible for mortality follow-up and free from	• ~10% of population died of cardiovascular disease (N=1870); majority were ischemic heart disease (IHD; 5.4%)	• Adjusted for many dietary and known risk factors for CVD

Citation	Study Design	Population	Findings	Considerations
	mortality linkage follow-up	history of heart disease (n=18,714)	• 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	• 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 Institutes of Health (NIH)–AARP Diet and Health Study); 12 y follow-up	388,229 men and women in the US aged 50–71 y	• 7904 CVD deaths in men; 3874 CVD deaths in women • 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	• Adjusted for dietary variables • No data on duration of supplement use • 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	• Details of supplement use were not described. • Relationship of serum calcium to calcium intake not described. • Concurrent assessment of intake and risk.

Citation	Study Design	Population	Findings	Considerations
			the relationship of serum calcium to estimated calcium intake.	
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 • 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)	• Quintiles of energy-adjusted total calcium intake (dietary and supplement) utilized for analysis. • Concurrent assessment of calcium intake and risk.
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				
Lappe et al 2017	Randomized, double-blinded, placebo-controlled trial	2,303 post-menopausal women ages 55+ y; subjects consumed 2,000 IU of vitamin D3 with 1,500 mg of calcium carbonate daily for 4 years	• No difference between CaD and placebo groups in total cancer incidence (HR = 0.70; 95% CI: 0.47, 1.02) nor breast cancer incidence (HR=0.79, 95% CI: 0.43, 1.43)	• Population of older women; findings may not be generalizable to the total population • No calcium only 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 • Total dietary intake of calcium is not measured
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).	• 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

Citation	Study Design	Population	Findings	Considerations
			• 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.	• 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.
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				
Yang et al, 2016	Prospective (Cancer Prevention Study II Nutrition Cohort)	73,079 women; 59,744 men (mean age =62.6 ±6.3 y) 43,186 deaths 13,157 cancer deaths 13,916 CVD deaths	• Total (diet + supplements) calcium intake: o $Q5_{men}$ >1100 mg/day o $Q5_{women}$ >1300 mg/day • Supplemental calcium intake and all-cause mortality • Men: $RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$ =1.17; 95%CI: 1.03-1.33; p-trend = 0.18. • Women: RR=0.93; 95%CI: 0.87-0.99; p-trend<0.01 • Total calcium intake and all-cause mortality	• Measured total calcium intake • Models for supplemental calcium were adjusted for dietary calcium intake and vice versa

Citation	Study Design	Population	Findings	Considerations
			• Men: $RR_{\geq 1000mg/d \text{ vs } 0 \text{ mg/d}}$ =1.00; 95%CI: 0.95-1.04 • No other significant associations were observed among men between supplemental or total calcium intake and cancer mortality as well as cancer-specific mortality endpoints including colorectal, prostrate, and lung cancer when comparing high to low intakes. • Among women, significant inverse (protective) associations between total calcium and all-cause, cancer, and colorectal cancer was observed with reductions ranging from 44% (95%CI: 25%-59%) for colorectal cancer when comparing the extreme	
Asemi et al, 2015	21 prospective studies; 1 nested case-control study (1992-2013)	2,346,368 male and female participants, 8 to >65 y 81,298 total deaths Total calcium and mortality: 559,666 participants 25-79 y Dietary calcium and mortality: 832,549 participants 8->65 y Supplemental calcium and mortality: 954,153 participants 35 - >65 y	• Total calcium and all-cause mortality: RR=1.16 (95%CI: 0.83-1.64) • Total calcium and cancer mortality: RR=1.49 (95%CI: 0.79-2.83) • Supplemental calcium and all-cause mortality: RR=0.91 (95%CI: 0.88-0.94) • Supplemental calcium and cancer mortality: RR=1.22 (95%CI: 0.81-1.84)	• Study did not find statistically significant association between calcium intake (total or supplemental) and all-cause or cancer mortality based on pooled RR.
Aune et al, 2015	Meta-analysis of prospective studies of dietary,	Total calcium: 750.275 participants (9 cohorts), 33,127 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;	• 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

