FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

SUPPORTING DOCUMENT 1

RISK ASSESSMENT REPORT

APPLICATION A1030
CALCIUM LIGNOSULPHONATE (40-65) AS A FOOD ADDITIVE

Executive Summary

This risk assessment was undertaken to establish the technological aspects and properties of calcium lignosulphonate (40-65), and whether this substance is suitable for addition to water-based foods as requested by the Applicant for Application A1030. The risk assessment also investigated any risk to public health and safety from the proposed use, including nutritional safety. Summaries of the findings from these assessments are provided below.

Technological Function

Question: Is the substance (in the quantity and form proposed by the Applicant) able to achieve the stated purpose; that is, is its use technologically justified?

The evidence presented in support of the Application provides adequate assurance that calcium lignosulphonate (40-65) is technologically justified, as an emulsifier and stabiliser in the addition of encapsulated fat-soluble active ingredients to water-based foods.

Safety Assessment

Question: Is there a need to establish a reference health standard for calcium lignosulphonate (40-65) in order to protect public health and safety? If so, what should this be?

An acceptable daily intake (ADI) is necessary based on the available toxicological data. An ADI of 0-20 mg/kg bw (rounded value) for calcium lignosulphonate (40-65) has been established based on a 13-week dietary study in rats that obtained a NOAEL of 1978 mg/kg bw/day for males and 2040 mg/kg bw/day for females. This ADI includes 10-fold safety factors for both intra- and inter-species variability giving an overall 100-fold safety factor. An additional safety factor for the absence of a chronic toxicity study of calcium lignosulphonate (40-65) was not considered to be necessary because of the poor absorption of calcium lignosulphonate (40-65) and the absence of any adverse effects in a 13-week study.

Dietary Exposure Assessment

Questions: What is the estimated dietary exposure to calcium lignosulphonate (40-65) for the Australian and New Zealand populations? Will Australian and New Zealand population intakes of calcium lignosulphonate (40-65) exceed the reference health standard as a result of this Application?

Predicted dietary exposures to calcium lignosulphonate (40-65) were low using the proposed food groups and concentration data provided by the Applicant, and the best available consumption data for the Australian and New Zealand populations. Predicted mean dietary exposures were less than 20% of the reference health standard, while 90th percentile exposures were less than 30% of the reference health standard for all population groups

assessed. These estimates were based on very conservative assumptions so as not to underestimate the potential exposures.

Given the conservative nature of this dietary exposure assessment, and the low exposures that have been obtained, FSANZ does not expect that intakes will exceed the ADI for calcium lignosulphonate (40-65).

Nutritional Assessment

Question: Are there any adverse nutritional outcomes associated with the Applicant's proposed use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients?

Data from a suitable animal model show that the use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients is likely to result in the same gastrointestinal absorption of these nutrients as occurs with the use of another common carrier agent. There is also indirect evidence suggesting that calcium lignosulphonate (40-65) presents fat-soluble nutrients to the gastrointestinal system such that normal digestion and absorption of these nutrients can occur.

The use of calcium lignosulphonate (40-65) could result in the capture of fat-soluble nutrients from other dietary sources into a calcium lignosulphonate (40-65) oil mixture. However, this scenario is unlikely to result in any interference with the normal digestion and absorption of these nutrients.

On the basis of this evidence, FSANZ concludes that the use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients is unlikely to result in any adverse nutritional outcomes.

Conclusions of the risk assessment

The findings of the risk assessment for Application A1030 show that calcium lignosulphonate (40-65) is technologically justified and safe for use in water-based foods as proposed by the Applicant.

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1. Introduction

Calcium lignosulphonate (40-65) is not permitted for use as a processing aid within the *Australia New Zealand Food Standards Code (the Code)*. In this Application, the proposed use of calcium lignosulphonate (40-65) is to function as a transferring carrier of fat-soluble nutrients (carotenoids, vitamins A, D, E, and K) into water-based foods.

1.1 Objective of the Assessment

FSANZ is undertaking this assessment to determine the following aspects associated with the use of calcium lignosulphonate (40-65) as a carrier of fat soluble nutrients:

- the technological properties of calcium lignosulphonate (40-65); and
- the risk to public health and safety, including nutritional safety.

1.2 Terminology

The Applicant has proposed that their request to use calcium lignosulphonate as a processing aid applies only to the molecular weight range of 40-65 for this substance. This assessment report will therefore use the term “calcium lignosulphonate (40-65)” in reference to the substance that is the subject of this Application, to distinguish it from other calcium lignosulphonates.

FSANZ has also used ‘calcium lignosul**ph**onate’ in the spelling of this substance instead of ‘calcium lignosul**f**onate’. Overseas spelling uses ‘f’, however the ‘ph’ spelling is already found within the Code for other lignosulphonate permissions (i.e. lignosulphonic acid), and so this document will maintain the ‘ph’ for reasons of consistency. FSANZ has maintained the ‘f’ spelling when referring to overseas documentation, or for the titles of scientific publications.

2. Characterisation

2.1 Sources and uses

Calcium lignosulphonate (40-65) is a member of a larger group of substances called lignosulfonates or lignosulphonates, which are derived from lignin, the second largest component of wood, and are available in the form of a number of salts. Synonyms include lignin sulfonic acids, lignin sulfonates, or ligninsulfonates. Calcium lignosulphonate (40-65) is a complex polymer which is soluble in water, but not in any common organic solvents.

Lignosulphonates (E 565) are approved as feed additives for all animal species and categories in the European Community (70/524/EEC as amended). No maximum levels for the amount added to feed are specified.

Lignosulphonates, including calcium lignosulphonate, are approved in the US as indirect additives to animal feed up to 4% (21 CFR 573.600), as food packaging adhesives (21 CFR 175.105; 21 CFR 172.715), and as stabilisers and dispersion agents in pesticides for preharvest or postharvest application to bananas.

2.2 Chemical identification

The name calcium lignosulphonate (40-65) includes the range of weight-average molecular weight, which is 40 to 65 kDa, and reflects the considerations by JECFA about the need to distinguish this product from other food-grade calcium lignosulphonates (FAO 2008). The molecular weight range of this product is the key differentiator between this product and other food-grade calcium lignosulphonates. Calcium lignosulphonate (40-65) also has a

higher purity, lower content of reducing sugars and higher degree of polymerisation than other calcium lignosulphonates.

In March 2009, the 41st Session of the Codex Committee on Food Additives (CCFA) assigned calcium lignosulphonate (40-65) the INS number of 1522 and functional class of 'carrier, encapsulating agent' (Codex, 2009). The CCFA did not take any further action relating to calcium lignosulphonate (40-65) at the 42nd session in March 2010, since no proposals for use of the substance for inclusion in the Codex General Standard for Food Additives (GSFA) had been forwarded in response to a request for information on uses and use levels of the substance sent to the members of the CCFA.

Chemical name according to IUPAC nomenclature:	Calcium lignosulphonate
C.A.S number:	8061-52-7
Other names:	Calcium lignosulphonate (40-65) Calcium lignosulphonate Lignosulfonic acid, calcium salt Lignosulphonic acid, calcium salt Calcium lignin
Marketing Name:	BorreGum (trademark) Ultrazine FG-R (previous product name)
Appearance:	light yellow brown powder

2.3 Chemical structure

Calcium lignosulphonate (40-65) is a complex, random biopolymer of phenyl propane monomers covalently linked through a variety of chemical bonds. It is derived from lignin, which is a polymer of highly irregular structure. The principal unit of lignin is coniferyl alcohol; other units, also both aromatic alcohols, are p-coumaryl alcohol and sinapyl alcohol. Calcium lignosulphonate (40-65) is randomly polymerized so a well-defined structural formula is not possible. A proposed approximate structure is provided in *Figure 1*. An elemental composition would equate to $C_{10}H_{12}O_5S_{0.5}$.

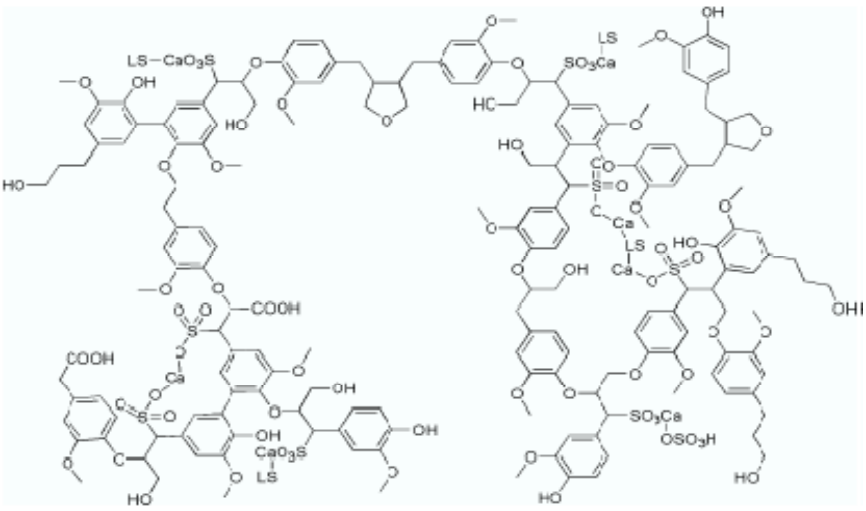

Figure 1: Structural formula of calcium lignosulphonate (40-65) proposed by the Applicant

2.4 Specifications for identity and purity

The specifications for the identity and purity of calcium lignosulphonate (40-65) were prepared at the 69th meeting of JECFA in 2008 and are published in the *FAO JECFA Monographs 5, 2008* (JECFA, 2008). Specifications for the purity of the commercial product, as provided by the Applicant, are presented in **Table 1**. These specifications meet those of JECFA.

Table 1: Specifications for the identity and purity of the commercial calcium lignosulphonate (40-65) preparation (as provided by the Applicant)

Identity	Specification
Solubility	Soluble in water but practically insoluble in organic solvents.
IR spectrum	The IR spectrum of a potassium bromide pellet of dried sample has characteristic signals at approx 1210-1220cm^{-1}, 1037cm^{-1}, and 655cm^{-1}.
UV spectrum	The UV spectrum of a 0.05% sample diluted 1:10 and adjusted to a pH of 2.0-2.2 by the addition of 3 drops of 5M HCl has an absorption maximum at 280 nm.
Weight-average molecular weight	40,000 to 65,000 Da with >90% sample ranging from 1,000 to 250,000 Da
pH	2.7-3.3 (10% solution)
Calcium	Passes test
Degree of sulfonation	0.3-0.7, on the dried basis
Purity	**Specification**
Calcium	Not more than 5.0% on the dried basis
Loss on drying	Not more than 8.0% (105°, 24h)
Total ash	Not more than 14.0% on the dried basis
Reducing sugars	Not more than 5.0%, on the dried basis
Sulfite	Not more than 0.5%, on the dried basis
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2mg/kg

As the JECFA specifications are a primary source of specifications in clause 2 of Standard 1.3.4 – Identity and Purity, no new specification is required to be written for the specific substance.

2.5 Production

Calcium lignosulphonate (40-65) is produced from the sulphite pulping of softwood from Spruce trees. Wood chips are digested in calcium bisulphite solution. The sulphite ions react with the lignin in the wood to produce lignosulphonate, which is more water soluble than lignin. The calcium ions then stabilise the sulphonate groups of the lignosulphonate, forming calcium lignosulphonate. Filtration, pH adjustment with sulphuric acid, evaporation, ultrafiltration, and spray drying steps are then used to achieve a product of desirable properties and purity. The process of manufacture from digestion of wood chips in calcium bisulfite to spray drying of the final product is shown in *Figure 2*.

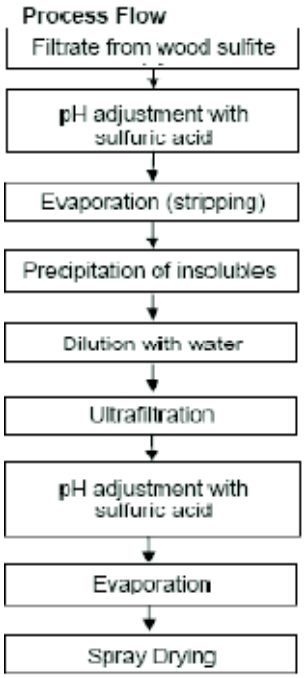

Figure 2: Manufacturing process of calcium lignosulphonate (40-65)

3. Key Risk Assessment Questions

1. Is the substance (in the quantity and form proposed by the Applicant) able to achieve the stated purpose; that is, is it use technologically justified?

2. Is there a need to establish a reference health standard for calcium lignosulphonate (40-65) in order to protect public health and safety? If so, what should this be?

