SCIENTIFIC OPINION

Scientific Opinion on re-evaluation of calcium carbonate (E 170) as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of calcium carbonate (E 170). Calcium carbonate is an inorganic salt authorised as a food additive in the EU, and is also included in the list of substances that may be added for specific nutritional purposes in foods for particular nutritional uses and in Directive 2002/46/EC relating to food supplements. Calcium carbonate was previously evaluated by JECFA in 1965, when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that the available toxicological database on calcium carbonate is limited, but does not give rise to concern. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes, and are also seen with other calcium salts. The Panel agrees with the group ADI "not specified" assigned by the SCF to a group of carbonates including calcium carbonate, when considering the use of calcium carbonate as a food additive. The Panel notes that the estimated exposures to calcium from all sources, including the use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and from food fortification are below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. The Panel concludes that trace levels of adventitious nanoscale material within macroscale calcium carbonate are not of toxicological concern.

KEY WORDS

Calcium carbonate, E 170, CAS Registry Number 471-34-1, calcite, chalk, carbonic acid calcium salt, CI Pigment White 18, food additive. food colouring substance, food supplement, food fortification.

1 On request from the European Commission, Question No EFSA-Q-2011-00363, adopted on 5 July 2011.

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Suggested citation: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on re-evaluation of calcium carbonate (E 170) as a food additive. EFSA Journal 2011;9(7):2318 [73 pp.]. doi:10.2903/j.efsa.2011.2318. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of calcium carbonate (E 170) when used as a food additive[4].

Calcium carbonate is a food additive generally authorised in foodstuffs under Directive 95/2/EC[5] on food additives and is a permitted food colouring substance under Directive 94/36/EC[6] on colours for use in foodstuffs. It is also included in Directive 2001/15/EC[7] on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC[8] relating to food supplements and it can be used in fortified foods according to Regulation 1925/2006[9] on the addition of vitamins and minerals and of certain other substances in food.

Calcium carbonate was previously evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1965, when the Committee established an Acceptable Daily Intake (ADI) not limited. The EU Scientific Committee for Food (SCF) evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified.

Specifications for calcium carbonate have been defined in Commission Directive 2008/128/EC[10] and by JECFA. The purity is specified as not less than 98% calcium carbonate. The Panel noted that calcium carbonate can have an amorphous or microcrystalline structure, and that particle size varies according to the form and manufacturing conditions. Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 µm diameter crystals typical of the crystalline forms of calcium carbonate. Nanoform calcium carbonate (as used in some of the toxicological studies reported in this opinion) was reported to have a particle size of 60 - 100 nm when examined by Scanning Electron Microscopy (SEM). The typical average particle size (d50) of food grade calcium carbonate is stated by industry to be about 5 µm, with an upper range (d98%) of 65 µm and less than 1% of particles having a diameter below 100 nm; the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded.

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion, but a large proportion (up to 80 %) is reconverted to calcium carbonate and other insoluble calcium salts and excreted in the faeces. Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20-40%. The Panel noted however that recent toxicokinetic data in mice indicate that calcium from nanoparticulate calcium carbonate is more readily absorbed than the microparticulate form, as evidenced by significantly higher serum calcium concentrations in the nano calcium carbonate group than in the micro calcium carbonate group. A slight increase in calcium bioavailability (by 38%) from nanonised pearl powder form by comparison with the corresponding

[4] The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent, release agent.
[5] European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1–63.
[6] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.94, p. 13-29.
[7] Commission Directive 2001/15/EC of February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52,2 2.22.2001, p 19.
[8] Commission Directive 2002/46/EC of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183 of 12.7.2002 p.51.
[9] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26-38.
[10] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

micronised form of calcium carbonate has also been observed in humans. In view of the small increases observed in these studies, the Panel considered that the absorption of calcium from calcium carbonate in nanoparticles will not be markedly different than that from calcium carbonate microparticles.

After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration.

Limited toxicological data are available on calcium carbonate. A number of short term studies (generally less than 90 days) have been carried out with calcium carbonate in rats, mice and cats, to investigate the effects of high calcium diets on various physiological and biochemical parameters. These studies have also assessed the toxicity of high levels of calcium carbonate in the diet, as manifest by effects on growth and food consumption, and have overall not demonstrated any evidence of toxicity attributable to calcium carbonate.

The only notable treatment-related effect seen in a 91-day feeding study with calcium carbonate in rats at dietary calcium levels equivalent to 250 or 500 mg/kg bw/day was nephrocalcinosis, seen in both males and (more marked) in females. The Panel considered however that the nephrocalcinosis finding in rats is not relevant for human safety assessment because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis. Results from a 91-day toxicity study in Beagle dogs fed calcium carbonate at doses to achieve equivalent calcium levels in the diet as in the rat study (~ 250 and 500 mg calcium/kg bw/day) did not report any signs of nephrocalcinosis.

Nephrocalcinosis was also not observed in a recent combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM) carried out in Wistar rats at dose levels of up to 1000 mg/kg bw/day for up to 48 days. The only changes seen in this study were slight but statistically significant haematological and biochemical effects in males receiving 1000 mg/kg bw/day, and significant reductions in plasma phosphate levels in all male treated groups. The Panel considered that these changes were non-adverse and agreed with the NOAEL of 1000 mg/kg bw/day identified by the authors of the study, the highest dose tested. No evidence of toxicity was reported in a study in which mice were administered calcium carbonate (described by the authors as nano calcium carbonate) by oral gavage at dose levels up to 1300 mg/kg bw/day for 28 days. The Panel noted however that only limited investigations were carried out in this study.

Calcium carbonate (including calcium carbonate having a particle size of 60 - 100 nm when examined by SEM) has given negative results in a range of *in vitro* genotoxicity assays. No data are available on the chronic toxicity or carcinogenicity of calcium carbonate. The Panel considered however that it is very unlikely that calcium carbonate has carcinogenic potential, given that both calcium and carbonate are natural constituents of the body and normal metabolites of man, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans.

Several reproductive and developmental toxicity studies in rats and mice have been carried out with calcium carbonate. In rats fed diets containing calcium carbonate at levels up to approximately 1500 mg/kg bw/day, no dose-related changes indicative of developmental toxicity were reported. In a recent OECD guideline-compliant combined repeat dose oral toxicity/reproduction study, no effects on reproduction, including developmental toxicity, were reported at dose levels of nanoparticulate calcium carbonate up to 1000 mg/kg bw/day, the highest dose tested. Other studies have shown evidence of fetotoxicity of calcium (as calcium carbonate) when administered during pregnancy at levels in the diet greater than 1500 mg/kg bw/day calcium carbonate. Overall, the Panel noted that in

rodents high doses of calcium carbonate (> 1500 mg/kg bw/day) causing hypercalcaemia during gestation can result in adverse effects on reproduction, fetotoxicity and elemental imbalances in the offspring. However a recent repeat dose oral toxicity/reproduction study provided a NOAEL of 1000 mg/kg bw/day, the highest dose tested, for developmental toxicity and the Panel considered overall that there is no concern for the reproductive effects of calcium carbonate at intakes below 1500 mg/kg bw/day.

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

The Panel noted that a number of studies have been carried out with calcium carbonate in humans, showing that hypercalcaemia and alkalosis can occur when calcium carbonate (between 2.0 and 16.5 g/day of supplementary calcium) is taken with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome). The hypercalcaemia is often associated with renal dysfunction, metastatic calcification and other symptoms, and similar changes have been reported in individuals taking large amounts of calcium carbonate and other calcium-containing antacids or large amounts of calcium food supplements.

The Panel also noted overall that although a recent meta-analysis reported by Bolland et al. in 2010 and 2011, showing an increased risk of myocardial infarction in individuals given regular calcium supplementation in the management of osteoporosis, was a very large and robust study, the trends reported were very modest, and are not supported by the findings in a similar study carried out by Lewis et al. and reported in 2010. The Panel also noted that the trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that a plausible mode of action could be described to explain these differences, based on serum levels of calcium following dietary intake and supplementation.

Overall the Panel considered that the available toxicological database on calcium carbonate is limited, but it does not give rise to concern. The Panel considered that further toxicological studies on calcium carbonate are not necessary for the reasons already stated by the SCF in 1991, namely that calcium carbonate is a natural constituent of man, animals and plants, and therefore occurs naturally in foodstuffs. Calcium and carbonate/bicarbonate also constitute some of the major electrolytes present in all biological materials. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes, and are also found after large intakes of other calcium salts. The Panel considered that these high-calcium intake effects are not relevant to the evaluation of calcium carbonate as a food additive, and that the SCF established an UL for calcium in 2003, taking these effects into account.

The Panel therefore agreed with the group ADI “not specified” established by the SCF in 1991 for a group of carbonates including calcium carbonate and considers that the JECFA definition of “ADI not specified” is applicable to calcium carbonate when used as a food additive.

The Panel considered however that intakes of calcium resulting from the use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and from food fortification, should be below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

Based on data made available to the Panel by industry and Member States, the Panel estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) as ranging from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are estimated at 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

Estimated exposure to calcium from all sources based on data presented in the literature for six Member States showed that mean calcium intakes (including food supplements) in all population groups were of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium /day for adults,. The range of high percentile intakes were 1070 – 1184 mg calcium/day for toddlers, 1124-1453 mg calcium/day for children, 1306 – 1905 mg calcium/day for adolescents and 1233 – 1851 mg calcium/day for adults. Mean intake for the elderly (>64 years old) ranges from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The Panel noted that both the intake estimates calculated by the Panel, which are considered to be conservative, and the total dietary intake of calcium reported in literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. Therefore, the Panel concluded that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern.

The Panel noted that calcium carbonate is currently permitted at *quantum satis* in the vast majority of food categories in which it is allowed (generally permitted in all foods with certain exceptions), however information gathered by the Panel for this re-evaluation shows that calcium carbonate is only used at defined amounts in a number of food categories. The Panel thus recommended that the legislation be updated to reflect actual usage levels evaluated in this opinion.

The Panel also noted that a range of studies have been performed with calcium carbonate described as nanoform (having a particle size of 60 - 100 nm when examined by SEM), however none of these provided comprehensive data on characterisation of the nano material. Nor do these studies provide a full toxicological database in line with the guidance provided in the EFSA Scientific Committee Opinion on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. According to the information provided by the industry, the presence of unintentional nanoscale particles at trace levels in calcium carbonate when used as a food additive cannot be excluded. Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel considered that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within macroscale calcium carbonate would not be an additional toxicological concern.

The Panel also noted that the JECFA specification for lead in calcium carbonate is $\leq$ 3 mg/kg whereas the EC specification is $\leq$10 mg/kg.

Additionally the Panel noted that limestone (a source of calcium carbonate) may contain variable amounts of aluminium. The Panel noted that aluminium intake from use of calcium carbonate as a food additive could significantly contribute (50-100%) to the weekly intake of aluminium, for which a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw/week has been established, and therefore specifications for the maximum level of aluminium in calcium carbonate may be required

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by the EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[11]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[12] of 2001. The report "Food additives in Europe 2000[13]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[11] OJL 80, 26.03.2010, p19
[12] COM(2001) 542 final.
[13] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. INTRODUCTION

The present opinion deals with the re-evaluation of the safety of calcium carbonate (E 170) when used as a food additive. The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent and release agent.

Calcium carbonate is a food additive generally authorised in foodstuffs under Directive 95/2/EC[14] on food additives and is a permitted food colouring substance under Directive 94/36/EC[15] on colours for use in foodstuffs. Calcium carbonate was previously evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1965 (JECFA, 1965, 1966). The EU Scientific Committee for Food (SCF) evaluated calcium carbonate in 1990 as part of a group of carbonates (SCF, 1991), and a review of carbonates as food additives has been carried out by TemaNord (TemaNord, 2002). Calcium carbonate has also been registered under the REACH Regulation 1907/2006[16] (ECHA, 2011).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data[17,18].

2. TECHNICAL DATA

2.1. Identity of the substance

Calcium carbonate is an inorganic salt with CAS Registry Number 471-34-1, Colour Index No. 77220 and EINECS No. 207-439-9. Synonyms are carbonic acid calcium salt, calcite, chalk, CI Pigment White 18, INS No. 170(i). The chemical formula is $CaCO_3$ and the molecular weight is 100.1 g/mol. Calcium carbonate is an odourless white micro-crystalline or amorphous powder with six known forms (polymorphs). Five of these are crystalline and one is amorphous (hydrated amorphous calcium carbonate (Meiron et al., 2011). Of the five crystalline forms, three are anhydrous crystalline (i.e., calcite, aragonite, and vaterite) and two are hydrated crystalline (i.e., crystalline monohydrocalcite and ikaite) (Meiron et al., 2011). The most thermodynamically stable of these forms is calcite, whereas the least stable is the amorphous form (Nebel et al., 2008; Meiron et al., 2011).

Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 µm diameter crystals typical of the other forms (Meiron et al., 2011). According to the Calcium Carbonate Association (CCA-Europe) engineered nanoscale calcium carbonate is not used as a food additive. According to CCA-Europe, the typical average particle size (d50) of food grade calcium carbonate is stated by CCA-Europe to be about 5 µm, with an upper range (d98%) of 65 µm, but the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded (CCA-Europe, 2011). According to CCA-Europe, in two samples of food grade calcium carbonate the total number of particles with a diameter of less than 100 nm was

[14] European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1–63.
[15] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.94, p. 13-29.
[16] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006, p. 1-849.
[17] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm
[18] Call for scientific data on calcium carbonate (E 170i). Published: 10 December 2010. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans101210.htm.

estimated to be less than 1% (0.3% and 0.65% respectively, and 0.04% and 0.13% when a correction factor was applied, to allow for the maximum possible dissolved content arising as a result of the determination method). Some recent toxicological studies have been carried out with nanoparticulate calcium carbonate, as reported in Section 3 of this opinion. Information on the particle size of the calcium carbonate used in these studies has been provided in this opinion, when available.

The Panel noted that in characterising the particle size of calcium carbonate as a food additive, the considerations laid out in the EFSA Scientific Committee Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain should be taken into account (EFSA, 2011).

Calcium carbonate is practically insoluble in water and ethanol (FCC 7, 2010), but is soluble with effervescence in hydrochloric and other acids (Commission Directive 2008/128/EC[19]). According to the available information in the ECHA database the solubility of calcium carbonate (conventional) in water (OECD Guideline 105) is 16.6 mg/l (20°C, pH 9-9.4), while that of the aragonite and calcite forms is 6.6 mg/l (20°C) and of the valerite form 11 mg/l (20°C) (ECHA, 2011). The amorphous form of calcium carbonate, with particles predominately in the nanoscale range, is reported to be approximately 10 times more soluble than crystalline calcium carbonate (Meiron et al., 2011).

2.2. Specifications

Specifications have been defined in Commission Directive 2008/128/EC and by JECFA (JECFA, 2006) (Table 1).

Table 1: Specifications for calcium carbonate according to Commission Directive 2008/128/EC and JECFA (2006)

	Commission Directive 2008/128/EC	**JECFA, 2006**
Assay (on anhydrous basis)	≥ 98%	≥ 98%
Loss on drying (200 °C, 4 hours)	≤ 2.0%	≤ 2%
Acid-insoluble substances	≤ 0.2%	≤ 0.2%
Free alkali	-	≤ 0.05%
Magnesium and alkali salts	≤ 1.5%	≤ 1.0%
Fluoride	≤ 50 mg/kg	≤ 50 mg/kg
Antimony (as Sb) Copper (as Cu) Chromium (as Cr) Zinc (as Zn) Barium (as Ba)	≤ 100 mg/kg, singly or in combination	≤ 0.03 %
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 10 mg/kg	≤ 3 mg/kg
Cadmium	≤ 1 mg/kg	-

The Panel noted that the JECFA specification for lead in calcium carbonate is ≤ 3 mg/kg, whereas the EC specification is ≤ 10 mg/kg.

The Panel noted that there is no available information indicating that calcium carbonate is used as an aluminium lake for colouring purposes. The Panel noted that limestone (a source of calcium carbonate) may contain variable amounts of aluminium, one report indicating approximate

[19] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

concentrations of between 1 and 134 mg/kg (EPA, 1995). Additionally, the results of the analysis of some samples of food grade calcium carbonate were provided to the Panel, showing a range of aluminium concentrations from 10 to 190 mg/kg (Chemische Fabrik Budenheim KG, 2011). The Panel noted that aluminium intake from use of calcium carbonate as a food additive could add to the weekly intake of aluminium, for which a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008a), and therefore specifications for the maximum level of aluminium in calcium carbonate may be required.