Citation	Study Design	Population	Findings	Considerations
	supplemental, and total calcium	Dietary calcium: 800,879 participants (15 cohorts), 35,493 cases	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)	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 - 2010, every 4 years	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.
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	227 male and female participants age >60	• Users of calcium supplements (yes/no), after controlling for dietary calcium intake, had significantly greater lesion	• No dose response established; daily supplement intake ranged from 37-1130 mg/day (mean 744.2 mg/day)

Citation	Study Design	Population	Findings	Considerations
	and brain lesion volume	y;149 supplement users, and 78 non-users	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)	• 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.

Safety Conclusion

Calcium lactate is approved for use as a firming agent, flavor enhancer, flavoring agent or adjuvant, leavening agent, nutrient supplement, and a stabilizer and thickener in food with no limitations other than GMPs (21CFR § 184.1207). Likewise, lactic acid and other salt forms, including ferrous lactate, potassium lactate, and sodium lactate, are affirmed GRAS as direct human food ingredients with no limitations other than GMPs. JECFA has established an ADI for "not limited" for lactic acid and all common salts of lactate including calcium lactate (JECFA, 1973, 2001, 1974a,b,c).

The current 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, as measured by the USDA) and calcium from dietary supplements, as well as calcium intake from the proposed use of calcium lactate in potato and vegetable snacks and sweetened crackers. 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 and vegetable snacks and sweetened crackers will contain calcium lactate. 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. 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 older women 71+ y (2,064 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 1,269 mg/day among females 71+ y to 1,681 mg/day 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 50% 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 lactate at a level up to 4 % in potato and vegetable snacks and sweetened crackers 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 lactate contributes from 5-9% (98 – 164 mg/day additional calcium) at the per user 90th percentile, and among non-calcium-supplement users: 7-9% (95-145 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 1,236 mg/day to 1,639 mg/ among females and males age 51 years and older, all well below the IOM UL of 2,000 mg/day.

Overall, the *per user* 90th percentile cumulative calcium intakes for the subpopulations of infants 6-11 months, children, adolescents and majority of adult subpopulations were below the IOM

UL. For the older adults 51+ y the per user 90th percentile cumulative calcium intake for males were below the exposure limit range (2,000 – 2,500 mg/day). For women 51+ 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 (<10%) from the proposed use of calcium lactate in potato and vegetable snacks and sweetened crackers 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 lactate at a maximum level 4% in potato and vegetable snacks and sweetened crackers 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 lactate in potato and vegetable snacks and sweetened crackers meeting appropriate specifications and used according to GMP, is GRAS.

Basis for Conclusion that there is Consensus Regarding Safety

The intended use of calcium lactate 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.

Part 7. List of Supporting Data and Information in GRAS Notice

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Appendices

Appendix A. Specifications

CALCIUM LACTATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS INS No. 327

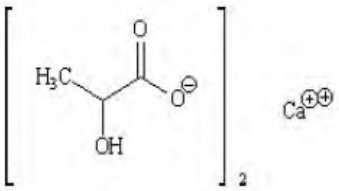
DEFINITION

Chemical names: Calcium dilactate, calcium dilactate hydrate, 2-Hydroxypropanoic acid calcium salt

C.A.S. number: 814-80-2

Chemical formula: $C_6H_{10}CaO_6 \cdot xH_2O$ (x = 0 - 5)

Structural formula:



Formula weight: 218.22 (anhydrous)

Assay: Not less than 98.0% of on the dried basis

DESCRIPTION White to cream coloured, almost odourless, crystalline powder or granules. The pentahydrate is somewhat efflorescent.