3. If a reference health standard is established for calcium lignosulphonate (40-65), then the following questions also apply:
   - What is the estimated dietary exposure to calcium lignosulphonate (40-65) for the Australian and New Zealand populations?
   - Will Australian and New Zealand population intakes of calcium lignosulphonate (40-65) exceed the reference health standard as a result of this Application?

4. Are there any adverse nutritional outcomes associated with the Applicant's proposed use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients?
   - Does calcium lignosulphonate (40-65) release the carried fat-soluble nutrient for use by the human body?
   - Will calcium lignosulphonate (40-65) encapsulate and decrease the availability of free nutrients in the food matrix once the carried fat-soluble nutrient has been delivered into the food?
   - Will the gastrointestinal absorption of fat-soluble nutrients be impaired by the use of calcium lignosulphonate (40-65) as their carrier?

4. Food Technology Assessment

4.1 Technological function

Encapsulation is a process in which an active ingredient (e.g. a carotenoid or a fat-soluble vitamin) is finely dispersed and embedded in a protective, stabilising and bulking matrix. One encapsulation method involves emulsification of a lipophilic phase (for example a fat-soluble nutrient and an antioxidant stabiliser in oil) with a hydrophilic phase (for example a suitable matrix material such as gelatine, gum acacia, or sucrose dissolved in water), creating a liquid that can then be dried through various processes to produce small dry particles of the formed emulsion. The physico-chemical properties of calcium lignosulphonate (40-65), being a hydrophobic basic lignin skeleton with hydrophilic sulphonate groups, allow it to act as an emulsifier in this particular encapsulation process. As an emulsifier, it stabilises the interaction of the surface of the oil droplets in the aqueous solution.

As a specific example, the carotenoid or other fat-soluble substance is dissolved in corn oil along with an antioxidant, and added to water containing calcium lignosulphonate (the emulsifier), corn starch and glucose syrup. This mixture is then homogenised to form a stable mini emulsion with oil droplets of the desired size (diameters 0.2-0.4µm). This process is illustrated in *Figure 3*.

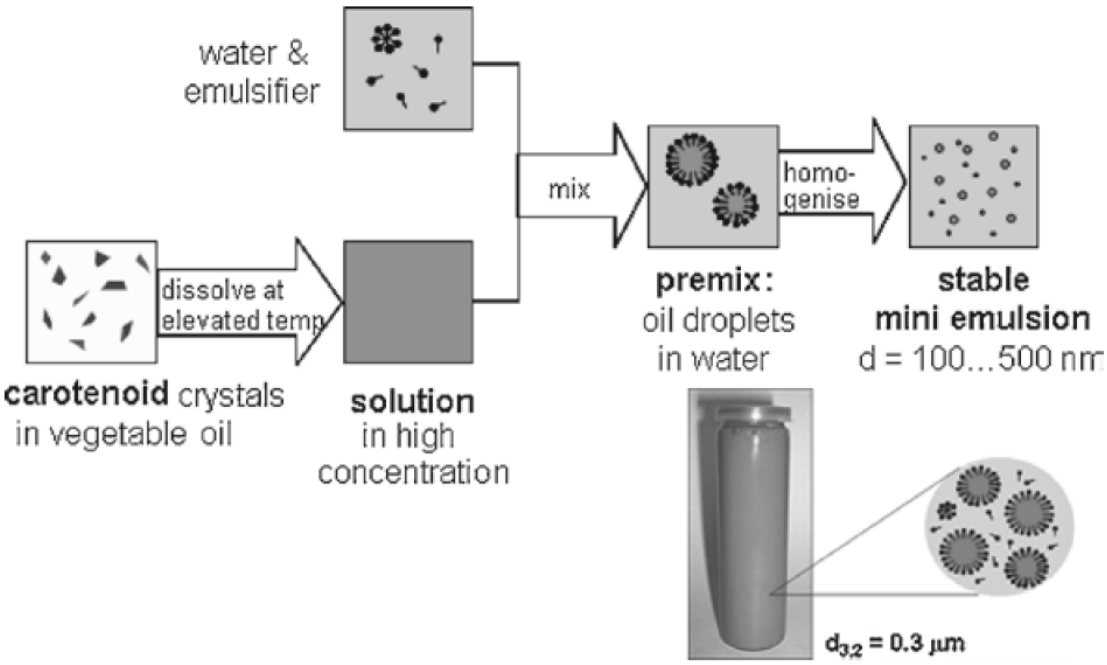

Figure 3: Formation of a stable mini emulsion using calcium lignosulphonate (40-65) as the emulsifier (Ribeiro et al. 2010)

This emulsion is then dried via a spray drying or powder catch process. If spray dried, the emulsion is sprayed into hot air, forming droplets and then dried particles of the desired size (0.1mm). A schematic of such a spray-dried microparticle is shown in Figure 4.

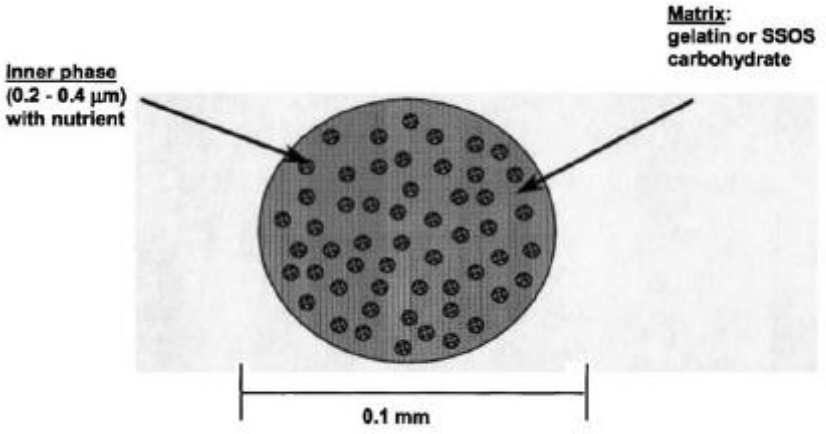

Figure 4: Schematic cross-section of spray-dried microparticle

The alternative method of production is the powder catch process. In this process, wet emulsion droplets are collected in a bed of starch powder, which then coats the matrix to form a slightly larger beadlet as shown in Figure 5.

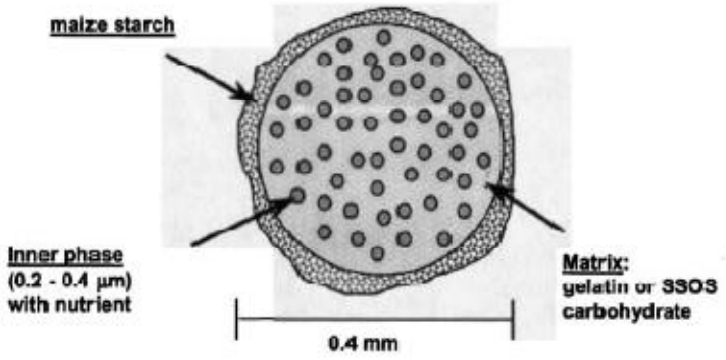

Figure 5: Schematic of a beadlet produced via the powder catch process

When the microparticles or beadlets are added to aqueous foods, the matrix of glucose, corn starch and some calcium lignosulphonate (40-65) dissolves, releasing the oil droplets stabilised by a thin layer of the dissolved matrix. This thin stabilising and emulsifying layer consists mainly of calcium lignosulphonate (40-65). Similarly to the way in which milk fat droplets are stabilised by a thin membrane of phospholipids and proteins, this layer forms a membrane-like weak structure that mediates between the hydrophobic droplet and the aqueous solution, rather than a rigid capsule.

This stabilising and emulsifying layer acts to allow uniform dispersal of the oil droplets in the aqueous media, without aggregation into larger droplets or clumps. At the pH range expected in the use conditions, the sulphonate function groups of calcium lignosulphonate (40-65) are negatively charged, and ensure the small droplets stay apart due to ionic repulsion. The oil droplets containing the active ingredient therefore stay dispersed as an emulsion of oil in water in the food during processing and in the final food. The Applicant explains that the layer stays attached to the oil droplets containing the nutrients even when the food is consumed.

The calcium lignosulphonate (40-65) layer surrounding the oil droplets does not interact chemically with the nutrient, and is held together not by chemical covalent bonds but by weak bonds between the molecules (e.g. hydrogen bonds and Van der Waals forces). The structure of these oil droplets in foods is shown in Figure 6.

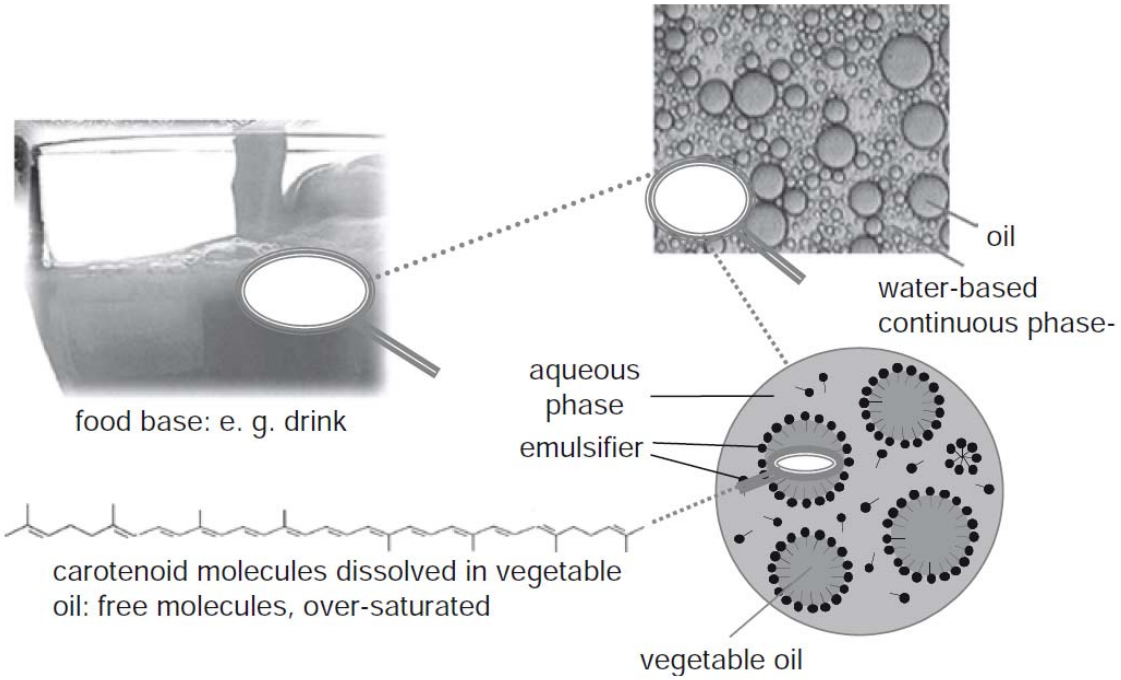

Figure 6: Structure of oil droplets containing the fat-soluble active ingredient (carotenoid molecules in this example) in foods (Ribeiro et al. 2010)

Calcium lignosulphonate (40-65) is proposed by the Applicant as an alternative to other suitable additives/ingredients, such as gelatine, gum acacia, soy protein hydrosylate and starches. Calcium lignosulphonate has the desired property of being able to produce particles of embedded oil droplets of the appropriate size for the encapsulation process. Gelatines, being of animal origin, have disadvantages in not being kosher or halal, are perceived as having potential BSE issues in the market, and may require allergen labelling if sourced from fish. Soy protein hydrosylate and some starches require allergen labelling and may also require GM labelling.

The Applicant has provided information on the expected ratio of calcium lignosulphonate (40-65) to active ingredients as listed in **Table 2**.

Table 2: Ratio by weight of active ingredient to calcium lignosulphonate (40-65) expected for use in foods

Active Ingredient:	Calcium lignosulphonate (40-65)
Carotenoids	1: 5
Vitamin E	1: 5
Vitamin A	1: 4
Vitamin D	1: 200
Vitamin K	1: 18

4.2 Classification as a processing aid vs an additive

Calcium lignosulphonate (40-65) performs a technological function in the final food and not just during the production and manufacture of the food. This function can be variously described as an emulsifier and stabiliser to ensure stable incorporation and emulsion of water insoluble phases in an aqueous media and their uniform dispersal by preventing aggregation of the small particles and droplets. Emulsifier and stabiliser are two technological functions of food additives listed in Schedule 5 (Technological functions which may be performed by food additives) in Standard 1.3.1.