2.3. Manufacturing process

According to Directive 2008/128/EC calcium carbonate is the product obtained from ground limestone or by the precipitation of calcium ions with carbonate ions. The most common industrial process for obtaining calcium carbonate involves the following steps (Domingo et al., 2004): (a) calcination of limestone (natural calcium carbonate rocks) to produce quicklime (CaO) and carbon dioxide (CO_2); (b) the slaking process, in which the quicklime is transformed to slaked lime slurry (a $Ca(OH)_2$ suspension) by controlled addition of water; and, finally (c) the carbonation reaction, in which CO_2 is bubbled through an aqueous slurry of slaked lime. The carbonation reaction is the crucial step determining the morphology of the obtained product. Particle size of the product can be varied by manipulation of the production conditions, such as partial pressure of CO_2, temperature, pH and presence of additives such as carboxylic acids (Tai and Chen, 1995; Franke and Mersmann 1995; García-Carmona et al., 2003; Domingo et al., 2004; Reiger et al., 2007; Henderson et al., 2008, Graham et al., 2008).

2.4. Methods of analysis in food

Determination of either calcium or the carbonate anion as result of use as a food additive in foodstuffs is only possible to a limited extent, since both occur endogenously in most foods. The most commonly used analytical techniques for determining the calcium carbonate content in foods rely on the measurement of total calcium by Atomic Absorption Spectroscopy (AAS), Atomic Emission Spectroscopy (AES) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or other related techniques following acid digestion of the sample (Scotter, 2011). Carbonate can be determined by measurement of evolved carbon dioxide volumetrically following acidification with hydrochloric acid and capture in a Chittick apparatus or gravimetrically after precipitation with barium hydroxide solution. In beer and wine carbonate can be determined by Ion Chromatography (IC) using conductivity detection (Scotter, 2011).

2.5. Reaction and fate in food

Calcium carbonate can be anticipated to be unstable in acidic foods.

2.6. Case of need and proposed uses

Authorised use and use levels have been defined in Directive 94/36/EC on colours for use in foodstuffs and in Directive 95/2/EC on food additives other than colours and sweeteners, as amended. The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent and release agent.

In accordance with Directive 94/36/EC, calcium carbonate is permitted *quantum satis* in all foodstuffs except for some foodstuffs in which the use of colours (in general) is specifically prohibited or restricted to food colours other than calcium carbonate.

In accordance with Directive 95/2/EC, calcium carbonate is generally permitted *quantum satis* in foodstuffs not referred to in Article 2 (3) of Directive 95/2, unless specifically provided for (see Table 2).

Table 2 summarises those beverages and foodstuffs that are permitted to contain calcium carbonate up to specified Maximum Permitted Levels (MPLs) set by Directive 94/36/EC and Directive 95/2/EC.

Table 2: Maximum Permitted Levels (MPLs) of use of calcium carbonate in beverages and foodstuffs according to European Parliament and Directives 94/36/EC and 95/2/EC and maximum reported use levels of calcium carbonate in beverages and foodstuffs used for the refined exposure assessment

Foodstuff Category	Legislation	Maximum Permitted Levels	Maximum Reported Use Level
Generally permitted in foodstuffs not referred to in Article 2 (3) of Directive 95/2	Directive 95/2/EC	*quantum satis*	See Annex B
Cocoa and chocolate products as defined in Directive 73/241/EEC[20]	Directive 95/2/EC	7 % on dry matter without fat expressed as potassium carbonates	
Grape juice as defined in Directive 93/77/EEC[21]	Directive 95/2/EC	*quantum satis*	
Ripened cheese	Directive 95/2/EC	*quantum satis*	
Sliced and grated ripened cheese	Directive 95/2/EC	*quantum satis*	
Carriers or carrier solvents for food additives	Directive 95/2/EC		
Processed cereal based foods and baby foods	Directive 95/2/EC	*quantum satis* (only for pH adjustment)	
Foodstuffs mentioned in Annex V Part 2 and in all other foodstuffs other than those listed in Annexes II and III of Directive 96/34/EC	Directive 96/34/EC	*quantum satis*	See Annex B

Actual levels of use of Calcium Carbonate

In the framework of Regulation (EC) No 1333/2008[22] on food additives and of Commission Regulation (EU) No 257/2010[23] regarding the re-evaluation of approved food additives, EFSA issued a public call for scientific data on calcium carbonate (E 170i)[19] related to:

- The conditions of use (function, uses and use levels) other than as a food colour of calcium carbonate as a miscellaneous food additive.

[20] Council Directive 73/241/EEC of 24 July 1973 on the approximationof the laws of the Member States relating to coca and chocolate products intended for human consumtion. OJ L 228, 16.8.1973, p 23.
[21] Council Directive 93/77/EEC of 21 September 1993 relating to fruit and certain similar products. OJ L 244, 30.9.1993, p23.
[22] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p16-33.
[23] Commission Regulation (EU) No 257/2010 of 15 March 2010 stting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parlieament and of the Council on food additives.

- The use and use levels of calcium carbonate as a nutrient source added for specific nutritional purposes in foods for particular nutritional uses (PARNUTs) and in food supplements.

Table 7 in Annex B summarises the information on current use levels of calcium carbonate made available to the Panel for several food categories following this call for data[19].

2.7. Information on existing authorisations and evaluations

Calcium carbonate is authorised as a food colour in the EU under Directive 94/36/EC, and is also authorised as an additive generally permitted in foodstuffs under Directive 95/2/EC. It is also included in Directive 2001/15/EC[24]on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC[25] relating to food supplements, and it can be used in fortified foods according to Regulation 1925/2006[26] on the addition of vitamins and minerals and of certain other substances in food. Calcium carbonate has been evaluated by JECFA in 1965 (JECFA, 1965, 1966), when the Committee established an Acceptable Daily Intake (ADI) not limited. The SCF evaluated calcium carbonate as part of a group of carbonates, and assigned a group ADI not specified (SCF, 1991). Calcium carbonate together with other carbonates has also been reviewed by TemaNord, who concluded that there was no need for a re-evaluation (TemaNord, 2002). The SCF allocated a Tolerable Upper Intake Level (UL) for calcium of 2500 mg/person/day as a nutrient, and also established a Population Reference Intake (PRI) of 700 mg calcium/day (range 400-1200 mg/day depending on age and physiological status) (SCF, 2003).

Calcium carbonate is included in Commission Decision 2006/257[27],establishing an inventory of ingredients in cosmetic products. In pharmaceuticals calcium carbonate is used as an excipient and as an active ingredient of antacids (Ph. Eur. Comment., 2009). A monograph on calcium carbonate is part of the European Pharmacopoeia (Ph. Eur., 2010). It is also included in Directive 91/414[28] concerning the placing of plant products on the market. Calcium carbonate has been registered under the REACH Regulation 1907/2006[29] (ECHA, 2011). EFSA have previously provided opinions on the calcium salt calcium citrate malate as a source for calcium for use in foods for particular nutritional uses and in foods for the general population (including food supplements) (EFSA, 2007) and on calcium sulphate as a source of calcium in food supplements (EFSA, 2008b). Calcium carbonate is included in the Food and Drug Administration (FDA) list of food additives that are GRAS for use in nutrient and dietary supplements, and is also certified by the FDA for use in amounts consistent with good manufacturing practice to colour drugs generally (FDA, 2006).

2.8. Exposure Assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the Scientific Co-operation (SCOOP) Task 4.2, to estimate additives' intakes (EC, 1998). In the tiered approach, Tier 1 is based on theoretical food consumption data and MPLs for food additives as permitted by relevant Community legislation. The Second and Third Tiers refer to assessment at the

[24] Commission Directive 2001/15/EC of February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 2 2.22.2001, p 19.
[25] Commission Directive 2002/46/EC of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183 of 12.7.2002, p.51
[26] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26-38.
[27] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p.1-528.
[28] Council Directive 91/414/EEC concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1-32.
[29] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006, p. 1-849.

level of individual Member States, combining national data on food consumption with the maximum permitted usage levels for the food additive (Tier 2) and with its actual usage patterns (Tier 3).

2.8.1. Crude estimates (Budget Method)

As calcium carbonate is permitted at *quantum satis* in all foodstuffs, the Panel could not estimate the dietary exposure using the Budget method (Tier 1).

2.8.2. Refined estimates

As with the exception of cocoa and cocoa products all applications are *quantum satis*, no Tier 2 calculations were performed.

Tier 3

Exposure estimates have been performed by the Panel based on the EFSA Comprehensive European Food Consumption Database (EFSA, 2011), which provides summary statistics for a number of European countries. For the purposes of this assessment, exposure estimates have been calculated based on data suitable for long-term (chronic) intake assessment for the population groups mentioned in Table 3:

Table 3. Population groups considered for the exposure estimates of calcium carbonate

Population	Age range	Countries with chronic food consumption statistics
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, UK
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Children	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Toddlers	from 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, Netherlands, Spain

In order to refine the exposure assessment for children and adults to food additives, the Panel has defined some rules to identify maximum reported use levels based on maximum actual usage or maximum analytical data. The rules followed in order to deal with *quantum satis* authorisation (for use as colour), with use level data or observed analytical data, are given in Annex A.

As calcium carbonate is generally permitted at *quantum satis* in all foodstuffs, exposure estimates are based on food-categories for which usage information was received (see Table 7 in Annex B). A number of assumptions had to be made in assigning concentration values to food categories, which are detailed in Annex B.

Usage data in the following food categories (Table 4) were provided and were included in the exposure assessment taking into account *quantum satis* rules for colours (see Annex A) and assumptions detailed in Annex B.

Table 4. Concentration levels of calcium carbonate used for exposure estimates from its use as a food additive and added nutrient source (fortification)

Food category	Calcium carbonate as a food additive (mg/kg)	Calcium carbonate as added nutrient source (fortification) (mg/kg)
Grain milling products	6000[1]	3650[1]
Bread and rolls	3000[1]	9800[1]
Breakfast cereals	2250[1]	13 500[1]
Fine bakery wares	1970[1]	1825[1]
Cocoa beans and cocoa products	1250[2]	11 250[1]
Fish products	2250[1]	0
Chocolate (cocoa) products	1250[2]	11 250[1]
Soft drinks	500[3]	0
Baking ingredients	12 000[1]	0
Cereal-based food for infants and young children	100[1]	1000[4]
Ready-to-eat meal for infants and young children	100[1]	1000[4]
Ready to eat soups	500[3]	0
Salt	20 000[1]	0
Food Supplements	0	800 mg calcium/day

[1] Based on usage data received from industry or Member States following the call for data (see Annex B for individual data contributions)

[2] According to Directive 95/2/EC and based on Directive 73/241/EEC (see Annex B for assumptions made)

[3] According to *quantus satis* rules for food colours in Directive 96/34/EC

[4] Use of calcium based on Directive 2006/15 (see Annex B for assumptions made)

Three exposure estimates based on the Comprehensive Food Consumption Database and usage data provided by industry and Member States have been calculated taking into account different sources of exposure. These were:

Scenario 1 Exposure to calcium carbonate/calcium from the use of calcium carbonate as a food additive

Scenario 2 Exposure to calcium carbonate/calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification)

Scenario 3 Exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification) and from consumption of food supplements

Table 5 summarises the anticipated exposure to calcium from the use of calcium carbonate in the three scenarios mentioned above in the different population groups (adults, adolescents, children and toddlers). For full details of the estimates for scenarios 1-3 see Tables 8-10 in Annex C. In order to facilitate comparison with the UL for calcium established by the SCF in 2003, all exposure values calculated for calcium carbonate in scenarios 1-3 have also been converted to calcium.

None of these three scenarios, however, take into account intake of naturally occurring calcium. To provide information on calcium intake from all dietary sources, data from the literature on total

calcium intake available from various Member States and for different population groups has also been provided (see Table 6).

Table 5. Anticipated exposure to calcium from the use of calcium carbonate in the three scenarios in adult, adolescent, children and toddler populations.

	Daily intake of calcium (mg/day)							
	Adults (60 kg)		**Adolescents (50 kg)**		**Children (30 kg)**		**Toddlers (15 kg)**	
	Mean	**95th percentile**	**Mean**	**95th percentile**	**Mean**	**95th percentile**	**Mean**	**95th percentile**
Scenario 1	200-320	320-560	230-430	380-680	230-410	350-650	170-260	245-425
Scenario 2	440-740	620-1580	530-930	930-1580	470-980	650-1700	305-575	470-905
Scenario 3	1240-1540	1420-2380	1330-1730	1730-2380	1270-1780	1450-2500	1105-1375	1270-1705

Table 5 provides the ranges for mean and 95th percentile intake estimates for scenarios 1-3, calculated for adults, adolescents, children and toddlers, based on food consumption data contained in the comprehensive food consumption database provided by a number of different Member States (EFSA, 2011).

The estimated mean calcium intake from the use of calcium carbonate as food additive (scenario 1) ranges from 170-260 mg calcium/day for toddlers, 230-410 mg calcium/day for children, 230-430 mg calcium/day for adolescents and 200-320 mg calcium/day for adults. The ranges of high percentile intakes are 245-425 mg calcium/day for toddlers, 350-650 mg calcium/day for children, 380-680 mg calcium/day for adolescents and 320-560 mg calcium/day for adults

The estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) (scenario 2) ranges from 305-575 mg calcium/day for toddlers, 470-980 mg calcium/day for children, 530-930 mg calcium/day for adolescents and 440-740 mg calcium/day for adults. The ranges of high percentile intakes are 470-905 mg calcium/day for toddlers, 650-1700 mg calcium/day for children, 930-1580 mg calcium/day for adolescents and 620-1580 mg calcium/day for adults

The estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) ranges from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

As can be seen in Table 5, the use of calcium carbonate as added nutrient source (fortification) in addition to its use as food additive, doubles or in some cases trebles intake estimates of calcium across all population groups (scenario 2 compared to scenario 1). This is expected, as reported use levels of calcium carbonate as added nutrient source are, for the majority of food categories, much higher than reported use levels for its use as additive (see Table 4). The intake of calcium-containing supplements (estimated at 800 mg calcium/day) contributes between 32-72% to the overall estimated intake of calcium from use as a food additive, nutrient source and intake from food supplements (scenario 3), whilst food additive only application (scenario 1) contributes between 15-30%.

2.8.3. Total estimated intake of calcium from all sources (food additive, fortification, supplement and natural sources) based on published dietary survey data

Further information on total estimated intake of calcium from all sources, including naturally occurring calcium, is available for a number of Member States, based on compositional information of foodstuffs consumed. For all countries, compositional nutrient data used to derive total calcium intake originate from analyses conducted in laboratories, supplemented by other sources. Other data sources include scientific publications, food composition tables, derived nutrients from comparable foods, calculations from recipes and estimations and information from manufacturers. Albeit not all encompassing, the so estimated calcium intake already takes into account use as a food additive and added nutrient source to a large degree. Table 6 provides a summary of these data, presenting the ranges of mean and 95th percentile intakes (for full details see Table 11, Annex D).

Information on major dietary contributors to calcium intake was available for some countries listed in Table 11. For Dutch children (Ocke et al, 2008), dairy products were the main sources of calcium (69%). For UK adults (Henderson et al, 2003), milk and milk products contributed 43% and cereal and cereal products contributed 30%. For Irish adults (IUNA, 2011), milk, yoghurt and cheese contributed 39%, followed by breads and breakfast cereals (25%). In France, (AFSSA, 2009), milk and dairy products contributed 43% to total calcium intake in adults and 49% in children and adolescents (3-17yrs), respectively. These findings suggest that dairy products are the single most important contributor to calcium intake from the diet.

Table 6. Anticipated exposure to calcium from all dietary sources, based on published dietary survey data

Daily intake of calcium (mg/day)							
Adults (60 kg)		**Adolescents (50 kg)**		**Children (30 kg)**		**Toddlers (15 kg)**	
Mean	**95th percentile**	**Mean**	**95thpercentile**	**Mean**	**95th percentile**	**Mean**	**95th percentile**
730-1164	1233-2106	738-1070	1306-1905	749-869	1124-1453	664-795	1070-1184

Table 6 shows that mean calcium intakes (including from food additives, fortification and food supplements) in all population groups are of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium/day for adults. The range of high percentile intakes are 1070 – 1184 mg calcium/day for toddlers, 1124-1453 mg calcium/day for children, 1306 – 1905 for adolescents and 1233 – 1851 for adults. Mean intake for the elderly (>64 years old) ranges from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The range of mean calcium intakes from use as a food additive, added nutrient source (fortification) and intake of food supplements calculated for the different age groups in this opinion (Scenario 3) are somewhat higher than the values reported in Table 6. This is expected as exposure estimates calculated in this opinion are rather conservative, as they are based on maximum reported use levels and are assumed to always be present in all foods covered by the assessment, which in practice is unlikely.

2.8.4. Dietary exposure to aluminium

The Panel derived anticipated exposure to aluminium arising from the intake of calcium from all sources, as estimated by the Panel and presented in Table 6. Assuming that calcium carbonate contributes approximately 20% to the total calcium intakes provided in Table 6 (Panel estimate), and assuming a concentration of 200 mg aluminium/kg calcium carbonate, the ranges of the mean and 95th percentile of the aluminium intakes across the population groups are 0.07-0.09 and 0.11-0.16 mg/kg bw/day, respectively, which is equivalent to an aluminium mean intake of 0.49-0.63 mg/kg bw/week

and 95th percentile intake of 0.77-1.12 mg/kg bw/week. Hence, the aluminium intake contributions from the consumption of calcium carbonate to the TWI for aluminium of 1 mg/kg bw/week would be 49-63% at the mean and 77-112% at the 95th percentile.