FUNCTIONAL USES Buffer, dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, practically insoluble in ethanol

Test for lactate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 30% (120°, 4 h)

pH (Vol. 4): 6.0-8.0 (1 in 20 soln)

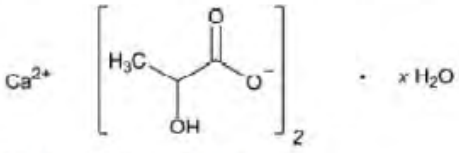
Acidity: Dissolve 1 g of the sample in 20 ml of water, add 3 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Not more than 0.5 ml should be required.

Magnesium and alkali salts	Not more than 1% mg/kg Dissolve 1 g of the sample in 40 ml of water, add 0.5 g of ammonium chloride, boil, and add about 20 ml of ammonium oxalate TS. Heat the solution on a water bath for 1 h, cool, add water to 100 ml, and filter. To 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, and ignite to constant weight. The residue should not exceed 5 mg.
Fluoride (Vol. 4)	Not more than 30 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
METHOD OF ASSAY	Dissolve about 350 mg of previously dried sample, accurately weighed, in 150 ml of water containing 2 ml of dilute hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml buret. Then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy-naphthol blue indicator, and continue the titration to a blue end-point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 10.91 mg of $C_6H_{10}CaO_6$.

Calcium Lactate

Published in: FCC 10 2S | FCC 10 3S
First Published: Prior to FCC 6

2-Hydroxypropanoic Acid, Calcium Salt



$C_6H_{10}CaO_6 \cdot xH_2O$
Formula wt: anhydrous 218.22
INS: 327
CAS: [814-80-2]
UNII: 2URQ2N32W3 [calcium lactate]

DESCRIPTION

Calcium Lactate occurs as a white to cream-colored, crystalline powder or granules. It contains up to five molecules of water of crystallization. The pentahydrate is somewhat efflorescent and at 120° becomes anhydrous. It is soluble in water and practically insoluble in alcohol.
Function: Buffer; dough conditioner; yeast nutrient
Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Calcium, *Appendix IIIA***
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests
- **Lactate, *Appendix IIIA***
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes test

ASSAY

- **PROCEDURE**

  Sample: Amount equivalent to 350 mg of $C_6H_{10}CaO_6$

  Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 2.7 N hydrochloric acid. 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 with the disodium EDTA to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 10.91 mg of $C_6H_{10}CaO_6$.

  Acceptance criteria: NLT 98.0% and NMT 101.0% of $C_6H_{10}CaO_6$, calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **FLUORIDE, *Fluoride Limit Test, Method I or Method III, Appendix IIIB***

  Sample: 3.3 g for *Method I* or 1.0 g for Method III

  Acceptance criteria: NMT 0.0015%

- **LEAD, *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIB***

  Sample: 3 g

  Acceptance criteria: NMT 2 mg/kg

- **MAGNESIUM AND ALKALI SALTS**

  Sample: 1 g

  Analysis: Mix the *Sample* with 40 mL of water and carefully add 1 mL of hydrochloric acid. Boil the solution for 1 min and rapidly add 40 mL of *oxalic acid TS*, followed immediately by 2 drops of *methyl red TS*. Then add 6 N ammonium hydroxide, dropwise from a buret, until the mixture is just alkaline. Cool the mixture to room temperature and transfer it into a 100-mL graduate cylinder. Dilute with water to 100 mL, mix, and allow the mixture to stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, transfer 50 mL of the clear filtrate to a tared platinum dish, and add 0.5 mL of sulfuric acid. Evaporate the contents of the dish to a small volume on a steam bath; then carefully heat over a free flame to dryness, and continue

heating to complete decomposition and volatilization of the ammonium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: The weight of the residue does not exceed 5 mg. (NMT 1%)

SPECIFIC TESTS

- **Acidity (as Lactic Acid)**

  Sample solution: 1 g in 20 mL of water

  Analysis: Add 3 drops of *phenolphthalein TS* to the *Sample solution* and titrate with 0.1 N sodium hydroxide.