As explained in section 3.3 the Codex Committee on Food Additives (CCFA) determined that calcium lignosulphonate (40-65) was a food additive with a food additive number of 1522, and functional class as carrier and encapsulating agent.

4.3 Addition to water-based foods

Although there are permissions in the Code for the addition of fat soluble vitamins or carotenoids to food, it is up to food manufacturers to determine how and when these nutrients are added, and whether they intend to use calcium lignosulphonate (40-65) for this purpose. There will need to be a step in the food manufacturing process that allows for the incorporation of the nutrients into an aqueous phase, or a step that uses water as a component of one of the ingredients of the food. Foods that do not utilise an aqueous media during processing will not accommodate the addition of fat soluble vitamins or carotenoids to food via calcium lignosulphonate (40-65), even if the Code permits the use of calcium lignosulphonate (40-65) for this purpose.

The Applicant has stated that they intend to use calcium lignosulphonate (40-65) as a carrier for fat-soluble vitamins (vitamins A, D, E and K) and carotenoids (β-apo-8'-carotenal, β-carotene, lutein and lycopene) to facilitate their introduction into water-based foods. However, these vitamins and carotenoids must not be added to a food unless their addition is specifically permitted in the Code (i.e. in Standard 1.3.2 – Vitamins and Minerals), and they are in a permitted form specified in the Schedule to Standard 1.1.1. An exception is made if there are other permissions in specific Standards of the Code, and Schedule 3 of Standard 1.3.1 does permit the addition of β-apo-8'-carotenal, β-carotene, lutein and lycopene as colours in accordance with Good Manufacturing Practice (GMP) to processed foods.

Therefore, in practice the permissions for the addition of fat-soluble vitamins and colours will limit where these nutrients can be added to foods, as will the manufacturing limitations associated with the use of calcium lignosulphonate (40-65) mentioned above. Fat-soluble substances may be directly added to high-fat foods without the need for encapsulation, therefore calcium lignosulphonate (40-65) serves no technological purpose in foods with a high fat content.

4.4 Analytical methods for determining the presence of calcium lignosulphonate (40-65) in food

The Applicant states in their Application that there is no analytical method available that quantifies the amount of calcium lignosulphonate (40-65) that would be present in the final food. The Chemical and Technical Assessment Report written by JECFA on the calcium lignosulphonate (40-65) makes the same statement.

The Applicant further argues that there will only be small amounts of the substance in the final food. An analytical method has not been developed due to the difficulties inherent in detecting a complex polymer of varying size, in low concentrations, within complex and varying food matrices.

FSANZ agrees that an analytical method to determine the presence of the substance in the final food is neither appropriate nor necessary. Various analytical methods are however available to determine the presence of the active ingredient (eg the fat soluble vitamin or carotenoid) encapsulated using calcium lignosulphonate (40-65). It is possible that the analytical methods to check for compliance with the JECFA specification could be adapted to check for the presence of the substance in the food, but this seems unlikely to be a practical method to discriminate the substance from the complex food matrix.

4.5 Response to Risk Assessment Question 1

Question: Is the substance (in the quantity and form proposed by the Applicant) able to achieve the stated purpose; that is, is its use technologically justified?

The evidence presented in support of the Application provides adequate assurance that calcium lignosulphonate (40-65) is technologically justified, as an emulsifier and stabiliser in the addition of encapsulated fat-soluble active ingredients to aqueous foods.

5. Hazard Characterisation

FSANZ has assessed the submitted data on calcium lignosulphonate (40-65) that includes studies on absorption, distribution, excretion, acute toxicity, repeat-dose toxicity, genotoxicity and reproductive toxicity. The submitted data are considered suitable for hazard assessment and assignment of a reference health standard for calcium lignosulphonate (40-65).

Absorption studies were conducted using the *in vitro* Caco-2 cell model and *in vivo* using radiolabeled calcium lignosulphonate. Distribution and excretion were also investigated in the *in vivo* study. Metabolism studies were not conducted because the absorption studies showed that absorption of calcium lignosulphonate is very low.

Data for acute toxicity of calcium lignosulphonate (40-65) were not submitted. A summary of acute toxicity data for other lignosulphonates was included in the submission. Acute toxicity data for this particular lignosulphonate are not considered to be necessary, because there is evidence that it is very poorly absorbed from the gastrointestinal tract and because data from several subchronic studies were included in the submission.

5.1 Absorption, distribution and excretion studies

5.1.1 Absorption

Analytical detection of lignosulphonates in biological samples is problematic, because the hydrophilic nature of lignosulphonate prevents efficient extraction from biological samples, and the lignosulphonate also precipitates readily with protein. To overcome the analytical problems, radiolabeled calcium lignosulphonate (40-65) was used for these studies. Studies of absorption were limited to *in vitro* and laboratory animal models.

5.1.1.1 Caco-2 cell monolayer.

Beck *et al.* (2006), amended by Beck (2008). **Study titles**: In vitro intestinal absorption of (3H)-Lignosulfonate using the CaCo-2 monolayer model; *and* Amendment 1 to REPORT No. 2500301 **Report no.**: RDR No. 2500301 **Report date**: Unpublished report. Amendment 1 dated 17 June 2008 **Laboratory**: Not stated **GLP**: No

The Caco-2 cell (human colon adenocarcinoma) monolayer model was used to predict intestinal absorption of ^{3}H-labelled calcium lignosulphonate (40-65), at concentrations of 1, 3, 10 and 30 mg/mL (Beck *et al.* 2006, amended by Beck 2008). Radioactivity in the permeated samples was determined after incubation times of 0.5 h, 1 h, 1.5 h, 2 h and 3 h. Permeation of radioactivity was low at a mean of 1.7% (±0.25%) per hour, and essentially the same at all concentrations. Less than 1% of the permeated radio-labelled products had a molecular weight greater than 200 Da, as determined by size exclusion chromatography. It was concluded that most of the permeated radioactivity was present as tritiated water formed by tritium-hydrogen exchange from ^{3}H-labelled calcium lignosulphonate (40-65). The apparent permeability coefficient (P_{app}) of lignosulphonate calculated from these data is less than 0.005×10^{-6} cm/s. On the basis of these results, it was concluded that no intestinal absorption of calcium lignosulphonate (40-65) and no systemic exposure would be expected *in vivo*.

5.1.1.2 Rats

Beck and Rossi (2005) as amended by Beck (2008b). **Study title**: Absorption, distribution and excretion of tritium labelled Lignosulfonate after single oral administration in rats. *and* Amendment 1 to REPORT No. 2500147. **Report no.**: RDR No. 2500147 **Report date**: Unpublished report. Amendment 1 dated 17 June 2008. **Laboratory**: Not stated **GLP**: No

Absorption, distribution and excretion of ^{3}H-labelled calcium lignosulphonate (40-65) were studied in 3 male and 3 female rats following administration of a single dose by oral gavage, at a dose of 10 mg/kg bw. The kinetics of radioactivity were studied in an additional 3 male rats from which blood samples were collected at 1 h, 2 h, 4 h, 6 h and 24 h. The overall fate of radioactivity was examined in all 9 rats. Urine and faeces were collected in two intervals, from 0 to 24 hours and from 24 to 48 hours. Rats were sacrificed at 48 h, blood collected, and the weights of various organs determined. Radioactivity was determined in all biological materials collected. Aliquots of samples of faeces, blood and tissues were analysed both before and after drying, in order to determine the presence of tritiated water.

Evidence of tritium-hydrogen exchange between water and ^{3}H-labeled calcium lignosulphonate (40-65) was noted during storage of the test article prior to use, and continuous formation of tritiated water during study periods was anticipated. Consequently, aliquots of samples of faeces, blood and tissues were analysed both before and after drying, in order to determine the presence of tritiated water, and molecular weight distribution of radiolabeled substance in urine and plasma was determined by size exclusion chromatography.

Results are summarised in Table 3 below.

The overall recovery of administered radioactivity was 98.4(±7)%. Seventy-five percent of the administered radioactivity was excreted via the faeces, and 90% of that was excreted in the first 24 hours. Faecal excretion was slightly slower in female rats than in males, but no significant gender difference was noted in radioactivity levels in other samples.

Removal of water from samples other than faeces resulted in significantly lower radioactivity levels than in the corresponding wet sample, consistent with much of the radioactivity being present as tritiated water. Of the radiolabeled components in the liquid samples, urine and plasma, 97% eluted in very low molecular weight fractions (<200 Da) and the majority co-eluted with tritiated water.

After 48 hours, only 0.8% of the administered radioactivity was found in urine (0.05%), liver (0.08%), blood (0.01%) and remaining carcass (0.66%). It was concluded that absorption of calcium lignosulphonate (40-65) is very low, and systemic exposure is less than 1%.

Table 3: Radioactivity distribution at 48 h in rats receiving ^{3}H-labeled calcium lignosulphonate (40-65)

Material/tissue	Radioactivity (% of total)	
	Sample as collected	Sample after removal of water
Faeces	74.56	71.04
Gastro-intestinal tract (including contents)	2.10	0.15
Skin/fur	4.42	0.44
Blood	0.61	0.01
Liver	0.73	0.08
Remaining carcass	13.06	0.66
Urine	2.84	0.05
Recovery	98.37	
Systemic radioactivity (absorbed/excreted)	<18.0	<1.0

5.1.2 Metabolism

Because the absorption of calcium lignosulphonate (40-65) was found to be negligible (< 1%), metabolism of absorbed compound was not studied.

5.1.3 Acute dose toxicity studies

Results of acute toxicity studies of calcium lignosulphonate (40-65) were not submitted. A summary of findings from animal toxicity studies on various lignosulphonate salts, of unspecified molecular weight range, was included in the references supporting the application. According to this summary, the estimated oral LD50 for lignosulphonates in rats is between 10 and 20 g/kg bw.

5.1.4 Repeat dose toxicity studies

Reports of three repeat-dose toxicity studies in rats, comprising two 28-day studies and one 13-week study, were submitted in support of the application.

Weber and Ramesh (2005). **Study title**: Repeated Dose (28-Day) Oral Toxicity Study with Calcium Lignosulfonate in Wistar Rats **Report no.**: TOXI-4091/04 **Report date**: 20 September 2005 **Laboratory**: Advinus Therapeutics Private Limited, Plot No.s 21 and 22, Peenya, Phase II, Bangalore 560 058, India **GLP**: OECD

This study was conducted using four groups of male and female Wistar rats, comprising 6/sex/group. Calcium lignosulphonate (40-65) was mixed in powdered diet to achieve dose levels of 500, 1500 or 4000 mg/kg bodyweight/day. The control group received the powdered diet without calcium lignosulphonate. Actual intakes of calcium lignosulphonate (40-65), as estimated from body weight and food consumption data, were somewhat lower than target dose levels, as shown in Table 4.

Table 4: Estimated mean intake (mg/kg bw/day) of calcium lignosulphonate (40-65)

Group	Target Dose	Gender	Estimated Dose			
			Week 1	Week 2	Week 3	Week 4
2 (low dose)	500	Male	388	428	446	445
		Female	420	453	466	475
3 (mid dose)	1500	Male	1239	1249	1351	1364
		Female	1190	1369	1387	1451
4 (high dose)	4000	Male	3493	3399	3560	3529
		Female	3225	3742	3709	3759

All rats survived to scheduled termination at the end of the study. No treatment-related effects were noted in clinical observations, body weights, body weight changes, food consumption, ophthalmoscopic findings, clinical pathology parameters, organ weights or organ weight ratios or gross pathology findings. Microscopically, an increased incidence of chronic inflammation in the rectum of high-dose males was noted and was considered to be treatment-related. The inflammation was of minimal severity and was focal or multifocal in distribution. On the basis of this finding in male rats, the authors identified the No Observed Effect Level as the target dose of 1500 mg/kg. The actual mean doses at this target level were calculated to be up to 1364 mg/kg/day for males and 1451 mg/kg/day for females. Females tolerated mean daily doses up to 3759 mg/kg/day with no treatment-related effects.

Wolz *et al.* (2004). **Study title**: AXN-DMS: 28-Day Range-finding Oral Toxicity (feeding) study in the Wistar Rat. **Report no.**: 853022 **Report date**: 29 July 2004 **Laboratory**: RCC Ltd, Toxicology Division, CH-4452 Itingen, Switzerland **GLP**: Not stated

In this study, calcium lignosulphonate of unspecified grade was used as a carrier for the test article under investigation, AXN-DMS. Han Wistar rats were assigned to one of five groups, each group comprising 5 rats/sex. The control group, Group 1, was fed diet containing neither calcium lignosulphonate nor AXN-DMS. Group 2, the placebo group, were fed diet containing 84700 ppm calcium lignosulphonate of unspecified grade, but no AXN-DMS. Groups 3, 4 and 5 were fed diet containing both calcium lignosulphonate of unspecified grade and AXN-DMS, to a total replacement of 84700 ppm for the two. Thus, Group 2 received the highest level of calcium lignosulphonate of unspecified grade in the diet. Intakes of calcium lignosulphonate of unspecified grade in this group were 5.4 to 6.4 g/kg bw/day by males and 5.8 to 6.9 g/kg bw/day by females.