3. BIOLOGICAL AND TOXICOLOGICAL DATA

No toxicological or biological information was submitted for the re-evaluation of calcium carbonate following a public call for data[17], prior to the start of this re-evaluation. A literature search was conducted on the most commonly available online databases for toxicological and biological information (PubMed, Science Direct, Toxline and Web of Knowledge), to cover recent published literature on calcium carbonate. An additional source of information was the registration dossier provided by industry for calcium carbonate under the REACH Regulation 1907/2006, published by the European Chemicals Agency (ECHA, 2011) and unpublished study reports provided by CCA-Europe (2011).

The present opinion briefly summarises a number of the most relevant toxicological studies on calcium carbonate that were reviewed by the SCF in its 2003 opinion, establishing an UL for calcium (SCF, 2003). It describes in more detail any new toxicological data published since the SCF opinion (2003), or earlier published studies that were not included in that opinion, including new information available in the ECHA registration dossier (ECHA, 2011). It also provides a brief overview of the adverse effects of high calcium intakes in humans as described in the SCF opinion (2003) establishing a UL for calcium (SCF, 2003). Given that the opinion is directed towards an evaluation of the safety of calcium carbonate as a food additive and that the safety of calcium in terms of amounts that may be consumed is outside the remit of this Panel, further details of the human studies on which the UL is based are not included, and the SCF opinion should be consulted for this information. However, details of some new human studies published since the SCF review in 2003 that are considered relevant to the safety assessment of calcium carbonate as a food additive are included.

3.1. Absorption, distribution, metabolism, excretion

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion, the bulk (89-90%) of unabsorbed calcium is complexed to bile acids, free fatty acids and oxalic acid and excreted with the faeces (Heaney, 2002). As reported by EFSA, absorption of calcium from calcium carbonate in rats and humans is comparable to that from calcium citrate malate, average absorption of calcium from calcium carbonate over a range of studies being in the range of 20-40% (EFSA, 2007).

As reported by SCF (2003), after intestinal absorption, calcium and carbonate/bicarbonate ions can enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also via faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration (SCF, 2003).

Animal studies not included in the SCF opinion (2003)

Meiron and co-workers have examined the bioavailability of calcium from a stable amorphous calcium carbonate (ACC) (Meiron et al. 2011). ACC was prepared by a novel synthetic method using phosphoaminoacids to stabilise this form of calcium carbonate, as described by Bentov et al. (2010). The study compared the solubility and fractional absorption of ACC (particle diameter 40 to 120 nm), ACC with chitosan (ACC-C), and crystalline calcium carbonate (CCC) (particle diameter 1 to 10 µm). Solubility was evaluated by dissolving these preparations in dilute phosphoric acid. The results demonstrated that both ACC and ACC-C were more soluble than CCC, ACC being reported to be approximately 10 times more soluble than CCC (see also section 2.1). Fractional absorption was

evaluated by intrinsically labelling calcium carbonate preparations with ^{45}Ca, which were then orally administered (12.5 mg of CCC, 15 mg of ACC, or 18.5 mg of ACC-C) to rats (17 per group) using gelatin capsules. Fractional absorption was determined by evaluating the percentage of the administered radioactive dose per millilitre in the serum, calcium deposition in the femur, and whole-body retention over a 34-hour period. Calcium serum analysis revealed that calcium absorption from ACC and ACC-C preparations was up to 40% higher than from CCC, whereas retention of ACC and ACC-C was up to 26.5% higher than from CCC. Absorbed calcium in the femurs of rats given ACC was 30% higher than in CCC-treated animals, whereas 15% more calcium was absorbed following ACC-C treatment than following CCC treatment. The authors considered that their study demonstrated the enhanced solubility and bioavailability of ACC compared with CCC (Meiron et al. 2011). The Panel noted that the information provided on the particle size of the tested amorphous calcium carbonate indicates that this preparation contained particles with sizes within the nanoscale range.

The absorption of calcium from calcium carbonate in microparticulate form (particle diameter 3.77 ± 0.76 μm) or in nanoparticulate form (as described by the authors, particle diameter 0.15 ± 0.02 μm, prepared from the microparticulate form with the aid of a pulsed air-flow pulverizer) was compared in ovariectomised ICR mice (Huang et al., 2009). Female mice (n=6 per group) were given vitamin D3 (261 U/kg bw) together with either micro calcium carbonate or nano calcium carbonate daily by gavage for 28 days at a dose of 1300 mg/kg bw. Two additional groups of mice were either ovariectomised or sham-ovariectomised, and received Vitamin D^3 but no calcium salt supplementation. Calcium absorption was measured by determination of serum calcium concentrations at 2, 6, 12 or 24 hours after the last calcium supplementation, and bone mineral density (BMD) of the whole body was determined by dual-energy x-ray absorptiometry (pDEXA). At 6 hours post-administration, increased serum calcium concentrations were observed in the nano calcium carbonate group when compared to the micro calcium carbonate group. The mean increase was small (9.1%), and no significant differences were observed at 2, 12 or 24 hours post-administration. The BMD of the whole body in ovariectomised mice was significantly lower than in sham-ovariectomised mice, and was greater in both the micro- and nano calcium carbonate-treated mice than in the controls. The BMD of the nano calcium carbonate-treated mice was significantly increased (by 7.1%) when compared to the micro calcium carbonate-treated mice (Huang et al., 2009). In view of the small increases in serum calcium observed in this study, the Panel considered that the absorption of calcium from nanoparticulate calcium carbonate would be not markedly different than that of microparticulate calcium carbonate.

Human studies not included in the SCF opinion (2003)

Absorption of calcium from radiolabelled (^{45}Ca) calcium carbonate or calcium citrate was compared in 37 healthy adult men and women given a single dose of either 300 mg (17 women only) or 1000 mg calcium (10 men and 10 women), ingested as part of a light breakfast meal (Heaney et al., 1999). The authors did not provide details on the form of calcium carbonate used. Absorption of calcium was measured following the 1000 mg calcium dose by tracer appearance in serum and by the absorptive increment in urinary calcium and at the 300 mg calcium dose by the tracer method only. Mean tracer absorption (for both salts combined) was 36.0% at the 300 mg calcium load and 28.4% at the 1000 mg calcium load. When the extent of absorption of the two salts of calcium was compared, the mean difference in absorption was small, calcium from calcium carbonate being significantly better absorbed than from calcium citrate at the 1000 mg calcium dose, while at the 300 mg calcium dose the reverse was reported. There was no significant difference between the men and the women tested in the 1000 mg calcium load experiment.

In contrast, in a study in 18 postmenopausal normal women given 500 mg calcium either as calcium carbonate or calcium citrate, the investigators found that calcium from calcium citrate was 2.5 fold more bioavailable than from calcium carbonate, based on measurement of AUC for serum calcium

(Heller et al, 1999). The authors did not provide details on the form of calcium carbonate used, other than it was a commercial calcium carbonate supplement.

In a study designed to evaluate the effect of calcium carbonate and sevelamer hydrochloride (SEV), a calcium-free phosphate binder, on serum calcium levels and urinary calcium excretion, twelve healthy male individuals were given calcium carbonate (500 mg, no details on the form of calcium carbonate used was indicated by the authors) or placebo daily for 6 days with 1-week washout between the treatment periods (Heinrich et al., 2008). SEV (800 mg) was administered in parallel to either calcium carbonate or placebo. During the study, weekly blood samples were taken and 24-hour urine was collected each day for measurement of calcium, magnesium, phosphorous, chloride and intact parathyroid hormone. Intake of calcium carbonate as compared to placebo was associated with an increase in the total amount of calcium absorbed and increased urinary calcium excretion in healthy individuals while serum calcium concentrations remained unaffected.

The calcium bioavailability of orally administered pearl powder (PP, a form of calcium carbonate prepared with a dry grinder) in nanonised form (NPP, pearl powder nanonised, particle diameter 0.04 to 0.4 μm) and micronised form (MPP, pearl powder micronised, particle diameter 4 to 300 μm) was compared in human subjects (Chen et al., 2008). A group of 28 adults (14 females, 14 males) free from hyperthyroidism, hypercalcemia and hypocalcemia were recruited as the subjects for the study. The bioavailability of NPP and MPP was measured by determination of serum calcium concentrations at 2, 4 and 6 hours after administration of either preparation. Serum intact parathyroid hormone (iPTH) was also measured at 0 and 4 hours after administration, and urinary creatinine was measured within 1 hour of administration, for determination of urinary calcium/creatinine ratio. At 2 hours post-administration, increased serum calcium concentrations were observed in the nano calcium carbonate (NPP) group when compared to the micro calcium carbonate (MPP) group. No significant differences were observed at 4 or 6 hours post-administration. The comparative bioavailability, $AUC_{0–6\ hours}$ for NPP was 38% greater than that for MPP, indicating better absorption of calcium from NPP than from MPP. According to the authors, the results showed better absorption and retention of calcium from NPP than from MPP, as reflected by a shorter elapsed time before the maximum concentration of calcium appeared in the serum and higher maximum calcium concentration (C_{max} at 2 hours post-administration) in serum after administration. iPTH was reduced after administration of pearl powder, the reduction being greater after administration of NPP than after MPP. However, no significant differences in calcium/creatinine ratio were observed between the NPP and MPP treatments. The authors concluded that nanonisation of pearl powder improves calcium bioavailability from this source in humans. Although a conventional form of calcium carbonate used was not directly tested in this study, the Panel noted that these results would support the data in ovariectomised mice from Huang et al. (2009) described above, which indicated a small increase in absorption of calcium from calcium carbonate in nanoparticles by comparison to the corresponding microparticles. However, the Panel noted that both studies were of short duration.

3.2. Toxicological data

3.2.1. Acute toxicity

The LD_{50} of calcium carbonate has been reported to be 6450 mg/kg bw, indicating that it is of low acute toxicity (Marhold, 1972).

In a study comparing the acute toxicity of nanoparticulate versus microparticulate calcium carbonate, groups of 8 male and 8 female ICR mice were administered a single dose of 1300 mg/kg bw of either the nano or the micro form by gavage, together with 261 U vitamin D3/kg bw, and observed for a 7 day period after dosing (Huang et al., 2009). No mortality or other treatment-related changes were observed in either group.

In a recent acute toxicity study, no treatment-related effects were seen in female Sprague-Dawley rats (n=5) administered a single oral dose of 2000 mg calcium carbonate/kg bw by gavage. The substance was described as calcium carbonate, with no further details on the form that was tested. The LD_{50} of calcium carbonate was estimated to be > 2000 mg/kg bw (SafePharm Laboratories, 2009).

3.2.2. Short-term and subchronic toxicity

In a study designed to induce chronic hypercalcaemia, groups of ten male Sprague Dawley rats were given increased amounts of calcium carbonate in their diet (levels of 4% or 8% elemental calcium w/w, equivalent to approximately 5000 or 10 000 mg/kg bw/day calcium carbonate, compared with the normal dietary content of 0.71%) (Puerro Vicente et al., 1993). No details on the form of calcium carbonate used were indicated by the authors. Other groups of rats were given calcium chloride in drinking water (1.5% or 2%) or were given a single intramuscular injection of vitamin D_1 on the day on which calcium treatment commenced. One group received 4% elemental calcium in the diet together with 1.5% calcium chloride in drinking water. Groups of 10 rats were killed after 2 and 14 days of treatment respectively, and blood samples taken for determination of total calcium. Other than determination of total calcium in blood, the only parameters investigated in the study were food consumption and body weight gain. Increases in blood calcium were seen after 2 days of treatment with either 4% or 8% elemental calcium (from approximately 100 mg/l to approximately 130 mg/l for either treatment), showing that calcium from calcium carbonate and/or calcium chloride was bioavailable; levels dropped slightly after 14 days of treatment. A reduction in food consumption in the calcium-treated animals resulted in a reduction in body weight gain, manifest throughout the experimental period. No other treatment-related changes were reported by the authors.

The effects of a high calcium carbonate diet or a high calcium citrate diet in comparison to a control diet on mineral concentrations in several tissues of rats were examined (Takasugi et al., 2005). Male Wistar rats (n= 5 per group) aged 5 weeks were fed one of the experimental diets for 4 weeks. The control diet contained 0.5% calcium as calcium carbonate. The authors did not provide information on the form of calcium carbonate used. High calcium diets contained 2.5% calcium as calcium carbonate or calcium citrate (1250 mg calcium/kg bw/day, equivalent to 3125 mg/kg bw/day calcium carbonate). Feed intake was measured daily and body weight was measured weekly. Blood samples were collected from the abdominal aorta, and the testis, kidney, liver and femur were taken for analysis of calcium content. Calcium, phosporus, magnesium, iron, copper and zinc concentrations in the tissues and plasma were determined using inductively coupled plasma emission spectrometry. Neither of the high calcium diets affected feed intake but body weight gain was significantly lower in both of the high calcium diet groups (approximately 15% lower, $p< 0.05$) than in the control group. Feed efficiency was also significantly lower in the high calcium groups than in the control group. The high calcium diets did not affect the calcium concentration in the plasma and tissues. Both of the high calcium diets produced a decrease in plasma phosphorus and also in iron levels in the testis, liver and femur. Hepatic copper increased in rats fed the high calcium diets whereas renal copper decreased. The high calcium carbonate diet increased femoral zinc and decreased femoral magnesium but in contrast the high calcium citrate diet did not affect these minerals in the femur. The authors considered that these effects might be due to different effects of the two calcium salts on intestinal pH, with calcium citrate causing a decrease in intestinal pH and thus enhancing the uptake of iron and other minerals. In contrast, as argued by the authors, calcium carbonate may increase the intestinal pH in rats (Koba et al., 2001), thereby reducing the availability of iron (Prather and Miller, 1992). Takasugi and co-workers, based on their results, suggest overall that the effect of excess calcium on minerals partly depends on its form (Takasugi et al., 2005).

As part of a series of studies designed to examine the bioavailability, acute and subacute toxicity of nanoparticulate (as described by the authors, 151 ± 19 nm) calcium carbonate and calcium citrate (396 ± 41 nm) ICR male and female mice (n=8 per group) were given 0, 13, 130 or 1300 mg/kg bw/day of nano calcium carbonate or calcium citrate orally by gavage for 28 days. The animals were monitored

for clinical signs of toxicity, body weights were recorded at the commencement of the study and at termination, and a macroscopic pathological examination was carried out. No treatment-related changes in any of these parameters were reported in any group (Huang et al., 2009).

A combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with "nano" calcium carbonate was carried out in Wistar rats (Han™:HsdRccHan™:WIST strain, n= 10 animals/sex/group), in accordance with OECD Guideline 422 (Harlan Laboratories, 2010a). According to CCA-Europe the material tested had a particle size of 60 - 100 nm when examined by SEM, although particle size determination using other methods such as Sedigraph and Malvern showed higher apparent particle size due to aggregation (CCA-Europe, 2011). A particle size analysis submitted by CCA-Europe for the nano material using Sedigraph indicated a median particle diameter of 0.58 $\pm$ 0.6 µm. Animals were administered the nano calcium carbonate by gavage as a suspension in distilled water at dose levels of 100, 300 and 1000 mg/kg bw/day for up to 48 days, including a two week maturation phase, pairing, gestation and early lactation for females. The study was preceded by a 14-day preliminary toxicity study using levels of up to 1000 mg/kg bw/day nano calcium carbonate administered by gavage, in which no toxicologically significant treatment-related changes were seen. Males were killed on day 43 of the study, while females were killed on day 5 post partum. Full clinical, behavioural, biochemical, haematological gross pathological and histopathological examinations were carried out on 5 males and 5 females per group. One male treated with 1000 mg/kg bw/day was killed *in extremis* on day 39, due to misdosing. Otherwise, no treatment-related effects were seen on clinical behaviour and in the functional observation battery. Body weights and body weight gain, food and water consumption and food efficiency were comparable between control and treated groups throughout the study. Males treated with 1000 mg calcium carbonate/kg bw/day showed a statistically significant reduction in mean corpuscular haemoglobin (5.4% decrease) and mean corpuscular volume (5.8% decrease), but individual values were within the normal ranges for rats of the strain and age used and were considered by the study authors not to be of toxicological importance. All other haematological results were comparable in test animals and controls. Males treated with 1000 mg calcium carbonate/kg bw/day also showed a statistically significant reduction in total plasma protein (5.4% decrease) and a statistically significant increase in plasma chloride concentration (1.9% increase), while males from all treatment groups also showed statistically significant reductions in plasma phosphorus levels (from 1.74 mmol/l in controls to 1.28, 1.24 and 1.22 mmol/l respectively in the groups receiving 100, 300 or 1000 mg calcium carbonate/kg bw/day). Individual values for these parameters were however within the normal ranges for rats of the strain and age used, and were considered by the study authors not to be of toxicological importance. Similar changes were not reported in the female rats receiving nano calcium carbonate, other than a slight, not statistically significant decrease in plasma phosphorus levels in females receiving either 300 or 1000 mg calcium carbonate/kg bw/day. While isolated statistically significant differences were seen in organ weights (absolute and relative) in treated groups relative to controls, none of these were dose-related and they were considered by the study authors to be unrelated to treatment. No treatment-related macroscopic or microscopic abnormalities were reported. The authors concluded that the No-Observed-Adverse-Effect-Level (NOAEL) for systemic toxicity was 1000 mg calcium carbonate/kg bw/day, the highest dose tested. The Panel agreed with this NOAEL, considering the minor changes in haematological parameters in males treated with 1000 mg calcium carbonate/kg bw/day to be non-adverse. The results of the reproductive/developmental phase of this study are reported in Section 3.2.5.