  Acceptance criteria: NMT 0.5 mL of titrant is required. (About 0.45%, as lactic acid)

- **Loss on Drying, *Appendix IIC*:** 120° for 4 h

  Sample: 1.5 g

  Acceptance criteria

  Pentahydrate: Between 22.0% and 27.0%

  Trihydrate: Between 15.0% and 20.0%

  Monohydrate: Between 5.0% and 8.0%

  Dried Form: NMT 3.0%

Please check for your question in the FAQs (http://www.usp.org/frequently-asked-questions/food-chemicals-codex-fcc) before contacting USP.

Topic/Question	Contact	Expert Committee
CALCIUM LACTATE	Gina Clapper (mailto:Gina.clapper@usp.org) Senior Scientific Liaison 301-692-3626	FI2015 Food Ingredients 2015

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- FCC 8 - page 172

Appendix B. Certificates of Analysis

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Comp reg. NL003253235B01

CERTIFICATE OF ANALYSIS

Order nr	1026903	**Cust order Ref**	CB122383
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	675631		
Lot No	1603001828		
Manufacturing Date	17-Mar-2016	**Retest Date**	16-Mar-2021

Test	Units	Specification	Results
Colour fresh (10% solution)	ppm K2Cr2O	<=3	<1
Odor		Almost odorless	Almost odorless
Taste		Neutral	Neutral
Assay	%	99.0 - 101.0	99.8
Assay Calcium	%	13.4 - 14.5	13.7
Identification on calcium		Passes test**	Passes test
Identification on lactate		Passes test**	Passes test
Form		Agglomerated powder	Agglomerated powder
Loss on drying	%	22.0 - 27.0	25.5
pH (5 g product + 95 g water)		6.0 - 8.0	6.9
Sieve analysis 75 um - 425 um	%	>=90.0	94.8
Sieve analysis <= 500 um	%	>=98.0	100.0
Dirt (visual, ref)		<=6 particles	<=6 particles

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color		White
Hydrate form		Penta hydrate
Solubility in water (1g/30ml)		Passes test**
Solubility in ethanol (95%)		Passes test**
Heavy metals total	ppm	<=7
Mercury	ppm	<=1
Iron	ppm	<=25
Arsenic	ppm	<=1
Chloride	ppm	<=40
Sulphate	ppm	<=200
Phosphate	ppm	<=50
Barium		Passes test**
Lead	ppm	<=0.2
Stereochemical purity (L-isomer)	%	>=98
Fluoride	ppm	<=15
Reducing substances		Passes test**
Volatile fatty acids		Passes test**
Acidity (as lactic acid)	%	<=0.19

Order nr	1026903	**Cust order Ref**	CB122383
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	675631		
Lot No	1603001828		
Manufacturing Date	17-Mar-2016	**Retest Date**	16-Mar-2021

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Mg. and alkalisalts	%	<=0.6
Alkalinity		Passes test**
Clarity of solution		Passes test**
Color of solution		Passes test**
Coliforms		Absent 1G
TAMC	counts/g	<=10
TYMC	counts/g	<=10
Bromide	ppm	<=20

**Passes test of latest version of FCC, JSFA, 231/2012/EC, EP and USP
This lot complies with: Latest version USP, JSFA, FCC, 231/2012/EC,JECFA, EP

This document is generated by a validated system and therefore not signed.

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Comp reg. NL003253235B01

CERTIFICATE OF ANALYSIS

Order nr	1020090	**Cust order Ref**	553302
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	410577 47973		
Lot No	1607000561		
Manufacturing Date	12-July-2016	**Retest Date**	11-July-2021

Test	Units	Specification	Results
Colour fresh (10% solution)	ppm K2Cr2O	<=3	<1
Odor		Almost odorless	Almost odorless
Taste		Neutral	Neutral
Assay	%	99.0 - 101.0	99.3
Assay Calcium	%	13.4 - 14.5	13.7
Identification on calcium		Passes test**	Passes test
Identification on lactate		Passes test**	Passes test
Form		Agglomerated powder	Agglomerated powder
Loss on drying	%	22.0 - 27.0	25.2
pH (5 g product + 95 g water)		6.0 - 8.0	7.1
Sieve analysis 75 um - 425 um	%	>=90.0	94.8
Sieve analysis <= 500 um	%	>=98.0	100.0
Dirt (visual, ref)		<=6 particles	<=6 particles