All rats survived to scheduled termination. Dark faecal discoloration was noted in the placebo group (Group 2) from treatment week 1 through to the end of the study, and soft faeces were noted in the same group from treatment week 3 through to the end of the study. These findings were attributed to the high content of calcium lignosulphonate of unspecified grade in the diet of this group. No other clinical signs were noted in this group, and there were no treatment–related effects on food consumption, body weights, body weight changes, clinical pathology parameters, organ weights, organ weight ratios, gross pathology findings or histopathological findings. In conclusion, a dietary intake of calcium lignosulphonate of unspecified grade at up to 6.4 g/kg bw in males, and up to 6.9 g/kg bw in females, for 28 days had no toxicological effects.

Thiel *et al.* (2007) **Study title**: Ultrazine FG-R (Food Grade Lignosulphonate): 13-Week Oral Toxicity (Feeding) Study in the Wistar Rat. **Report number:** A29553 **Report date**: 25 May 2007 **Laboratory**: RCC Ltd., CH-4452 Itingen, Switzerland **GLP**: OECD

Han Wistar rats were assigned to one of four groups. Within each group were two or three cohorts, Allocations A, B and C. All groups included Allocation A rats, 20/sex/group, which were scheduled for termination at 13 weeks. The control and high-dose groups, Groups 1 and 4 respectively, included Allocation B rats, 10/sex/group, which were treated for 13 weeks and then underwent a 28-day recovery period prior to scheduled termination. All groups included a cohort of Allocation C rats, 6/sex/group, that were used to assess primary immunological response after 13 weeks of treatment.

Ultrazine FG-R, which is calcium lignosulphonate (40-65), was mixed in the feed to target dose levels of 0, 500, 1000 or 2000 mg/kg/day.

Toxicological endpoints assessed included survival, clinical signs, food consumption, body weights and body weight changes, ophthalmoscopic findings, clinical pathology parameters, organ weights and organ weight ratios, gross pathology and histopathology. Faecal pH was measured in Weeks 2, 6 and 13 of treatment and during recovery in Week 17. Functional observational battery, locomotor activity and grip strength were assessed during Weeks 13 and 17. Sperm count, motility and morphology were examined in all Allocation A males at 13 weeks and all Allocation B males at 17 weeks. The oestrous cycle was assessed over a two-week period in all Allocation A and B females from Week 10, and in all Allocation B females in Week 15.

Estimated mean test article intake was very close to the target dose level for all groups. Males consumed -0.8%, -0.6% and -1.13% of the target dose levels of 500, 1000 and 2000 mg/kg bw/day, respectively. Corresponding percentages for females were +1.3%, +1.8% and +2.0%, respectively.

No changes attributable to calcium lignosulphonate (40-65) were noted in survival, clinical signs, food consumption, body weights or body weight changes, ophthalmoscopic findings, gross pathology, faecal pH, functional observational battery results, grip strength, sperm characteristics, or oestrous cycles.

A number of statistically significant differences between treated rats and gender-matched controls were noted after 2, 6 or 13 weeks' treatment, but these were inconsistent between genders and over time. Most were considered to be unrelated to treatment, but some may represent physiological adaptation responses.

No changes attributable to calcium lignosulphonate (40-65) were noted in absolute or relative organ weights. The primary immune response to sheep erythrocytes, determined in the Allocation C cohort at 13 weeks, was comparable between treated groups and controls. Consistent with this, there were no treatment-related effects on group mean total and differential leukocyte counts, or on group mean T-cell type distributions.

Microscopic findings attributable to calcium lignosulphonate (40-65) ingestion were limited to the kidneys and the mesenteric lymph nodes. The rectal inflammation observed in the 28–day study of Weber and Ramesh (2005) was not observed in any rats in this study.

The presence of tubular vacuolation in the kidneys was considered to be treatment-related but, since there was no evidence of tubular damage or other renal lesions, and no evidence of related changes in clinical pathology parameters, the finding was considered to be non-adverse. The incidence and mean severity of these findings is summarised in Table 5.

Table 5: Incidence and mean severity of vacuolation of renal tubules at termination in Week 13 (Allocation A rats).

Group	Target Dose (mg/kg/day)	Gender	Number examined	Incidence	Mean Severity*
1	0	Male	20	0	-
		Female	20	0	-
2	500	Male	20	0	-
		Female	20	0	-
3	1000	Male	20	0	-
		Female	20	5	1.0
4	2000	Male	20	3	1.0
		Female	20	13	1.0

* Severity scale: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

Minimal vacuolation of renal tubules was also still present in treated rats after the recovery period, with unchanged mean severity. There were no other signs of renal impairment. The incidence and severity of the finding is summarised in Table 6.

Table 6: Incidence and mean severity of vacuolation of renal tubules at termination in Week 17 (Allocation B rats).

Group	Target Dose (mg/kg/day)	Gender	Number examined	Incidence	Mean Severity*
1	0	Male	10	0	-
		Female	10	0	-
4	2000	Male	10	4	1.0
		Female	10	5	1.0

* Severity scale: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

The presence of large focal or multifocal aggregates of foamy histiocytes in mesenteric lymph nodes of treated rats at all dose levels was considered to be treatment-related. The incidence and mean severity of the finding increased with dose, but no tissue damage was noted, and the findings were therefore considered to be non-adverse. The incidence and mean severity of these findings is summarised in Table 7.

Table 7: Incidence and mean severity of foamy histiocytosis of mesenteric lymph nodes at Week 13 (Allocation A rats).

Group	Target Dose (mg/kg/day)	Gender	Number examined	Incidence	Mean Severity*
1	0	Male	20	0	-
		Female	20	0	-
2	500	Male	20	4	1.0
		Female	20	3	1.0
3	1000	Male	20	17	1.3
		Female	20	8	1.3
4	2000	Male	20	20	2.3
		Female	20	19	2.1

* Severity scale: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

At scheduled termination of Allocation B rats in Week 17, after 4 weeks without treatment, foamy histiocytes were present in the mesenteric lymph nodes of treated rats but the mean severity of the finding was reduced, consistent with partial regression. No tissue damage was evident and the finding was considered to be non-adverse. The incidence and severity of the finding is summarised in Table 8.

Table 8: Incidence and mean severity of foamy histiocytosis of mesenteric lymph nodes at termination in Week 17 (Allocation B rats).

Group	Target Dose (mg/kg/day)	Gender	Number examined	Incidence	Mean Severity*
1	0	Male	10	0	-
		Female	10	0	-
4	2000	Male	10	10	2.1
		Female	10	10	1.8

* Severity scale: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

Lymphoid hyperplasia was observed in the mandibular and mesenteric lymph nodes, in the Peyer's patches of the gastrointestinal tract. The great majority of the instances of lymphoid hyperplasia of lymphoid tissues were also Grade 1, although a few individuals had Grade 2, or slight, hyperplasia. In addition, lymphoid infiltration was observed in the liver in rats at all dose levels, including control rats. All the findings of lymphoid infiltration of the liver were graded Grade 1, or minimal. These observations were not commented upon in the pathology report. There was no evidence from clinical pathology parameters or primary immune response that the immune systems of the rats were in any way adversely affected, there was no evidence, from serum chemistry results, that the lymphoid infiltration of the liver had any effect on liver function.

In conclusion, because the treatment-related findings in mesenteric lymph nodes and renal tubules were considered to be non-adverse, the No Observed Adverse Effect Level (NOAEL) for dietary calcium lignosulphonate is 1978 mg/kg bw/day in males and 2040 mg/kg bw/day in females. For additional discussion surrounding the toxicological significance of foamy histiocytes refer to the discussion section below.

5.1.5 Genotoxicity studies

Calcium lignosulphonate (40-65) was tested for genotoxic potential by means of a reverse mutation test, including metabolic activation, in two bacterial species, and also by means of the chromosomal aberration test in Chinese hamster V79 lung fibroblast cells.

Thiel *et al.* (2005). **Study title**: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Ultrazine FG-R (Food Grade Lignosulphonate). **Report no.**: 899101 **Report date**: 13 December 2005 **Laboratory**: RCC-Cytotest Cell Research GmbH (RCC-CCR) , In den Leppsteinswiesen 19, D-64380 Rossdorf, Germany **GLP**: OECD

Thiel *et al.* (2006b) **Study title**: In Vitro Chromosome Aberration Test in Chinese Hamster V79 Cells with Ultrazine FG-R (Food Grade Lignosulphonate). **Report no.**: 899102 **Report date**: 9 May 2006 **Laboratory**: RCC-Cytotest Cell Research GmbH (RCC-CCR) , In den Leppsteinswiesen 19, D-64380 Rossdorf, Germany **GLP**: OECD

Calcium lignosulphonate (40-65) did not induce reverse mutation in bacteria or clastogenesis in mammalian cells. Positive controls gave expected results in these assays. In light of these findings and the lack of any systemic exposure, *in vivo* genotoxicity testing following oral or intraperitoneal administration was considered unnecessary.

The results of the tests are summarised in Table 9.

Table 9: Summary of results of genotoxicity assays of calcium lignosulphonate (40-65)

Test type	Test system	Concentrations	Result	Reference
Bacterial reverse mutation[a]	*S. typhimurium* TA 98, TA 100, TA1535 and TA1537 and *E. coli* WP2 uvrA (±S9)	33, 100, 333, 1000, 2500 and 5000 µg/plate[b,c]	Negative	Thiel *et al.* (2005)
Chromosomal aberration *in vitro*.	Chinese hamster V79 lung fibroblast cells	4 h, -S9 625, 1250 and 2500 µg/mL 18 h, -S9 156.3, 312.5 and 625 µg/mL 28 h, -S9 1500 µg/mL 4 h, +S9 2500, 3750 and 5000 µg/mL	Negative	Thiel *et al.* (2006b)

[a]In the presence and absence of Phenobarbital/β-Naphthoflavone-induced rat liver S9 mix
[b]Plate incorporation method
[c]Pre-incubation method

5.1.6 Carcinogenicity studies

None submitted.

5.1.7 Developmental toxicity study

Thiel *et al.* (2006a). **Study title**: Ultrazine FG-R (Food Grade Lignosulphonate): Prenatal Developmental Toxicity Study in the Han Wistar Rat. **Report no.** A29992 **Report date**: 23 October 2006 **Laboratory**: RCC Ltd Toxicology, CH-4414 Füllinsdorf, Switzerland **GLP**: OECD, USFDA

Female Han Wistar rats, 22/group, were fed diets containing calcium lignosulphonate (40-65) formulated at target doses of 0, 100, 300 or 1000 mg/kg bw/day from the end of Day 5 post-coitum through to Day 21 post-coitum. Because of technical failures resulting in some foetuses being unsuitable for examination, an additional group of 22 control females and an additional group of high dose (1000 mg/kg bw/day) females were also used. All dams were terminated on Day 21 post-coitum and foetuses were removed.

Based on food consumption data the actual mean doses achieved were 101, 309 and 1007 mg/kg bw/day for the low-, mid- and high-dose groups respectively.

Endpoints for toxicity were maternal survival, maternal clinical signs, maternal body weight, maternal food consumption, uterine weight at termination, number of live and dead foetuses, number of corpora lutea, number of implantation sites, number of early and late resorptions. Live foetuses were weighed, sexed, and examined for external anomalies. Foetuses were subject to visceral and skeletal examinations.

All dams survived to scheduled termination, and there were no test article-related effects on clinical signs, food consumption, or maternal body weights or body weight changes as corrected for gravid uterine weight. Conception rates for all treated groups were similar to those of controls, as were post implantation loss rates, numbers of foetuses, and embryo-foetal resorption rates. No abnormal findings were noted on necropsy of the dams.

There were no treatment-related effects on litter sizes, foetal body weights, sex ratios or findings on external examinations, visceral examinations or skeletal examinations of foetuses. The NOAEL was the high dose of 1007 mg/kg bw/day.

5.2 Discussion of available studies

The submitted data are considered adequate to define the hazard of calcium lignosulphonate (40-65) and to establish a reference health standard. Adequate numbers of animals per group were evaluated in the main toxicity studies and appropriate investigations were conducted. The pivotal *in vivo* studies were conducted under GLP conditions.