As reported by EFSA, a 91-day feeding study (preceded by a 28-day study of similar design) in Sprague-Dawley rats compared the effects of calcium carbonate to those of calcium citrate malate (EFSA, 2007). The authors did not provide any details on the form of calcium carbonate used. One hundred (50 male and 50 female) rats divided into 5 treatment groups were fed a semi-purified diet. Three groups were fed calcium sources to deliver 0.5% calcium in the diet (equivalent to ~250 mg calcium/kg bw/day): a calcium carbonate group (group 1), a calcium carbonate plus citric and malic acids group (group 2) receiving the same levels of the individual acids as group 3, and a calcium

citrate malate group (group 3). Two more groups were fed calcium sources to deliver 1% calcium in the diet (equivalent to ~500 mg calcium/kg bw/day), a calcium carbonate plus calcium citrate malate group (group 4) and a calcium carbonate group (group 5). Parameters evaluated included body weight and food consumption, in-life and gross necropsy observations, clinical chemistry, haematology, and urinalysis, kidney calcium content; spleen and liver iron content, fat-free dry weight, ash, and magnesium and phosphorus content of the left femur; selected organ weights, and histology of kidneys, thyroid glands, parathyroid glands, and left tibia. Daily and weekly observations revealed no treatment-related effects. No statistically significant differences in body weight gain or feed efficiency were reported in rats of either sex supplemented with calcium as calcium carbonate, compared to those receiving calcium citrate malate. Male and female rats in group 4 and female rats in group 5 showed increases in feed consumption compared to group 1 animals (low calcium carbonate). These were attributed to differences in utilisation efficiency of the diets since increased feed consumption was not associated with an increase in body weight. In female rats supplemented with calcium carbonate plus calcium citrate malate phosphorus content of femurs was significantly higher. The histopathological evaluation of kidneys revealed nephrocalcinosis in all groups for both sexes. In males, this condition was less severe in groups 1, 2, and 3 (fed ~250 mg calcium/kg bw/day) compared to groups 4 and 5 (fed ~500 mg calcium/kg bw/day). Nephrocalcinosis was observed in all females except one animal in group 3 and was more severe than that of the males in the corresponding treatment groups (EFSA, 2007).

In a study designed to examine the effects of increasing calcium on apoptosis and cell proliferation in normal murine colonic crypt epithelium, 21-day old male mice (n= 10 per group) were fed either control diet or one supplemented with 1% calcium carbonate (equivalent to 1500 mg/kg bw/day) for 12 weeks (Penman et al., 2000). Initial and final body weights and body weight changes were similar between the two groups, and no signs of toxicity were noted in the calcium-supplemented group. Calcium treatment was associated with an increase in apoptotic index in the distal but not proximal colon. In the proximal colon of the calcium treated group there was also a minor but significant shift towards the crypt base as the mean position at which apoptotic cells were recorded (Penman et al., 2000).

As reported by EFSA (EFSA, 2007), a 91-day feeding study comparing the effects of calcium carbonate to those of calcium citrate malate was conducted in groups of five male and five female Beagle dogs using a similar experimental design as that for the rat study described above. No treatment-related effects of biological significance were observed in any of the groups. Notably, contrary to the findings in the rat studies, it was reported that there were no apparent treatment-related histopathological lesions (e.g. kidney) and that any pathology seen was either of low incidence or present across all groups equally (EFSA, 2007).

Adult cats and kittens were maintained on high calcium (17.7 mmol calcium/MJ) diets or normal calcium diets (9.5 mmol calcium/MJ) for up to 31 weeks, in a study designed to compare the efficacy of calcium carbonate and calcium chloride as sources of calcium (Pastoor et al., 1994). In both cats and kittens fed high calcium diets, urinary concentrations of magnesium and phosphorus and apparent absorption of these minerals were lower than after feeding the normal calcium diets. Urinary pH and phosphorus concentration were lower in cats and kittens fed diets with calcium chloride instead of calcium carbonate. Body weight gain and tibia growth in the kittens tended to be greater after feeding the diets with calcium chloride. Calcium chloride versus calcium carbonate and also supplemental calcium chloride in the high calcium diet significantly stimulated femur density and reduced renal calcium concentrations (Pastoor et al., 1994).

3.2.3. Genotoxicity

Nanoform calcium carbonate was tested in three in vitro assays according to OECD guidelines and in compliance with GLP (CCA-Europe, 2011). According to CCA-Europe the material tested had a

particle size of 60 - 100 nm when examined by SEM, although particle size determination using Sedigraph showed higher apparent particle size due to aggregation (CCA-Europe, 2011). This was the same material as was tested in the combined repeat dose oral toxicity/reproduction/developmental toxicity screening study described in Section 3.2.2. This nanoform calcium carbonate gave negative results in a bacterial reverse mutation assay according to OECD TG 471, in *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A and using both the direct plate incorporation and pre-incubation methods (Harlan Laboratories, 2010b). In this study nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations of 50, 150, 500, 1500 or 5000 µg/plate, with and without metabolic activation. No evidence of toxicity was seen at any of the concentrations tested, and there was no precipitation of test material when the direct plate incorporation method was used. However, when the pre-incubation method was used precipitation of test material was evident at both the 1500 and 5000 µg/plate test concentrations, in the absence and presence of S9-mix, respectively. There was no evidence of a treatment-related increase in revertants in any of the treatment groups (Harlan Laboratories, 2010b).

A negative result was also obtained for this nanoform calcium carbonate in a mammalian cell gene mutation assay (OECD TG 476) using mouse lymphoma L5178Y TK+/- cells (Harlan Laboratories, 2010c). In this study nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations in the range of 7.81 to 250 µg/ml, and exposure periods of 4 hours with and without metabolic activation and 24 hours without metabolic activation were used. In a preliminary toxicity test there was no evidence of marked toxicity of the test substance and maximum test concentrations achievable were limited by precipitation of calcium carbonate, which was observed at and above 62.5 µg/ml at the 4 hour exposure period and above 15.6 µg/ml at the 24 hour exposure period. No evidence of a dose-related increase in mutant frequency was seen at any dose level in any of the 3 exposure groups (Harlan Laboratories, 2010c).

Nanoparticulate calcium carbonate (median particle diameter of 0.58 $\pm$ 0.6 µm) was not clastogenic in human lymphocytes in an *in vitro* mammalian chromosome aberration test conducted in accordance with OECD TG 473 (Harlan Laboratories, 2010d). Three treatment conditions were used in the study, namely a 4 hour exposure period in the absence of metabolic activation followed by a 20 hour expression period, a 4 hour exposure period in the presence of metabolic activation followed by a 20 hour expression period, and a 24 hour continuous exposure period in the absence of metabolic activation. Nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations in the range of 31.25 to 1000 µg/ml for each of the three treatment conditions. Toxicity was not marked up to the highest concentration level of 1000 µg/ml, and there was no marked dose-related inhibition of mitotic index. Although precipitation of calcium carbonate was observed at concentrations of 125 µg/ml which persisted onto the slides at and above 500 µg/ml in the 4- and 20-hour group without S9 and at 1000 µg/ml in the 4- and 20-hour group with S9 and in the 24-hour group without S9, this did not impede the scoring of chromosome aberrations except at the highest test concentration of 1000 µg/ml calcium carbonate. There was no evidence of a dose-related increase in chromosome aberrations up to test concentrations of 500 µg/ml calcium carbonate (Harlan Laboratories, 2010d).

Overall, the Panel considered that based on these data there is no concern regarding the genotoxicity of calcium carbonate

3.2.4. Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies on calcium carbonate have been reported in the open literature or were provided to EFSA.

3.2.5. Reproductive and developmental toxicity

As reported in section 3.2.2, a combined repeat dose oral toxicity/reproduction/developmental toxicity screening study was carried out in Wistar rats (n= 10/sex/group), in accordance with OECD Guideline 422 (Harlan Laboratories, 2010a). Animals were administered nanoform calcium carbonate (characteristics as already described in Section 3.2.2) by gavage at dose levels of 100, 300 and 1000 mg/kg bw/day for up to 48 days, including a two week maturation phase, pairing, gestation and early lactation for females. Pregnant females were allowed to give birth and maintain their offspring until termination on day 5 post partum. Evaluations of each litter size, litter weight, mean offspring weight by sex, clinical observations and landmark developmental signs were also performed during the post partum period. At termination, all offspring, including those dying during the study, were subjected to a full external and internal examination and any macroscopic abnormalities were recorded.

No evidence of treatment-related effects was seen in the parental animals during the study, other than the minor haematological and biochemical changes in males receiving 1000 mg/kg bw/day, as already reported in section 3.2.2. No treatment-related effects were detected on the fertility or mating performance of animals receiving calcium carbonate when compared to controls. All treated animals mated within the first four days of pairing. No treatment-related effects were detected in the length of gestation for treated females when compared to controls. All animals showed gestation lengths between 22 to 23.5 days. All treated females gave birth to a live litter and successfully reared young to day 5 of age. Corpora lutea, implantation counts, litter sizes and pup viability was comparable in all treated groups and controls. There were no differences in litter weights or mean pup body weights between control and treated animals, and no clinical signs of toxicity were detected in pups from treated females when compared to controls. No treatment-related effects were detected for surface righting reflex for pups from treated animals when compared to controls. The incidence, type or distribution of macroscopic findings in pups dying in the post partum period or in pups killed at scheduled termination (day 5 of age) did not indicate any adverse effect of maternal treatment with calcium carbonate. The authors considered that no treatment-related effects were observed on reproduction in this study, and that the NOAEL for reproductive toxicity was 1000 mg/kg bw/day, the highest dose tested. The Panel agreed with this NOAEL.

Fairney and Weir maintained female rats throughout pregnancy on a high calcium diet (3% calcium as calcium carbonate in the chow (equivalent to 1500 mg calcium/kg bw/day and 3750 mg calcium carbonate/kg bw/day) and 4% calcium lactate added to the drinking water (equivalent to 882 mg calcium/kg bw/day and 4800 mg calcium lactate/kg bw/day). The number of dams was not given, but the growth rate of 41 offspring from the resulting litters from hypercalcaemic dams was compared with that from 38 offspring from dams maintained on a normal calcium content diet. The mean plasma calcium level in the high calcium group was 12.7 $\pm$ 1.41/100 ml in the 24 hours after parturition, compared with 10.1 $\pm$ 0.59/100 ml in the control group. The resulting litters were stated by the authors to be smaller in number (data not provided) and the offspring had lower body weights compared with controls. On macroscopic examination the offspring from hypercalcaemic dams showed alopecia, and the kidneys, liver and heart were paler in appearance compared with control offspring. On histopathological examination the kidneys from the former group showed pyelonephritic scarring, but no soft tissue calcification or nephrocalcinosis was seen on radiological examination (Fairney and Weir, 1970).

In a study designed to evaluate the developmental effects of increased dietary calcium in rats fed otherwise nutritionally adequate diets, female Charles River CD/VAF Plus were given dietary levels of 0.5% (control level), 0.75%, 1% or 1.25% calcium as calcium carbonate for 6 weeks before mating, during mating and up to Gestation Day (GD) 20 (Shackelford et al. 1993). These levels were equivalent to 375, 500 or 625 mg/kg bw/day calcium and 937.5, 1250 or 1562.5 mg/kg bw/day calcium carbonate. No dose related changes were observed in maternal clinical findings, average numbers of implantations, fetal resorptions and viable fetuses, nor on fetal length and weight. The

authors concluded that dietary calcium (given as calcium carbonate) was not fetotoxic or teratogenic at the concentrations used in the study. The Panel agreed with this conclusion and considered that 1562.5 mg/kg bw/day calcium carbonate, the highest dose tested, was a NOAEL for reproductive effects in this study. Dose-related increases of the femoral calcium content were however measured in both pregnant and non-pregnant rats receiving increased dietary calcium, and there were also increases in the phosphorus, magnesium and zinc content of the liver in non-pregnant control rats on the same diets (Shackelford et al., 1994). There were dose-related decreases of the iron and copper contents in kidneys of non-pregnant animals and of iron in the liver of pregnant rats. Fetuses showed dose-related decreases in the whole body content of phosphorus, magnesium, iron and copper Changes in mineral levels in rats receiving increased dietary calcium were seen in all dose groups compared with controls, although not all minerals in the organs examined were equally affected (Shackelford et al., 1994).

Richards and Greig evaluated the effect of dietary calcium carbonate (0.3%, 0.5%, 0.7% and 1.1%, equivalent to approximately 450, 750, 1050 or 1650 mg calcium carbonate/kg bw/day) on reproductive performance in Swiss mice and on survival and organ pathology of the litters (Richards and Greig, 1952). Mice were given the experimental diets one week before mating and up to GD 21, when the offspring were killed. All diets with a calcium carbonate content of 1.1% resulted in a decreased number of litters and decreased total litter weight. Litters at this dietary level showed hypertrophy of the heart and atrophy of the thymus when killed at age 21 days. Increased heart weights were negatively correlated with haemoglobin levels. Addition of iron to high-calcium diets reduced the degree of cardiac hypertrophy, and the authors concluded that the effects seen were due to a calcium:iron interaction, with consequential reductions in maternal iron levels and anaemia. However, the authors did not provide any information on maternal toxicity in the study, other than an observation of increased heart weight in the mothers and maternal anaemia.

The effect of diet-induced maternal hypercalcaemia on fetal development was investigated in the CD-1 mouse (Liebgott et al., 1989). The diet for the control group contained 1.2 % calcium, while that for the experimental group contained 3% calcium as calcium carbonate in the chow (1500 mg calcium/kg bw/day, equivalent to 3750 mg/kg bw/day calcium carbonate) and 4% calcium lactate added to the drinking water. The experimental group was maintained on the high calcium diet for at least 10 days before mating, and during pregnancy until GD-18. The number of litters in both the control and experimental group was identical (n=13). However, fetuses from the treated litters showed significantly lower birth weights and higher frequencies of missing calcification centres in the developing skeletons and dentitions. The authors concluded that high maternal calcium levels can result in fetotoxic effects (Liebgott et al., 1989).

3.2.6. Allergenicity, sensitivity and intolerance

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

3.2.7. Other studies

3.2.7.1. Effects of calcium carbonate and other sources of calcium in humans

A number of studies on the effects of calcium carbonate in humans have been published, as summarised by the SCF in their opinion establishing an UL for calcium (SCF, 2003). In summary, calcium carbonate like other calcium salts can cause constipation if taken in large amounts as an antacid (Martindale, 2002). Hypercalcaemia and alkalosis can occur in individuals taking calcium carbonate (between 2.0 and 16.5 g/day of supplementary calcium) with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome), often associated with renal dysfunction, metastatic calcification and other symptomology in the absence of hypercalciuria (Orwoll, 1982; Abreo et al., 1993, as reported in SCF, 2003). Acute hypercalcaemia and recurrent

nephrolithiasis were reported in three subjects regularly consuming large quantities (7 to 15 g daily) of calcium carbonate-sodium bicarbonate powders over a period of 10 years (Robson and Heading, 1978).