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color		White
Hydrate form		Penta hydrate
Solubility in water (1g/30ml)		Passes test**
Solubility in ethanol (95%)		Passes test**
Heavy metals total	ppm	<=7
Mercury	ppm	<=1
Iron	ppm	<=25
Arsenic	ppm	<=1
Chloride	ppm	<=40
Sulphate	ppm	<=200
Phosphate	ppm	<=50
Barium		Passes test**
Lead	ppm	<=0.2
Stereochemical purity (L-isomer)	%	>=98
Fluoride	ppm	<=15
Reducing substances		Passes test**
Volatile fatty acids		Passes test**
Acidity (as lactic acid)	%	<=0.19

Order nr	1020090	**Cust order Ref**	553302
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	410577 47973		
Lot No	1607000561		
Manufacturing Date	12-July-2016	**Retest Date**	11-July-2021

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Mg. and alkalisalts	%	<=0.6
Alkalinity		Passes test**
Clarity of solution		Passes test**
Color of solution		Passes test**
Coliforms		Absent 1G
TAMC	counts/g	<=10
TYMC	counts/g	<=10
Bromide	ppm	<=20

**Passes test of latest version of FCC, JSFA, 231/2012/EC, EP and USP
This lot complies with: Latest version USP, JSFA, FCC, 231/2012/EC,JECFA, EP

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Comp reg. NL003253235B01

CERTIFICATE OF ANALYSIS

Order nr	1029358	**Cust order Ref**	4502790641
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	1035790		
Lot No	1602000397		
Manufacturing Date	03-Feb-2016	**Retest Date**	01-Feb-2021

Test	Units	Specification	Results
Colour fresh (10% solution)	ppm K2Cr2O	<=3	<1
Odor		Almost odorless	Almost odorless
Taste		Neutral	Neutral
Assay	%	99.0 - 101.0	99.9
Assay Calcium	%	13.4 - 14.5	13.7
Identification on calcium		Passes test**	Passes test
Identification on lactate		Passes test**	Passes test
Form		Agglomerated powder	Agglomerated powder
Loss on drying	%	22.0 - 27.0	25.4
pH (5 g product + 95 g water)		6.0 - 8.0	7.1
Sieve analysis 75 um - 425 um	%	>=90.0	93.8
Sieve analysis <= 500 um	%	>=98.0	100.0
Dirt (visual, ref)		<=6 particles	<=6 particles

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color		White
Hydrate form		Penta hydrate
Solubility in water (1g/30ml)		Passes test**
Solubility in ethanol (95%)		Passes test**
Heavy metals total	ppm	<=7
Mercury	ppm	<=1
Iron	ppm	<=25
Arsenic	ppm	<=1
Chloride	ppm	<=40
Sulphate	ppm	<=200
Phosphate	ppm	<=50
Barium		Passes test**
Lead	ppm	<=0.2
Stereochemical purity (L-isomer)	%	>=98
Fluoride	ppm	<=15
Reducing substances		Passes test**
Volatile fatty acids		Passes test**
Acidity (as lactic acid)	%	<=0.19

Order nr	1029358	**Cust order Ref**	4502790641
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	1035790		
Lot No	1602000397		
Manufacturing Date	03-Feb-2016	**Retest Date**	01-Feb-2021

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Mg. and alkalisalts	%	<=0.6
Alkalinity		Passes test**
Clarity of solution		Passes test**
Color of solution		Passes test**
Coliforms		Absent 1G
TAMC	counts/g	<=10
TYMC	counts/g	<=10
Bromide	ppm	<=20

**Passes test of latest version of FCC, JSFA, 231/2012/EC, EP and USP
This lot complies with: Latest version USP, JSFA, FCC, 231/2012/EC,JECFA, EP