5.2.1 Absorption, distribution and excretion

Since calcium lignosulphonate (40-65) cannot be readily extracted from tissue or body fluid samples because it readily binds very tightly with proteins through hydrophobic domains it was necessary to use tritiated calcium lignosulphonate (40-65) for these studies. *In vitro* absorption studies, using tritiated calcium lignosulphonate (40-65), were conducted in Caco-2 cell (human colon adenocarcinoma) monolayers. Unfortunately, the well known problem of tritium exchange was encountered which necessitated various strategies, such as size exclusion chromatography and dehydrating tissue and fluid samples, to be used to 'correct' for the exchange.

From the results of the Caco-2 cell monolayer study, it was concluded that no intestinal absorption of calcium lignosulphonate (40-65) was likely to occur and therefore systemic exposure was unlikely. The results of the absorption study in rats are broadly consistent with this conclusion in that the systemic exposure was less than 1%.

Because systemic absorption of calcium lignosulphonate (40-65) is very low, metabolism studies were not conducted.

5.2.2 Single dose toxicity

No single dose study data for calcium lignosulphonate (40-65) were submitted. This is not considered to be a critical omission because adequate data from subchronic studies were submitted.

5.2.3 Repeat dose toxicity

Three repeat dose toxicity study reports were submitted, of which the study considered to be pivotal is a 13-week study.

The Weber and Ramesh (2005) 28-day dietary study, which was GLP-compliant, reported only chronic focal or multifocal inflammation with minimal severity in the rectum of high-dose males at the highest tested dose of calcium lignosulphonate (40-65), an estimated mean daily intake of 3909 mg/kg bw/day. However, this effect was not observed in an earlier 28-day study in which much higher dietary concentrations of calcium lignosulphonate of unspecified grade (resulting in doses of 6400 mg/kg bw/day in males and 6900 mg/kg bw/day in females) were administered (Wolz et al., 2004). In the Wolz study the only finding attributable to administration of calcium lignosulphonate of unspecified grade, was dark faecal discoloration and soft faeces. Dark faecal discoloration was observed in male rats receiving ≥ 5.4 g/kg bw/day and female rats receiving ≥ 5.8 g/kg bw/day. Soft faeces were observed in male rats receiving ≥ 3.6 g/kg bw/day and female rats receiving ≥ 3.9 g/kg bw/day.

In the 13-week GLP-compliant dietary toxicity study treatment-related effects were observed in the mesenteric lymph nodes and the renal tubules. Foamy histiocytes were noted in mesenteric lymph nodes in all treated groups of both sexes, but not in controls. Incidence of this finding increased with dose, and mean severity also increased, although no associated tissue damage was evident, other lymphoid organs were unaffected, and there was no

evidence of changes to total leukocyte numbers, leukocyte differential counts, T cell differentials or primary immune response to sheep erythrocytes. There was limited evidence of regression after a four-week recovery period. Minimal vacuolation of renal tubules was found in the males at 2000 mg/kg and in females at 1000 and 2000 mg, with an increase in incidence but not mean severity observed in the females with increasing dose. No evidence of regression after a four-week recovery period was observed. There was no evidence of inflammation or degenerative changes in renal tissue, and no evidence of impairment of renal function as measured by clinical pathology parameters. The conclusion that these changes are non-adverse is therefore accepted. It is noted that these findings indicate that some systemic absorption of calcium lignosulphonate (40-65) does occur.

Foamy histiocytosis is not unique to calcium lignosulphonate (40-65), but has been found in other studies of polymers administered in the diet or by oral gavage. Reports of chronic oral studies of polymers in which similar changes were found were included in the application. Polypentosan sulphate sodium (Elmiron®), which has a molecular weight between 1500 and 4000 Da, was administered by oral gavage to rats in GLP-compliant chronic and chronic/carcinogenicity studies. Vacuolated histiocytes were observed in lymph nodes and other tissues in the 3-month study, and also in the 2-year chronic/carcinogenicity study, but there was no evidence that they led to neoplastic changes in any organ (NTP 2004). There was no evidence that the accumulations of histiocytes impaired the functions of any organs in which they were found. Accumulations of vacuolated histiocytes were also found in the mesenteric lymph nodes of beagle dogs administered Kollidon VA 64 copovidone in the diet for 52 weeks. Copovidone is a copolymer of vinylpyrrolidone and vinyl acetate, which is supplied in various molecular weight ranges. The molecular weight for Kollidon VA 64 is 45 000–70 000 Da. The histiocytes stained with chlorazol-fast-pink, a test for polyvinyl pyrrolidones. There were no inflammatory or degenerative changes found in association with the histiocytes (Mellert *et al*., 2004).

It is considered unlikely that the foamy histiocytosis of mesenteric lymph nodes or the vacuolation in renal tubules would progress to more adverse lesions with time. It is considered most probable that the foamy histiocytes and the vacuolated renal tubule cells contain relatively insoluble material derived from lignosulphonate. Dosing of rats with material of relatively high molecular weight can cause renal tubular vacuolation that is slow to resolve. Bendele et al (1998) showed that incidence of renal tubular vacuolation was directly correlated with the molecular weight of PEG-linked proteins administered by oral gavage to Sprague Dawley rats, and that such vacuoles were only partially resolved two months after the end of a three-month dosing period.

5.2.4 Genotoxicity

The genotoxicity study data submitted, comprising reverse mutation assays, including metabolic activation, in two bacterial species, and the chromosomal aberration test in Chinese hamster V79 lung fibroblast cells, are considered to be adequate. Under the conditions specified in the studies there was no evidence that calcium lignosulphonate (40-65) had a genotoxic potential.

5.2.5 Carcinogenicity

Although no carcinogenicity or chronic toxicity studies were submitted this was not considered to be a deficiency because it was shown that polymeric calcium lignosulphonate (weight average molecular weight range; M_w=40-65 kDa) is poorly absorbed (<1%), had no evidence of genotoxicity, and did not show any treatment-related pre-neoplastic or neoplastic lesions in the subchronic study.

5.2.6 Developmental toxicity

A GLP-compliant developmental toxicity study in rats showed that calcium lignosulphonate (40-65) at doses up to 1007 mg/kg bw/day had no effects on either dams or foetuses.

5.3 Response to Risk Assessment Question 2

Question: Is there a need to establish a reference health standard for calcium lignosulphonate (40-65) in order to protect public health and safety? If so, what should this be?

An acceptable daily intake (ADI) is necessary based on the available toxicokinetic data. An ADI of 0-20 mg/kg bw (rounded value) for calcium lignosulphonate (40-65) has been established based on a 13-week dietary study in rats that obtained a NOAEL of 1978 mg/kg bw/day for males and 2040 mg/kg bw/day for females. This ADI includes 10-fold safety factors for both intra- and inter-species variability giving an overall 100-fold safety factor. An additional safety factor for the absence of a chronic toxicity study of calcium lignosulphonate (40-65) was not considered to be necessary because of the poor absorption of calcium lignosulphonate (40-65) and the absence of any adverse effects in a 13-week study.

6. Dietary Exposure of Calcium Lignosulphonate (40-65)

A dietary exposure assessment (DEA) estimates the amount of a food chemical consumed by a population, or population sub-group. To assess the risk of the chemical from dietary intake, the estimate is compared to a reference health standard. In the case of calcium lignosulphonate (40-65), this reference health standard is an ADI of less than 20 mg/kg bw/day.

6.1 Approach to estimating the dietary exposure

The approach for this DEA was to use chemical concentration data as proposed by the Applicant combined with food consumption data available from the most recent Australian and New Zealand national nutrition surveys. The dietary exposure assessment was conducted using FSANZ's custom built dietary modelling computer program, DIAMOND. A summary of the FSANZ approach to conducting dietary exposure assessments is at Appendix 1. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

6.1.1 Proposed food list and chemical concentration data

The Applicant has indicated the maximum levels of calcium lignosulphonate (40-65) that, if permitted, would occur in foods available in Australia and New Zealand if calcium lignosulphonate (40-65) was used as a carrier of fat soluble vitamins (A[1],D,E and K) and carotenoids[2]. This DEA is based on the nominated foods and calcium lignosulphonate (40-65) levels provided by the Applicant. The requested range of foods and associated maximum concentrations of calcium lignosulphonate (40-65) are detailed in Table A1 of Appendix 1.

[1] Permitted forms of vitamin A in the Code include the *Retinol Forms:* Vitamin A (retinol), Vitamin A acetate (retinyl acetate), Vitamin A palmitate (retinyl palmitate), Vitamin A propionate (retinyl propionate), and *Carotenoid Forms:* beta-apo-8'-carotenal, beta -carotene-synthetic, carotenes-natural, beta -apo-8'-carotenoic acid ethyl ester.

[2] Permitted forms of carotenoids when used as a colouring include only the carotenoid forms of vitamin A.

For several food groups, permission was requested to add calcium lignosulphonate (40-65) at different levels, for different uses. To ensure conservative (worst case) estimates of dietary exposure, the rounded sum of the highest levels proposed for each use was considered for these food groups. For example, the Applicant requested permission for calcium lignosulphonate (40-65) to be added in "cheese and cheese products", as a carrier for several permitted vitamins. Depending on the vitamin being added, calcium lignosulphonate (40-65) could be used in concentrations of up to 20 mg/kg as a Vitamin A carrier and up to 12.8 mg/kg as Vitamin D carrier. In addition, calcium lignosulphonate (40-65) could be added to cheese and cheese products in concentrations up to 50 mg/kg as a carotenoids carrier (where used as a colouring). For the DEA, the concentration of calcium lignosulphonate (40-65) in cheese and cheese products was assumed to be the sum of maximum use for these proposed permissions, 85 mg/kg (20 + 12.8 + 50 = 82.8, rounded to 85 for the purposes of the DEA).

6.1.2 Population consumption data

This DEA has been conducted for both Australian and New Zealand populations. Food consumption data used were the best available for this assessment, and were extracted from the following four national nutrition surveys:

- The 2007 Australian National Children's Nutrition and Physical Activity Survey (also known as '*Kids Eat Kids Play*') (2007 Aus NCS)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ NCS)
- The 1997 New Zealand National Nutrition Survey (1997 NZ NNS)
- The 1995 Australian National Nutrition Survey (1995 Aus NNS)

Further information on each national nutrition survey is provided in Appendix 1.

The safety assessment did not identify any population sub-group for which there was a specific safety consideration in relation to calcium lignosulphonate (40-65). Consequently, taking into account the data available, estimates of dietary exposure are presented for the following population groups:

- Australians children aged 2 to 6 years (2007 Aus NCS)
- Australians children aged 2 to 16 years (2007 Aus NCS)
- Australian population aged 2 years and above (1995 Aus NNS)
- New Zealand children aged 5 to 14 years (2002 NZ NCS)
- New Zealand population aged 15 years and above(1997 NZ NNS)

Children aged 2-6 years are included as they have the highest food intake on a per kilogram body weight basis, due to their lower body weights and proportionally higher energy needs as they are growing and developing (FSANZ, 2009). Consumption data for this age group were available for the Australian population only.

6.1.3 Assumptions and limitations of the dietary exposure assessment.

Assumptions made in the dietary exposure assessment include:

- where permission for calcium lignosulphonate (40-65) was given to a food classification code, all foods in that group contained calcium lignosulphonate (40-65) at the same concentration (as set out in Table A1 of Appendix 1), whether or not calcium lignosulphonate (40-65) would be used in that food

- where a food has a specified calcium lignosulphonate (40-65) concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient
- where a food was not included in the exposure assessment, it was assumed to contain a zero concentration of calcium lignosulphonate (40-65)
- there are no reductions in calcium lignosulphonate (40-65) concentration from food preparation or due to cooking
- there is no contribution to calcium lignosulphonate (40-65) exposure through the use of dietary supplements. There is currently no permission to use calcium lignosulphonate (40-65) in dietary supplements and the Applicant has advised that the use of calcium lignosulphonate (40-65) as a carrier for fat-soluble vitamins and carotenoids is intended as a food application only.

These assumptions are likely to lead to a considerable over-estimate for calcium lignosulphonate (40-65) dietary exposure.

In addition to the specific assumptions made in relation to this DEA, there are a number of limitations associated with the dietary survey data upon which the DEA is based. A discussion of these limitations is included in Section 6 of the FSANZ publication: *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

6.2 Results: estimated dietary exposure of the Australian and New Zealand populations to calcium lignosulphonate (40-65).

Estimated dietary exposures to calcium lignosulphonate (40-65) were expressed on a per kilogram of body weight basis, taking into account each consumer's food consumption and individual body weight. Calcium Lignosulphonate (40-65) dietary exposures were also expressed as a percentage of the 20 mg/kg bw/day ADI.