Bolland and co-workers have assessed the risk of myocardial infarction and cardiovascular events in individuals given regular calcium supplementation (without co-administered vitamin D) in the management of osteoporosis in a recent study, using a meta-analysis of 15 randomised clinical trials (Bolland et al., 2010). Only 15 of 190 trials originally identified met the pre-specified inclusion (randomised, double blind, placebo controlled trials; elemental calcium was administered at a dose of ≥500 mg/day; the participants' mean age at baseline was more than 40 years old; 100 or more participants were randomised; participants of either sex were studied; and the trial duration was more than one year) and exclusion (calcium and vitamin D given together with a placebo comparator; trials in which calcium was administered in the form of dietary modification or a complex nutritional supplement; and trials in which most participants had a major systemic disease other than osteoporosis) criteria. The calcium dose was generally greater than 1000 mg/person (2 studies used doses of 500 and 750 mg/person respectively and one had two dose levels of 600 and 1200 mg/person) whilst dietary calcium ranged from 406 to 1240 mg/day (generally below 1000 mg/day). Five of these trials used patient level data from 8151 participants and eleven used trial level data, involving 11 921 participants with a mean duration of 4 years. In the five studies contributing patient level data, 143 people given calcium supplementation had a myocardial infarction compared with 111 given placebo. These data provided a hazard ratio (HR) of 1.31 (95% confidence interval 1.02 to 1.67, P=0.035). Non-significant increases occurred in the incidence of stroke (HR 1.20, 0.96 to 1.50, P=0.11), the composite end point of myocardial infarction, stroke, or sudden death (HR 1.18, 1.00 to 1.39, P=0.057), and death (HR 1.09, 0.96 to 1.23, P=0.18). The meta-analysis of trial level data showed similar results: 296 people had a myocardial infarction (166 in individuals provided with calcium supplementation compared with 130 in individuals given placebo), with an increased incidence of myocardial infarction in the former group (pooled relative risk 1.27, 95% confidence interval 1.01 to 1.59, P=0.038). Pre-specified subgroup analyses showed a significant interaction between treatment allocation and dietary calcium intake for myocardial infarction. There was an increased risk of myocardial infarction in people with dietary calcium intake above the median of 805 mg/day (hazard ratio 1.85, 95% confidence interval 1.28 to 2.67) but no increased risk in those with dietary calcium intake below the median (0.98, 0.69 to 1.38, P for interaction 0.01). The increased risk of myocardial infarction reported by Bolland and co-workers occurred early after calcium supplementation (median follow-up of 3.6 years). The authors concluded that calcium supplementation (without co-administered vitamin D) is associated with an increased risk of myocardial infarction, and noted that given the wide use of calcium supplements by the population, the small increases in risk of cardiovascular disease could translate into a large burden of disease in the population (Bolland et al., 2010).

The authors noted that, in contrast to their findings on supplemental calcium, observational studies do not show increased cardiovascular risks with higher dietary calcium intake (Bolland et al., 2010). Although not part of the meta-analysis, the authors have previously reviewed the mechanisms by which calcium supplements might increase the risk of myocardial infarction (Reid et al., 2010). As argued by the authors, calcium supplements acutely increase serum calcium levels to a modest degree, and serum calcium levels have been positively associated with an increased incidence of myocardial infarction in large observational studies. Primary hyperparathyroidism, a condition in which serum calcium levels are raised, has also been associated with an increased risk of cardiovascular events and death. Ingestion of equivalent doses of calcium from dairy products has a much smaller effect than calcium supplements on serum calcium levels, which might account for the absence of a detrimental vascular effect of dietary calcium intake in the observational studies reviewed.

In a recent update of this study Bolland et al. reanalysed the data used in their earlier studies and unpublished data from two other studies, with the objective of investigating the effect of personal

calcium supplement use on cardiovascular risk (Bolland et al., 2011). The authors had hypothesised that the personal use of calcium supplements by women in the trials evaluated in their earlier study might have obscured an adverse effect of calcium and vitamin D on cardiovascular risk. They concluded from this most recent metanalysis that women who were given regular calcium supplementation (with or without co-administered vitamin D) but who were not taking personal calcium supplements were still at increased risk of cardiovascular event, confirming their basic premise that calcium supplements used with or without vitamin D modestly increase cardiovascular risk (Bolland et al., 2011).

A 5-year, randomised double-blinded placebo controlled trial (Calcium Intake Fracture Outcome Study, CAIFOS) showed no risk of atherosclerotic vascular disease (Lewis et al., 2010). The participants were 1460 women aged 75.1 +/- 2.7 years at baseline (1998), recruited from the general population and randomised to receive 1200 mg of calcium carbonate daily or an identical placebo. All hospital admission and deaths during the 5-year study and the 4.5-year follow-up were derived from the Western Australian Data Linkage Service (WADLS). Hazard ratios for the combined endpoint of atherosclerotic vascular mortality or first-hospitalisation were calculated using pre-specified intention-to-treat and per-protocol models. The intervention group that received calcium supplementation did not have a higher risk of death or first-time hospitalisation from atherosclerotic vascular disease in either the 5-year study (multivariate-adjusted HR 0.938 95% CI 0.690-1.275) or during the total 9.5 years of observational study (multivariate-adjusted HR 0.919 95% CI 0.737-1.146). Further analysis suggested calcium supplementation may reduce the risk of hospitalisation and mortality in patients with pre-existing atherosclerotic cardiovascular disease. The authors concluded that their study provided strong evidence that calcium supplementation of 1200 mg daily does not significantly increase the risk of atherosclerotic vascular disease in elderly women.

The Panel noted that although the meta-analysis carried out by Bolland et al. (2010) was a large and robust study with specified inclusion and exclusion criteria, the trends reported were very modest following supplementation with calcium in addition to dietary exposure, and are not supported by the findings of Lewis et al. (2010). The Panel also noted that the trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that the authors considered that a plausible mode of action could be described to explain these differences, based on serum levels of calcium following dietary intake and supplementation.

3.2.7.2. Biochemical changes in the colon of rats following administration of calcium carbonate

Awad and co-workers examined biochemical changes in the apical membranes of the colon as a result of feeding excess calcium to rats maintained on a saturated fatty acid-rich diet Weanling rats (n=12 per group) were fed a semi-synthetic diet (high fat content plus basal calcium content of 0.5%) and excess calcium (1% calcium carbonate, equivalent to 1000 mg/kg bw/day) for 4 weeks. Animals fed the high calcium diet weighed significantly more than the controls after 4 weeks of feeding. By the end of the 4th week on the diet, significant increases in serum calcium were observed, but there were no clinical signs of toxicity. Excess dietary calcium intake resulted in a 54% reduction in faecal water bile acids and a 44% reduction in faecal water free fatty acids. The calcium-supplemented diet had no effect on either the lipid content or the fatty acid composition of the colonic apical membranes, but protein composition was altered. The authors suggested that the observed alterations in protein patterns of these membranes may be due to either the reduction of faecal water bile acids and free fatty acids or may be a direct effect of dietary calcium on membrane protein (Awad et al., 1990).

4. DISCUSSION

The Panel was not provided with a newly submitted dossier on calcium carbonate and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Calcium carbonate is a food additive generally permitted in foodstuffs under Directive 95/2/EC on food additives and is an authorised food colouring substance under Directive 94/36/EC on colours for use in foodstuffs. It is also included in Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC relating to food supplements and it can be used in fortified foods according to Regulation 1925/2006 on the addition of vitamins and minerals and of certain other substances in food.

Calcium carbonate was previously evaluated by JECFA in 1965 (JECFA, 1965, 1966), when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified (SCF, 1991). A review of carbonates as food additives has also been carried out by TemaNord (TemaNord, 2002). The SCF concluded that both calcium and carbonate were natural constituents of man, animals and plants, and occur naturally in foodstuffs. The Committee noted that these cations and anions constitute the major electrolytes present in all biological materials and, while noting that exhaustive systematic toxicological studies had not been carried out with the individual ions (or their salts), they concluded that "*no safety problems are likely to arise from their use in food, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body*." (SCF, 1991). The SCF based their ADI not specified for carbonates on these considerations. The SCF have also allocated an UL for calcium, from the diet and from supplements, of 2500 mg/person/day as a nutrient (SCF, 2003).

Specifications for calcium carbonate have been defined in Commission Directive 2008/128/EC and by JECFA (JECFA, 2006). The purity is specified as not less than 98% calcium carbonate. The Panel noted that the JECFA specification for lead in calcium carbonate is $\leq$ 3 mg/kg whereas the EC specification is $\leq$10 mg/kg. The Panel also noted that calcium carbonate can have an amorphous or microcrystalline structure, and that particle size varies according to the form and manufacturing conditions. Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 µm diameter crystals typical of the crystalline forms (Meiron et al., 2011). Nanoform calcium carbonate (as used in some of the toxicological studies reported in this opinion) was reported to have a particle size of 60 - 100 nm when examined by SEM, although particle size determination using Sedigraph showed higher apparent particle size due to aggregation (CCA-Europe, 2011). The typical average particle size (d50%) of food grade calcium carbonate is stated by CCA-Europe to be about 5 µm, with an upper range (d98%) of 65 µm and less than 1% of particles having a diameter below 100 nm; the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded (CCA-Europe, 2011).

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion (SCF, 2003), the bulk of unabsorbed calcium is complexed to bile acids, free fatty acids and oxalic acid and excreted with the faeces (Heaney, 2002). Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20-40% (EFSA, 2007). A recent 28-day oral feeding study in mice has shown that calcium from nanoparticulate calcium carbonate is 9.1% more bioavailable (mean of 9.1% higher plasma levels) than from the microparticulate form, as evidenced by significantly higher serum calcium concentrations in the nano calcium carbonate group than in the micro calcium carbonate group. The bone mineral density in the nano calcium carbonate-treated mice was increased by 7.1% when compared to the micro calcium carbonate-treated mice (Huang et al., 2009). A slight increase in calcium bioavailability (by 38%) from nanonised pearl powder form by comparison with the corresponding micronised form of calcium carbonate was also observed in humans (Chen et al., 2008).

In view of the small increases in calcium absorption observed in these studies, the Panel considered that the absorption of calcium from calcium carbonate in nanoparticles would be not markedly different than that from calcium carbonate microparticles.

After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration (SCF, 2003).

A number of short term studies (generally less than 90 days) have been carried out with calcium carbonate in rats and mice, to investigate the effects of high calcium diets on calcium levels in the body (Puerro-Vicente et al., 1993) and on homeostasis of other minerals (Takasugi et al., 2005). Effects of high calcium on membrane biochemistry in the colon (Awad et al, 1990) or effects on apoptosis and cell proliferation in the colon (Penman et al., 2000) have also been investigated. These studies have also assessed the toxicity of high levels of calcium carbonate in the diet, as manifest by effects on growth and food consumption, and have overall not demonstrated any evidence of toxicity attributable to calcium carbonate. Similarly, a 31-week study in cats and kittens, comparing the effects of high calcium carbonate and high calcium chloride diets revealed changes in homeostasis of calcium and other minerals in the body, but no overt toxicity (Pastoor et al., 1994). The Panel considered that the investigations carried out in these studies were of limited value in assessing the safety of calcium carbonate.

The only notable treatment-related effect seen in a 91-day feeding study designed to compare the effects of calcium carbonate to those of calcium citrate malate in rats at dietary calcium levels equivalent to 250 or 500 mg calcium/kg bw/day was nephrocalcinosis, seen in both males and (more marked) in females (EFSA, 2007). These dietary doses correspond to approximately 625 or 1250 mg calcium carbonate/kg bw/day. The Panel considered however that the nephrocalcinosis finding in rats is not relevant for human safety assessment because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis (Ritskes-Hoitinga et al., 1989, 1991, 1992). Results from a 91-day toxicity study in Beagle dogs designed to compare the effects of calcium carbonate to those of calcium citrate malate at equivalent calcium levels in the diet as in the rat study reported above (providing intakes of approximately 250 and 500 mg calcium/kg bw/day) did not report any signs of nephrocalcinosis or other evidence of toxicity (EFSA, 2007).

Nephrocalcinosis was also not observed in a recent combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with nanoform calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM), carried out in Wistar rats at dose levels of up to 1000 mg/kg bw/day (Harlan Laboratories, 2010a). The only changes seen in this study, in which dosing was continued for up to 48 days, were slight but statistically significant haematological and biochemical effects in males receiving 1000 mg/kg bw/day, and significant reductions in plasma phosphate levels in all male treated groups. The Panel considered that these changes were non-adverse and agreed with the NOAEL of 1000 mg/kg bw/day identified by the authors of the study, the highest dose tested.

No evidence of toxicity was reported in a study in which mice were administered calcium carbonate (described by the authors as nano calcium carbonate) by oral gavage at dose levels up to 1300 mg/kg bw/day for 28 days (Huang et al., 2009). The Panel noted however that only limited investigations were carried out in this study.

Calcium carbonate (with a particle size of 60 - 100 nm when examined by SEM) has given negative results in a range of *in vitro* genotoxicity assays. No data are available on the chronic toxicity or carcinogenicity of calcium carbonate. The Panel considered however that it is unlikely that calcium carbonate has carcinogenic potential, given that both calcium and carbonate are natural constituents of the body and normal metabolites of man, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans.

Several reproductive and developmental toxicity studies in rats and mice have been carried out with calcium carbonate. In rats fed diets containing calcium carbonate at levels up to approximately 1500 mg/kg bw/day, no dose-related changes indicative of developmental toxicity were reported (Shackelford et al., 1993). The Panel considered that a NOAEL of 1500 mg/kg bw/day could be identified for reproductive effects of calcium carbonate in this study. However, there were dose-related increases of the femoral calcium content and decreases in tissue phosphorus, magnesium, iron and copper in both dams and fetuses (Shackelford et al., 1994). The Panel also noted the results of a recent OECD guideline-compliant combined repeat dose oral toxicity/reproduction/ developmental toxicity screening study carried out with calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM) in Wistar rats at dose levels up to 1000 mg/kg bw/day in which no effects on reproduction, including developmental toxicity, were reported (Harlan Laboratories, 2010a). Other studies have shown evidence of fetotoxicity of calcium (as calcium carbonate) when administered during pregnancy at levels in the diet equivalent to 3750 mg/kg bw/day calcium carbonate, together with additional calcium intake from calcium lactate in drinking water (Fairney and Weir, 1970; Liebgott et al., 1989). In the study of Richards and Greig in mice reported in 1952, diets with a calcium carbonate content of 1.1% (1650 mg calcium carbonate/kg bw/day) resulted in a decreased number and total weight of litters and in cardiac hypertrophy and thymic atrophy in the offspring when killed at age 21 days Overall, the Panel noted that in rodents high doses of calcium carbonate (> 1500 mg/kg bw/day) causing hypercalcaemia during gestation can result in adverse effects on reproduction, fetotoxicity and elemental imbalances in the offspring. However a recent repeat dose oral toxicity/reproduction study provided a NOAEL of 1000 mg/kg bw/day, the highest dose tested, for developmental toxicity and the Panel considered overall that there is no concern for the reproductive effects of calcium carbonate at intakes below 1500 mg/kg bw/day.

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

The Panel noted that hypercalcaemia and alkalosis can occur in humans taking calcium carbonate with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome), often associated with renal dysfunction, metastatic calcification and other symptoms. Similar changes have been reported in individuals taking large amounts of calcium carbonate and other calcium-containing antacids or large amounts of calcium food supplements. The SCF (2003) reported that one third of the cases had consumed both alkali and calcium (between 2.0 and 16.5 g/day of supplementary calcium), while one third developed symptoms as a result of high calcium carbonate intakes alone (between 2 to 10.8 g additional calcium per day from several months to 30 years).

The Panel additionally noted that while the meta-analysis carried out by Bolland and co-workers (Bolland et al., 2010, 2011), showing an increased risk of myocardial infarction in individuals given regular calcium supplementation in the management of osteoporosis, was a very large and robust study, the trends reported were very modest, and are not supported by the findings in a similar study carried out by Lewis et al. (2010). The Panel also noted that the trends in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that the authors considered that a plausible mode of action could be identified to explain these differences, based on serum levels of calcium following dietary intake and supplementation (Bolland et al., 2010, 2011).

Overall the Panel considered that the available toxicological database on calcium carbonate is limited, but it does not give rise to concern. The Panel considered that further toxicological studies on calcium carbonate are not necessary for the reasons already stated by the SCF in 1991, namely that calcium carbonate is a natural constituent of man, animals and plants, and therefore occurs naturally in foodstuffs. Calcium and carbonate/bicarbonate also also constitute some of the major electrolytes present in all biological materials. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes (2 – 16 g/person/day in humans and > 1500 mg/kg

bw/day in animals), and are also found after large intakes of other calcium salts. The Panel considered that these high-calcium intake effects are not relevant to the evaluation of calcium carbonate as a food additive, and that the SCF established an UL for calcium taking these effects into account (SCF, 2003).

The Panel therefore agrees with the group ADI "not specified" established by the SCF for a group of carbonates including calcium carbonate (SCF, 1991), and considers that the definition of ADI "not specified"[30] is applicable to calcium carbonate when used as a food additive.

The Panel considered however that intakes of calcium resulting from use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and food fortification, should be below the UL of 2500 mg/day for calcium from all food sources established by the SCF (SCF, 2003).

In assessing exposure to calcium carbonate as a food additive, since it is permitted at *quantum satis* in all foodstuffs, the Panel could not estimate the dietary exposure using the Budget method (Tier 1). No Tier 2 calculations were performed, as with the exception of cocoa and cocoa products all applications are *quantum satis*. Refined exposure estimates have thus been performed only for Tier 3 using the maximum reported use levels presented in Annex B, for adult adolescent, children and toddler populations.

The Panel has derived estimates for three exposure scenarios taking into account different sources of exposure, namely Scenario 1: exposure to calcium carbonate/calcium from use of calcium carbonate as a food additive, Scenario 2: exposure to calcium carbonate/calcium from use of calcium carbonate as a food additive and added nutrient source (fortification), and Scenario 3: exposure to calcium from use of calcium carbonate as a food additive and added nutrient source (fortification) and from consumption of food supplements. In addition, the Panel has estimated exposure to calcium from all sources based on published data from dietary surveys presented in the literature for six Member States.

Anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements) in adult, adolescent, children and toddler populations were calculated by the Panel (Table 5).

Based on data made available to the Panel by industry and Member States, the Panel estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) as ranging from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are estimated at 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

[30] JECFA definition of "ADI not specified: "*A term applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance". Available at http://www.who.int/foodsafety/chem/jecfa/glossary.pdf*

The Panel noted that the use of calcium carbonate as added nutrient source (fortification) in addition to its use as food additive, doubles or in some cases trebles intake estimates of calcium across all population groups (scenario 2 compared to scenario 1). This is expected, as reported use levels of calcium carbonate as added nutrient source are, for the majority of food categories, much higher than reported use levels for its use as additive (Table 4). The intake of calcium-containing supplements (estimated at 800 mg calcium/day) contributes between 32-72% to the overall estimated intake of calcium from use as a food additive, nutrient source and intake from food supplements (scenario 3), whilst additive only application (scenario 1) contributes between 15-30%.

Estimated exposure to calcium from all sources based on data presented in the literature for six Member States (Table 6) showed that mean calcium intakes (including food supplements) in all population groups were of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium/day for adults,. The range of high percentile intakes were 1070 – 1184 mg calcium/day for toddlers, 1124-1453 mg calcium/day for children, 1306 – 1905 mg calcium/day for adolescents and 1233 – 1851 mg calcium/day for adults. Mean intake for the elderly (>64 years old) ranged from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The range of mean calcium intakes from use as a food additive and added nutrient source (fortification) calculated for the different age groups in this opinion (Scenario 3, Table 5) are somewhat higher than the values provided for the estimated exposure to calcium from all sources based on data presented in the literature for six Member States (Table 6). This is expected as exposure estimates calculated in this opinion are rather conservative, as they are based on maximum reported use levels and are assumed to always be present in all foods covered by the assessment, which in practice is unlikely.

The Panel noted that both the intake estimates calculated by the Panel, which are considered to be conservative, and the total dietary intake of calcium reported in literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. Therefore, the Panel concluded that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern.

The Panel noted that a range of studies have been performed with calcium carbonate described as nanoform (having a particle size of 60 - 100 nm when examined by SEM), however none of these provided comprehensive data on characterisation of the nano material. Nor do these studies provide a full toxicological database in line with the guidance provided by EFSA Scientific Committee Opinion on Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA, 2011). According to the information provided by the industry the presence of unintentional nanoscale particles at trace levels in calcium carbonate when used as a food additive cannot be excluded (CCA-Europe, 2011). Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel considered that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within microscale calcium carbonate would not be an additional toxicological concern.

The Panel additionally noted that limestone may contain variable amounts of aluminium, reported concentrations ranging between 1 and 134 mg/kg (EPA, 1995), and that analysis of food grade calcium carbonate has shown aluminium concentrations from 10 to 190 mg/kg (Chemische Fabrik Budenheim KG, 2011). Assuming that calcium carbonate contributes approximately 20% to the total calcium intake shown in Table 6 (Panel estimate), and assuming a concentration of 200 mg aluminium/kg calcium carbonate, the Panel estimated a mean intake for aluminium from calcium carbonate of 0.49-0.63 mg/kg bw/week and 95th percentile intake of 0.77-1.12 mg/kg bw/week. Hence, the aluminium intake contributions from the consumption of calcium carbonate to the TWI for aluminium of 1mg/kg bw/week would be 49-63% at the mean and 77-112% at the 95th percentile.

CONCLUSIONS

Calcium carbonate is an inorganic salt authorised as a food additive and food colour in the EU and previously evaluated by JECFA in 1965, when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee also assigned a group ADI not specified.

The Panel agrees with the group ADI "not specified" assigned by the SCF when considering the use of calcium carbonate as a food additive and food colour. The Panel concurs with the JECFA definition of "ADI not specified" and considers it applicable to calcium carbonate when used as a food additive.

The Panel therefore concludes that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern provided that the total intake of calcium from all sources does not exceed the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

The Panel notes that both the estimated intake of calcium calculated by the Panel, considered to be conservative and the total dietary intake of calcium reported in the literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

The Panel noted that calcium carbonate is currently permitted at *quantum satis* in the vast majority of food categories in which it is allowed (generally permitted in all foods with certain exceptions), however information gathered by the Panel for this re-evaluation shows that calcium carbonate is only used at defined amounts in a number of food categories. The Panel thus recommends that the legislation be updated to reflect actual usage levels evaluated in this opinion.

The Panel notes that the presence of unintentional nanoscale particles at trace levels in food additive grade calcium carbonates cannot be excluded. Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel concluded that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within microscale calcium carbonate would not be an additional toxicological concern.

The Panel also notes that the JECFA specification for lead in calcium carbonate is $\leq$ 3 mg/kg whereas the EC specification is $\leq$10 mg/kg.

Additionally the Panel notes that limestone (a source of calcium carbonate) may contain variable amounts of aluminium. The Panel noted that aluminium intake from use of calcium carbonate as a food additive could significantly contribute (50-100%) to the weekly intake of aluminium, for which a TWI of 1 mg aluminium/kg bw/week has been established, and therefore specifications for the maximum level of aluminium in calcium carbonate may be required

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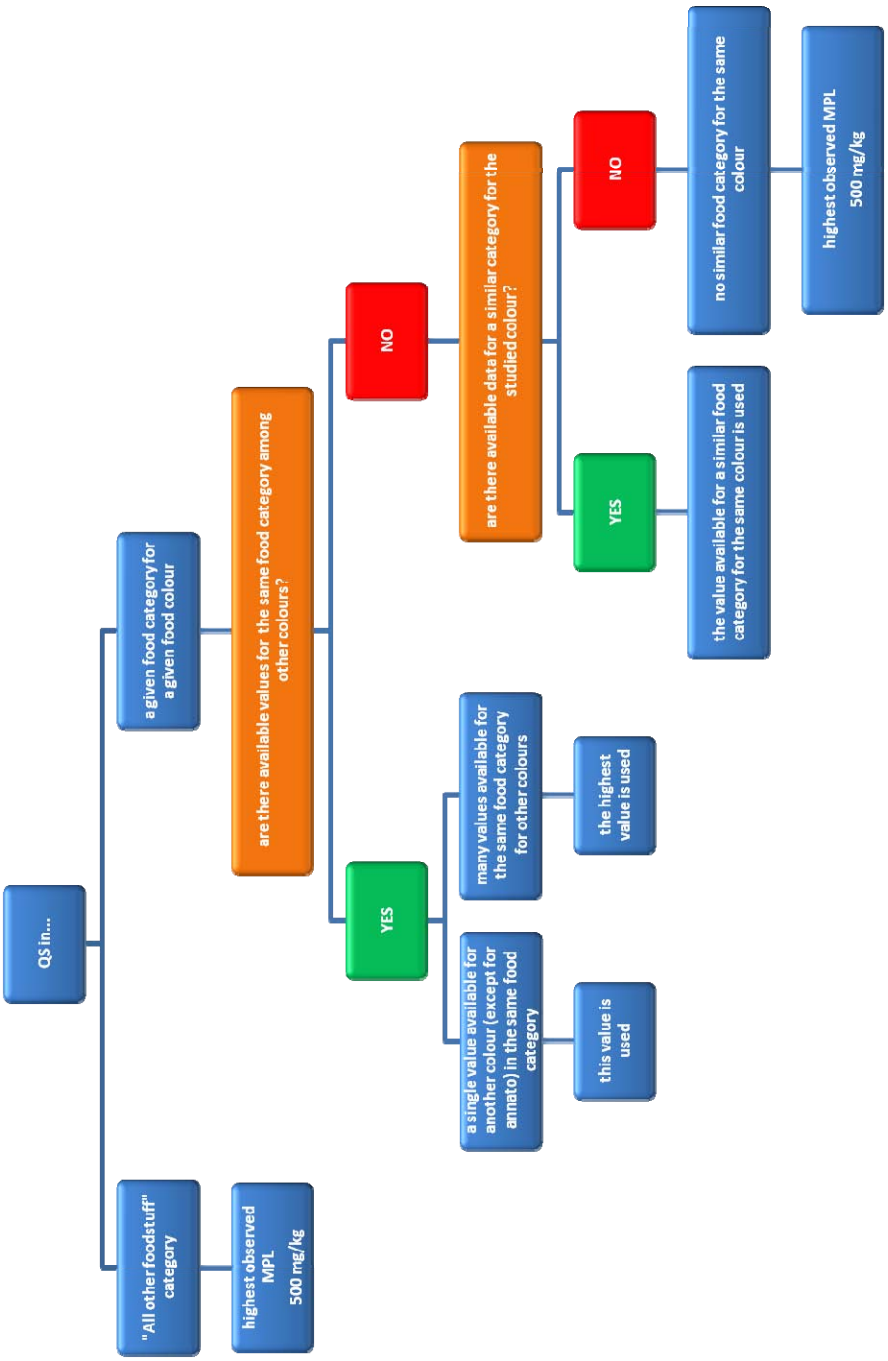
ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for for all regulated food additives to be re-evaluated.

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

Figure 2: Rules defined by the Panel to deal with *quantum satis* (QS) authorisation.



ANNEX B

Table 7. Overview of information provided on usage of calcium carbonate as a food additive or added nutrient source.

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
Tennant (2008)	Additive		Non-alcoholic beverages		No use	No use		
SYFAB	Additive	Anti-caking agent	Baking powder	g/kg	n.s.	6		6000
Belgium Authority/CIAA	Additive	Anti-caking agent, Acidity regulator	Baking powders for production of fine bakery wares		n.s.	n.s.		
GEMEF	Additive	Anti-caking agent	Baking improvers for bread in countries outside the EU with shipping and storage at high temperature (final product)	%	0.02		200	
CIAA	Additive	n.s.	Baking powders for production of fine bakery wares (rarely used)	mg/kg	1000	900-12 000	1000	12 000
CIAA	Additive	n.s.	Fine bakery ware filled croissants (Limited representation of the European Market)	mg/kg	1970	n.s.	1970	
CIAA	Additive	n.s.	Fine bakery ware stol (limited representation of the Dutch Market)	mg/kg	1000	n.s.	1000	
CIAA	Additive	n.s.	Melkbrood (representative for Dutch market)	mg/kg	100	500	100	500
DTU	Additive	Acidity regulator	Fine bakery ware (nougat and almond cream containing biscuits: Nougat Pie Royal		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			Biscuit, Mazariner Royal Biscuit, Mazariner Royal Biscuit)					
DTU	Additive	Acidity regulator	Fine bakery ware (dessert waffles: Dessertvafler u. sukker (frost))		n.s.	n.s.		
Ireland, INFID	Additive	Anti-caking agent	Hot cross buns		n.s.			
CIAA	Additive	n.s.	Confectionery coating for fine bakery wares (very rarely used)	mg/kg	n.s.	85 000		85 000
CIAA	Additive	n.s.	Rusk (limited representation of the German market)	mg/kg	5000	12 000	5000	12 000
CIAA	Additive	n.s.	Fine bakery ware biscuits (Limited representation of the European Market)	mg/kg	<10 000	n.s.	10 000	
CIAA	Nutrient	Nutrient	Fine bakery ware		n.s.	n.s.		
Hovis	Nutrient	Mandatory Fortification (UK)	White and brown flour (excluding wholemeal)	mg/kg	n.s.	3650		3650
Hovis	Nutrient	Nutrient	Bread	mg/kg	9800	n.s.	9800	
Hovis	Nutrient	Nutrient	Rolls	mg/kg	8900	n.s.	8900	
Ireland, INFID	Nutrient	Nutrient	Bread (McCann's White Sliced Pan, Irish Sliced Stoneground Wheaten Rankin selection)		n.s.	n.s.		
Belgium Authority/CIAA	Additive	Anti-caking agent	Breakfast cereals (Use during the production of some of the extruded products such as rings, crisps, shells, spheres,		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			etc. to improve the flowing properties of the flour. Also e.g. rice crisps to be included in some kinds of muësli.)					
CIAA	Additive	n.s.	Muesli (limited to German market)	mg/kg	600	1500	600	1500
GEMEF	Additive	Acidity regulator	Cereal bars (extruded cereal component)	%	0.2	0.5	2000	5000
CIAA	Nutrient	Fortification	Breakfast Cereals	mg/kg	13 000	15 000	13 000	15 000
Polish Agency	Nutrient	Nutrient	Breakfast Cereals	mg/g (as Ca)	120	264-450	3000	11 250
Ireland, INFID	Nutrient	Nutrient	Breakfast Cereals		n.s.	n.s.		
CIAA	Nutrient	Fortification	Cereal bars	mg/kg	12 000	15 000	12 000	15 000
Ireland, INFID	Nutrient	Nutrient	Cereal Bars		n.s.	n.s.		
Ireland, INFID	Nutrient	Nutrient	Chocolate bar		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Chocolate (to give products less sweet taste (to replace sugar)		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Chocolate/chocolate fillings (E.g. a hazelnut filling containing calcium carbonate (+/- 5%) to give products less sweet taste)	%	5	n.s.	50 000	
CIAA	Additive	n.s.	Chocolate fillings (hazelnut fillings)	mg/kg	50 000	n.s.	50 000	

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
SYFAB	Additive	Release Agent	Confectionery (residual amount)	mg/kg	400	n.s.	400	
Belgium Authority/CIAA	Additive	Colour	Confectionery: Surface of citric jellified confectionery (limited representation of Belgian market)	mg/kg	n.s.	7200		7200
GEMEF	Additive	Acidity regulator	Chocolate confectionary (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Chocolate bars with crispy cereals (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Toppings for ice creams (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Chocolate coatings (extruded cereal products)	%	0.2	0.5	2000	5000
Ireland, INFID	Additive	Glazing agent	Chocolate covered peanuts		n.s.	n.s.		
Ireland, INFID	Additive	Glazing agent	Chocolate covered peanuts		n.s.	n.s.		
Polish Agency	Nutrient	Nutrient	Cocoa	mg/100g as calcium	120	264-450	3000	11 250
CIAA	Additive	Firming agent	Fruit and vegetable preparations excluding compote (limited use on German		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			market). The acid liberates Ca and enables complexation with natural tomato pectin					
DTU	Additive	Acidity Regulator	Frugt muckis kvark dessert (Milk based dessert with fruit)		n.s.	n.s.		
Ireland, INFID	Nutrient	Nutrient	Edible ice		n.s.	n.s.		
Polish Agency	Additive	colour acidity regulator, stabiliser, carrier or anticaking agent	Not specified		n.s.	n.s.		
Belgium Authority	Additive	Hardening Agent	Oils and fats (to improve texture of oils (hardening effect), to allow for the use of less saturated fat. (1 to 5% calcium carbonate).		currently not used	currently not used		
Südsalz	Additive	Anti-caking agent	salt and salt substitutes	g/kg	10	20	10 000	20 000
Belgium Authority	Additive	Colour	Prepared salads (as bread spread) In the surimi ingredient, to make it white when otherwise greyish		n.s.	n.s.		
CIAA	Additive	Colour	Few surimi applications	mg/kg		1500-2000		
DTU	Additive	n.s.	Seafood mix, surimi		n.s.	n.s.		
CIAA	Additive	Colour	Meat curing surface treatment in dry sausages (limited representation of Belgian and German market)	g/kg		7		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
Heiploeg	Additive	Gelling agent/Thickener/Colour	Surimi	%	n.s.	1.5		15 000
Belgium Authority/CIAA	Nutrient	Nutrient	Water and water based beverages (added mineral salt)		n.s.	n.s.		
GSK Nutritional Healthcare	Nutrient	Nutrient	Malt beverage	g/25g powder	1	n.s.	40	
Ireland, INFID	Nutrient	Nutrient	Drinking chocolate powder		n.s.	n.s.		
Belgium Authority	Additive	Colour	Soft drink		n.s.	n.s.		
CIAA	Additive	Colour	Soft drink (limited representation)	mg/l	100	1000		
Ireland, INFID	Additive	Colour	Cup-a-soup (mushroom)		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Food supplement	%	1	n.s.	10 000	
CIAA	Nutrient	n.s.	Food supplements	mg/kg	10 000	n.s.	10 000	
EHPM	Additive	Bulking Agent	Food supplements (limited use)	g/kg	n.s.	500		500 000
EHPM	Additive	Colour	Food supplements (very limited use)	mg/kg	n.s.	7500		7500
Belgium Authority	Nutrient	Nutrient	Food supplements (effervescent tablets, tablets, chewable tablets, capsules, drinks, gelules, powder)	mg daily intake as calcium	120	1600	300	4000
EHPM	Nutrient	Nutrient	Food supplements (Ca min RDA at 120 mg=300 mg CaCO3) max 100% RDA =	mg daily intake	300	2000	300	2000

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			2000 mg CaCO3					
CIAA	Additive	Acidity Regulator	Weaning foods for infants and young children in good health (for pH adjustment)	mg/kg	n.s.	100		100
Belgium Authority	Nutrient	Nutrient	Food for particular nutritional uses (e.g. infant formulae, follow up formulae, cereal based foods for infants and young children, baby food, foods for special medical purposes, replacement meals).		n.s.	n.s.		
Belgium Authority	Nutrient	Nutrient	Biscuits nutritional substance in baby biscuits (according to Directive 2006/125[31])		n.s.	n.s.		
CIAA	Nutrient	Nutrient	Biscuits nutritional substance in baby biscuits (according to Directive 2006/125)		n.s.	n.s.		
Belgium Authority	Nutrient	Nutrient	Breakfast cereals, biscuits, soy drinks and other beverages, cereal bars, confectionary, desserts, …)	mg daily intake as calcium	120	1600		

n.s. = not specified

[31] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 5.12.2006, 16-35..