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Comp reg. NL003253235B01

CERTIFICATE OF ANALYSIS

Order nr	993517	**Cust order Ref**	4502652140
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	1035790		
Lot No	1503002601		
Manufacturing Date	25-Mar-2015	**Retest Date**	23-Mar-2020

Test	Units	Specification	Results
Colour fresh (10% solution)	ppm K2Cr2O	<=3	<1
Assay	%	99.0 - 101.0	100.0
Assay Calcium	%	13.4 - 14.5	13.8
Identification on calcium		Passes test**	Passes test**
Identification on lactate		Passes test**	Passes test**
Loss on drying	%	22.0 - 27.0	25.1
pH (5 g product + 95 g water)		6.0 - 8.0	6.9
Sieve analysis 75 um - 425 um	%	>=90.0	96.0
Sieve analysis <= 500 um	%	>=98.0	100.0
Dirt (visual, ref)		<=6 particles	<=6 particles

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color		White
Odor		Neutral
Taste		Neutral
Solubility in water (1g/30ml)		Passes test**
Solubility in ethanol (95%)		Passes test**
Heavy metals total	ppm	<=7
Mercury	ppm	<=1
Iron	ppm	<=25
Arsenic	ppm	<=1
Chloride	ppm	<=40
Sulphate	ppm	<=200
Phosphate	ppm	<=50
Barium		Passes test**
Lead	ppm	<=0.2
Fluoride	ppm	<=15
Reducing substances		Passes test**
Volatile fatty acids		Passes test**
Acidity (as lactic acid)	%	<=0.19
Mg. and alkalisalts	%	<=0.6
Alkalinity		Passes test**
Clarity of solution		Passes test**

Order nr	993517	**Cust order Ref**	4502652140
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Customer item number	1035790		
Lot No	1503002601		
Manufacturing Date	25-Mar-2015	**Retest Date**	23-Mar-2020

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color of solution		Passes test**
TAMC	counts/g	<=10
TYMC	counts/g	<=10
Bromide	ppm	<=20

**Passes test of latest version of FCC, JSFA, 231/2012/EC, EP and USP
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Comp reg. NL003253235B01

CERTIFICATE OF ANALYSIS

Order nr	1030517	**Cust order Ref**	V1609261
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Lot No	1601000469		
Manufacturing Date	07-Jan-2016	**Retest Date**	05-Jan-2021

Test	Units	Specification	Results
Colour fresh (10% solution)	ppm K2Cr2O	<=3	<1
Odor		Almost odorless	Almost odorless
Taste		Neutral	Neutral
Assay	%	99.0 - 101.0	99.9
Assay Calcium	%	13.4 - 14.5	13.8
Identification on calcium		Passes test**	Passes test
Identification on lactate		Passes test**	Passes test
Form		Agglomerated powder	Agglomerated powder
Loss on drying	%	22.0 - 27.0	24.7
pH (5 g product + 95 g water)		6.0 - 8.0	7.0
Sieve analysis 75 um - 425 um	%	>=90.0	95.3
Sieve analysis <= 500 um	%	>=98.0	100.0
Dirt (visual, ref)		<=6 particles	<=6 particles

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Color		White
Hydrate form		Penta hydrate
Solubility in water (1g/30ml)		Passes test**
Solubility in ethanol (95%)		Passes test**
Heavy metals total	ppm	<=7
Mercury	ppm	<=1
Iron	ppm	<=25
Arsenic	ppm	<=1
Chloride	ppm	<=40
Sulphate	ppm	<=200
Phosphate	ppm	<=50
Barium		Passes test**
Lead	ppm	<=0.2
Stereochemical purity (L-isomer)	%	>=98
Fluoride	ppm	<=15
Reducing substances		Passes test**
Volatile fatty acids		Passes test**
Acidity (as lactic acid)	%	<=0.19
Mg. and alkalisalts	%	<=0.6

Order nr	1030517	**Cust order Ref**	V1609261
Product	PURACAL® PP/USP Calcium-L-Lactate 5-hydrate powder		
Lot No	1601000469		
Manufacturing Date	07-Jan-2016	**Retest Date**	05-Jan-2021