6.2.1 Dietary exposure for each population group assessed

Mean estimated exposure ranged from 1 mg/kg bw/day (Australian population aged 2 years & above and New Zealand population aged 15 years and above) to 4 mg/kg bw/day (Australian population aged 2 to 6 years). The estimated exposure for high consumers at the 90th percentile of exposure ranged from 2 mg/kg bw/day (New Zealand population aged 15 years and above) to 6 mg/kg bw/day (Australian population aged 2 to 6 years). The dietary exposure estimate for each population group assessed is detailed in Table A2 of Appendix 1. These results are similar to estimated dietary exposures to calcium lignosulphonate (40-65) calculated by JECFA. The dietary exposure assessment for European populations conducted by JECFA estimated that "the maximum potential dietary exposure to calcium lignosulphonate (40–65) was low and not expected to exceed 7 mg/kg bw/day from use as a carrier of fat-soluble vitamins and carotenoids in food and supplements" (JECFA, 2008).

Comparisons with the ADI are summarised in Figure 7, with the detailed assessment provided in Table A2 of Appendix 1. Mean estimated exposures ranged from 5% (for the New Zealand population aged 15 years and above) to 18% of the ADI (Australian population aged 2 to 6 years). For these same population groups, estimated exposures for the 90th percentile "high consumers" ranged from 9% to 28% of the ADI, respectively.

Importantly, there was no National Nutrition Survey respondent whose dietary exposure to calcium lignosulphonate (40-65) would exceed the ADI even with the conservative assumptions made in this DEA.

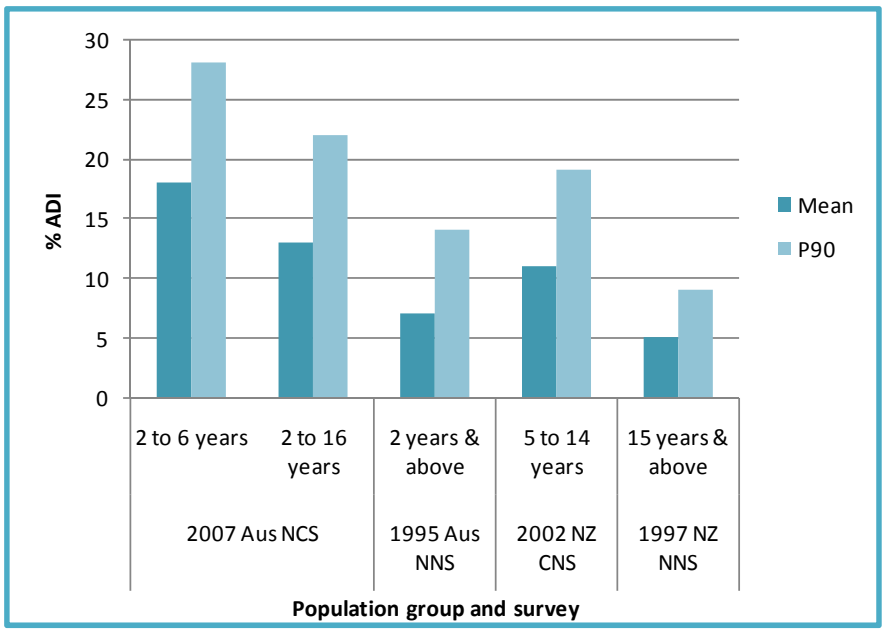

Figure 7: Mean and 90th percentile estimated dietary exposure to Calcium lignosulphonate (40-65) as a proportion of the ADI (20 mg/kg bw/day) for all population groups assessed

6.2.2 Major foods contributing to calcium lignosulphonate (40-65) exposure

Major foods contributing to calcium lignosulphonate (40-65) estimated dietary exposure were calculated from consumers' mean intake of foods proposed to contain the additive. These data give an indication only of the food groups most likely to contribute to dietary exposure, as an assumption has been made that all foods in a food group would contain calcium lignosulphonate (40-65) at the maximum permitted level. It is unlikely that this would be the case under normal food consumption conditions.

For the purposes of illustrating the major foods predicted to contribute to total calcium lignosulphonate (40-65) exposure, similar foods were combined. For example all food codes relating to milk, flavoured milk, yoghurt, cream, cheese etc were combined under the group 'dairy products'.

Major food groups contributing to the estimated calcium lignosulphonate (40-65) dietary exposure for each population group assessed are summarised in Figures 8 a-e, and Table A3 of Appendix 1. Three broad food groups, "Cereal & cereal products", "Non-alcoholic beverages" and "Breads and bakery products" contributed the most to the estimated dietary exposure to Calcium Lignosulphonate (40-65) for all population groups assessed, although in different proportions for each group. Non-alcoholic beverages group was the highest contributor to dietary exposure for Australians aged 2-16 years (26%), Australians aged 2 years and above (31%), and New Zealanders aged 5-14 years (27%), while the cereals and cereal products group was the highest contributor for Australian children aged 2-6 years (25%) and breads and bakery products were the highest contributor for New Zealanders aged 15 years and above (29%).

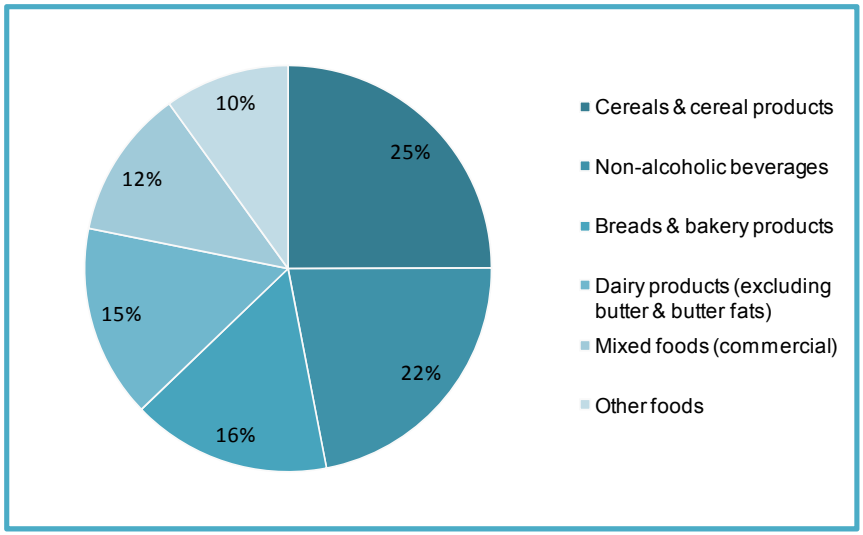

Figure 8a: Major contributors to estimated dietary exposure for Australian children aged 2-6 years

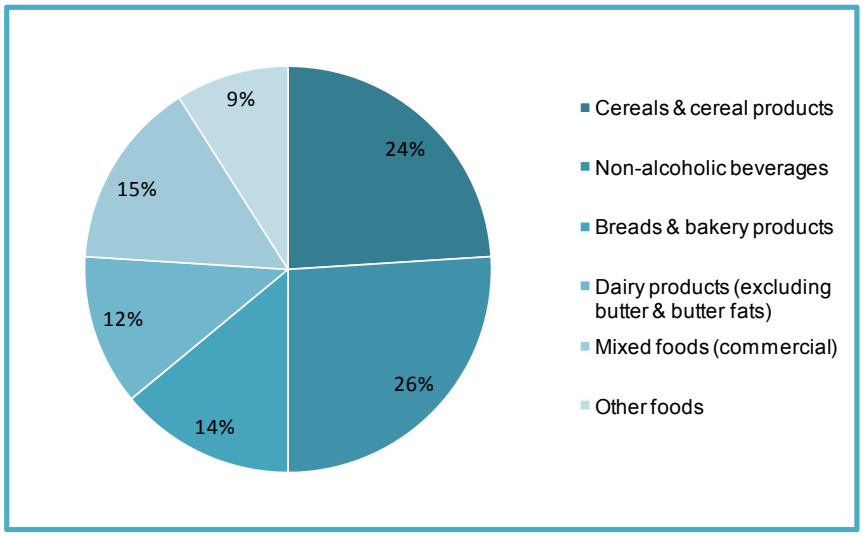

Figure 8b: Major contributors to estimated dietary exposure for Australian children aged 2-16 years

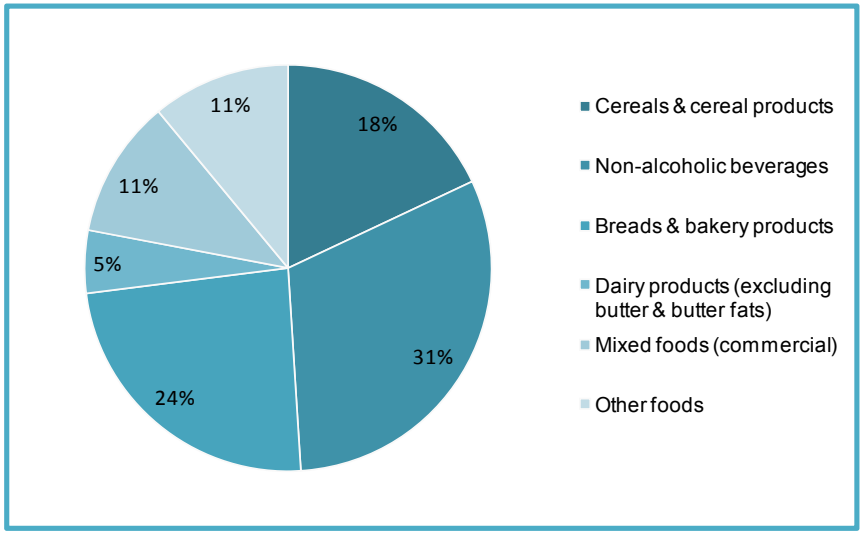

Figure 8c: Major contributors to estimated dietary exposure for Australians aged 2 years and above

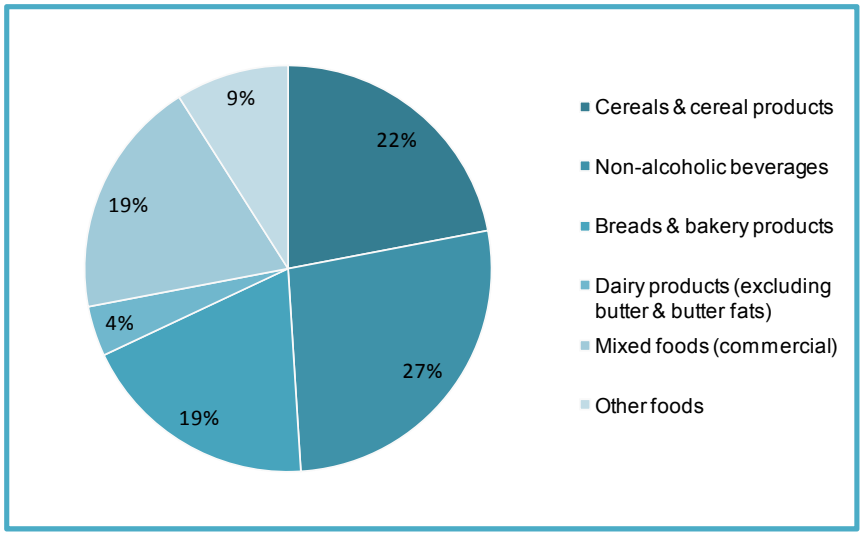

Figure 8d: Major contributors to estimated dietary exposure for New Zealand children aged 5-14 years

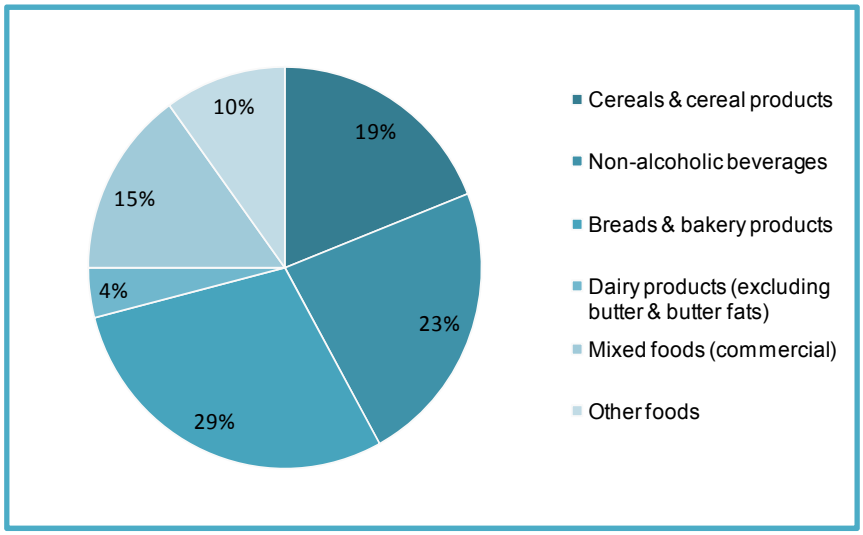

Figure 8e: Major contributors to estimated dietary exposure for New Zealand adults aged 15 years and above

6.3 Response to Risk Assessment Question 3

Question: If a reference health standard is established for calcium lignosulphonate (40-65), then the following questions also apply:

- *What is the estimated dietary exposure to calcium lignosulphonate (40-65) for the Australian and New Zealand populations?*
- *Will Australian and New Zealand population intakes of calcium lignosulphonate (40-65) exceed the reference health standard as a result of this Application?*

Predicted dietary exposures to calcium lignosulphonate (40-65) (Tables A2 and A3 in the Appendix) were low using the proposed food groups and concentration data provided by the Applicant, and the best available consumption data for the Australian and New Zealand populations. Predicted mean dietary exposures were less than 20% of the reference health standard of 20 mg/kg bw/day for all population groups assessed, while 90th percentile exposures were less than 30% of the reference health standard for all population groups assessed. These estimates were based on very conservative assumptions so as not to underestimate the potential exposure.