Assumptions made:

Grain milling products

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent in flour at a concentration of 6 g/kg flour.	Anti caking use at 6000 mg/kg flour
Use as added nutrient source, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Mandatory fortification at 3650 mg/kg

The maximum reported use level as a food additive was 6000 mg/kg in bread.
The maximum reported use level as added nutrient source was 3650 mg/kg in bread/rolls.

Bread and rolls

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent in flour at a concentration of 6 g/kg flour.	Bread contains on average 50% flour, resulting in a concentration of 3000 mg/kg bread
Use as anti-caking agent in bread in countries outside the EU with shipping and storage at high temperature. Concentration in the final product 0.02%	200 mg/kg residual amount in bread.
Use as nutrient, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Assuming a 50% content of flour in bread, the level in the final product calculates at 1825 mg/kg bread

Use as nutrient in bread (UK market). Typical use level 9800 mg/kg	Nutrient use at 9800 mg/kg
Use as nutrient in rolls (UK market). Typical use level 8900 mg/kg	Nutrient use at 8900 mg/kg

The maximum reported use level as a food additive was 3000 mg/kg in bread.
The maximum reported use level as added nutrient source was 9800 mg/kg in bread/rolls.

Breakfast cereals (including cereal bars)

Information received on the use of calcium carbonate	**Assumptions made**
Use as anti-caking agent breakfast cereals (use during production of extruded products such as rings, crisps, shells and spheres). Use in rice crisps to be included in muesli. No usage information reported	No usage level reported. Usage as anti-caking agent in flour reported at 6 g/kg. Assuming a 50% flour content, the usage level is estimated at 3000 mg/kg extruded breakfast cereal
Unspecified use in muesli (limited to German market). Typical use 600 m/kg, maximum use 1500 mg/kg.	The maximum use of 1500 mg/kg was used for exposure from muesli.
Use as acidity regulator in cereal bars in the extruded cereal component. Typical usage 0.2%, maximum use 0.5%	Based on labelled cereal content of a popular brand of cereal bars, cereal content was estimated at 25%, which calculates at a maximum usage of 1250 mg/kg cereal bar
Use as nutrient in cereal bars. Typical use 12 000 mg/kg, maximum use 15 000 mg/kg	Nutrient use at a maximum of 15 000 mg/kg
Use as nutrient in breakfast cereals. Typical use 13 000 mg/kg, max use 15 000 mg/kg	Nutrient use at a maximum of 15 000 mg/kg
Use as nutrient in breakfast cereals. Typical use 120 mg calcium/100 g food and maximum use of 264-450 mg calcium/100 g food.	Expressed as calcium carbonate per kg food: Typical 3000 mg/kg, maximum 11 250 mg/kg

The maximum reported use level as a food additive was 3000 mg/kg in extruded breakfast cereals.
The maximum reported use level as a food additive was 1500 mg/kg in muesli.
The maximum reported use level as a food additive was 1250 mg/kg in cereal bars.
The maximum reported use level as added nutrient source was 15 000 mg/kg in breakfast cereals and cereal bars.

For the purposes of estimating exposure of calcium carbonate from breakfast cereals including mueslis and cereal bars, the following fractions have been assigned based on the observed range of contributions of the individual foodgroups to the overall breakfast cereal foodgroup in the comprehensive food consumption database:

- Extruded breakfast cereals: 60% at a level of 3000 mg/kg (additive use)
- Mueslis and Cereal Bars: 30% at a level of 1500 mg/kg (additive use)
- Extruded Breakfast Cereaks, mueslis and Cereal Bars: 90% at a level of 15 000 mg/kg (use as added nutrient source)

Fine bakery wares

Information received on the use of calcium carbonate	**Assumptions made**
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg

Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this information was not used in the exposure estimate.
Information received	Assumptions made
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg
Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this

	information was not used in the exposure estimate.
Information received	Assumptions made
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg
Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this information was not used in the exposure estimate.
Unspecified use in rusk (limited representation of the German market). Typical use 5000 mg/kg, maximum use 12 000 mg/kg	Maximum use level of 12 000 mg/kg. As this usage is very specific and not considered representative for fine bakery ware (it is a very specific double baked item commonly available only in Germany) it was not used in the exposure estimate.
Unspecified use in biscuits (limited representation of the European market). Typical	Typical use level <1000 mg/kg

use less than 1000 mg/kg	
Use as nutrient in fine bakery ware. No usage level reported	-
Use as nutrient, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Assuming a 50% content of flour in bakery ware, the level in the final product calculates at 1825 mg/kg

The maximum reported use level as a food additive was 1970 mg/kg in fine bakery ware.
The maximum reported use level as added nutrient source was 1825 mg/kg in fine bakery ware.

Cocoa beans and cocoa products (Cocoa beans, powder, mass and powder for beverage preparation)

Information received on the use of calcium carbonate	**Assumptions made**
Use as added nutrient source in cocoa. Typical use 120 mg calcium/100 g food, maximum use 264 – 450 mg calcium/100 g food	Expressed as calcium carbonate per kg food: typical 3000 mg/kg food, maximum 11 250 mg/kg food
Use as added nutrient sourcein drinking chocolate powder. No usage level reported	

No information on the use of calcium carbonate in this food category was provided by industry. Directive 95/2/EC permits a usage of 7% expressed as potassium carbonate based on the dry weight excluding fat. The equivalent amount of calcium carbonate calculates at 5g/kg dry cocoa excluding fat. Based on a minimum content of 2.5% dry non-fat cocoa solids in milk chocolate (as required by legislation, Directive 73/241/EEC), a usage of 1250 mg/kg calcium carbonate in chocolate can be calculated.
The maximum reported use level as added nutrient source in cocoa was 11 250 mg/kg

Fish products

Information received on the use of calcium carbonate	**Assumptions made**

Use as colour in prepared salads as bread spread in the surimi ingredient. Maximum concentration 1500 mg-2000 mg/kg	
Use as gelling agent, thickener and colour in Surimi. Typical use 1.5%	Calculated at 15 000 mg/kg surimi

The maximum reported use level as a food additive was 15 000 mg/kg surimi.

For the purposes of estimating exposure of calcium carbonate from surimi, a factor of 15% was assigned to the fish product group, as it contains a number of different food groups (such as fish fingers, fish cakes, etc), which do not contain calcium carbonate (E170).

Dried cured sausages covering

Use as a food colour for meat curing surface tratment in dry sausages, maximum use of 7g/kg.	Maximum use level 7 g/kg. As no information on consumption of sausage and the amount that is covering the sausage (probably the skin of the sausage is not eaten) is available, this information was not used in the exposure estimate.

Fermented milk products

Information received on the use of calcium carbonate	**Assumptions made**
Use as acidity regulator in milked based desserts containing fruit (Frugt Muckis Kvark dessert). No usage level reported	

No usage level was reported and there was insufficient information to approximate likely usage levels. No exposure from this foodgroup could be calculated.

Cheese

This food category is specifically mentioned in Directive 95/2/EC (ripened cheese and sliced and grated ripened cheese, *quantum satis*). Industry did not submit any information on usage of calcium carbonate in cheese and therefore no exposure from this foodgroup was calculated.

Fruit juice

This food category is specifically mentioned in Directive 95/2/EC (grape juice as defined in Directive 93/77/EEC, *quantum satis*). Industry did not submit any information on usage of calcium carbonate in grapefruit juice and therefore no exposure from this foodgroup was calculated.

Chocolate (Cocoa) products (Chocolate, filled chocolate, chocolate bars, etc)

Information received on the use of calcium carbonate	**Assumptions made**
Use as bulking agent in chocolate (to replace sugar). No usage level reported	-
Use as bulking agent in chocolate/chocolate fillings (e.g. hazelnut filling containing calcium carbonate (+/- 5%) to give products a less sweet taste.	Assuming 50% filling, the concentration in the final product calculates at 25 000 mg/kg hazelnut filled chocolate confectionery
Use as release agent in confectionery with a residual amount of 400 mg/kg	Typical residual amount of 400 mg/kg
Use as colour in confectionery (surface of citric jellified confectionery). Maximum use 7200 mg/kg	Maximum use level 7200 mg/kg. As no information on consumption of citrified jellies with whitened surface powder is available, this information was not used in the exposure estimate
Unspecified use in chocolate fillings (hazelnut fillings). Typical use 50 000 mg/kg	Assuming 50% filling, the concentration in the final product calculates at 25 000 mg/kg
Use as acidity regulator in chocolate confectionary and chocolate bars (in the extruded cereal part). Typical levels 0.2%, maximum levels 0.5%	Based on labelled cereal content of a popular brand of chocolate bars (3.5-11%), cereal content was estimated at 10%, which calculates at a maximum usage of 500

	mg/kg cereal containing chocolate bar
Use as acidity regulator for chocolate coatings (extruded cereal products). Typical use 0.2%, maximum use 0.5%	Maximum use level 5000 mg/kg (coating). As no information on type and amount of quantity of coating is known, this information was not used in the exposure estimate.
Use as glazing agent in chocolate covered peanuts. No usage level reported	Assuming 50% chocolate coating, the use level can be calculated based on 5 g/kg permitted in dry non fat cocoa solids. Assuming a minimum content of 2.5% dry non fat cocoa solids, the estimated usage calculates at 625 mg/kg chocolate covered peanuts.
Use as nutrient in malt beverage. Typical use 1 g/25 g powder (to be reconstituted with 200 ml milk)	Calculates at 40 mg/kg powder or 5 g/l reconstituted beverage

The maximum reported use level as a food additive was 25 000 mg/kg in filled chocolate
The maximum reported use level as a food additive was 500 mg/kg in chocolate bars.
The maximum reported use level as a food additive was 625 mg/kg in chocolate coated peanuts.

Directive 95/2/EC permits a usage level of 7% expressed as potassium carbonate based on the dry weight excluding fat. The equivalent amount of calcium carbonate calculates at 5g/kg dry cocoa excluding fat. Based on a minimum content of 2.5% dry non-fat cocoa solids in milk chocolate (as required by legislation Directive 73/241/EEC), a usage level of 1250 mg/kg calcium carbonate in chocolate can be calculated. This level was applied to the food category chocolate confectionery as the reported usage referred to very specific food items only.

Soft drinks

Information received on the use of calcium carbonate	**Assumptions made**
Use as food colour in soft drinks-Typical use level 100 mg/l, maximum use level 1000 mg/l	500 mg/kg use (in accordance with *quantum satis* rules for colours) was assigned.

Use as added nutrient source in water and water based beverages as added mineral salt. No usage data reported.	

No usage data for calcium carbonate used as food colour in soft drink was provided. In accordance with *quantum satis* rules for food colours (see Annex A), an MPL of 500 mg/kg has been assigned for the exposure estimate. The usage data reported by industry only became available at a very late stage and after exposure estimates had already been finalised. The exposure estimates are based on an MPL of 500 mg/kg based on *quantum satis* rules for food colours (see Annex A). In view of the very limited use reported by industry, the Panel was satisfied that the applied level of 500 mg/kg was sufficient to estimate exposure from soft drinks. Since calcium carbonate is a white colour and its use is assumed to be limited (due to limited availability of white soft drinks), a factor of 10% has been assigned to avoid gross overestimation from this food category.

Seasoning or extracts

Information received on the use of calcium carbonate	**Assumptions made**
Use as anti-caking agent in salt. Typical use level 10 g/kg, maximum use level 20 g/kg	Maximum use level 20 000 mg/kg

The maximum reported use level as a food additive was 20 000 mg/kg.

As the comprehensive food consumption database does not provide detailed information on salt intake, exposure to calcium carbonate from salt consumption has been estimated based on an average daily intake of 10 g of salt per day, which is in line with reported salt intake in the European Union (EC, 2008).

Baking ingredients

Information received on the use of calcium carbonate	**Assumptions made**
Use as anti-caking agent and as acidity regulator in bread improvement products. No usage level supplied	-

Unspecified rare use in baking powders for production of fine bakery wares. Typical use level 1000 mg/kg. Maximum use 900-12 000 mg/kg	Maximum use level 12 000 mg/kg

The maximum reported use level as a food additive was 12 000 mg/kg.

Ready to eat soups

Information received on the use of calcium carbonate	**Assumptions made**
Use as food colour in mushroom soup. No usage level reported	500 mg/kg use (in accordance with *quantum satis* rules for food colours) was assigned.

No usage level for calcium carbonate used as food colour in soups was provided. In accordance with *quantum satis* rules for colours (see Annex A), an MPL of 500 mg/kg has been assigned for the exposure estimate. Since calcium carbonate is a white colour and its use is assumed to be limited, a factor of 10% has been assigned to avoid gross overestimation from this food category.

Vegetable products (pureed, mashed pickled, etc)

Information received on the use of calcium carbonate	**Assumptions made**
Use as firming agent in fruit and vegetable preparations excluding compote (to enable complexation with natural tomato pectin). Limited use on German market. No usage level reported.	

No usage level was reported and there was insufficient information to approximate likely usage levels. No exposure from this food category could be calculated.

Animal and vegetable fats and oils

Information received on the use of calcium carbonate	Assumptions made
Potential use as hardening agent in oils and fats to improve texture and allow for the use of less saturated fat. Usage 1-5% .Currently not used.	As currently not in use, this has not been included in the exposure estimates.

The maximum reported potential use level as a food additive was 500 mg/kg, however, as this application is currently not in use it has not been included in the exposure estimate.

Dietary supplements

Information received on the use of calcium carbonate	Assumptions made
Use as bulking agent in food supplements at 1%	Calculates at 10 000 mg/kg food supplement
Limited use as bulking agent in food supplements. Maximum use 500 g/kg	Maximum usage 500 000 mg/kg
Very limited use as colour in food supplements. Typical use 7500 mg/kg	Typical use 7500 mg/kg
Use as nutrient in food supplements (effervescent tablets, tablets, chewable tablets, capsules, drinks, gelules, powder, etc). Typical daily intake 120 mg calciuma/day maximum intake 1600 mg calcium/day.	Converted to calcium carbonate. Usual daily intake 300 mg. Maximum daily intake 4000 mg.
Use as nutrient in food supplements. Typical daily intake 120 mg calcium/day, maximum intake 800 mg calcium/day	Converted to calcium carbonate. Usual daily intake 300 mg. Maximum daily intake 2000 mg.

The maximum reported use level asa food additive was 500 000 mg/kg.

The maximum reported use level as added nutrient source was expressed as daily intake at 4000 mg/day

The use as bulking agent was not considered representative for estimation of exposure from consumption of food supplements. Estimation of intake from food supplements was based on a daily intake of 4000 mg as reported.

Cereal-based food for infants and young children

Information received on the use of calcium carbonate	**Assumptions made**
Use as acidity regulator in weaning foods for infants and young children in good health (for pH adjustment). Maximum usage 100 mg/kg	Maximum usage 100 mg/kg
Use as nutrient in food for particular nutritional uses as specified by legislation	
Use as nutrient in baby biscuits according to Directive 2006/125	Use of calcium according to Directive 2006/125: Rusks and biscuits which are to be used either directly or, after pulverisation, with the addition of water, milk or other suitable liquids; (min 50mg/100 kcal (for milk biscuits) max 100 mg/100 kcal)

The maximum reported use level as a food additive was 100 mg/kg
The maximum reported use level as added nutrient source was not specified. According to Directive 2006/125 a maximum of 100 mg calcium per 100 kcal baby biscuit is permitted. Based on labelled ingredient information of a popular brand, 100 kcal equates to 24 g of biscuit, giving a total of 416 mg calcium/kg biscuit. This can be converted to 1040 mg calcium carbonate/kg biscuit.