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Alkalinity		Passes test**
Clarity of solution		Passes test**
Color of solution		Passes test**
Coliforms		Absent 1G
TAMC	counts/g	<=10
TYMC	counts/g	<=10
Bromide	ppm	<=20

**Passes test of latest version of FCC, JSFA, 231/2012/EC, EP and USP
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Acc.nr. 47.22.15.248
Vat nr: NL003253235B01

CERTIFICATE OF ANALYSIS

Product	PURACAL PP/USP Calcium-L-Lactate 5-hydrate powder
Test period	2013-2016

Test	Units	Specification	Average	St dev
Acidity (as lactic acid)	%	<=0.19	<0.19	N.A.
Arsenic	ppm	<=1	< 0.3	N.A.
Assay	%	99.0 - 101.0	99.7	0.27
Assay Calcium	%	13.4 - 14.5	13.7	0.08
Bromide	ppm	<=20	<20	N.A.
Chloride	ppm	<=80	<40	N.A.
Colour fresh (10%) solution)	ppm K2Cr2O	<=3	1	N.A.
Fluoride	ppm	<=15	<5	N.A.
Heavy metals total	ppm	<=7	<2	N.A.
Iron	ppm	<=25	2.3	1.60
Lead	ppm	<=0.2	<0.2	N.A.
Loss on drying	%	22.0 - 27.0	25.1	0.41
Mercury	ppm	<=1	<0.005	N.A.
Mg. and alkalisalts	%	<=0.6	0.1	0.05
pH (5 g product + 95 g water)		6.0 - 8.0	7.0	0.11
Phosphate	ppm	<=50	5.2	N.A.
Sieve analysis <= 500 um	%	>=98.0	99.99	0.07
Sieve analysis 75 um - 425 um	%	>=90.0	94.1	1.8
Stereochemical purity (L-isomer)	%	>=98	99.6	0.2
Sulphate	ppm	<=200	89	32
TAMC	counts/g	<=10	<10	N.A.
TYMC	counts/g	<=10	<10	N.A.

This product complies with: Latest version USP, JSFA, FCC, 231/2012/EC, JECFA, EP

(b) (6)

Simon Jager
Friday 18 November 2016 11:07 +01:00

GoSign

Simon Jager
Manager QA/QC

Printed on: 18 November 2016

Page 1 of 1

Appendix C. Analytical Methods

Pages 000099-000102 of business confidential information were removed.

Appendix D. Food Codes Included in Analysis

Food Code	Food Description
Potato snacks	
54402610	Salty snacks, multigrain and potato chips (made with rice flour, dried potatoes, corn flour, and wheat starch)
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
71202500	White potato chips, lightly salted
71205000	White potato, sticks
71211000	White potato skins, chips
Vegetable snacks	
71220000	Vegetable chips
71905410	Plantain chips
71980200	Taro chips
73410210	Sweet potato, chips
Sweetened crackers	
51184000	Bread sticks, hard
51187000	Melba toast
51188500	Zwieback toast
54001000	Crackers, NS as to sweet or nonsweet
54101010	Cracker, animal
54102010	Crackers, graham
54102020	Crackers, graham, chocolate covered
54102100	Crackers, graham, lowfat
54102110	Crackers, graham, fat free
54203010	Crackers, toast thins (rye, wheat, white flour), low sodium
54204010	Cracker, 100% whole wheat, low sodium
54205010	Cracker, snack, low sodium
54205100	Cracker, snack, lowfat, low sodium
54207010	Crispbread, wheat, low sodium
54210010	Cracker, multigrain, low sodium
54301000	Cracker, snack