Given the conservative nature of this dietary exposure assessment, and the low exposures that have been obtained, FSANZ does not expect that intakes will exceed the ADI for calcium lignosulphonate (40-65).

7. Nutritional Safety

Calcium lignosulphonate (40-65) is proposed for use as a carrier of various fat-soluble nutrients (carotenoids, vitamins A, D, E and K). FSANZ has therefore undertaken an assessment to determine any adverse nutritional outcomes associated with the use of this carrier.

Specifically, there are three aspects of human digestion that require investigation to determine the potential for adverse nutritional outcomes. These are whether:

- the use of calcium lignosulphonate (40-65) as a carrier allows for the release and luminal digestion of the carried fat-soluble vitamins and carotenoids.
- calcium lignosulphonate (40-65) binds to or interacts with any fat-soluble nutrients that may be consumed concurrently from other dietary sources.
- there is any impairment of the gastrointestinal absorption of fat-soluble nutrients carried by calcium lignosulphonate (40-65).

These aspects are discussed in the sections below.

7.1 Release of carried fat-soluble vitamins and carotenoids in the gastrointestinal system

As discussed previously, the technological action of calcium lignosulphonate (40-65) is to emulsify and disperse oil droplets (that contain fat-soluble vitamins) in water-based foods. As a result, the carried nutrients are presented into the gastrointestinal system in a structure where the oil particle is surrounded by an oil-water interface membrane composed of calcium lignosulphonate (40-65).

This situation is similar to the usual gastrointestinal presentation of fat-soluble nutrients that are available from the dietary sources. Once fat-soluble nutrients (including vitamins and pro-vitamin compounds) are released from a food matrix during digestion, they preferentially migrate and solubilise into any available hydrophobic medium (Deming and Erdman, 1999; Institute of Medicine, 2000a; Institute of Medicine, 2000b; Institute of Medicine, 2001). The concurrent presence of fat in a meal is especially important for providing lipid particles that act as this solubilising medium. As the fat content of a meal increases, there is a proportional increase in the absorption of fat-soluble vitamins/carotenoids across the gastrointestinal lumen (Borel, 2003). Since the use of calcium lignosulphonate (40-65) requires that the carried vitamins/carotenoids are already solubilised into an oil particle, it is therefore likely that these nutrients would also be available for gastrointestinal absorption in a manner similar to non-carried fat-soluble vitamins/carotenoids.

Once fat-soluble vitamins/carotenoids are solubilised into a lipid medium, bile salts and lipases then act on these lipid particles to form micelle structures, which are then taken up by the small intestinal lumen (along with the solubilised nutrients). It is unlikely that the presence of calcium lignosulphonate (40-65) would adversely affect this process, because a recent *in vitro* study demonstrates that oil/water interface membranes (such as those created by calcium lignosulphonate (40-65)) do not impair the digestive actions of pancreatic lipases or the micelle-forming action of bile salts (Ribero *et al*., 2010). This study simulated the effects of a two-compartment environment (pH, electrolyte and enzymatic environment of the

stomach and small intestine) on emulsified lipid particles versus dispersed lipid particles, and showed that after an 8 hour incubation time, the liposome size was lower and more dispersed with the addition of the emulsifying agent (0.1µm versus 0.8µm).

In respect to other fat-soluble nutrients that are released from a food matrix in the presence of calcium lignosulphonate (40-65), the chemistry described in Section 4 of this risk assessment indicates that it may be possible, although unlikely, that they could be captured within the calcium lignosulphonate (40-65) bound oil mixture. Because fat-soluble nutrients migrate to available lipid sources, it is expected that a calcium lignosulphonate/oil matrix would provide an attractive medium to these nutrients. However, as described above, it is expected that bile salts will still be able to act on these lipid-calcium lignosulphonate structures, and so will be able to direct even dietary sources of fat-soluble nutrients from these structures into micelles.

7.3 Gastrointestinal absorption of carried fat-soluble nutrients

FSANZ sought data specifically on calcium lignosulphonate (40-65) as it relates to the gastrointestinal absorption of the carried fat-soluble nutrients, to determine if the considerations (above) on the release of these nutrients translate into quantifiable physiological outcomes. In response, the Applicant supplied FSANZ with one unpublished animal feeding study that provides data on a carotenoid dispersed by calcium lignosulphonate (40-65) into a water-based medium. FSANZ's own literature searches have been unable to locate any other studies that examine the use of calcium lignosulphonate (40-65) and the gastrointestinal absorption of fat-soluble nutrients (see Appendix 2 for details and outcomes from FSANZ's search strategy).

7.3.1 Animal feed data

Philipps *et al.* (2007) **Study title**: Efficiency of two different apo-ester formulations in broiler pigmentation compared to a control treatment **Report no.** 2500308 **Report date**: 10 January 2007 **Study location**: DSM Nutritional Products Ltd, Switzerland

The study by Philipps *et al.* (2007) is an unpublished 36-day broiler chicken feeding study comparing the intake of a carotenoid using calcium lignosulphonate (40-65) as the carrier in the feed, versus gelatin as a carrier, or no carrier. The aim of the study was to assess whether the different intakes affected the pigmentation of skin and flesh of the chickens. The carotenoid used in the study was 'Carophyll® Yellow', a colouring agent manufactured by the Applicant (DSM). Carophyll® Yellow consists of apocarotenoic acid ethyl ester (CAS 1109-11-1) solubilised into a vegetable oil matrix (DSM Nutritional Products, 2010). Apocarotenoic acid ethyl ester is approved in the *Australia New Zealand Food Standards Code* for general use as a form of β-carotene.

A summary of the study protocol is provided in Figure 9 below. The chickens were provided with a low carotenoid basal diet *ad libitum* for two weeks, and were then divided into seven groups of 20 chickens each: a control (no carrier), and the carotenoid carried by either gelatin or the calcium lignosulphonate (40-65) at three doses (20, 40, and 80 mg/kg of feed). The feed consumption and weight gain were monitored and the feed adjusted for weight variations at days 1, 15 and 36. On day 36 of the trial, half of the chickens per group were randomly selected and labelled, sampled for blood (jugular vein), and then slaughtered at a commercial abattoir. The dorsal skin and abdominal fat were collected on the following day. Each of the plasma, skin and abdominal fat samples were pooled by group allocation and analysed for carotenoids by high pressure liquid chromatography (HPLC).

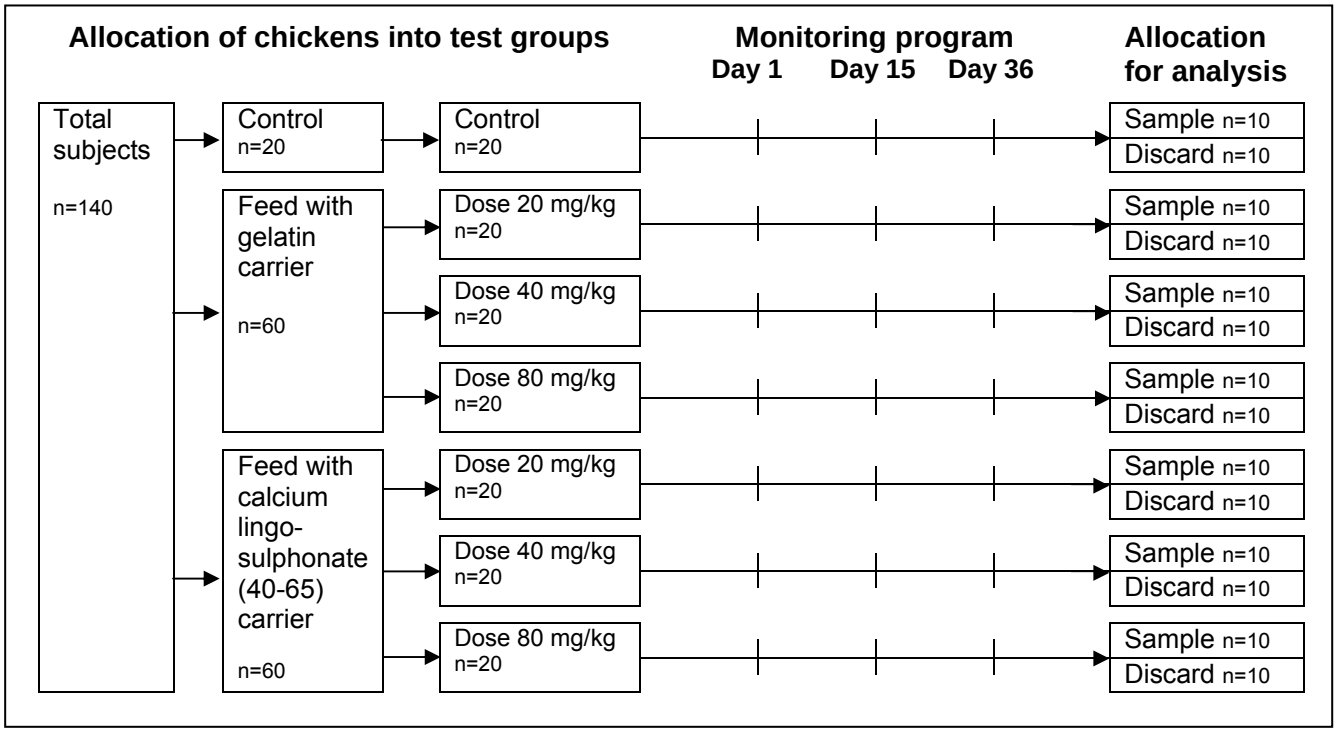

Figure 9: Summary of the study protocol for Philipps et al. (2007)

The results from Philipps *et al.* (2007) are presented in Table 10. The results from the abdominal fat, skin and plasma samples all show a linear dose-response for both the gelatin and calcium lignosulphonate (40-65) carrier. There was no statistical difference in these linear responses in an analysis of variance across the two different carrier groups (p=0.16 skin, p=0.75 abdominal fat, p=0.98 plasma).

Table 10: Comparison of the distribution of apocarotenoic acid ethyl ester using either calcium lignosulphonate (40-65) or gelatin as a carrier in the feed of chickens

Treatment Group	Dose of apocarotenoic acid ethyl ester (mg/kg feed)	Skin (mg/kg sample)		Abdominal fat (mg/kg sample)		Plasma (mg/kg sample)	
		Concentration	±SD	Concentration	±SD	Concentration	±SD
Control	0	<0.01	-	<0.01	-	<0.01	-
Gelatin 20	20	3.30	0.2	3.44	0.4	10.70	0.0
Gelatin 40	40	6.34	0.3	6.63	0.1	23.20	0.7
Gelatin 60	80	11.85	1.1	13.00	1.7	38.35	11.0
CL 20	20	2.74	0.3	2.96	0.7	10.09	1.3
CL 40	40	5.82	0.2	6.97	0.6	20.00	2.6
CL 60	80	11.55	0.5	12.60	1.3	42.40	5.4

CL = calcium lignosulphonate (40-65) group

7.3.2 Discussion

The study by Philipps *et al.* (2007) shows that the intake of a carotenoid carried by calcium lignosulphonate (40-65) results in the same physiological uptake of the carotenoid as occurs with the use of another standard carrier preparation (gelatin). Unlike standard studies on bioavailability, there are no data on the immediate physiological responses (e.g. post-prandial) to the ingested caroteinoids, as the study was not designed to specifically test for gastrointestinal absorption (the aim was to assess tissue pigmentation).