Ready-to-eat meal for infants and young children

Information received on the use of calcium carbonate	**Assumptions made**
Use as acidity regulator in weaning foods for infants and young children in good health (for pH adjustment). Maximum usage 100 mg/kg	Maximum usage 100 mg/kg
Use as nutrient in food for particular nutritional uses as specified by legislation	Use of calcium according to Directive 2006/125 (a) 'processed cereal-based foods' which are divided into the following four categories: (i) simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids; (maximum 180 mg/100kcal) (ii) cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid (minimum 80 mg/100 kcal, maximum 180 mg/100kcal) (iii) pastas which are to be used after cooking in boiling water or other appropriate liquids(maximum 80 mg/100 kcal) (iv) rusks and biscuits which are to be used either directly or, after pulverisation, with the addition of water, milk or other suitable liquids (minimum 50 mg/100 kcal for milk biscuits maximum 100 mg/100 kcal) (b) 'baby foods' other than processed cereal-based foods. (max 80 mg/100kcal)

The maximum reported use level as a food additive was 100 mg/kg.

The maximum reported use level as added nutrient source was assumed to be of similar range as calculated for biscuits and a value of 1040 g/kg was assigned.

Ices and Desserts

Use as acidity regulator in toppings for ice creams (extruded cereals), typical use 0.2%, maximum use 0.5%	Maximum use level 5000 mg/kg. As no information on type and amount of quantity of topping is known, this information was not used in the exposure estimate.

ANNEX C

Exposure Scenarios (1 - 3)

Table 8 summarises the anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from use as a food additive, Table 9 summarises the total anticipated exposure from its use as a food additive and added nutrient source (food fortification) and Table 10 summarises the anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements). For all scenarios, exposure from consumption of salt was calculated separately, as consumption information in the comprehensive database was not sufficiently detailed to allow for incorporation of this food group in the calculation.

Exposure to calcium carbonate from salt consumption has been estimated based on an average daily intake of 10 g of salt per day, which is in line with reported salt intake in the European Union (EC, 2008). However, no specific information on salt intake by children or toddlers was specified in the former mentioned report and the assumed intake of 10 g/day may present an over-estimate in these population groups.

Scenario 1 - Exposure from use of calcium carbonate as a food additive

Table 8. Anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from its use as a food additive

Daily intake (mg/kg bw) as CaCO3 from use as a food additive only								
	Adults		**Adolescents**		**Children**		**Toddlers**	
	Mean	**P95**	**Mean**	**P95**	**Mean**	**P95**	**Mean**	**P95**
BEL	8	14	10	16	23	35	27	43
BUL					28	48	28	43
CZE	11	18	19	28	24	34		
CYP			8	22				
DK	10	15	15	23	26	38		
FIN	11	20			17-23	29-39	29	57
F	7	14	12	18	18	27		
DE	7	13	9	17	18-19	27-28	18-18	27-32
HU	10	17						
IRL	8	14						
GRE					13	23		
ITA	9	15	14	26	21	34	16	41
LV	9	17	12	30	16	40		
NL	9	14			20	31	23	37
ES	6-6	11-11	8-12	14-23	16-16	26-28		
SE	8	11	10	15	15	23		
UK	8	12						
ALL	6-11	11-20	8-19	14-30	13-28	23-48	14-29	27-57
Daily intake (mg) as CaCO3								
	Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ALL	360-660	660-1200	400-950	700-1500	390-840	690-1440	210-435	405-855
ALL including salt	560-860	860-1400	600-1150	900-1700	590-1040	890-1640	410-635	605-1055

Daily intake (mg) converted to Ca								
	Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ALL	120-240	240-480	150-350	300-600	150-330	270-570	90-180	165-345
ALL including salt	**200-320**	**320-560**	**230-430**	**380-680**	**230-410**	**350-650**	**170-260**	**245-425**

Estimates calculated for the adult population give a range of mean dietary exposure to calcium carbonate of 560 - 680 mg/day (200-320 mg calcium /day) and a range of 860 – 1400 mg/day (320 - 560 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the adolescent give a range of mean dietary exposure to calcium carbonate of 600-1150 mg/day (230 - 430 mg calcium /day) and a range of 900 – 1700 mg/day (380 - 680 mg calcium a/day) for high level (95th percentile) consumers.

Estimates calculated for the children give a range of mean dietary exposure to calcium carbonate of 590-1040 mg/day (230-410 mg calcium /day) and a range of 890-1640 mg/day (350-650 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the toddler give a range of mean dietary exposure to calcium carbonate of 410-635 mg/day (170-260 mg calcium /day) and a range of 605-1055 mg/day (245-425 mg calcium /day) for high level (95th percentile) consumers.

For all population groups, potential exposure to calcium carbonate from intake of 10 g salt per day has been calculated at 200 mg/day which can be considered a significant contributor to the overall exposure.

For adults, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (53.3 to 72.6% in 14 countries). Grain milling products accounted for 8.8 to 89.4% in 11 countries, breakfast cereals accounted for 5.1 to 18.3% in 4 countries, fine bakery wares accounted for 9.5 to 21.9% in 12 countries and baking ingredients accounted for 5.3% in 1 country.

For adolescents, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (42.2 to 71.2%). Grain milling products accounted for 6.8 to 34.2% in 7 countries, breakfast cereals accounted for 6.4 to 9.1% in 5 countries and fine bakery wares accounted for 13.6 to 28.6% in 11 countries.

For children, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (34.4 to 67.3% in 16 countries). Grain milling products accounted for 8 to 83.9% in 11 countries, breakfast cereals accounted for 5.7 to 27.5% in 10 countries, fine bakery wares accounted for 10.1 to 38.4% in 15 countries and chocolate (cocoa) products accounted for 5.1% in 1 country.

For toddlers, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (42.4 to 71.6% in 8 countries). Grain milling products accounted for 5.5 to 92.6% in 8 countries, breakfast cereals accounted for 5.7 to 7.1% in 2 countries, fine bakery wares accounted for 8.2 to 22.1% in 8 countries, chocolate (cocoa) products accounted for 5.4% in 1 country and ready-to-eat meals for infants and young children accounted for 5.6% in 1 country.

Scenario 2 - Exposure from use of calcium carbonate as additive and added nutrient source

Table 9. Anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from its use as food additive and added nutrient (excluding exposure from food supplements)

Daily intake mg/kg bw as CaCO3 from use as additive and added nutrient source								
	Adults		Adolescents		Children		Toddlers	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BEL	23	45	31	51	68	109	84	137
BUL					69	135	67	137
CZE	26	49	43	75	57	136		
CYP			26	45				
DK	27	44	40	66	74	112		
FIN	14	23			33-59	48-131	37	65
F	22	41	31	51	47	75		
DE	22	41	28	53	58-59	86-91	61-62	91-106
HU	26	47						
IRL	26	43						
GRE					34	66		
ITA	21	41	32	60	49	92	38	92
LV	25	52	35	68	48	110		
NL	26	45			62	98	71	117
ES	17-18	32-34	24-36	41-71	51-51	81-91		
SE	25	62	29	48	45	92		
UK	24	38						
ALL	14-27	23-62	24-43	41-75	33-75	48-136	37-84	65-137

Daily intake mg as CaCO3								
	Adults (60kg)		Adolescents (50kg)		Children (30kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
All	840-1620	1380-3720	1200-2150	2050-3750	990-2250	1440-4080	555-1260	975-2055
All including salt	1040-1820	1580-3920	1400-2350	2250-3950	1190-2450	1640-4280	755-1460	1175-2255
Daily intake mg converted to Ca								
	Adults (60kg)		Adolescents (50kg)		Children (30kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
All	360-660	540-1500	450-850	850-1500	390-900	570-1620	225-495	390-825
All including salt	**440-740**	**620-1580**	**530-930**	**930-1580**	**470-980**	**650-1700**	**305-575**	**470-905**

The use of calcium carbonate as added nutrient source (in fortification) in addition to its use as a food additive results in an up to 3 fold increased intake estimate of calcium carbonate.

Estimates calculated for the adult population give a range of mean dietary exposure to calcium carbonate of 1040 - 1820 mg/day (440 - 740 mg calcium /day) and a range of 1580 - 3920 mg/day (620 - 1580 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the adolescent give a range of mean dietary exposure to calcium carbonate of 1400 - 2350 mg/day (530 - 930 mg calcium/day) and a range of 2250 - 3950 mg/day (930 - 1580 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the children give a range of mean dietary exposure to calcium carbonate of 1190 - 2,450 mg/day (470 - 980 mg calcium /day) and a range of 1640 - 4280 mg/day (650 - 1700 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the toddler give a range of mean dietary exposure to calcium carbonate of 755-1460 mg/day (305 - 575 mg calcium /day) and a range of 1175 - 2255/day (470 - 905 mg calcium /day) for high level (95th percentile) consumers.

For all population groups, potential exposure to calcium carbonate from intake of 10 g salt per day has been calculated at 200 mg/day which can be considered a significant contributor to the overall exposure.

For adults, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (54.6 to 80.3% in 14 countries). Grain milling products accounted for 5.4 to 70.6% in 6 countries, breakfast cereals accounted for 5.4 to 33.3% in 11 countries, fine bakery wares accounted for 5.3 to 8.1% in 10 countries and chocolate (cocoa) products accounted for 5.6 to 9.2% in 8 countries

For adolescents, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (51.6 to 76.1%). Grain milling products accounted for 5.5 to 15.4% in 4 countries, breakfast cereals accounted for 6 to 18.3% in 10 countries, fine bakery wares accounted for 5.8 to 10.8% in 10 countries, cocoa beans and cocoa products accounted for 5.5% in 1 country and chocolate (cocoa) products accounted for 5.4 to 14% in 10 countries.

For children, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (6.7 to 77.8%). Grain milling products accounted for 5.2 to 59.5% in 6 countries, breakfast cereals accounted for 6.6 to 47.9% in 15 countries, fine bakery wares accounted for 5.9 to 14.6% in 12 countries, cocoa beans and cocoa products accounted for 7.2% in 1 country and chocolate (cocoa) products accounted for 5.4 to 15% in 14 countries.

For toddlers, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (48.4 to 78.5% in 8 countries). Grain milling products accounted for 7.3 to 73.5% in 4 countries, breakfast cereals accounted for 5.4 to 15% in 7 countries, fine bakery wares accounted for 5.2 to 9.1% in 5 countries, cocoa beans and cocoa products accounted for 12.5% in 1 country, chocolate (cocoa) products accounted for 5.2 to 15.6% in 4 countries, cereal-based food for infants and young children accounted for 6.5% in 1 country and ready-to-eat meals for infants and young children accounted for 6.7 to 16.3% in 4 countries.

Scenario 3 - Exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification) including food supplements

Estimation of exposure to calcium carbonate/calcium from food supplements was hampered by lack of detail in the comprehensive food consumption database and did not permit calculation of reliable intake estimates. It is also not known how much of the calcium contained in food supplements is in the form of calcium carbonate. Information received from the Belgium authorities indicated a daily intake range between 15-200% of the RDA for calcium (120 – 1600 mg/day). Therefore for the purposes of

estimating long-term additional exposure to calcium from consumption of food supplements in this opinion, an intake of 800 mg calcium/day (100% RDA) in all population groups was assumed.

Table 10 summarises the total anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (from fortification) as calculated in scenario 2 (see Table 9) and from consumption of calcium-containing food supplements.

Table 10. Anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements) in adult, adolescent, children and toddlers populations.

Daily intake of calcium (mg)							
Adults (60 kg)		**Adolescents (50 kg)**		**Children (30 kg)**		**Toddlers (15 kg)**	
Mean	**95th percentile**	**Mean**	**95th percentile**	**Mean**	**95th percentile**	**Mean**	**95th percentile**
1240-1540	1420-2380	1330-1730	1730-2380	1270-1780	1450-2500	1105-1375	1270-1705

Estimates calculated for the adult population give a range of mean dietary exposure to calcium of 1240 - 1540 mg/day and a range of 1420 – 2380 mg/day for high level (95th percentile) consumers.

Estimates calculated for adolescents give a range of mean dietary exposure to calcium of 1330 - 1730 mg/day and a range of 1730 – 2380 mg/day for high level (95th percentile) consumers.

Estimates calculated for children give a range of mean dietary exposure to calcium of 1270 – 1780 mg/day and a range of 1450 - 2500 mg/day for high level (95th percentile) consumers.

Estimates calculated for toddlers give a range of mean dietary exposure to calcium of 1105 – 1375 mg/day and a range of 1270 - 1705 mg/day for high level (95th percentile) consumers.

ANNEX D

Total estimated dietary intake of calcium from all sources based on published dietary survey data

Table 11. Mean and 95th/97.5th percentile calcium intakes (mg/day) from food and supplements (where available)

Toddlers										
					plus supplements			without supplements		
Country	**Year**	**N**	**sex**	**age**	**mean**	**P95**	**P97.5**	**mean**	**P95**	**P97.5**
					(mg/kg)					
NL	2005/2006	327	Male	2-3	795	1184		788	1180	
		313	Female	2-3	740	1099		734	1089	
ITA	2005/2006	52	All	0-2.9	664	1070				
UK	2008/2009	120	All	1.5-3				771		1310
Children										
					plus supplements			without supplements		
Country	**Year**	**N**	**sex**	**age**	**mean**	**P95**	**P97.5**	**mean**	**P95**	**P97.5**
					(mg/kg)					
NL	2005/2006	327	Male	4-6	863	1345		854	1335	
		312	Female	4-6	756	1124		748	1113	
F	2006/2007	570	All	3-10				804	1219	1339
IRL	2003/2004	145	Male	5-8	869	1453	1603	852	1414	1538
		151	Female	5-8	815	1213	1425	810	1213	1338
ITA	2005/2006	193	All	3-9.9	749	1197				
UK	2008/2009	238	All	4-10				787		1318
Adolescents										
					plus supplements			without supplements		
Country	**Year**	**N**	**sex**	**age**	**mean**	**P95**	**P97.5**	**mean**	**P95**	**P97.5**
F	2006/2007	874	All	11-17				809	1299	1432
IRL	2005/2006	224	Male	13-17	1070	1905				
		217	Female	13-17	738	1383				
	2003/2004	148	Male	9-12	965	1507	1655	961	1507	1655
		151	Female	9-12	800	1393	1487	798	1425	1487
ITA	2005/2006	108	Male	10-17.9	892	1435				
		139	Female	10-17.9	770	1306				
UK	2008/2009	224	All	11-18				813		1522
		548	All	19-64				824		1492
Adults										
					plus supplements			without supplements		
Country	**Year**	**N**	**sex**	**age**	**mean**	**P95**	**P97.5**	**mean**	**P95**	**P97.5**
UK	2000/2001	833	Male	19-64	1016		1794	1007		1783
		891	Female	19-64	809		1550	777		1372
F	2006/2007	1918	All	18-79				914	1488	1652
IRL	2008-2010	1274	All	18-64	941	1690	1872			
		226	All	>65	954	2106	2337			
ITA	2005/2006	1068	Male	18-64.9	799	1433				
		1245	Female	18-64.9	730	1233				
		202	Male	>65	825	1403				
		316	Female	>65	754	1285				
UK	2008/2009	548	All	19-64				824		1492
NL	2003	398	Female	19-30	1008	1626				
		352	Male	19-30	1164	1851				

N: Number of survey population
P95: 95th percentile
P97.5: 97.5th percentile
Netherlands (NL): Ocké et al., 2008; Hulshof et al., 2004.
United Kingdom (UK): Henderson et al., 2003; FSA, 2010.
France (F): AFFSA, 2009
Ireland (IRL): IUNA, 2006, 2008, 2011
Italy (ITA): Sette et al., 2010.

GLOSSARY AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ACC	Amorphous Calcium Carbonate
ADI	Acceptable Daily Intake
AFSSA	L'Agence Francaise de Sécurité Sanitaire des Aliments
ANS	Panel on Food Additives and Nutrient Sources added to Food
BMD	Bone Mineral Density
CAIFOS	Calcium Intake Fracture Outcome Study
CAS	Chemical Abstracts Service
CCA-Europe	Calcium Carbonate Association-Europe
CCC	Crystalline Calcium Aarbonate
CIAA	Confederation of the Food and Drink Industries of the EU
DTU	Danmarks Tekniske Universitet
EC	European Commission
EFSA	European Food Safety Authority
EHPM	European Federation of Associations of Health Product Manufacturers
EPA	US Enviromental Protection Agency
EU	European Union
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FAO/WHO	Food and Agriculture Organization/World Health Organization
FDA	Food and Drug Administration
GD	Gestation Day
GRAS	Generally recognized as safe
HR	Hazard Ratio
ICP	Inductively Coupled Plasma
IC	Ion Chromatography
INFID	Irish National Food Ingredient Database
iPTH	intact Parathyroid Hormone

IUNA	Irish Universities Nutrition Alliance
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level
MJ	MegaJoule
MPL	Maximum Permitted Level
MPP	Micronised Pearl Powder
MS	Mass Spectrometry
NOAEL	No Observed Adverse Effect Level
NPP	Nanosised Pearl Powder
Ph. Eur.	European Pharmacopoeia
PRI	Population Reference Intake
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
SCF	Scientific Committee for Food
SYFAB	Syndicat national des Fabricants de Produits intermédiaires pour boulangerie, pâtisserie et biscuiterie
UL	Tolerable Upper Intake Level
WADLS	Western Australian Data Linkage Service