Food Code	Food Description
54301100	Cracker, snack, reduced fat
54301200	Cracker, snack, fat free
54304500	Cracker, high fiber, no added fat
54305000	Crispbread, wheat, no added fat
54305500	Crispbread, wheat or rye, extra crispy
54307000	Crackers, matzo
54308000	Crackers, milk
54326000	Crackers, multigrain, made with whole wheat, wheat, oat, and other flours
54334000	Crackers, toast thins (rye, pumpernickel, white flour)
54336000	Crackers, water biscuits
54337000	Cracker, 100% whole wheat
54337050	Cracker, 100% whole wheat, reduced fat
54337100	Crackers, whole wheat and bran
54338000	Crackers, wheat
54338100	Crackers, wheat, reduced fat
54402700	Pita chips
54440010	Bagel chip

* Represents extruded

Appendix D. Literature Search

		Hits (n)				
Search Terms	**Limits**	**Initial search (10/31/13)**	**Updated search (6/20/2014)**	**Updated Search (10/2015)**	**Updated Search (10/2016)**	**Updated Search (10/2017)**
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	98	188
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	122	143
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	291	540
Total citations reviewed	-	3317	246	938	421	705

Thomas, Joseph

From: Nga Tran <ntran@exponent.com>
Sent: Tuesday, February 20, 2018 5:57 PM
To: Thomas, Joseph
Subject: RE: Questions for GRN 747- calcium lactate

Follow Up Flag: Follow up
Flag Status: Flagged

Dr. Thomas:

I have inserted our responses right below questions 1 and 2 of your email (blue fonts).

Please let me know if we can provide any other clarifications.

Best regards,

Nga Tran
Principal Scientist
Exponent
1150 Connecticut Ave., NW
Suite 1100
Washington, DC 20036
Direct +1-202-772-4915 • Email ntran@exponent.com
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From: Thomas, Joseph [mailto:Joseph.Thomas@fda.hhs.gov]
Sent: Friday, February 16, 2018 8:09 AM
To: Nga Tran <ntran@exponent.com>
Subject: Questions for GRN 747- calcium lactate

Dear Dr. Tran,

We would like to clarify a couple points identified during review of GRAS Notice GRN No. 747 for calcium lactate in potato and vegetable snacks and sweetened crackers.

1) We would like to clarify the date of the literature searches related to the safety of calcium lactate in GRN No. 747. During evaluation of GRN 747, we noted several discrepant numbers and dates. On page 37, the notifier states that the "initial search was conducted on October 31, 2013 with updated searches conducted in June 2014, October 2015, February 2016, October 2016, and October 2017." These search strategies are then outlined in Appendix D on page 104. In Appendix D, the notifier does not include the February 2016 literature search, for which the notifier states an additional 56 publications were identified on page 37. It also does not appear that these publications were included in the additional 421 publications identified from February 2016 to October 2016. A similar sentence on literature searches on page 32 does not include the October 2017 search date and uses an end date of January 2017. In addition, on page 27, a date of November 2016 is used for the safety of calcium lactate and lactic acid. We ask that the notifier please clarify the dates and numbers to be considered for literature searches on pages 37, 104, 32, and 27.
Response: The full description of the literature searches conducted to evaluate the safety of calcium among humans using epidemiological data and number of articles is correctly outlined on page 37 of GRN 747. In

Appendix D on page 104, the February 2016 search was mistakenly left out of that table. As correctly stated on page 37, there was a search conducted in February 2016 that yielded 56 additional articles. Further, the text outlining the search on page 32 should read:

> *To capture human health information on calcium that have been published since the IOM and EFSA reviews and publications, a PubMed search 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, October 2015, February 2016, October 2016, and October 2017 for human health information published between June 2014 and January 2018 (to capture publications ahead of print).*

The date of November 2016 reported on page 27 is the correct date relating to the search for safety data on calcium lactate and lactic acid.

2) On page 14, the last line of the summary states "…summarized in Figure 4 below" yet the figure below is Figure 3. Please confirm that the last line of the summary is referring to Figure 3.

   Response: Yes- we confirm that the last line of the summary is referring to Figure 3.

We ask that the notifier please respond to the above points within 14 days.

Thank you and please let me know if you have any questions,

Joseph M. Thomas, Ph.D.
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