As Philipps *et al.* (2007) is a study on chickens, its applicability to humans has been assessed by FSANZ. Chickens are able to absorb carotenoids (especially β-carotene) from their gastrointestinal systems at a more efficient rate than humans and are able to convert greater proportions of these carotenoids into retinol (Baker, 2008; Biehler and Bohn, 2010; van Vliet, 1996). Chickens also utilise the liver as a storage site for carotenoids, which is not the case for humans (Biehler and Bohn, 2010). However, like humans, chickens also use adipose tissues as carotenoid storage sites, and distribute absorbed carotenoids through the lipoprotein transport system to these sites in a similar manner (Parker, 1996). Therefore the use of a chicken model in this instance is considered to be relevant for human comparisons, given the similar distribution and storage of carotenoids, and that Philipps *et al.* (2007) investigates the distribution endpoints rather than the metabolic endpoints of absorbed carotenoids (e.g. retinol activity).

The higher standard deviations experienced with the plasma endpoint in Philipps *et al.* (2007) are also consistent with human plasma responses, as the circulating levels of carotenoids (especially β-carotene) are known to vary between human individuals when consumed in the same amount (Parker *et al.*, 1999).

7.4 Response to Risk Assessment Question 4

Question: Are there any adverse nutritional outcomes associated with the Applicant's proposed use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients?

- *Does calcium lignosulphonate (40-65) release the carried fat-soluble nutrient for use by the human body?*
- *Will calcium lignosulphonate interfere with the normal digestion and absorption of fat-soluble nutrients that are consumed concurrently from other dietary sources?*
- *Will the gastrointestinal absorption of fat-soluble nutrients be impaired by the use of calcium lignosulphonate (40-65) as their carrier?*

Data from a suitable animal model show that the use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients is likely to result in the same gastrointestinal absorption of these nutrients as occurs with the use of another common carrier agent. There is also indirect evidence suggesting that calcium lignosulphonate (40-65) presents fat-soluble nutrients to the gastrointestinal system in an arrangement that allows for normal digestion and absorption of these nutrients.

The use of calcium lignosulphonate (40-65) could result in the capture of fat-soluble nutrients from other dietary sources into a calcium lignosulphonate (40-65) oil mixture. However, this scenario is unlikely to result in any interference with the normal digestion and absorption of these nutrients.

On the basis of this evidence, FSANZ concludes that the use of calcium lignosulphonate (40-65) as a carrier of fat-soluble nutrients is unlikely to result in any adverse nutritional outcomes.

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Appendix 1

Dietary Exposure Methodology

A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, consumes. Dietary exposure to (or intake of) food chemicals is estimated by combining food consumption data with food chemical concentration data. The process of doing this is called 'dietary modelling'.

Dietary exposure = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating dietary exposure to food chemicals. Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments, FSANZ uses the food consumption data of each person from the national nutrition surveys to estimate their individual dietary exposure. Population summary statistics such as the mean exposure or a high percentile exposure are derived from each individual person's exposure.

An overview of how dietary exposure assessments are conducted and their place in the FSANZ Risk Analysis Process is provided on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/dietaryexposureandin4438.cfm

FSANZ has developed a custom built computer program 'DIAMOND' to calculate dietary exposures. More information on DIAMOND is available on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/fsanzdietaryexposure4439.cfm

Further detailed information on the principles and practices of conducting dietary exposure assessments at FSANZ is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009), available at:
http://www.foodstandards.gov.au/_srcfiles/Principles%20&%20practices%20exposure%20assessment%202009.pdf

A1. Survey Data used by FSANZ

A1.1 2007 Australian Children's Nutrition & Physical Activity Survey (2007 Aus NCS)

The 2007 Aus NCS collected data on nutrition and physical activity for 4,487 children aged 2-16 years across Australia. The survey was conducted over a seven month time period, from February to August 2007.

In the 2007 Aus NCS each respondent completed two 24-hour recalls on non-consecutive days. The availability of two days of food consumption data provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes account of those who may eat a food on one day of the survey but not on the other. Using one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat.

In this assessment, exposure to calcium lignosulphonate (40-65) was estimated from each consumer's average exposures from foods containing calcium lignosulphonate (40-65) across Day 1 and Day 2. The results of the 2007 Aus NCS were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used.

A1.2 1995 Australian National Nutrition Survey (1995 Aus NNS)

The 1995 Aus NNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged from 2 years and above. It is the most recent NNS for Australians aged 17 years and above. The survey used a 24-hour recall method for all respondents, with 10% of respondents also completing a second 24-hour recall on a second, non-consecutive day. Food frequency data are available for a subset of the national sample (respondents aged 12 years and above) as are responses to a series of short dietary questions about food habits. These data are used unweighted in DIAMOND.

A1.3 1997 New Zealand National Nutrition Survey (1997 NZ NNS)

The 1997 NZ NNS provides comprehensive information on the dietary patterns of a sample of 4,636 respondents aged from 15 years and above. The survey was conducted on a stratified sample over a 12 month period. The survey used a 24-hour recall methodology with 15% of respondents also completing a second 24-hour recall with an additional food frequency questionnaire and questions on food consumption patterns. These data are used unweighted in DIAMOND.

A1.4 2002 New Zealand National Children's Nutrition Survey (2002 NZ NCS)

The 2002 New Zealand Children's National Nutrition Survey provides comprehensive information on the dietary patterns of a nationally representative sample of 3,275 New Zealand children aged 5-14 years, including sufficient numbers of children in the Mäori and Pacific groups to enable ethnic-specific analyses. The survey was conducted using a 24-hour recall methodology and collected data on dietary supplements as well as foods and beverages. A repeat 24-hour diet recall was obtained from a subsample, which enabled the statistical adjustment of the data to present the 'usual' intake distribution for nutrients by subgroup.

Further information on how the Australian National Nutrition Surveys are used to conduct dietary exposure assessments is available on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/foodconsumptiondatau4440.cfm

Further information on the New Zealand National Nutrition Surveys is available from the New Zealand Ministry of Health website at:
http://www.moh.govt.nz/moh.nsf/indexmh/dataandstatistics-subjects-nutrition

A2. Change in approach for 'high consumers'

Exaggeration occurs at the extremes of consumption, where estimates of dietary exposure are based on food consumption data from one or two days of single 24-hour recall from NNSs. FSANZ has therefore adopted a policy that a high consumer's chronic dietary exposure is best represented by the 90th percentile of exposure. This replaces the previous standard use of the 95th percentile and is in line with international best practice. For further information on the use of the 90^{th} percentile for dietary exposure assessments, refer to the FSANZ information paper: Protecting 'high consumers', available on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/protectinghighconsum4441.cfm

A3. Limitations of dietary exposure assessments

Dietary exposure assessments based on food consumption data from national nutrition surveys provide the best estimate of actual consumption of a food and the resulting estimated dietary exposure assessment for Australian and New Zealand populations. However, it should be noted that national nutrition survey data do have limitations. Further details of the limitations relating to dietary exposure assessments undertaken by FSANZ are set out in Section 6 of the FSANZ document, *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

A4. Requested food groups and maximum concentrations of calcium lignosulphonate (40-65)

The Applicant requested approval to add calcium lignosulphonate (40-65) in a range of foods. These foods were provided in two lists, based on current permissions to add specific fat soluble vitamins (A, D, E and K) and carotenoids when used as a colouring, as set out in Standard 1.3.1 – Food Additives, and Standard 1.3.2 – Vitamins and Minerals, of the *Australia New Zealand Food Standards Code*.

To perform the dietary exposure assessment, foods requested to contain calcium lignosulphonate (40-65) must be matched against food codes used in DIAMOND. As DIAMOND's food codes are marginally different for surveys which occurred after 2008 and for earlier surveys, two series of DIAMOND food codes have been generated for either the 1995 Australian and 1997 New Zealand National Nutrition Surveys, or the 2002 New Zealand and 2007 Australian National Children's Nutrition Surveys.

The Applicant also provided information on the maximum calcium lignosulphonate (40-65) levels for each food group and for each use, as per the current permissions set out in Standards 1.3.1 and 1.3.2. For several food groups, permission was requested to add calcium lignosulphonate (40-65) at different levels, for different uses. The data have been compiled to establish the total concentration of calcium lignosulphonate (40-65) that could potentially occur in the specified foods should all requested uses occur at the maximum level. To calculate the calcium lignosulphonate (40-65) concentrations, all liquids were assigned a density of "1" (1 mL weighs 1 g); the serving size of "Cereals & cereal products" was estimated to be 30 g and that of solid "Foods intended for particular dietary uses" to be 4 g. Estimation of food serving size was based on a conservative review of FSANZ's food labels database.

The two lists of requested permissions provided by the Applicant have been consolidated to form a unique food list detailed in Table A1 below.

Table A1: Applicant's requested food uses, resulting calcium lignosulphonate (40-65) concentration and corresponding DIAMOND food codes.

Requested food use	DIAMOND food code		Calcium Lignosulphonate requested concentration
	1995 Aus NNS 1997 NZ NNS	2002 NZ NCS 2007Aus NCS	(mg/kg)
Liquid milk prod & flavoured liquid milk	1.1.2	1.1.2	55
Fermented & rennetted milk prod, flavoured	1.2.1, 1.2.2	1.2.1, 1.2.2	
Condensed milk & evaporated milk	1.3	1.3	
Cream & cream products	1.4.2	1.4.2	70
Dried milk, milk powder, cream powder	1.5	1.5	55
Cheese & cheese products	1.6	1.6	85
Butter	2.2.1.1	2.2.1.1	85
Ice cream & edible ices	3	3	50
Processed fruit & veges	4.3	4.3	85
Confectionery	5.2, 5.4	5.2, 5.4	50
Cereals & cereal products	6	6	160
Breads & bakery products	7	7	125
Meat & meat products	8.2, 8.3, 8.4, 8.5	8.2, 8.3, 8.4, 8.5	50
Fish & fish products	9.2, 9.3, 9.4	9.2, 9.3, 9.4	50
Sugars, honey & related products	11.1.1, 11.4	11.1.1, 11.4	50
Salts & condiments	12.1.2, 12.1.3, 12.6	12.1.2, 12.1.3, 12.6	50
Foods intended for particular dietary uses	13.3.1, 13.3.3, 13.4.1	13.3.1, 13.4.1	2030 (dry powders)
	13.3.2, 13.3.4, 13.4.2	13.3.2, 13.4.2	65(ready to consume)
Non-alcoholic & alcoholic beverages	14.1.0.1, 14.1.0.2, 14.1.1.2, 14.1.2, 14.1.3, 14.1.5.6, 14.2.3, 14.2.4, 14.2.5, 14.3	14.1.1.2, 14.1.2, 14.1.3, 14.1.4, 14.1.7, 14.1.8, 14.2.3, 14.2.4.1, 14.2.5, 14.3	65
Mixed Foods Commercial	20	20	50

Table A2: Estimated dietary exposure to calcium lignosulphonate (40-65)

Survey & age group	Number of consumers*	% Consumers/ Respondents#	Exposure (mg/kg bw/day)		% ADI (ADI = 20mg/kg bw/day)	
2007 Aus NNS			Mean	P90	Mean	P90
2 to 6 years	1463	100	4	6	18	28
2 to 16 years	4487	100	3	4	13	22
1995 Aus NNS						
2 years & above	13843	100 (99.89)	1	3	7	14
2002 NZ NCS						
5 to 14 years	3275	100	2	4	11	19
1997 NZ NNS						
15 years & above	4622	100 (99.69)	1	2	5	9

* Consumers only include the people who have consumed a food which would contain calcium lignosulphonate (40-65)
# respondents include all members of the survey population, whether or not they consumed food which would contain calcium lignosulphonate (40-65)

Table A3: Percentage contribution of major food groups to estimated calcium lignosulphonate (40-65) exposure

	Australia			New Zealand	
Food Group	2-6 YO	2 to 16 YO	>2YO	5-14 YO	>15 YO
Cereals & cereal products	25	24	18	22	19
Non-alcoholic beverages	22	26	31	27	23
Breads & bakery products	16	14	24	19	29
Dairy products (ex. butter & butter fats)	15	12	5	4	4
Commercial Mixed Foods	12	15	11	19	15

Calculated from the proportion (% of total exposure) to which all food groups contributing at least 10% of the total estimated exposure for any population group.

Appendix 2

Nutritional Safety Literature Search Details

Search strategy for studies or data relating to the gastrointestinal absorption fat-soluble nutrients carried by calcium lignosulphonate